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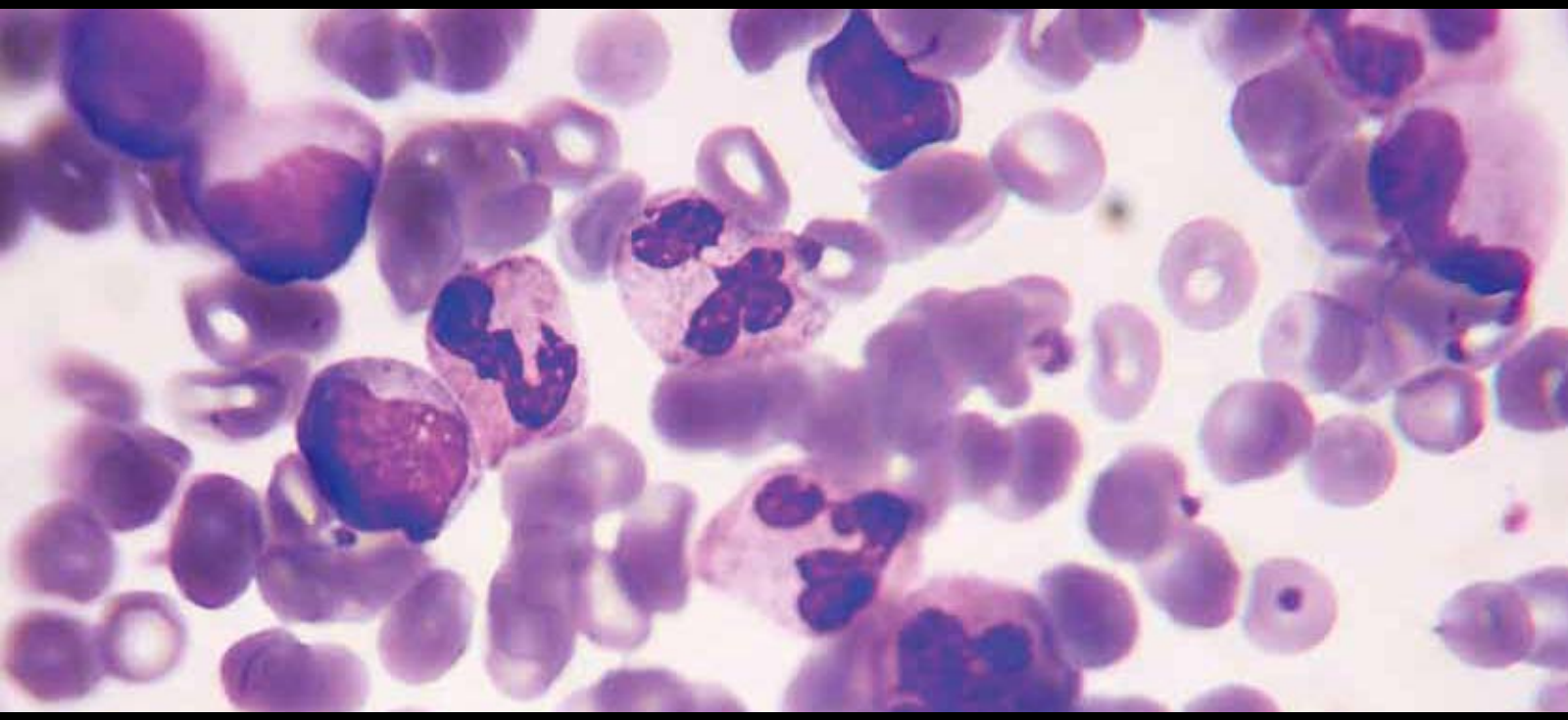
Molecular Pathology of Gastrointestinal Cancer

Guest Editors: Rhonda K. Yantiss, Alyssa M. Krasinskas, Wade Samowitz, and Brian Rubin



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Review Article

Serrated Polyposis: An Enigmatic Model of Colorectal Cancer Predisposition

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Serrated polyposis has only recently been accepted as a condition which carries an increased personal and familial risk of colorectal cancer. Described over four decades ago, it remains one of the most underrecognized and poorly understood of all the intestinal polyposes. With a variety of phenotypic presentations, it is likely that serrated polyposis represents a group of diseases rather than a single entity. Further, neoplastic progression in serrated polyposis may be associated with premature aging in the normal mucosa, typified by widespread gene promoter hypermethylation. From this epigenetically altered field, arise diverse polyps and cancers which show a range of molecular features. Despite a high serrated polyp count, only one-third of colorectal cancers demonstrate a *BRAF* V600E mutation, the molecular hallmark of the canonical serrated pathway, suggesting that though multiple serrated polyps act as a marker of an abnormal mucosa, the majority of CRC in these patients arise within lesions other than BRAF-mutated serrated polyps.

1. Introduction

Serrated polyposis [1], a condition also known as hyperplastic polyposis, was described in the early seventies [2] and remains to the present day the most under-recognized and least understood of the colorectal polyposes. Its defining characteristic, specifically the presence of numerous colorectal epithelial polyps with serrated architecture (Figure 1, Text Box 1), placed it, until relatively recently, among conditions without significant clinical consequence, based upon the perception that all serrated polyps were innocuous. For decades, the malignant transformation of conventional adenomas was considered to be the single mechanism underlying the genesis of colorectal cancer (CRC) [3]. In the late nineties, a number of important observations set in motion a major paradigm shift in the way the initiation and progression of CRC were viewed. These observations carefully detailed at a molecular level that some serrated polyps may act as the precursor lesions in an alternative developmental pathway for CRC, existing alongside the traditional adenoma-carcinoma sequence [4]. As with the histological observations suggesting that a subset of serrated polyps may develop features associated with malignancy [5–7], clear molecular evidence for the malignant transformation of serrated polyps was also first observed in a patient with serrated polyposis [8], suggesting that this condition could serve as a model for the malignant conversion of serrated polyps, a mechanism which has become known as the *serrated pathway*. Today the clinical significance of serrated polyposis rests upon consistent observations in relatively large studies of an increased personal and familial risk of CRC [9–12]. To include serrated polyposis as a CRC predisposition is a concept whose time has come [13].

2. Definition and Features

Clinical criteria for the recognition of serrated polyposis were first established in 2000 [14] and were proposed for

Figure 1: Colectomy specimen from a patient with serrated polyposis showing multiple flat polyps on mucosal folds, measuring less than 10 mm, distributed throughout the colon (courtesy of Dr. Andrew Clouston, Envoi Pathologists, Brisbane).

two major reasons. Firstly, to delineate it from the clearly penetrant and clinically severe condition familial adenomatous polyposis (FAP). Secondly, necessarily stringent criteria ensured that diminutive serrated polyps observed relatively often in the distal colorectum of older patients, including those which cluster around rectal cancers, were not included in the definition [15, 16]. In serrated polyposis, the serrated polyps demonstrate features that distinguish them from sporadic serrated polyps in that they are unusually numerous. They also may be large and proximal and may exhibit atypical histological architecture. While large polyps are preferentially located in the proximal colon, small sessile polyps are often distributed throughout the colorectum [14, 17]. An example of the gross appearance of a serrated polyposis colon is shown in Figure 1.

The current revised criteria, published in 2010 [1], are

(1) at least five serrated polyps proximal to the sigmoid colon with two or more of these being >10 mm,

(2) any number of serrated polyps proximal to the sigmoid colon in an individual who has a first-degree relative with serrated polyposis,

(3) >20 serrated polyps of any size, but distributed throughout the colon. The implied meaning of this last criterion is that the polyps are not all present in the rectum (Text Box 2).

The individual criteria to define serrated polyposis have been difficult to delineate due to the phenotypic plasticity of this condition, which overlaps with the occurrence of common sporadic serrated polyps found in the population. Serrated polyposis may represent a group of diseases or a continuum which is influenced by a variety of genetic and environmental modifiers, rather than a single disease. Therefore, at the time of writing, the current criteria remain empirical in nature [1].

When establishing the serrated polyp count for an individual, Higuchi and Jass have suggested that the polyp count be cumulative over time [18]. Serrated polyposis affects both sexes and is most likely to be identified in persons aged between 55 and 65 years. It may become apparent considerably earlier [7, 19–21], particularly if symptoms such as bleeding, bowel habit alterations, and abdominal pain result from the polyp burden or advanced neoplasia. The range of ages at presentation in the literature varies from 11 to 83 years [7, 19–21].

Estimates of the prevalence of serrated polyposis suggest that it is relatively rare. A large population-based screening trial of over 40,000 asymptomatic patients aged 55–64 years prospectively identified serrated polyposis at a rate of 1 in 3000, with 50% of these demonstrating at least one synchronous conventional adenoma [22]. Rubio et al. reported that only 10 cases were observed in a 1026-bed Scandinavian hospital over a 16-year period [23], whilst Leggett et al. identified 12 cases from a similar institution during a 5-year period [24]. A family history of CRC is a relatively frequent finding with figures of up to 59% reported [25–27], though other publications suggest that this is rare [28, 29]. A recent large multicentre study from The Netherlands has estimated the risk to first-degree relatives of CRC to be approximately fivefold that of the general population adjusted for demographic variables: RR 5.4 (95% CI 3.7 to 7.8) [10]. Studies of ethnicity suggest that, in contrast to conditions such as FAP and Lynch syndrome which can occur in many ethnic groups, serrated polyposis is a condition largely of north-western Europeans. Observations from a multiethnic New Zealand gastroenterology service demonstrated that, in a 24-case series of serrated polyposis patients, all cases were derived from the European component, despite only 46% of the attending patients having European ancestry [17]. Buchanan et al. reported a prevalence of 95% northern Europeans in a case series of 126 serrated polyposis patients [11]. Similarly, Kalady et al. reported that, in a large series of serrated polyposis patients ($n = 115$) collected in Ohio, 97% were white [27]. The fundamental defect in serrated polyposis has yet to be elucidated and may involve defects in inflammation and/or apoptosis. The involvement of widespread DNA methylation in the normal mucosa of patients with this condition [30] suggests deregulation of an epigenetic control mechanism, either directly or as a consequence of upstream genetic events.

3. History of Its Recognition as a Colorectal Cancer Predisposition

Described in some earlier reports primarily in order to distinguish it from FAP, serrated polyposis was originally considered to have no important clinical consequences [29]. However, today it is recognized as a condition with substantial risk of CRC. The early literature contains much to interest those who study serrated polyposis, and the serrated pathway to carcinoma in general. Serrated polyposis was described as early as 1970 by Goldman et al. [2]. In this case report,

Epithelial polyps with serrated architecture arising in the large intestine were until very recently collectively known as hyperplastic polyps. A modern classification has now been proposed which uses the descriptive umbrella term serrated polyps for all epithelial polyps with serrated architecture and the term hyperplastic polyps for the subset of small common lesions without evidence of abnormal proliferation. A detailed description of the WHO classification of serrated polyps is given in Section 3 of this paper.

Text Box 1: Serrated polyps or hyperplastic polyps? [1].

(1) At least five serrated polyps proximal to the sigmoid colon with two or more of these being >10 mm.
(2) Any number of serrated polyps proximal to the sigmoid colon in an individual who has a first-degree relative with serrated polyposis.
(3) >20 serrated polyps of any size, but distributed throughout the colon.

Text Box 2: Clinical criteria for the identification of serrated polyposis [1].

a 42-year-old man presented with 30 serrated polyps ranging in size between 0.75 and 1.5 cm. Of particular interest was the observation of adenomatous transformation within the polyps, a concept now readily accepted, but somewhat controversial in those earlier times. Despite several reports relating serrated polyps to the development of adenomatous change, villous components, and even adenocarcinoma [31–33], the bulk of investigations published around this time returned a classification of nonneoplastic for serrated polyps [3, 34–36].

In 1978, Cooke described serrated polyposis as a variant of FAP [5]. A further report describing cancer and dysplasia in a background of serrated polyposis was presented in 1979 [37]. In this publication, the authors, whilst suggesting that hyperplastic polyps were "benign, nonneoplastic proliferations which unlike tubular and villous adenomas did not predispose the patient to colonic cancer," went on to demonstrate a case of serrated polyposis where hyperplastic adenomatous transformation and cancer "had probably occurred," and recognized that cancer could be associated with unusual cases of multiple hyperplastic polyps [6]. In 1980, seven cases of serrated polyposis were recorded from a London hospital [29], and this became an influential landmark paper in the confusion surrounding serrated polyposis. Six of the seven cases were male, and there was an average age at presentation of 37 years. The presence of larger metaplastic polyps was noted, and the possibility that "metaplastic polyposis" was a pathological entity was raised [29]. With followup, however, no cases of CRC were observed, thereby designating serrated polyposis as a low-risk condition of young males. This paper influenced thinking on serrated polyposis for over a decade. However, during the 1990s association with CRC was revisited by Torlakovic and Snover [7], Burt and Samowitz [38], and Jeevaratnam and colleagues in a familial setting [39] and in a small series of patients who were instrumental in demonstrating the malignant transformation of serrated polyps [8]. Many series and case reports have now been published [5–8, 19–21, 23, 24, 26, 28, 39–60]. In the three largest series published to date, 25–38% of patients presented with at least one CRC [9, 12, 27], and multiple CRCs were common [11]. However, the problem remains that published series are predominantly composed of retrospective clinic-based records, and therefore the estimation of CRC risk is likely to be inflated and to reflect a CRC risk associated with symptomatic patients. In addition, the wide variety of phenotypic presentations within serrated polyposis has the potential to be associated with varying risk magnitudes. At the time of writing, no prospectively collected population-based risk estimates for CRC are available. A summary of the findings in published series of serrated polyposis cases is presented in Table 1.

4. Serrated Polyp Subtypes

Serrated polyps are the second most common type of colorectal polyps, after conventional adenomas, found during population colonoscopy. Serrated polyps are also the most prominent phenotypic feature in serrated polyposis usually ranging in number from 5 to greater than 150, and varying greatly in size. In addition, a diverse range of dysplastic lesions of both conventional and serrated lineages may also be present.

In a study reporting the prevalence of each polyp type diagnosed from 179,111 consecutive population colonoscopies in the United States, Lash and colleagues found that epithelial benign polyps were classified as conventional adenomas in 58.6% and as serrated polyps in 41.4% [62]. The terminology and histologic classification of serrated polyps have been a matter of debate for some years. The most clinically relevant feature is the presence of dysplasia that increases the risk of developing CRC and impacts colonoscopy surveillance intervals. Therefore, the classification of serrated polyps into dysplastic polyps and nondysplastic polyps is the most meaningful division. However, there is still some degree of confusion in diagnosing serrated polyps with reported significant variation in the detection rate and in the histologic classification, justifying the need for increased awareness and education [63]. All of the following subtypes are observed in serrated polyposis.

Table 1: Summary findings in serrated polyposis from publications reporting more than five cases (adapted from Buchanan et al., 2010) [11].

Author	Year	Cases (n)	Mean age at diagnosis (years)	Number of polyps observed	% with CRC	Family history of CRC
Kalady et al. [27]	2011	115	62	2-multiple	25%	38%
Buchanan et al. [11]	2010	126	49	5–150	40%	59%*
Boparai et al. [9]	2009	77	56	2–53	35%	NS
Carvajal-Carmona et al. [25]	2007	32	46	11-multiple	25%	59%
Chow et al. [26]	2006	38	44	20-multiple	26%	50%
Renaut et al. [61]	2001	28	58	20-multiple	29%	39%
Yeoman et al. [17]	2007	24	61	5-multiple	54%	17%
Ferrández et al. [28]	2004	15	53	15-multiple	0%	0%
Lage et al. [48]	2004	14	58	19–100	43%	33%
Hyman et al. [43]	2004	13	62	20-multiple	54%	38%
Rashid et al. [54]	2000	13	58	multiple	77%	38%
Leggett et al. [24]	2001	12	57	30–>100	58%	17%
Rubio et al. [23]	2006	10	61	6–159	70%	10%
Spjut and Estrada [56]	1977	9	53	Multiple	11%	NS
Williams et al. [29]	1980	7	37	50–150	0%	14%
Torlakovic and Snover [7]	1996	6	57	50–100	67%	NS
Place and Simmang [53]	1999	6	60	50–100	50%	14%

NS: not specified or unknown.
*Genetics clinic series.

4.1. Nondysplastic Serrated Polyps. Nondysplastic serrated polyps comprise hyperplastic polyps and sessile serrated adenomas/polyps, representing the vast majority of all serrated polyps. The use of new high-definition endoscopes in association with chromoendoscopy or narrow-band imaging has led to a higher detection of these polyps.

4.1.1. Hyperplastic Polyps (HPs). More than 75% of serrated polyps are HPs [62, 64, 65]. Most often measuring 5 mm or less, HPs are sessile pale lesions, usually found on the tip of mucosal folds in the distal colorectum, with normal architecture and normal proliferation characteristics. In the proximal colon, HPs are often larger and more difficult to visualize. The prevalence of HPs in asymptomatic adults aged 40 years or more has been estimated to be around 10% in Western populations [66–68]. In autopsy studies, the prevalence rate of HPs ranged from 5% in a Cretan study to 40% in a British study [69–71]. While HPs develop at an earlier age than conventional adenomas, its incidence does not seem to significantly increase after 50 years, contrasting with the positive correlation between increased age and the prevalence of conventional adenoma [72].

Histologically, HP is the prototypical example of serrated polyps of the colon with a saw-toothed appearance caused by in-foldings of the crypt lining epithelium in the upper half of the crypts. All types of HP are characterized by elongated crypts, with maturation of cells towards the surface, and proliferation activity limited to the lower portion of the crypts. HPs are further divided into 3 histologic subtypes: microvesicular, goblet-cell, and mucin-poor, without clinical relevance as yet. Microvesicular HP is the most common type, characterized by the presence of columnar cells with abundant apical vesicular mucin and by a decreased number of goblet cells. In contrast, goblet-cell HPs show elongated crypts with numerous goblet cells and minimal serration limited to the most upper portion of the crypts. These polyps may be overlooked and are often underdiagnosed and therefore may occur more frequently than reports suggest. Finally, mucin-poor HPs are very rare and display prominent serration, regenerative changes, and mucin-depleted columnar cells. It is still debated whether mucin-poor HPs are a true separated subtype or an irritated form of microvesicular HPs.

4.1.2. Sessile Serrated Adenomas/Polyps (SSA/P). First designated "serrated polyps with abnormal proliferation" by Torlakovic et al. in 2003, SSA/Ps comprise 15–20% of all serrated polyps [65]. SSA/Ps are flat or slightly elevated lesions most commonly found in the proximal colon and usually measuring more than 5 mm. Histologically, SSA/Ps differ from HPs by the presence of abnormal architectural features secondary to abnormal proliferation. Whereas the proliferative zone in HPs is located in the base of the crypts, it is usually on the sides of the crypts in SSA/Ps, leading

to asymmetrical growth in crypts with an inverted T shape or L shape. Other histologic features of SSA/Ps include the presence of mature goblet cells at the bases of the crypts, hyperserration at the base or throughout the crypts, and pseudoinvasion of the muscularis propria. Dysplasia is not present.

4.2. Dysplastic Serrated Polyps

4.2.1. Traditional Serrated Adenomas (TSAs). TSA is a relatively uncommon polyp, comprising up to 5% of serrated polyps in Western countries with a higher prevalence in Asia, particularly in Korea [73]. Compared to SSA/Ps, TSAs are protuberant polyps more frequently found in the left colon and in older individuals. The architecture of TSA is often more complex than villous or tubulovillous adenoma, with prominent serration and finger-like projections. The presence of ectopic crypt foci in TSAs, defined by the crypts with their base not seated at the level of the muscularis propria, is useful to distinguish them from SSA/P [74]. The neoplastic cells are characterized by an abundant eosinophilic cytoplasm and elongated pencillate nuclei. Dysplasia in TSA is usually mild, with a different appearance of the dysplasia associated with conventional adenoma and low proliferative characteristics. However, conventional adenoma-type dysplasia can also be present and sometimes with high-grade features.

4.2.2. Sessile Serrated Adenomas/Polyps with Dysplasia (SSA/P-D). The presence of dysplastic crypts in a SSA/P was often reported as part of the "mixed polyp" group and is now better recognized as a specific category of dysplastic serrated polyps with malignant potential. In most cases, dysplasia in SSA/P is similar to dysplasia in conventional adenoma and is well demarcated from the nondysplastic areas. Dysplasia in SSA/Ps is rare, found in 14% of all SSA/P in a recent study by Lash et al. [62]. In this study of over 2000 patients, the median age for presenting with a nondysplastic SSA/P was 61, increasing to 66 for SSA/P with low-grade dysplasia, 72 for SSA/P with high-grade dysplasia, and 76 for SSA/P with invasive carcinoma (a span of 15 years). In contrast, the span between tubular adenoma and non-SSA/P carcinoma is only 5 years. Examples of serrated polyp subtypes are given in Figure 2.

5. Phenotypic and Molecular Heterogeneity

Table 2 shows the rates of molecular alterations for the major serrated polyp subtype categories, after adding all available results from various large studies [73, 75–84]. Somatic molecular alterations associated with serrated polyps have been well described in previous publications [54, 76, 79, 85–87] and include *BRAF* (V600E) mutation, *KRAS* (codons 12 and 13) mutations, *MLH1* methylation, *MGMT* methylation, and CpG island methylator phenotype (CIMP). The prevalence of these alterations varies according to the subtype of serrated polyp. *BRAF* mutation is the most common alteration in all polyp types, with the highest rate in SSA/Ps (83.9%) and the lowest rate in goblet-cell HPs (20%). In contrast, *KRAS* mutation is most commonly detected in goblet-cell HPs (48.4%) and TSA (22.4%). *MLH1* methylation ranges from 14.3% in goblet-cell HPs to 47.5% in TSA. Interestingly, while *MLH1* methylation does occur quite frequently, a high-level microsatellite instability phenotype is very rarely encountered in serrated polyps [76, 88]. MSI-H is a late and probably an important pivotal event in the serrated pathway, occurring at the transition between high-grade dysplasia and invasive carcinoma [76, 89]. *MGMT* methylation ranges from 0% in goblet-cell HPs to 74.2% in TSA. A high level of CIMP, defined by ≥2/5 methylated markers, occurs in 39% of HPs, 76% of SSA/Ps, and 79% of TSA [76].

The question of phenotypic heterogeneity in serrated polyposis can be further considered with an examination of the reported molecular changes of serrated polyps as they apply to the syndromic patient. Though there appear to be at least two phenotypic subtypes which correspond to the first and last criteria (Text Box 2), a previous report from the UK clearly demonstrates that the predominant molecular change in the polyps is that of *BRAF* mutation, even in patients with numerous polyps [25], and therefore suggesting that oncogene mutations are of little value in subtyping this disorder. However, rare cases of serrated polyposis are reported where *KRAS* mutations predominate [25], and current evidence suggests that, in at least *some* of these patients, biallelic germline mutation of *MUTYH* may be responsible [25, 26, 90, 91].

Although the possibility of these two types of serrated polyposis was first raised over 10 years ago [92], the application of such a classification to CRC risk may not be readily implemented. Even though large and dysplastic polyps are likely to be an indicator of high malignant potential, the presence of CRC in cases with multiple small HPs [61], as well as the absence of CRC in many cases of serrated polyposis [11, 12] with large and dysplastic polyps, argues against a nonoverlapping classification.

6. Epigenetic Field Defect in Establishment of Neoplasia

CRC in general develops through one of two independent molecular pathways that involve sequences of genomic and epigenomic alterations associated with pathological and clinical features: the adenoma pathway in 70–80% and the serrated pathway in the remaining 20–30%. The somatic molecular features which characterize the serrated pathway to CRC include activating mutations in *BRAF* [81, 83, 86] and widespread hypermethylation of gene promoters (CIMP) [87] with or without MSI [8, 42]. In the serrated pathway, the earliest known event is somatic *BRAF* mutation, found in aberrant crypt foci [93], and with a high rate in microvesicular HP and SSA/P [76, 85]. The hypermature cells of the upper crypt in serrated polyps are thought to result from a mechanism of oncogene-induced senescence brought about by the presence of an activating *BRAF* mutation [94]. Escape from senescence is achieved subsequently

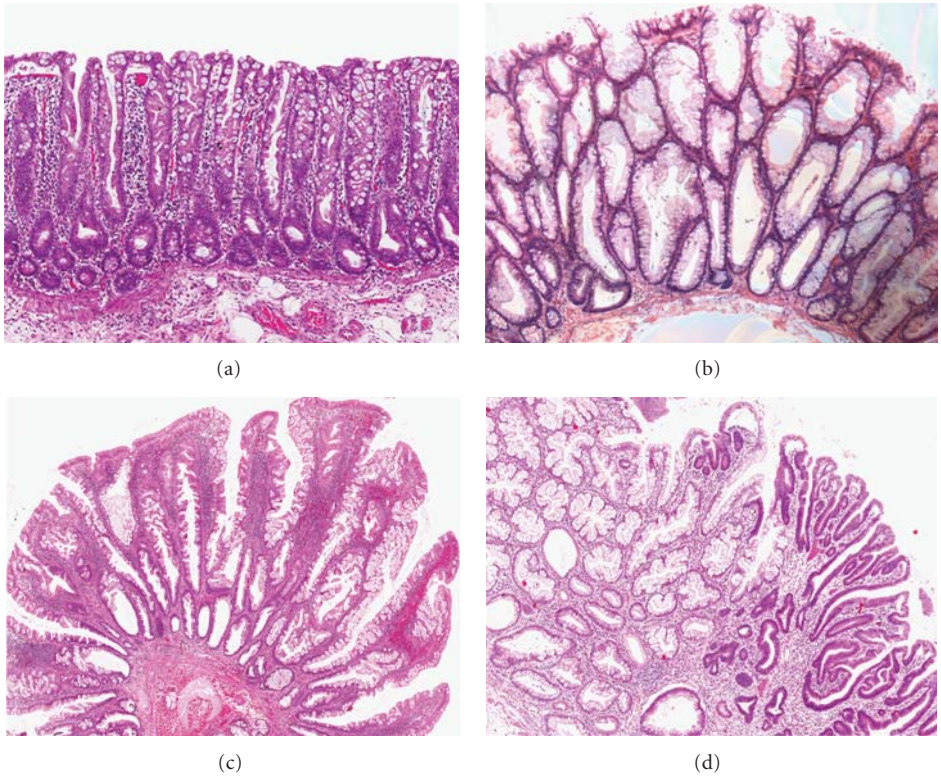


Figure 2: Showing 4 serrated polyp subtypes. (a) Microvesicular hyperplastic polyp with crypt serration and proliferative crypt bases. (b) Sessile serrated adenoma/polyp, showing asymmetrical serrated crypts and dilated crypts. (c) Traditional serrated adenoma, with prominent complex serration and hypereosinophilic cells. (d) Sessile serrated adenoma/polyp with high-grade dysplasia (right).

Table 2: Prevalence of genetic and epigenetic alteration in the different subtypes of serrated polyps by combining all comparable data from [73, 75–84].

Polyp type	*BRAF* mutation	*KRAS* mutation	CIMP-H*	*MLH1* methylation	*MGMT* methylation
HP					
Microvesicular	66.3%	12.3%	47.4%	39.5%	26.3%
Goblet-cell	20%	48.4%	14.3%	14.3%	0%
All subtypes	51.5%	22.1%	38.5%	27%	14.8%
SSA/P	83.9%	5.8%	75.9%	42.6%	25%
TSA	62.5%	22.4%	79.3%	47.5%	74.2%

* CIMP-H: high level of CIMP in polyps defined by ≥2/5 methylated markers from O'Brien et al. [76, 85].

by the inactivation by promoter methylation of tumour suppressors controlling senescence [95], thus allowing a lesion to progress to a more proliferative neoplasm. An epigenetic field defect present in serrated polyposis would facilitate this process more readily [30] with the consequent increase in polyp numbers which define the condition. When multiple lesions are examined, serrated pathway features of *BRAF* mutation and CIMP demonstrate a high rate of concordance between discrete lesions in individuals with serrated polyposis [86, 87]. As would be expected, most CRCs (70%) in serrated polyposis derive from the proximal colon [17]. An exception to this occurs in young-onset patients (<50 yrs old) where the CRCs are more likely to be distal [11, 12, 26]. This is an under-recognized feature of young-onset serrated polyposis though it has been mentioned in previous publications [96].

Hypermethylation of gene promoters is also observed in the normal mucosa of individuals with increasing age [97, 98] and is also more likely to be associated with synchronous proximal CRC with concordant molecular features [99, 100]. However, in serrated polyposis, increased methylation of gene promoters is evident even in the normal mucosa of younger individuals [87, 91, 101], indicating that an epigenetic regulatory defect may be present in the normal tissues of these patients and suggesting a prematurely aged mucosa associated with increased risk for the establishment of neoplasia. Of interest, in 1968, Arthur observed that metaplastic polyps were a marker of age in the normal mucosa

[35]. The concept of an epigenetic field defect in serrated polyposis was clearly demonstrated by Minoo et al. in 2006 [30]. Significantly the level of methylation in apparently normal mucosa was higher in serrated polyposis patients when compared to patients with sporadic serrated polyps.

7. Role of the Conventional Adenoma in Cancer Risk

The complex biology of serrated neoplasia and the plasticity of its developmental pathway can give rise to CRC with variable MSI status [8], and to small numbers of apparently conventional adenomas, in addition to multiple serrated polyps. It has been estimated that conventional adenomas are seen in up to 90% of serrated polyposis patients [23, 24], raising the notion that lesser numbers of conventional adenomas are part of the syndrome. Importantly, the risk for patients with serrated polyposis to present with a synchronous CRC at time of diagnosis is significantly higher when at least one conventional adenoma is present [11, 12, 24].

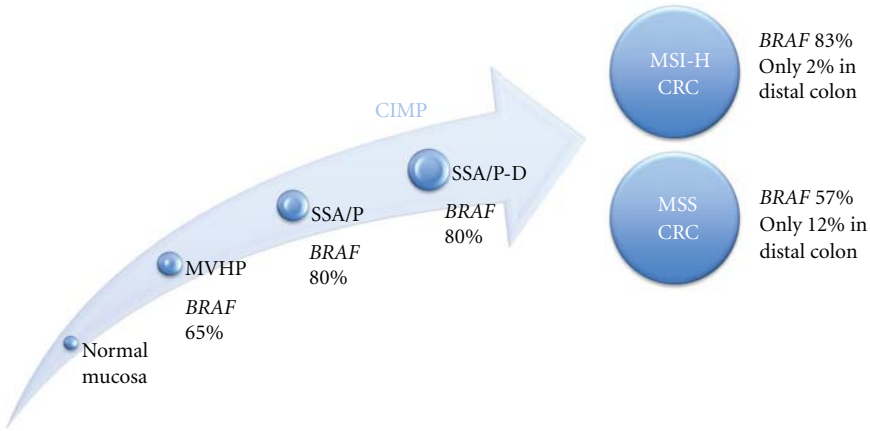
Adenomatous lesions in a serrated polyposis patient may either evolve from serrated polyps, progressing to a conventional type of dysplasia, or arise via an alternate mechanism. The presence of "mixed polyps," as they were previously known (now called SSA/P with dysplasia), which demonstrate a very high rate of somatic *BRAF* mutation (80–90%) [76] and therefore, by implication, origin in a serrated polyp, provides a plausible precursor lesion for the CRC which arises in serrated polyposis [6, 19, 50, 56, 57]. In contrast, conventional adenomas almost never harbor a *BRAF* mutation [84]. If most of the CRCs in serrated polyposis were to arise from advanced serrated polyps, a high rate of *BRAF*-mutated CRC would be expected. CRCs in serrated polyposis have shown somatic *BRAF* mutation in 33% of 6 cases in an early published report [86] and, consistently, in 19/58 (33%) of a recent case series, [102]. Whilst this level is still greater than that of a population series, (which ranges from 7% in southern Europeans to 21% in Anglo-Celts [103]), these observations suggest that the majority of CRCs arising in serrated polyposis develop within lesions not known to be involved in the *canonical serrated pathway* (see Figure 3).

The balance of CRC (BRAF wild-type) in serrated polyposis either demonstrates somatic *KRAS* mutation at a rate of approximately 19% which is half that of the population [102] or is oncogene mutation null. These CRCs may arise, in the manner of common CRC, from the conventional adenomas which frequently coexist in patients with serrated polyposis [17, 23]. Of interest, in reporting the first autosomal dominant family with serrated neoplasia, Jeevaratnam et al. made note of an adenomatous precursor in contiguity with a small CRC [39] and concluded that the CRCs in serrated polyposis arise through both the development of dysplasia in serrated polyps and through coincident conventional adenomas. In 2010, Pai et al. published a report which examined the adenomas which coexisted with SSA/P in the general (non-syndromic) population [105]. They observed that 35% of the polyps removed from population patients with an index SSA/P were conventional adenomas. Pai et al. observed 3 morphologic features which were more prevalent in the study adenomas compared to control adenomas in a population without SSA/P: eosinophilic cytoplasm, focal (rather than widespread) serration, and crypt dilatation. These features were seen in 30% of the study adenomas compared to 2.5% of controls. In addition, these atypical polyps demonstrated low levels of methylation, and increased staining for MUC6, properties more associated with serrated lesions than with adenomas. Importantly, none of these lesions showed *BRAF* mutation. Given previous findings from the same group of authors which demonstrated that patients with an index SSA/P had a significantly increased risk of having other serrated polyps, combined with the presence of atypical conventional adenomas, supports the presence of an epigenetic field defect in serrated polyp patients including those not meeting the criteria for serrated polyposis [105, 106].

Small numbers of serrated polyps as well as CRCs with somatic *KRAS* mutation are also observed in serrated polyposis patients. Somatic *KRAS* mutation straddles the division between serrated and adenomatous polyps and is mutually excluded in lesions bearing *BRAF* mutation [107]. It is observed in goblet-cell HPs [85] which rarely undergo malignant transformation; however, its presence, albeit less frequently, in advanced serrated polyps [84], in rare contiguous serrated polyps attached to population CRC (Young, unpublished observations) and in the lesions present in biallelic mutation carriers for *MUTYH* [90], suggests that serrated lesions with *KRAS* mutations are not completely devoid of malignant potential.

8. The Smoking Paradox and Serrated Polyposis

Risk factors for the development of serrated polyps in the population are similar to risk factors for the development of conventional adenomas, including alcohol consumption, low folate intake, and high body mass index [72, 108]. High calcium intake, hormone replacement therapy, and use of nonsteroidal anti-inflammatory drugs are associated with a reduced risk. Perhaps the most interesting of risk factor associations involves cigarette smoking. The relationship between smoking and colorectal neoplasia has become known as the *smoking paradox* in that smoking is associated significantly with polyps, but its relationship with CRC is much weaker. A consistent observation has been the relationship between smoking and serrated polyps, which has been analyzed in a number of population-based studies [109]. Three independent studies have demonstrated a concordant pattern of higher risk estimates for serrated (hyperplastic) polyps than for conventional adenomas [110–112] in long-term and current smokers. When both serrated polyps and conventional adenomas were present [110–112], risks were higher still. The association of current smoking with serrated polyps begins very early in serrated neoplasia [113] and is the greatest in the distal colon [114], where malignant potential of the serrated polyps is low. The weak association between smoking and CRC has been explained by the relationship being dominated by the serrated pathway subset with *BRAF* mutation [115], which accounts for only 15% of CRC.



Originally the canonical serrated pathway was devised to explain CRC with two highly associated molecular markers, *BRAF* mutation and CIMP. *BRAF* mutation is an early event in serrated polyps throughout the colon; however, CIMP is more highly concentrated in the proximal colon, and therefore most CIMP CRCs are found there. In contrast serrated polyps with *BRAF* mutation in the distal colon rarely progress to CRC. Increasing CIMP in progression to CRC is shown as a pale blue arrow.

Figure 3: The canonical serrated pathway showing progression through MVHP (microvessicular hyperplastic polyp) to SSA/P (sessile serrated adenoma/sessile serrated polyp) and SSA/P-D (SSA/P with dysplasia) to CRC with CIMP and high levels of BRAF mutation frequently arising in the proximal colon. CRC with CIMP can evolve into MSI-H and non-MSI-H subtypes. Though KRAS mutation can be observed in CIMP CRC, these are relatively rare [103, 104].

The role of smoking in serrated polyposis has not been extensively explored. In 2010, several reports added to the puzzle surrounding the smoking paradox. Initially it was demonstrated that current smoking is associated with a significantly higher polyp count in patients with serrated polyposis [11]. Also in 2010, a report suggesting a causative role for smoking was published on a small series of cases and showed a higher prevalence of current smokers amongst serrated polyposis patients than in the population [116]. Given that current smoking is associated with increased serrated polyps [114] and even serrated aberrant crypt foci in the general population [113], both the preceding observations in serrated polyposis patients are reasonable.

Later in 2010, the authors of this current review reported that, even though current smoking was associated with increased polyp numbers, there was no significant effect on the risk of CRC in a case series of 151 patients with serrated polyposis [12], once again highlighting the smoking paradox. Given that the major association between smoking and CRC is largely confined to those CRCs with somatic *BRAF* mutation, and less than one-third of CRCs in serrated polyposis harbor a *BRAF* mutation, this is perhaps not unexpected, as *BRAF*-mutated CRCs constitute a minority of serrated polyposis CRCs. Of interest, however, an unexpected finding emerged regarding currently smoking females, who had a significantly decreased risk of CRC, after correcting for age and adenomas [12] (O.R 0.10, 95% CI 0.02 to 0.47, $P = .004$). Further, female patients who had ever smoked had an average age of onset for CRC of 63 years compared to those who had never smoked (50 yrs). Though the results did not reach statistical significance due to low numbers, a trend for delayed onset of CRC in female smokers was evident. In the population, female smokers with serrated pathway subset CRC are elderly onset.

The preceding observation is consistent with a biological mechanism similar to that reported in ulcerative colitis patients [117] and suggests that perhaps an inflammatory process may be responsible for neoplastic progression in serrated polyposis in a *subset* of female patients and that, similarly to ulcerative colitis, smoking may be anti-inflammatory. In the population, a study of risk factors for serrated polyps demonstrated that aspirin use decreased the risk of advanced proximal polyps, lending indirect support to this hypothesis [114]. Alternatively, confounding factors may be responsible for this finding, including unspecified sex-specific factors related to body mass index (BMI) or hormonal factors, as observed in the protective effects of smoking on endometrial cancer. This finding whilst perplexing cannot be ignored because it could potentially lead to a CRC-preventive modality for female patients with serrated polyposis *independent* of cigarette smoking and its attendant harms.

9. Serrated Polyposis as a Genetic Predisposition Syndrome

Serrated polyposis has many hallmarks of a genetic predisposition. These include an earlier age of onset of CRC, polyp, and cancer multiplicity, increased CRC risk in both patients and their relatives, and restricted ethnicity. An important

clinical consequence associated with serrated polyposis is the increased risk of both CRC [26, 39, 43, 44, 54] and possibly extracolonic cancers [27, 118, 119] in the family setting of serrated polyposis patients. The risk to first-degree relatives of CRC has been estimated at fivefold greater than that of the general population [10]. WHO Criterion 2 (Text Box 2) addresses the evidence that serrated polyposis may occur in a familial context [25, 26, 40, 43, 48, 54, 61] and elevates the significance of smaller numbers of hyperplastic polyps in a first-degree relative of an individual with serrated polyposis. The genetic basis for serrated polyposis is yet to be determined, though small numbers of patients have reported mutations in *MUTYH* [90], *PTEN* [120], and *EPHB2* [121].

Biallelic *MUTYH* mutation is a phenotypically diverse disorder which appears to interact with the genetic background of the individual. In one-third of biallelic mutation carriers, there are no adenomas present [122]. In approximately 1% of patients with serrated polyposis, biallelic mutation of *MUTYH* can be demonstrated [123]. Conversely, when biallelic *MUTYH* mutation carriers are assessed, 18% meet the WHO criteria for serrated polyposis [90]. A recent report from Buchanan et al. [124] suggests that individuals with both *MUTYH*-associated polyposis (MAP) and serrated polyposis may be segregating two conditions with diverse modes of inheritance. The report describes a 56-year-old Caucasian male with >100 colonic polyps (approximately 50 conventional 10–15 mm adenomas predominating in the proximal colon and approximately 50 < 5 mm serrated polyps in the distal colon and rectum) who also demonstrated biallelic mutation for the two common European variants in *MUTYH* [124]. His mother had CRC of the sigmoid colon at 70 yrs. His 17-year-old symptomatic son who was not a biallelic mutation carrier had multiple 4 mm hyperplastic polyps in the rectosigmoid. The implications of this case report are that the risk to first-degree relatives of biallelic *MUTYH* mutation carriers (RR < 1.5) [125] is not as substantial as in serrated polyposis where the risk is fivefold greater than the population risk [10]. Therefore screening protocols in the setting described need also to consider the extra risk to first-degree relatives that serrated polyposis can pose. For this reason, detailed pathological examination of the polyps in patients with MAP is recommended to exclude coexisting serrated polyposis. Should serrated polyposis also be present, screening beyond siblings should be considered.

The evidence that serrated polyposis is a genetic predisposition is accumulating. Though multiple cases of serrated polyposis within a single family are rare [39, 54], the phenotype of multiple neoplasms, young-onset, and occasional affected sibships including consanguineous kindreds [26] suggest a pattern of inheritance consistent with an autosomal recessive or codominant mode [13, 126]. Codominant modes of inheritance result in an intermediate phenotype when one variant risk allele is present and a significantly altered phenotype in those where both alleles are variants.

10. Surveillance and Cancer Prevention Approaches

Several reports have suggested that malignant transformation in the serrated pathway may be unusually rapid in some clinical settings. Despite these observations, the apparent rapid evolution to cancer of advanced serrated polyps remains unproven and may be due to the difficulty of visualizing flat serrated lesions at colonoscopy. SSA/P progress very slowly to dysplasia in a population cohort [62]; however, in the syndromic patient this progression may be more rapid in an epigenetically abnormal environment. Hyman and colleagues reported 3 cases of serrated polyposis where CRC developed despite colonoscopy every 2 years [43]. Similarly, Azimuddin and colleagues reported that colonoscopy every 3 years was inadequate for some families with atypical serrated polyps [40]. Boparai et al. reported that 5 of 77 serrated polyposis patients developed a CRC whilst under surveillance for 5 years [9]. In four of five, the CRC arose within 12 months of a previous colonoscopy; however, many polyps had been left *in situ*. Currently, the issue of rapid evolution in serrated polyposis remains unresolved.

There are no clear guidelines though recommendations are evolving [127]. Frequent surveillance colonoscopy in the initial period after diagnosis both to allow endoscopic control of the polyps and to determine the nature and progress of the disease appears justified. Appropriate subsequent surveillance intervals can then be determined, but to avoid interval CRC this is unlikely to extend beyond 2-3 year intervals. Referral to a tertiary centre should be planned particularly if the polyp burden is difficult to control endoscopically and surgery is being considered.

11. Summary and Future Directions

Serrated polyposis is a condition with an increased CRC risk to both individuals and their relatives. An understanding of the mechanism of malignant transformation in serrated polyposis is still evolving, along with the risk factors which influence it [96]. Without a known germline sequence variant and estimated genetic penetrance, the identification and management of individuals and their families with a CRC predisposition syndrome become increasingly problematical. The prospect of a syndrome with a codominant mode of inheritance presents particular difficulties in that, although some individuals will present with a florid phenotype, such as that seen in serrated polyposis, first-degree relatives may have only a few polyps or none at all. The role of genetics departments, pathologists, and endoscopists in understanding the clinical picture for such families is likely to become increasingly important and interdependent. The challenge will be to determine if we can confidently identify and assign CRC risks to the different phenotypes of serrated polyposis, thereby allowing tailored clinical management with regard to the frequency of colonoscopic surveillance, the aggressiveness of polyp removal, and consideration of colonic resection.

Conflict of Interests

The authors have no conflict of interests to declare with respect to this work.

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References

[1] D. Snover, D. Ahnen, R. Burt, and R. D. Odze, *Serrated Polyps of the Colon and Rectum and Serrated Polyposis*, WHO Classification of Tumours of the Digestive System, IARC, Lyon, France, 4th edition, 2010.

[2] H. Goldman, S. Ming, and D. F. Hickock, "Nature and significance of hyperplastic polyps of the human colon," *Archives of pathology*, vol. 89, no. 4, pp. 349–354, 1970.

[3] N. Lane, "The precursor tissue of ordinary large bowel cancer," *Cancer Research*, vol. 36, no. 7, pp. 2669–2672, 1976.

[4] J. R. Jass, J. Young, and B. A. Leggett, "Hyperplastic polyps and DNA microsatellite unstable cancers of the colorectum," *Histopathology*, vol. 37, no. 4, pp. 295–301, 2000.

[5] S. A. R. Cooke, "Polyposis coli. The clinical spectrum in adults," *South African Medical Journal*, vol. 53, no. 12, pp. 454–457, 1978.

[6] H. S. Cooper, A. S. Patchefsky, and G. A. S. Marks, "Adenomatous and carcinomatous changes within hyperplastic colonic epithelium," *Diseases of the Colon and Rectum*, vol. 22, no. 3, pp. 152–156, 1979.

[7] E. Torlakovic and D. C. Snover, "Serrated adenomatous polyposis in humans," *Gastroenterology*, vol. 110, no. 3, pp. 748–755, 1996.

[8] J. R. Jass, H. Lino, A. Ruszkiewicz et al., "Neoplastic progression occurs through mutator pathways in hyperplastic polyposis of the colorectum," *Gut*, vol. 47, no. 1, pp. 43–49, 2000.

[9] K. S. Boparai, E. M. H. Mathus-Vliegen, J. J. Koornstra et al., "Increased colorectal cancer risk during follow-up in patients with hyperplastic polyposis syndrome: a multicentre cohort study," *Gut*, vol. 59, no. 8, pp. 1094–1100, 2010.

[10] K. S. Boparai, J. B. Reitsma, V. Lemmens et al., "Increased colorectal cancer risk in first-degree relatives of patients with hyperplastic polyposis syndrome," *Gut*, vol. 59, no. 9, pp. 1222–1225, 2010.

[11] D. D. Buchanan, K. Sweet, M. Drini et al., "Phenotypic diversity in patients with multiple serrated polyps: a genetics clinic study," *International Journal of Colorectal Disease*, vol. 25, no. 6, pp. 703–712, 2010.

[12] D. D. Buchanan, K. Sweet, M. Drini et al., "Risk factors for colorectal cancer in patients with multiple serrated polyps: a cross-sectional case series from genetics clinics," *PLoS One*, vol. 5, no. 7, Article ID e11636, 2010.

[13] J. Young and J. R. Jass, "The case for a genetic predisposition to serrated neoplasia in the colorectum: hypothesis and review of the literature," *Cancer Epidemiology Biomarkers and Prevention*, vol. 15, no. 10, pp. 1778–1784, 2006.

[14] R. Burt and J. R. Jass, "Hyperplastic polyposis," in *Pathology and Genetics of Tumours of the Digestive System*, S. R. Hamilton and L. A. Aaltonen, Eds., pp. 135–136, IARC Press, Lyon, France, 2000.

[15] J. R. Jass, "Gastrointestinal polyposes: clinical, pathological and molecular features," *Gastroenterology Clinics of North America*, vol. 36, no. 4, pp. 927–946, 2007.

[16] J. R. Jass, "Colorectal polyposes: from phenotype to diagnosis," *Pathology Research and Practice*, vol. 204, no. 7, pp. 431–447, 2008.

[17] A. Yeoman, J. Young, J. Arnold, J. Jass, and S. Parry, "Hyperplastic polyposis in the New Zealand population: a condition associated with increased colorectal cancer risk and European ancestry," *New Zealand Medical Journal*, vol. 120, no. 1266, pp. 31–39, 2007.

[18] T. Higuchi and J. R. Jass, "My approach to serrated polyps of the colorectum," *Journal of Clinical Pathology*, vol. 57, no. 7, pp. 682–686, 2004.

[19] O. Bengoechea, J. M. Martinez-Penuela, and B. Larrinaga, "Hyperplastic polyposis of the colorectum and adenocarcinoma in a 24-year-old man," *American Journal of Surgical Pathology*, vol. 11, no. 4, pp. 323–327, 1987.

[20] S. M. Cohen, L. Brown, M. L. Janower, and F. J. McCready, "Multiple metaplastic (hyperplastic) polyposis of the colon," *Gastrointestinal Radiology*, vol. 6, no. 4, pp. 333–335, 1981.

[21] D. J. Keljo, A. G. Weinberg, N. Winick, and G. Tomlinson, "Rectal cancer in an 11-year-old girl with hyperplastic polyposis," *Journal of Pediatric Gastroenterology and Nutrition*, vol. 28, no. 3, pp. 327–332, 1999.

[22] M. J. Lockett and W. S. Atkin, "Hyperplastic polyposis: prevalence and cancer risk," *Gut*, vol. 48, article A4, 2001.

[23] C. A. Rubio, S. Stemme, E. Jaramillo, and A. Lindblom, "Hyperplastic polyposis coli syndrome and colorectal carcinoma," *Endoscopy*, vol. 38, no. 3, pp. 266–270, 2006.

[24] B. A. Leggett, B. Devereaux, K. Biden, J. Searle, J. Young, and J. Jass, "Hyperplastic polyposis: association with colorectal cancer," *American Journal of Surgical Pathology*, vol. 25, no. 2, pp. 177–184, 2001.

[25] L. G. Carvajal-Carmona, K. M. Howarth, M. Lockett et al., "Molecular classification and genetic pathways in hyperplastic polyposis syndrome," *Journal of Pathology*, vol. 212, no. 4, pp. 378–385, 2007.

[26] E. Chow, L. Lipton, E. Lynch et al., "Hyperplastic polyposis syndrome: phenotypic presentations and the role of MBD4 and MYH," *Gastroenterology*, vol. 131, no. 1, pp. 30–39, 2006.

[27] M. F. Kalady, A. Jarrar, B. Leach et al., "Defining phenotypes and cancer risk in hyperplastic polyposis syndrome," *Diseases of the Colon and Rectum*, vol. 54, no. 2, pp. 164–170, 2011.

[28] A. Ferrández, W. Samowitz, J. A. DiSario, and R. W. Burt, "Phenotypic characteristics and risk of cancer development in hyperplastic polyposis: case series and literature review," *American Journal of Gastroenterology*, vol. 99, no. 10, pp. 2012–2018, 2004.

[29] G. T. Williams, J. F. Arthur, H. J. R. Bussey, and B. C. Morson, "Metaplastic polyps and polyposis of the colorectum," *Histopathology*, vol. 4, no. 2, pp. 155–170, 1980.

[30] P. Minoo, K. Baker, R. Goswami et al., "Extensive DNA methylation in normal colorectal mucosa in hyperplastic polyposis," *Gut*, vol. 55, no. 10, pp. 1467–1474, 2006.

[31] J. T. Prior and E. Dunn, "Diffuse colonic mucosal hyperplasia. Morphology and significance," *Archives of Surgery*, vol. 109, no. 4, pp. 575–577, 1974.

[32] S. Kozuka, "Premalignancy of the mucosal polyp in the large intestine. I. Histologic gradation of the polyp on the basis

of epithelial pseudostratification and glandular branching," *Diseases of the Colon and Rectum*, vol. 18, no. 6, pp. 483–493, 1975.

[33] D. W. Crocker and F. J. Veith, "Focal primary mucosal hyperplasia of the colon: the case for its importance in the pathogenesis of cancer," *Annals of Surgery*, vol. 160, pp. 215–225, 1964.

[34] B. C. Morson, "Precancerous lesions of the colon and rectum. Classification and controversial issues," *The Journal of the American Medical Association*, vol. 179, pp. 316–321, 1962.

[35] J. F. Arthur, "Structure and significance of metaplastic nodules in the rectal mucosa," *Journal of Clinical Pathology*, vol. 21, no. 6, pp. 735–743, 1968.

[36] C. M. Fenoglio and N. Lane, "The anatomical precursor of colorectal carcinoma," *Cancer*, vol. 34, supplement 3, pp. 819–823, 1974.

[37] H. S. Cooper, A. S. Patchefsky, and G. A. S. Marks, "Adenomatous and carcinomatous changes within hyperplastic colonic epithelium," *Diseases of the Colon and Rectum*, vol. 22, no. 3, pp. 152–156, 1979.

[38] R. W. Burt and W. S. Samowitz, "Serrated adenomatous polyposis: a new syndrome?" *Gastroenterology*, vol. 110, no. 3, pp. 950–952, 1996.

[39] P. Jeevaratnam, D. S. Cottier, P. J. Browett, N. S. Van De Water, V. Pokos, and J. R. Jass, "Familial giant hyperplastic polyposis predisposing to colorectal cancer: a new hereditary bowel cancer syndrome," *Journal of Pathology*, vol. 179, no. 1, pp. 20–25, 1996.

[40] K. Azimuddin, J. J. Stasik, I. T. Khubchandani, L. Rosen, R. D. Riether, and M. Scarlatto, "Hyperplastic polyps: 'more than meets the eye'? Report of sixteen cases," *Diseases of the Colon and Rectum*, vol. 43, no. 9, pp. 1309–1313, 2000.

[41] C. Beusnel, N. Le Berre, M. Pagenault et al., "Giant hyperplastic polyposis with adenomatous tissue," *Gastroenterologie Clinique et Biologique*, vol. 20, no. 3, pp. 294–297, 1996.

[42] N. J. Hawkins, P. Gorman, I. P. M. Tomlinson, P. Bullpitt, and R. L. Ward, "Colorectal carcinomas arising in the hyperplastic polyposis syndrome progress through the chromosomal instability pathway," *American Journal of Pathology*, vol. 157, no. 2, pp. 385–392, 2000.

[43] N. H. Hyman, P. Anderson, and H. Blasyk, "Hyperplastic polyposis and the risk of colorectal cancer," *Diseases of the Colon and Rectum*, vol. 47, no. 12, pp. 2101–2104, 2004.

[44] J. R. Jass, D. S. Cottier, V. Pokos, S. Parry, and I. M. Winship, "Mixed epithelial polyps in association with hereditary nonpolyposis colorectal cancer providing an alternative pathway of cancer histogenesis," *Pathology*, vol. 29, no. 1, pp. 28–33, 1997.

[45] H. Jørgensen, A. Mellon Mogensen, and L. B. Svendsen, "Hyperplastic polyposis of the large bowel. Three cases and a review of the literature," *Scandinavian Journal of Gastroenterology*, vol. 31, no. 8, pp. 825–830, 1996.

[46] N. Koide, Y. Saito, T. Fujii, H. Kondo, D. Saito, and T. Shimoda, "A case of hyperplastic polyposis of the colon with adenocarcinomas in hyperplastic polyps after long-term follow-up," *Endoscopy*, vol. 34, no. 6, pp. 499–502, 2002.

[47] M. Kusunoki, S. Fujita, Y. Sakanoue et al., "Disappearance of hyperplastic polyposis after resection of rectal cancer: report of two cases," *Diseases of the Colon and Rectum*, vol. 34, no. 9, pp. 829–832, 1991.

[48] P. Lage, M. Cravo, R. Sousa et al., "Management of Portuguese patients with hyperplastic polyposis and screening of at-risk first-degree relatives: a contribution for future guidelines based on a clinical study," *American Journal of Gastroenterology*, vol. 99, no. 9, pp. 1779–1784, 2004.

[49] R. J. Lieverse, R. E. Kibbelaar, G. Griffioen, and C. B. H. W. Lamers, "Colonic adenocarcinoma in a patient with multiple hyperplastic polyps," *Netherlands Journal of Medicine*, vol. 46, no. 4, pp. 185–188, 1995.

[50] B. G. McCann, "A case of metaplastic polyposis of the colon associated with focal adenomatous change and metachronous adenocarcinomas," *Histopathology*, vol. 13, no. 6, pp. 700–702, 1988.

[51] B. I. Oberschmid, W. Dietmaier, A. Hartmann et al., "Distinct secreted frizzled receptor protein 1 staining pattern in zpatients with hyperplastic polyposis coli syndrome," *Archives of Pathology and Laboratory Medicine*, vol. 128, no. 9, pp. 967–973, 2004.

[52] S. Orii, S. I. Nakamura, T. Sugai et al., "Hyperplastic (metaplastic) polyposis of the colorectum associated with adenomas and an adenocarcinoma," *Journal of Clinical Gastroenterology*, vol. 25, no. 1, pp. 369–372, 1997.

[53] R. J. Place and C. L. Simmang, "Hyperplastic-adenomatous polyposis syndrome," *Journal of the American College of Surgeons*, vol. 188, no. 5, pp. 503–507, 1999.

[54] A. Rashid, P. S. Houlihan, S. Booker, G. M. Petersen, F. M. Giardiello, and S. R. Hamilton, "Phenotypic and molecular characteristics of hyperplastic polyposis," *Gastroenterology*, vol. 119, no. 2, pp. 323–332, 2000.

[55] N. A. Shepherd, "Inverted hyperplastic polyposis of the colon," *Journal of Clinical Pathology*, vol. 46, no. 1, pp. 56–60, 1993.

[56] H. J. Spjut and R. G. Estrada, "The significance of epithelial polyps of the large bowel," *Pathology Annual*, vol. 12, no. 1, pp. 147–170, 1977.

[57] H. W. Sumner, N. F. Wasserman, and C. J. McClain, "Giant hyperplastic polyposis of the colon," *Digestive Diseases and Sciences*, vol. 26, no. 1, pp. 85–89, 1981.

[58] H. Heng Teoh, B. Delahunt, and W. H. Isbister, "Dysplastic and malignant areas in hyperplastic polyps of the large intestine," *Pathology*, vol. 21, no. 2, pp. 138–142, 1989.

[59] A. B. Tulman, S. Bradford, E. Lee, and P. G. Brady, "Giant hyperplastic polyps associated with vasculitis of colon," *Journal of the Florida Medical Association*, vol. 69, no. 5, pp. 380–383, 1982.

[60] A. S. Warner, M. E. Glick, and F. Fogt, "Multiple large hyperplastic polyps of the colon coincident with adenocarcinoma," *American Journal of Gastroenterology*, vol. 89, no. 1, pp. 123–125, 1994.

[61] A. J. Renaut, P. R. Douglas, and G. L. Newstead, "Hyperplastic polyposis of the colon and rectum," *Colorectal Disease*, vol. 4, no. 3, pp. 213–215, 2002.

[62] R. H. Lash, R. M. Genta, and C. M. Schuler, "Sessile serrated adenomas: prevalence of dysplasia and carcinoma in 2139 patients," *Journal of Clinical Pathology*, vol. 63, no. 8, pp. 681–686, 2010.

[63] J. T. Hetzel, C. S. Huang, J. A. Coukos et al., "Variation in the detection of serrated polyps in an average risk colorectal cancer screening cohort," *American Journal of Gastroenterology*, vol. 105, no. 12, pp. 2656–2664, 2010.

[64] T. Higuchi, K. Sugihara, and J. R. Jass, "Demographic and pathological characteristics of serrated polyps of colorectum," *Histopathology*, vol. 47, no. 1, pp. 32–40, 2005.

[65] E. Torlakovic, E. Skovlund, D. C. Snover, G. Torlakovic, and J. M. Nesland, "Morphologic reappraisal of serrated colorectal polyps," *American Journal of Surgical Pathology*, vol. 27, no. 1, pp. 65–81, 2003.

[66] D. A. Johnson, M. S. Gurney, R. J. Volpe et al., "A prospective study of the prevalence of colonic neoplasms in asymptomatic patients with an age-related risk," *American Journal of Gastroenterology*, vol. 85, no. 8, pp. 969–974, 1990.

[67] T. F. Imperiale, D. R. Wagner, C. Y. Lin, G. N. Larkin, J. D. Rogge, and D. F. Ransohoff, "Results of screening colonoscopy among persons 40 to 49 years of age," *New England Journal of Medicine*, vol. 346, no. 23, pp. 1781–1785, 2002.

[68] D. A. Lieberman, S. Prindiville, D. G. Weiss, and W. Willett, "Risk factors for advanced colonic neoplasia and hyperplastic polyps in asymptomatic individuals," *Journal of the American Medical Association*, vol. 290, no. 22, pp. 2959–2967, 2003.

[69] L. G. K. Johannsen, O. Momsen, and N. O. Jacobsen, "Polyps of the large intestine in Aarhus, Denmark. An autopsy study," *Scandinavian Journal of Gastroenterology*, vol. 24, no. 7, pp. 799–806, 1989.

[70] G. A. Paspatis, N. Papanikolaou, E. Zois, and E. Michalodimitrakis, "Prevalence of polyps and diverticulosis of the large bowel in the Cretan population. An autopsy study," *International Journal of Colorectal Disease*, vol. 16, no. 4, pp. 257–261, 2001.

[71] A. R. Williams, B. A. W. Balasooriya, and D. W. Day, "Polyps and cancer of the large bowel: a necropsy study in Liverpool," *Gut*, vol. 23, no. 10, pp. 835–842, 1982.

[72] L. M. Morimoto, P. A. Newcomb, C. M. Ulrich, R. M. Bostick, C. J. Lais, and J. D. Potter, "Risk factors for hyperplastic and adenomatous polyps: evidence for malignant potential?" *Cancer Epidemiology Biomarkers and Prevention*, vol. 11, no. 10, pp. 1012–1018, 2002.

[73] K. M. Kim, E. J. Lee, Y. H. Kim, D. K. Chang, and R. D. Odze, "KRAS mutations in traditional serrated adenomas from Korea herald an aggressive phenotype," *American Journal of Surgical Pathology*, vol. 34, no. 5, pp. 667–675, 2010.

[74] E. E. Torlakovic, J. D. Gomez, D. K. Driman et al., "Sessile serrated adenoma (SSA) vs. traditional serrated adenoma (TSA)," *American Journal of Surgical Pathology*, vol. 32, no. 1, pp. 21–29, 2008.

[75] J. R. Jass, K. Baker, I. Zlobec et al., "Advanced colorectal polyps with the molecular and morphological features of serrated polyps and adenomas: concept of a 'fusion' pathway to colorectal cancer," *Histopathology*, vol. 49, no. 2, pp. 121–131, 2006.

[76] M. J. O'Brien, S. Yang, C. Mack et al., "Comparison of microsatellite instability, CpG island methylation phenotype, BRAF and KRAS status in serrated polyps and traditional adenomas indicates separate pathways to distinct colorectal carcinoma end points," *American Journal of Surgical Pathology*, vol. 30, no. 12, pp. 1491–1501, 2006.

[77] N. J. Carr, H. Mahajan, K. L. Tan, N. J. Hawkins, and R. L. Ward, "Serrated and non-serrated polyps of the colorectum: their prevalence in an unselected case series and correlation of BRAF mutation analysis with the diagnosis of sessile serrated adenoma," *Journal of Clinical Pathology*, vol. 62, no. 6, pp. 516–518, 2009.

[78] H. K. Yong, S. Kakar, L. Cun, G. Deng, and Y. S. Kim, "Distinct CpG island methylation profiles and BRAF mutation status in serrated and adenomatous colorectal polyps," *International Journal of Cancer*, vol. 123, no. 11, pp. 2587–2593, 2008.

[79] T. L. Chan, W. Zhao, S. Y. Leung, and S. T. Yuen, "BRAF and KRAS mutations in colorectal hyperplastic polyps and serrated adenomas," *Cancer Research*, vol. 63, no. 16, pp. 4878–4881, 2003.

[80] K. Otori, Y. Oda, K. Sugiyama et al., "High frequency of K-ras mutations in human colorectal hyperplastic polyps," *Gut*, vol. 40, no. 5, pp. 660–663, 1997.

[81] S. Yang, F. A. Farraye, C. Mack, O. Posnik, and M. J. O'Brien, "BRAF and KRAS mutations in hyperplastic polyps and serrated adenomas of the colorectum: relationship to histology and CpG island methylation status," *American Journal of Surgical Pathology*, vol. 28, no. 11, pp. 1452–1459, 2004.

[82] F. Fogt, T. Brien, C. A. Brown, C. J. Hartmann, R. L. Zimmerman, and R. D. Odze, "Genetic alterations in serrated adenomas: comparison to conventional adenomas and hyperplastic polyps," *Human Pathology*, vol. 33, no. 1, pp. 87–91, 2002.

[83] T. Kambara, L. A. Simms, V. L. J. Whitehall et al., "BRAF mutation is associated with DNA methylation in serrated polyps and cancers of the colorectum," *Gut*, vol. 53, no. 8, pp. 1137–1144, 2004.

[84] K. J. Spring, Z. Z. Zhao, R. Karamatic et al., "High prevalence of sessile serrated adenomas with BRAF mutations: a prospective study of patients undergoing colonoscopy," *Gastroenterology*, vol. 131, no. 5, pp. 1400–1407, 2006.

[85] M. J. O'Brien, S. Yang, J. L. Clebanoff et al., "Hyperplastic (serrated) polyps of the colorectum: relationship of CpG island methylator phenotype and K-ras mutation to location and histologic subtype," *American Journal of Surgical Pathology*, vol. 28, no. 4, pp. 423–434, 2004.

[86] R. Beach, A. O. O. Chan, T. T. Wu et al., "BRAF mutations in aberrant crypt foci and hyperplastic polyposis," *American Journal of Pathology*, vol. 166, no. 4, pp. 1069–1075, 2005.

[87] A. O. O. Chan, J. P. J. Issa, J. S. Morris, S. R. Hamilton, and A. Rashid, "Concordant CpG island methylation in hyperplastic polyposis," *American Journal of Pathology*, vol. 160, no. 2, pp. 529–536, 2002.

[88] E. J. Sawyer, A. Cerar, A. M. Hanby et al., "Molecular characteristics of serrated adenomas of the colorectum," *Gut*, vol. 51, no. 2, pp. 200–206, 2002.

[89] T. B. Sheridan, H. Fenton, M. R. Lewin et al., "Sessile serrated adenomas with low- and high-grade dysplasia and early carcinomas: an immunohistochemical study of serrated lesions "caught in the act"," *American Journal of Clinical Pathology*, vol. 126, no. 4, pp. 564–571, 2006.

[90] K. S. Boparai, E. Dekker, S. van Eeden et al., "Hyperplastic polyps and sessile serrated adenomas as a phenotypic expression of MYH-associated polyposis," *Gastroenterology*, vol. 135, no. 6, pp. 2014–2018, 2008.

[91] C. V. A. Wynter, M. D. Walsh, T. Higuchi, B. A. Leggett, J. Young, and J. R. Jass, "Methylation patterns define two types of hyperplastic polyp associated with colorectal cancer," *Gut*, vol. 53, no. 4, pp. 573–580, 2004.

[92] R. W. Burt and W. S. Samowitz, "Serrated adenomatous polyposis: a new syndrome?" *Gastroenterology*, vol. 110, no. 3, pp. 950–952, 1996.

[93] D. W. Rosenberg, S. Yang, D. C. Pleau et al., "Mutations in BRAF and KRAS differentially distinguish serrated versus non-serrated hyperplastic aberrant crypt foci in humans," *Cancer Research*, vol. 67, no. 8, pp. 3551–3554, 2007.

[94] L. A. S. Carragher, K. R. Snell, S. M. Giblett et al., "V600EBraf induces gastrointestinal crypt senescence and promotes tumour progression through enhanced CpG methylation of p16INK4a," *EMBO Molecular Medicine*, vol. 2, no. 11, pp. 458–471, 2010.

[95] T. Hinoue, D. J. Weisenberger, F. Pan et al., "Analysis of the association between CIMP and BRAF in colorectal cancer by DNA methylation profiling," *PloS One*, vol. 4, no. 12, p. e8357, 2009.

[96] J. P. Young and S. Parry, "Risk factors: hyperplastic polyposis syndrome and risk of colorectal cancer," *Nature Reviews Gastroenterology and Hepatology*, vol. 7, no. 11, pp. 594–595, 2010.

[97] N. Ahuja and J. P. J. Issa, "Aging, methylation and cancer," *Histology and Histopathology*, vol. 15, no. 3, pp. 835–842, 2000.

[98] N. Ahuja, Q. Li, A. L. Mohan, S. B. Baylin, and J. P. J. Issa, "Aging and DNA methylation in colorectal mucosa and cancer," *Cancer Research*, vol. 58, no. 23, pp. 5489–5494, 1998.

[99] K. Konishi, L. Shen, J. Jelinek et al., "Concordant DNA methylation in synchronous colorectal carcinomas," *Cancer Prevention Research*, vol. 2, no. 9, pp. 814–822, 2009.

[100] K. Nosho, S. Kure, N. Irahara et al., "A prospective cohort study shows unique epigenetic, genetic, and prognostic features of synchronous colorectal cancers," *Gastroenterology*, vol. 137, no. 5, pp. 1609–1620, 2009.

[101] P. Minoo, K. Baker, R. Goswami et al., "Extensive DNA methylation in normal colorectal mucosa in hyperplastic polyposis," *Gut*, vol. 55, no. 10, pp. 1467–1474, 2006.

[102] M. D. Walsh, C. Rosty, D. D. Buchanan et al., "Origin of colorectal cancers in high-risk serrated neoplasia patients," *Journal of Gastroenterology and Hepatology*, vol. 25, article A79, 2010.

[103] D. R. English, J. P. Young, J. A. Simpson et al., "Ethnicity and risk for colorectal cancers showing somatic BRAF V600E mutation or CpG island methylator phenotype," *Cancer Epidemiology Biomarkers and Prevention*, vol. 17, no. 7, pp. 1774–1780, 2008.

[104] D. J. Weisenberger, K. D. Siegmund, M. Campan et al., "CpG island methylator phenotype underlies sporadic microsatellite instability and is tightly associated with BRAF mutation in colorectal cancer," *Nature Genetics*, vol. 38, no. 7, pp. 787–793, 2006.

[105] R. K. Pai, A. C. MacKinnon, L. Joseph, A. Noffsinger, and J. Hart, "Identification of histologically distinct conventional adenomas that arise predominately in patients with sessile serrated adenomas," *American Journal of Surgical Pathology*, vol. 34, no. 3, pp. 355–363, 2010.

[106] R. K. Pai, J. Hart, and A. E. Noffsinger, "Sessile serrated adenomas strongly predispose to synchronous serrated polyps in non-syndromic patients," *Histopathology*, vol. 56, no. 5, pp. 581–588, 2010.

[107] S. T. Yuen, H. Davies, T. L. Chan et al., "Similarity of the phenotypic patterns associated with BRAF and KRAS mutations in colorectal neoplasia," *Cancer Research*, vol. 62, no. 22, pp. 6451–6455, 2002.

[108] J. Kearney, E. Giovannucci, E. B. Rimm et al., "Diet, alcohol, and smoking and the occurrence of hyperplastic polyps of the colon and rectum (United States)," *Cancer Causes and Control*, vol. 6, no. 1, pp. 45–56, 1995.

[109] J. Young, "Serrated neoplasia of the colorectum and cigarette smoking," *Gastroenterology*, vol. 135, no. 1, pp. 323–324, 2008.

[110] B. T. Ji, J. L. Weissfeld, W. H. Chow, W. Y. Huang, R. E. Schoen, and R. B. Hayes, "Tobacco smoking and colorectal hyperplastic and adenomatous polyps," *Cancer Epidemiology Biomarkers and Prevention*, vol. 15, no. 5, pp. 897–901, 2006.

[111] L. M. Morimoto, P. A. Newcomb, C. M. Ulrich, R. M. Bostick, C. J. Lais, and J. D. Potter, "Risk factors for hyperplastic and adenomatous polyps: evidence for malignant potential?" *Cancer Epidemiology Biomarkers and Prevention*, vol. 11, no. 10 I, pp. 1012–1018, 2002.

[112] M. J. Shrubsole, H. Wu, R. M. Ness, Y. Shyr, W. E. Smalley, and W. Zheng, "Alcohol drinking, cigarette smoking, and risk of colorectal adenomatous and hyperplastic polyps," *American Journal of Epidemiology*, vol. 167, no. 9, pp. 1050–1058, 2008.

[113] J. C. Anderson, D. C. Pleau, T. V. Rajan et al., "Increased frequency of serrated aberrant crypt foci among smokers," *American Journal of Gastroenterology*, vol. 105, no. 7, pp. 1648–1654, 2010.

[114] K. Wallace, M. V. Grau, D. Ahnen et al., "The association of lifestyle and dietary factors with the risk for serrated polyps of the colorectum," *Cancer Epidemiology Biomarkers and Prevention*, vol. 18, no. 8, pp. 2310–2317, 2009.

[115] W. S. Samowitz, H. Albertsen, C. Sweeney et al., "Association of smoking, CpG island methylator phenotype, and V600E BRAF mutations in colon cancer," *Journal of the National Cancer Institute*, vol. 98, no. 23, pp. 1731–1738, 2006.

[116] R. G. Walker, J. K. Landmann, D. G. Hewett et al., "Hyperplastic polyposis syndrome is associated with cigarette smoking, which may be a modifiable risk factor," *American Journal of Gastroenterology*, vol. 105, no. 7, pp. 1642–1647, 2010.

[117] S. Söderlund, F. Granath, O. Broström et al., "Inflammatory bowel disease confers a lower risk of colorectal cancer to females than to males," *Gastroenterology*, vol. 138, no. 5, pp. 1697–1703, 2010.

[118] A. W. Jarrar, J. M. Church, S. Fay, and M. F. Kalady, "Is the phenotype mixed or mistaken? Hereditary nonpolyposis colorectal cancer and hyperplastic polyposis syndrome," *Diseases of the Colon and Rectum*, vol. 52, no. 12, pp. 1949–1955, 2009.

[119] J. Vandrovcova, K. Lagerstedt-Robinsson, L. Påhlman, and A. Lindblom, "Somatic BRAF-V600E mutations in familial colorectal cancer," *Cancer Epidemiology Biomarkers and Prevention*, vol. 15, no. 11, pp. 2270–2273, 2006.

[120] B. Heald, J. Mester, L. Rybicki, M. S. Orloff, C. A. Burke, and C. Eng, "Frequent gastrointestinal polyps and colorectal adenocarcinomas in a prospective series of PTEN mutation carriers," *Gastroenterology*, vol. 139, no. 6, pp. 1927–1933, 2010.

[121] A. Kokko, P. Laiho, R. Lehtonen et al., "EPHB2 germline variants in patients with colorectal cancer or hyperplastic polyposis," *BMC Cancer*, vol. 6, Article ID 145, 2006.

[122] M. G. Dunlop and S. M. Farrington, "MUTYH-associated polyposis and colorectal cancer," *Surgical Oncology Clinics of North America*, vol. 18, no. 4, pp. 599–610, 2009.

[123] D. Buchanan and J. Young, "A perspective on Bi-allelic MUTYH mutations in patients with hyperplastic polyposis syndrome," *Gastroenterology*, vol. 136, no. 7, pp. 2407–2408, 2009.

[124] D. D. Buchanan, A. Roberts, M. D. Walsh, S. Parry, and J. P. Young, "Lessons from Lynch syndrome: a tumor biology-based approach to familial colorectal cancer," *Future Oncology*, vol. 6, pp. 539–549, 2010.

[125] S. P. Cleary, M. Cotterchio, M. A. Jenkins et al., "Germline MutY human homologue mutations and colorectal cancer: a multisite case-control study," *Gastroenterology*, vol. 136, no. 4, pp. 1251–1260, 2009.

[126] J. Young, M. Jenkins, S. Parry et al., "Serrated pathway colorectal cancer in the population: genetic consideration," *Gut*, vol. 56, no. 10, pp. 1453–1459, 2007.

[127] J. E. East, B. P. Saunders, and J. R. Jass, "Sporadic and syndromic hyperplastic polyps and serrated adenomas of the colon: classification, molecular genetics, natural history, and clinical management," *Gastroenterology Clinics of North America*, vol. 37, no. 1, pp. 25–46, 2008.

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Review Article

CpG Island Methylation in Colorectal Cancer: Past, Present and Future

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The concept of a CpG island methylator phenotype, or CIMP, quickly became the focus of several colorectal cancer studies describing its clinical and pathological features after its introduction in 1999 by Toyota and colleagues. Further characterization of CIMP in tumors lead to widespread acceptance of the concept, as expressed by Shen and Issa in their 2005 editorial, "CIMP, at last." Since that time, extensive research efforts have brought great insights into the epidemiology and prognosis of CIMP+ tumors and other epigenetic mechanisms underlying tumorigenesis. With the advances in technology and subsequent cataloging of the human methylome in cancer and normal tissue, new directions in research to understand CIMP and its role in complex biological systems yield hope for future epigenetically based diagnostics and treatments.

1. Introduction

In the October 29, 2010 issue, Science turned the spotlight on epigenetics—a term that encompasses histone modification, nucleosome location, noncoding RNA, and DNA methylation. Epigenetic processes do not involve changes to DNA sequence but rather are self-propagating molecular signatures that are potentially reversible, unlike changes in genetic information [1]. DNA methylation is the most widely studied epigenetic marker [2]. The discovery of global DNA hypomethylation in human tumors was followed by the identification of hypermethylated tumor-suppressor genes and recently, inactivation of microRNA (miRNA) by DNA methylation also has been described [3–5]. Growing interest in epigenetic systems stems from an inability to determine causative genetic variants in many disorders. It is hoped that a better understanding of these systems may provide insight into our understanding of complex diseases such as cancer.

Approximately half of all protein-encoding genes in the human genome contain CG-rich regions in their promoters or CpG islands. Aberrant DNA methylation, in the form of hypermethylation of CpG islands, results in repression of transcription in tumor suppressor genes. For example, inactivation of the mismatch repair gene *MLH1* by promoter methylation is the molecular basis for microsatellite instability in sporadic microsatellite unstable colon cancers [6]. This phenomenon of tumor alteration via epigenetic silencing associated with dense hypermethylation of CpG islands, and their complex interplay with modifications in histone structure, provides an alternate mechanism to genetic inactivation of tumor suppressor genes via loss or mutation [2]. The presence of widespread CpG island methylation in a tumor is termed the CpG island methylator phenotype, or CIMP, and this paper is focused on this specific aspect of epigenetics.

2. CIMP in Colorectal Cancer

The role of CIMP in colorectal carcinogenesis was originally postulated in 1999 by Toyota et al. [7]. Their pioneering study distinguished between age-related and cancer-related methylation and defined CIMP in terms of the latter. Recognition of CIMP as a phenomenon in colorectal cancer is relatively recent; more than a decade earlier, in 1988, Vogelstein and colleagues developed a model that hypothesized that

colorectal neoplasia occurs from a series of genetic alterations that includes activation of oncogenes and inactivation of tumor suppressor genes [8]. The concept of an epigenetic etiology in cancer introduced in the late 1990s was met with some controversy and resistance in the field of carcinogenesis [6, 9, 10]. However, the existence of CIMP has since gained wide acceptance, as the epidemiology characterizing this epigenetic alteration and its utility in understanding carcinogenic pathways support its significance in colorectal cancer biology [11–14].

Most sporadic microsatellite unstable colon tumors are CIMP positive, whereas CIMP is uncommon in Lynch syndrome-associated cancers which exhibit microsatellite instability (MSI), indicative of distinct underlying molecular processes [13, 15]. Based on a number of relatively large case-control and prospective cohort studies, ~30–40% of sporadic proximal-site colon cancers are CIMP positive, compared to 3–12% of distal colon and rectal cancers [16–21] as illustrated in Figure 1. Thus CIMP is significantly more frequent in tumors of the proximal colon, and this is independent of MSI status. CIMP is also associated with *BRAF* mutations in both microsatellite stable and unstable colon cancers [11, 18, 20]. CIMP is observed in proximal hyperplastic (serrated) polyps, suggesting this lesion may be a precursor to unstable tumors (and perhaps stable tumors) in the CIMP high pathway [22]. Once thought to lack potential for malignant progression, hyperplastic polyps are now considered to represent a heterogeneous group, most of which harbor *BRAF* mutations and some of which exhibit epigenetic alterations (both uncommon in colorectal adenomas). A subset of hyperplastic polyps has been defined by architectural features and renamed sessile serrated polyps (or sessile serrated sessile adenomas). Most of these polyps are right-sided and many show CIMP, supporting the notion that they may be a precursor lesion for CIMP high tumors [23, 24].

Some case-control and cohort studies have reported a poor prognosis associated with CIMP in combination with microsatellite stable tumors [19, 25–27], although this may reflect the co-occurrence of *BRAF* V600E mutations, which have been associated with significantly poorer survival in colon cancer [28, 29]. Relatively minor effects of CIMP on prognosis suggest that the effect of mutations in *BRAF* on survival in stable tumors is not dependent on CIMP [16, 28]. Indeed, Ogino et al. reported that CIMP-high appears to be an independent predictor of a *low* colon cancer-specific mortality [30]. These results suggest the need for a large sample size to determine the relative contributions of *BRAF* and CIMP on prognosis.

3. Characterization of CIMP

In contrast to the relatively straightforward determination of MSI tumor status, a consensus as to the optimal panel of CpG sites for CIMP determination is only starting to take shape (Table 1). Different panels may yield slightly different results, although a strong relationship to the presence of a *BRAF* V600E mutation is consistently observed with all panels. The so-called "classic" panel of Park et al. utilized to

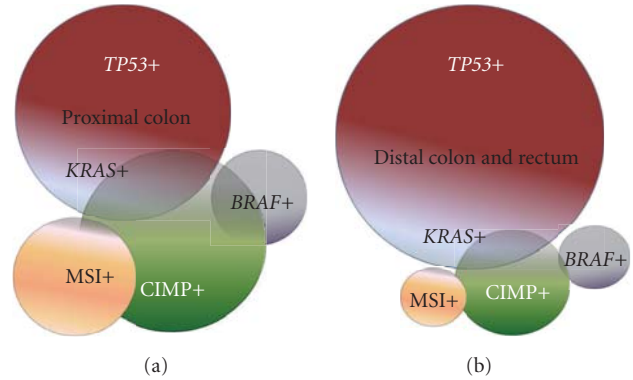

Figure 1: In colorectal cancer, CIMP+ occurs more frequently in tumors of the proximal colon (Figure 1(a)) and less frequently in tumors of the distal colon and rectum (Figure 1(b)). An approximate distribution of genetic and epigenetic tumor alterations is shown.

assess CIMP status consists of CpG sites in *MLH1*, *CDKN2A* (p16), and methylated in tumors (MINTS) 1, 2, and 31 [31]. It has been suggested that there are two general types of CIMP in sporadic tumors: CIMP high, related to *BRAF* mutations and *MLH1* methylation; and CIMP low, related to *KRAS* mutations [12, 32, 33]. Tumors characterized as CIMP positive (CIMP+) based on the classic panel include both CIMP high and CIMP low categories; therefore, a subset of CIMP+ associates with *BRAF* and another with *KRAS* mutations, somewhat surprising given mutations in these genes are typically mutually exclusive since both are members of the ras signal transduction pathway [6, 11].

Based on a systematic screen of 195 CpG sites and an unsupervised two-dimensional cluster analysis, Weisenberger et al. proposed a robust alternative to the classic panel to classify CIMP+ tumors consisting of *CACNA1G, IGF2, NEUROG1, RUNX3,* and *SOCS1* [13]; CIMP as defined by this panel did not show a relationship to *KRAS*. Using quantitative real-time PCR, Ogino et al. [34] selected a panel of markers to distinguish high from low levels of methylation including *MLH1* and *CDKN2A*, and three markers that differ from the classic panel: *CACNA1G*, *CRABP1*, and *NEUROG1*. Shen et al. examined genetic markers (*BRAF, KRAS*) and epigenetic markers at 27 promoter-associated CpG sites using clustering analysis to identify two distinct CIMP+ groups: CIMP1, characterized by MSI+ and *BRAF* mutations, and MINT1, *MLH1, RIZ1, TIMP3* methylation; and CIMP2, characterized by *KRAS* mutations and methylation of several MINT markers [33].

Using structural equation modeling to construct causal models of CIMP and locus-specific CpG island methylation and a large database of colorectal cancers, Tanaka et al. showed the correlation structures of 16 methylation markers and CIMP status differed between *BRAF* mutated, *KRAS* mutated, and wildtype *BRAF*/*KRAS* tumors [35]. They suggested a possible role of these mutations differentially modifying the propensity for locus-specific methylation at the cellular level. To examine the question of whether or not *BRAF* V600E plays a causal role in the development of

Table 1: A history of CIMP panels used to assess CpG island methylation in colorectal cancer.

Study	CIMP panel markers	Notes
Toyota et al. [7]	*CDKN2A (p16)*, MINT1, MINT2, MINT12, MINT17, MINT25, MINT27, MINT31, *MLH1, THBS1*	Pioneering work to identify markers that distinguish CIMP from age-related methylation
Park et al. [31]	*CDKN2A*, MINT1, MINT2, MINT31, *MLH1*	So-called "classic" or traditional panel
Weisenberger et al. [13]	*CACNA1G, IGF2, NEUROG1, RUNX3, SOCS1*	"New" panel based on stepwise screen of 195 markers
Ogino et al. [34]	*CACNA1G, CDKN2A, CRABP1, MLH1, NEUROG1*	Selected markers to distinguish high-level from low-level methylation
Shen et al. [33]	CIMP1: MINT1, *MLH1, RIZ1, TIMP3, BRAF* mutation; CIMP2: MINT2, MINT27, MINT31, Megalin, *KRAS* mutation	Examined 27 CpG sites, proposed optimal epigenetic and genetic markers to identify CIMP1, CIMP2, or CIMP-
Tanaka et al. [35]	*CACNA1G, CDKN2A, CHFR, CRABP1, HIC1, IGF2, IGFBP3, MGMT*, MINT1, MINT31, *NEUROG1, p14, RUNX3, SOCS1, WRN*	Correlation structures of markers and CIMP differ by *KRAS* and *BRAF* status
Ang et al. [36]	Total of 202 CpG sites differentially methylated between tumor and normal	Comprehensive DNA methylation profiling in 807 cancer genes
Kaneda and Yagi [37]	Group 1: *IGF2, LOX*, MINT1, MINT2, MINT31, *MLH1, RUNX3, SOCS1;* Group 2: *ADAMTS1, DUSP26, EDIL3, ELMO1, FBN2, HAND1, IGFBP3, NEUROG1, RASSF2, STOX2, THBD, UCHL1*	Comprehensive DNA epigenotyping of genomewide regions indentified two groups (high and intermediate to low methylation)

CIMP, Hinoue et al. determined 100 CIMP-associated CpG sites and examined changes in DNA methylation in eight stably transfected clones over multiple passages [38]. They observed that *BRAF* was not sufficient to induce CIMP in their system.

In contrast to evaluation of relatively small sets of CIMP markers, comprehensive DNA methylation profiling and unsupervised hierarchical clustering were recently used to identify several CpG sites that were differentially methylated between tumor and normal tissue [36]. Using a similar approach, the use of two methylation panels as classifiers of colorectal cancer has been proposed: the first to identify highly methylated tumors (strongly correlated with *BRAF*) and a second to distinguish between intermediate (associated with *KRAS*) and low methylation groups [37]. Since epigenetic therapy is in clinical use or trials for several cancers, efficient methods for epigenetic profiling are needed; Kondo and Issa provide a summary of available profiling techniques and their features [39]. As approaches to CIMP characterization in colorectal cancer continue to evolve, it is clear that *BRAF* and *KRAS* oncogene mutations will continue to refine any definition of CIMP. Although characterization of CIMP status depends on methylation markers and criteria used, classification of tumors by both CIMP and MSI status recently proposed by Jass [40] and further refined by Ogino and Goel [14] has become an increasingly common strategy to define the pathological and clinical features of colorectal cancer.

4. Epidemiology of CIMP

4.1. Characteristics. Relationships between CIMP and clinicopathologic features of colorectal tumors that have been widely reported include proximal location, older age at diagnosis, female gender, poor tumor differentiation, MSI (CIMP high cancer), *BRAF* mutations, *KRAS* mutations (microsatellite stable cancer), and wildtype *TP53* [11, 13, 16, 17, 34, 41]. Based on a large Australian cohort, English et al. reported that individuals of southern European ethnicity had lower risk of CIMP and *BRAF* mutation than those with origins in northern Europe [21].

Using actual data and a classification tree method to visualize carcinogenic pathways, Slattery et al. suggested that unique mutational pathways to colon and rectal cancer likely exist [18]. This method describes the probability of developing various alterations in proximal colon, distal colon, or rectal tumors given previously acquired mutations. Using bootstrap resampling, the probabilities of developing specific mutations differed across tumor sites. For example, the estimated proportion of tumors that will develop methylation at CpG sites decreased as one goes from proximal colon to rectal cancers. Regardless of site, a methylation pathway in which *BRAF* is subsequently acquired independent of MSI or *MLH1* methylation was predicted. This work supports previous observations that link CIMP and *BRAF* mutation, independent of MSI status [11, 12].

4.2. Smoking. The presence of methylation in human malignancies bears a relationship to a history of cigarette smoking. Cigarette smoking has been associated with CpG island methylation within the bronchial epithelium of smokers and in lung cancer, and activation of the aromatic hydrocarbon receptor by cigarette smoke has been associated with CpG methylation [42–44]. A significant relationship has been reported between cigarette smoking and CIMP (and closely related mutations in *BRAF*) in colon and rectal carcinomas in both prospective cohort and case-control studies [45–48]. Interestingly, the relationship between smoking and CIMP

provides an explanation for the previously observed association between cigarette smoking and MSI, as most of these tumors also exhibit CIMP [49]. Evidence also suggests that cigarette smoking (related to CIMP and *BRAF*) may be associated with hyperplastic polyps rather than adenomatous polyps; as mentioned above, a subset of hyperplastic polyps has been hypothesized to be the precursor to CIMP high colorectal carcinomas [50].

4.3. Other Risk Factors. S Adenosylmethionine (SAM), the universal donor of methyl groups in humans, and S Adenosylhomocysteine (SAH), the product of and an inhibitor of DNA methyltransferase (DNMT) enzymes, provide connections between folate metabolism and DNA methylation [51]. It has been hypothesized that diets low in folate and high in alcohol intake may disturb DNA methylation, which may result in global DNA hypomethylation concurrently with a greater risk of cancers with CpG island methylation [52, 53]. In contrast, other studies have shown that global DNA hypomethylation is inversely correlated with CIMP and may represent different pathways to colorectal cancer [54, 55]. Studies generally do not support a unique role for alcohol and folate in CIMP+ tumors [56, 57], although genetic polymorphisms in *MTHFR* 1298A > C (rs1801131), interacting with diet, and *TCN2* 776G > C (rs1801198) may be involved in the development of highly CpG-methylated colon and rectal cancers [58–60]. Conversely, *MTHFR* 1298A > C was not associated with CIMP+ tumors in the Netherlands cohort study [61]. Polymorphisms in DNA repair genes have been implicated in CIMP-positive colon cancer [62, 63]. A promoter polymorphism in *MLH1* (−93G > A) was associated with CIMP, *MLH1* methylation, and *BRAF* mutations in unstable sporadic colon tumors and not in stable tumors, suggesting the genetic variant may be acting at a relatively late stage of carcinogenesis to drive CIMP-positive tumors down the microsatellite instability pathway [63].

Overexpression of DNMT3B has been shown to be a risk factor for the development of CIMP in colorectal cancer [64, 65]. DNMT3B is important in establishing and maintaining genomic methylation patterns, and overexpression in mice can induce tumors with methylation in specific CpG islands. Recent findings indicate that DNA methylation changes occur sequentially during tumor progression, and DNMT3B expression levels increase during this progression [66].

The future of CIMP in colorectal cancer research may well take place in the evolving trans- and interdisciplinary field of "molecular pathological epidemiology" outlined by Ogino et al, which is designed to elucidate how genetic factors and lifestyle exposures interact with specific molecular subtypes of cancer [67]. Hughes et al. reported that severe caloric restriction was associated with decreased risk of developing a tumor characterized by CIMP. This study provides a potential link between early life conditions and epigenetic changes that later influence colorectal cancer development [68]. The work of Slattery et al. regarding differences in the etiologies of rectal-site and colon-site tumors, and the influence of genetic factors in the inflammatory pathway in the etiology of CIMP in both, is an example of this approach [69, 70].

5. Emerging Trends in CIMP Research

Although aberrant DNA methylation of promoter CpG islands in cancer genes as well as repressive chromatin are frequently involved in gene inactivation during tumorigenesis, the mechanisms underlying CIMP are poorly understood. Patterns of hypermethylation are specific to tumor type, and it is unclear why certain regions become hypermethylated; however, mapping of the human methylome as a result of technological advances has expanded our understanding of epigenetic mechanisms [71]. Inactivation of particular genes may confer a growth advantage, resulting in clonal selection [72]. Another possibility is that long-range epigenetic silencing by DNA methylation can span chromosome regions of 1 Mb in colorectal cancer, resembling the loss of heterozygosity often observed in tumors [73]. In a large cohort of sporadic colorectal cancers, Wong et al. reported a strong relationship between long-range silencing of chromosome region 3p22 and CIMP+ tumors [74].

Recent findings suggest that most DNA methylation alterations in colon cancer occur in CpG island shores, sequences up to 2 kb distant from CpG islands [75]. Hypermethylated CpG shores appear closer to their associated CpG islands, while hypomethylated shores occur further away from their associated islands and resemble noncolon normal tissues. These findings are consistent with an epigenetic progenitor model of cancer, in which epigenetic alterations affecting tissue-specific differentiation are the predominant mechanism by which epigenetic changes cause cancer. Alternative transcription may be a function of differential DNA methylation during differentiation, and one role of altered methylation in cancer may be to disrupt regulatory control of specific promoter usage [75].

Previous studies suggest a general model in which genes reposition away from the heterochromatin when activated and gravitate to heterochromatin when silenced [76]. However, Easwaran et al. demonstrated that aberrant silencing of cancer-related genes occurred without requirement for their being positioned at heterochromatic domains using immunostaining for active/repressive chromatin marks and fluorescence in-situ hybridization in CRC cell lines. Furthermore, CpG hypermethylation, even associated with long-range silencing of nearby genes, occurred independently of their heterochromatic or euchromatic location [77]. These findings have important implications for understanding relationships between gene expression patterns and nuclear organization in cancer.

Another area under investigation is the understanding of mechanisms underlying which tumor suppressor genes are targeted for inactivation in cancer. Studies suggest a stem cell origin linked to epigenetic control of gene expression patterns in precursor cells regulated by constituent proteins in PcG repressive complexes including Polycomb Repressive Complex PRC1 [78, 79]. It was subsequently shown that sustained expression of the PRC1 protein, CBX7 along with

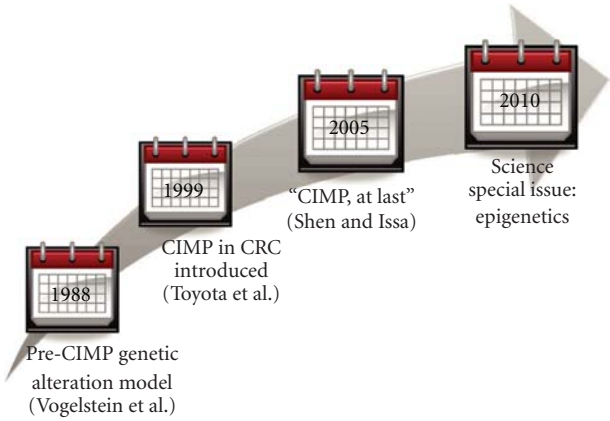

Figure 2: A decade of epigenetic research in colorectal cancer (CRC) has led to widespread recognition and acceptance of the CpG Island Methylator Phenotype.

other proteins, targets gene promoters in a progenitor-like embryonic tumor cell resulting in a cell population that models epigenetic characteristics of adult cancer (including aberrant CpG-island methylation) via inhibited response to differentiation cues [80].

DNA methylation markers have potential clinical use as diagnostic and prognostic tools. Hypermethylation of CpG islands can serve as a biomarker of cancer cells in tumor biopsies and other specimens. For example, quantitative assessment of methylation in CIMP-specific promoters of *MLH1*, *WRN*, and other DNA-repair genes in colon tumors, in comparison to paired normal tissues, may predict response to treatment [2]. Profiles of miRNA expression also differ between tumor and normal tissues; silencing of *miR-124a* in colon cancer cells activates expression of the oncogene *CDK6* [5]. Continued research involving detailed DNA methylomes in healthy and diseased tissues will help distinguish causal epigenetic alterations from "bystander changes" which are a consequence of cellular processes [71].

Unlike mutations in DNA sequence, epigenetic alterations such as CpG Island hypermethylation are potentially reversible by "reawakening" silenced tumor suppressor genes. Two nucleoside DNA methylation inhibitors, azacitidine and decitabine, are used clinically in low doses to treat myelodysplastic syndrome, providing proof of principal for epigenetic therapy [81]. Structurally, these agents mimic cytosine; during cell replication, fake cytosines replace real cytosines in growing DNA strands and then trap DNA methyltransferases to interfere with the ability of these enzymes to reproduce existing methylation in new cells. While inhibiting DNA methylation is a targeted molecular approach to therapy, downstream effects on neoplastic behavior are nonspecific and may result in cytotoxic cell death, making predictions of clinical outcomes difficult [81]. Clinical trials are being extended to test DNMT inhibitors in solid tumors of the breast, lung, and colon, in combination with histone deacetylase (HDAC) inhibitors which provide synergistic benefits in cell studies [82]. Other treatment avenues on the horizon include induced cellular programming to guide development of epigenetic-modifying drugs [83].

It has been a little over a decade since the concept of a CpG island methylator phenotype in colorectal cancer was introduced, and subsequent focus of several studies on describing the clinical and pathological features of CIMP as well as its characterization in tumors has supported widespread acceptance of the role of DNA methylation in cancer (Figure 2). The past few years have brought substantial insights as to the mechanisms underlying the CIMP pathway in cancer, and the future development of diagnostics and treatments based on our understanding of this epigenetic alteration are an exciting development in epigenetic research.

References

[1] R. Bonasio, S. Tu, and D. Reinberg, "Molecular signals of epigenetic states," *Science*, vol. 330, no. 6004, pp. 612–616, 2010.

[2] M. Esteller, "Molecular origins of cancer: epigenetics in cancer," *New England Journal of Medicine*, vol. 358, no. 11, pp. 1148–1096, 2008.

[3] A. P. Feinberg and B. Vogelstein, "Hypomethylation distinguishes genes of some human cancers from their normal counterparts," *Nature*, vol. 301, no. 5895, pp. 89–92, 1983.

[4] V. Greger, E. Passarge, W. Hopping, E. Messmer, and B. Horsthemke, "Epigenetic changes may contribute to the formation and spontaneous regression of retinoblastoma," *Human Genetics*, vol. 83, no. 2, pp. 155–158, 1989.

[5] A. Lujambio, S. Ropero, E. Ballestar et al., "Genetic unmasking of an epigenetically silenced microRNA in human cancer cells," *Cancer Research*, vol. 67, no. 4, pp. 1424–1429, 2007.

[6] W. S. Samowitz, "The CpG island methylator phenotype in colorectal cancer," *Journal of Molecular Diagnostics*, vol. 9, no. 3, pp. 281–283, 2007.

[7] M. Toyota, N. Ahuja, M. Ohe-Toyota, J. G. Herman, S. B. Baylin, and J. P. J. Issa, "CpG island methylator phenotype in colorectal cancer," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 96, no. 15, pp. 8681–8686, 1999.

[8] B. Vogelstein, E. R. Fearon, S. R. Hamilton et al., "Genetic alterations during colorectal-tumor development," *New England Journal of Medicine*, vol. 319, no. 9, pp. 525–532, 1988.

[9] J. P. J. Issa, L. Shen, and M. Toyota, "CIMP, at last," *Gastroenterology*, vol. 129, no. 3, pp. 1121–1124, 2005.

[10] P. W. Laird, "Cancer epigenetics," *Human Molecular Genetics*, vol. 14, no. 1, pp. R65–R76, 2005.

[11] W. S. Samowitz, H. Albertsen, J. Herrick et al., "Evaluation of a large, population-based sample supports a CpG island methylator phenotype in colon cancer," *Gastroenterology*, vol. 129, no. 3, pp. 837–845, 2005.

[12] J. R. Jass, "Colorectal cancer: a multipathway disease," *Critical Reviews in Oncogenesis*, vol. 12, no. 3-4, pp. 273–287, 2006.

[13] D. J. Weisenberger, K. D. Siegmund, M. Campan et al., "CpG island methylator phenotype underlies sporadic microsatellite instability and is tightly associated with BRAF mutation in colorectal cancer," *Nature Genetics*, vol. 38, no. 7, pp. 787–793, 2006.

[14] S. Ogino and A. Goel, "Molecular classification and correlates in colorectal cancer," *Journal of Molecular Diagnostics*, vol. 10, no. 1, pp. 13–27, 2008.

[15] A. McGivern, C. V. A. Wynter, V. L. J. Whitehall et al., "Promoter hypermethylation frequency and BRAF mutations distinguish hereditary non-polyposis colon cancer from sporadic MSI-H colon cancer," *Familial Cancer*, vol. 3, no. 2, pp. 101–107, 2004.

[16] N. Hawkins, M. Norrie, K. Cheong et al., "CpG island methylation in sporadic colorectal cancers and its relationship to microsatellite instability," *Gastroenterology*, vol. 122, no. 5, pp. 1376–1387, 2002.

[17] M. Van Rijnsoever, F. Grieu, H. Elsaleh, D. Joseph, and B. Iacopetta, "Characterisation of colorectal cancers showing hypermethylation at multiple CpG islands," *Gut*, vol. 51, no. 6, pp. 797–802, 2002.

[18] M. L. Slattery, K. Curtin, R. K. Wolff et al., "A comparison of colon and rectal somatic DNA alterations," *Diseases of the Colon and Rectum*, vol. 52, no. 7, pp. 1304–1311, 2009.

[19] L. Barault, C. Charon-Barra, V. Jooste et al., "Hypermethylator phenotype in sporadic colon cancer: study on a population-based series of 582 cases," *Cancer Research*, vol. 68, no. 20, pp. 8541–8546, 2008.

[20] K. Nosho, N. Irahara, K. Shima et al., "Comprehensive biostatistical analysis of CpG island methylator phenotype in colorectal cancer using a large population-based sample," *PLoS ONE*, vol. 3, no. 11, article e3698, 2008.

[21] D. R. English, J. P. Young, J. A. Simpson et al., "Ethnicity and risk for colorectal cancers showing somatic BRAF V600E mutation or CpG island methylator phenotype," *Cancer Epidemiology Biomarkers and Prevention*, vol. 17, no. 7, pp. 1774–1780, 2008.

[22] C. P. Vaughn, A. R. Wilson, and W. S. Samowitz, "Quantitative evaluation of CpG island methylation in hyperplastic polyps," *Modern Pathology*, vol. 23, no. 1, pp. 151–156, 2010.

[23] G. B. Baretton, F. Autschbach, and S. Baldus, "Histopathological diagnosis and differential diagnosis of colorectal serrated polys: findings of a consensus conference of the working group "Gastroenterological pathology of the German Society of Pathology"," *Pathologe*, vol. 32, no. 1, pp. 76–82, 2011.

[24] E. E. Torlakovic, J. D. Gomez, D. K. Driman et al., "Sessile serrated adenoma (SSA) vs. traditional serrated adenoma (TSA)," *American Journal of Surgical Pathology*, vol. 32, no. 1, pp. 21–29, 2008.

[25] L. Shen, P. J. Catalano, AL. B. Benson, P. O'Dwyer, S. R. Hamilton, and J. P. J. Issa, "Association between DNA methylation and shortened survival in patients with advanced colorectal cancer treated with 5-fluorouracil-based chemotherapy," *Clinical Cancer Research*, vol. 13, no. 20, pp. 6093–6098, 2007.

[26] S. Ogino, J. A. Meyerhardt, T. Kawasaki et al., "CpG island methylation, response to combination chemotherapy, and patient survival in advanced microsatellite stable colorectal carcinoma," *Virchows Archiv*, vol. 450, no. 5, pp. 529–537, 2007.

[27] A. M. Dahlin, R. Palmqvist, M. L. Henriksson et al., "The role of the CpG island methylator phenotype in colorectal cancer prognosis depends on microsatellite instability screening status," *Clinical Cancer Research*, vol. 16, no. 6, pp. 1845–1855, 2010.

[28] W. S. Samowitz, C. Sweeney, J. Herrick et al., "Poor survival associated with the BRAF V600E mutation in microsatellite-stable colon cancers," *Cancer Research*, vol. 65, no. 14, pp. 6063–6070, 2005.

[29] J. H. Kim, SO. H. Shin, H. J. Kwon, N. Y. Cho, and G. H. Kang, "Prognostic implications of CpG island hypermethylator phenotype in colorectal cancers," *Virchows Archiv*, vol. 455, no. 6, pp. 485–494, 2009.

[30] S. Ogino, K. Nosho, G. J. Kirkner et al., "CpG island methylator phenotype, microsatellite instability, BRAF mutation and clinical outcome in colon cancer," *Gut*, vol. 58, no. 1, pp. 90–96, 2009.

[31] S. J. Park, A. Rashid, J. H. Lee, S. G. Kim, S. R. Hamilton, and T. T. Wu, "Frequent CpG island methylation in serrated adenomas of the colorectum," *American Journal of Pathology*, vol. 162, no. 3, pp. 815–822, 2003.

[32] S. Ogino, T. Kawasaki, G. J. Kirkner, M. Loda, and C. S. Fuchs, "CpG island methylator phenotype-low (CIMP-low) in colorectal cancer: possible associations with male sex and KRAS mutations," *Journal of Molecular Diagnostics*, vol. 8, no. 5, pp. 582–588, 2006.

[33] L. Shen, M. Toyota, Y. Kondo et al., "Integrated genetic and epigenetic analysis identifies three different subclasses of colon cancer," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 104, no. 47, pp. 18654–18659, 2007.

[34] S. Ogino, M. Cantor, T. Kawasaki et al., "CpG island methylator phenotype (CIMP) of colorectal cancer is best characterised by quantitative DNA methylation analysis and prospective cohort studies," *Gut*, vol. 55, no. 7, pp. 1000–1006, 2006.

[35] N. Tanaka, C. Huttenhower, K. Nosho et al., "Novel application of structural equation modeling to correlation structure analysis of CpG island methylation in colorectal cancer," *American Journal of Pathology*, vol. 177, no. 6, pp. 2731–2740, 2010.

[36] P. W. Ang, M. Loh, N. Liem et al., "Comprehensive profiling of DNA methylation in colorectal cancer reveals subgroups with distinct clinicopathological and molecular features," *BMC Cancer*, vol. 10, article 227, 2010.

[37] A. Kaneda and K. Yagi, "Two groups of DNA methylation markers to classify colorectal cancer into three epigenotypes," *Cancer Science*, vol. 102, no. 1, pp. 18–24, 2011.

[38] T. Hinoue, D. J. Weisenberger, F. Pan et al., "Analysis of the association between CIMP and BRAF in colorectal cancer by DNA methylation profiling," *PloS one*, vol. 4, no. 12, p. e8357, 2009.

[39] T. Kondo and J. P. Issa, "DNA methylation profiling in cancer," *Expert Review of Proteomics*, vol. 12, article e23, 2010.

[40] J. R. Jass, "Classification of colorectal cancer based on correlation of clinical, morphological and molecular features," *Histopathology*, vol. 50, no. 1, pp. 113–130, 2007.

[41] M. Toyota, M. Ohe-Toyota, N. Ahuja, and J. P. J. Issa, "Distinct genetic profiles in colorectal tumors with or without the CpG island methylator phenotype," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 97, no. 2, pp. 710–715, 2000.

[42] S. D. Dertinger, A. E. Silverstone, and T. A. Gasiewicz, "Influence of aromatic hydrocarbon receptor-mediated events on the genotoxicity of cigarette smoke condensate," *Carcinogenesis*, vol. 19, no. 11, pp. 2037–2042, 1998.

[43] S. A. Belinsky, W. A. Palmisano, F. D. Gilliland et al., "Aberrant promoter methylation in bronchial epithelium and sputum from current and former smokers," *Cancer Research*, vol. 62, no. 8, pp. 2370–2377, 2002.

[44] S. S. Ray and H. I. Swanson, "Dioxin-induced immortalization of normal human keratinocytes and silencing of p53 and p16," *Journal of Biological Chemistry*, vol. 279, no. 26, pp. 27187–27193, 2004.

[45] W. S. Samowitz, H. Albertsen, C. Sweeney et al., "Association of smoking, CpG island methylator phenotype, and V600E BRAF mutations in colon cancer," *Journal of the National Cancer Institute*, vol. 98, no. 23, pp. 1731–1738, 2006.

[46] K. Curtin, W. S. Samowitz, R. K. Wolff, J. Herrick, B. J. Caan, and M. L. Slattery, "Somatic alterations, metabolizing genes and smoking in rectal cancer," *International Journal of Cancer*, vol. 125, no. 1, pp. 158–164, 2009.

[47] D. Limsui, R. A. Vierkant, L. S. Tillmans et al., "Cigarette smoking and colorectal cancer risk by molecularly defined subtypes," *Journal of the National Cancer Institute*, vol. 102, no. 14, pp. 1012–1022, 2010.

[48] L. S. Rozek, C. M. Herron, J. K. Greenson et al., "Smoking, gender, and ethnicity predict somatic BRAF mutations in colorectal cancer," *Cancer Epidemiology Biomarkers and Prevention*, vol. 19, no. 3, pp. 838–843, 2010.

[49] M. L. Slattery, K. Curtin, K. Anderson et al., "Associations between cigarette smoking, lifestyle factors, and microsatellite instability in colon tumors," *Journal of the National Cancer Institute*, vol. 92, no. 22, pp. 1831–1836, 2000.

[50] L. M. Morimoto, P. A. Newcomb, C. M. Ulrich, R. M. Bostick, C. J. Lais, and J. D. Potter, "Risk factors for hyperplastic and adenomatous polyps: evidence for malignant potential?" *Cancer Epidemiology Biomarkers and Prevention*, vol. 11, no. 10 I, pp. 1012–1018, 2002.

[51] C. M. Ulrich, "Nutrigenetics in cancer research—folate metabolism and colorectal cancer," *Journal of Nutrition*, vol. 135, no. 11, pp. 2698–2702, 2005.

[52] S. J. James, S. Melnyk, M. Pogribna, I. P. Pogribny, and M. A. Caudill, "Elevation in S-Adenosylhomocysteine and DNA hypomethylation: potential epigenetic mechanism for homocysteine-related pathology," *Journal of Nutrition*, vol. 132, no. 8, pp. 2361S–2366S, 2002.

[53] K. Kawakami, A. Ruszkiewicz, G. Bennett, J. Moore, GO. Watanabe, and B. Iacopetta, "The folate pool in colorectal cancers is associated with DNA hypermethylation and with a polymorphism in methylenetetrahydrofolate reductase," *Clinical Cancer Research*, vol. 9, no. 16 I, pp. 5860–5865, 2003.

[54] S. Ogino, T. Kawasaki, K. Nosho et al., "LINE-1 hypomethylation is inversely associated with microsatellite instability and CpG island methylator phenotype in colorectal cancer," *International Journal of Cancer*, vol. 122, no. 12, pp. 2767–2773, 2008.

[55] M. R. H. Estécio, V. Gharibyan, L. Shen et al., "LINE-1 hypomethylation in cancer is highly variable and inversely correlated with microsatellite instability," *PLoS ONE*, vol. 2, no. 5, article e399, 2007.

[56] M. L. Slattery, K. Curtin, C. Sweeney et al., "Diet and lifestyle factor associations with CpG island methylator phenotype and BRAF mutations in colon cancer," *International Journal of Cancer*, vol. 120, no. 3, pp. 656–663, 2007.

[57] M. Van Engeland, M. P. Weijenberg, G. M. J. M. Roemen et al., "Effects of dietary folate and alcohol intake on promoter methylation in sporadic colorectal cancer: the Netherlands cohort study on diet and cancer," *Cancer Research*, vol. 63, no. 12, pp. 3133–3137, 2003.

[58] K. Curtin, M. L. Slattery, C. M. Ulrich et al., "Genetic polymorphisms in one-carbon metabolismml: associations with CpG island methylator phenotype (CIMP) in colon cancer and the modifying effects of diet," *Carcinogenesis*, vol. 28, no. 8, pp. 1672–1679, 2007.

[59] K. Curtin, W. Samowitz, C. Ulrich et al., "Nutrients in folate-mediated, one-carbon metabolism and risk of rectal tumors in men and women," *Nutrition and Cancer*, vol. 31, no. 1, 2011.

[60] A. Hazra, C. S. Fuchs, T. Kawasaki, G. J. Kirkner, D. J. Hunter, and S. Ogino, "Germline polymorphisms in the one-carbon metabolism pathway and DNA methylation in colorectal cancer," *Cancer Causes and Control*, vol. 21, no. 3, pp. 331–345, 2010.

[61] S. De Vogel, K. A. D. Wouters, R. W. H. Gottschalk et al., "Genetic variants of methyl metabolizing enzymes and epigenetic regulators: associations with promoter CpG island hypermethylation in colorectal cancer," *Cancer Epidemiology Biomarkers and Prevention*, vol. 18, no. 11, pp. 3086–3096, 2009.

[62] K. Curtin, W. S. Samowitz, R. K. Wolff et al., "MSH6 G39E polymorphism and CpG island methylator phenotype in colon cancer," *Molecular Carcinogenesis*, vol. 48, no. 11, pp. 989–994, 2009.

[63] W. S. Samowitz, K. Curtin, R. K. Wolff et al., "The MLH1 −93G > A promoter polymorphism and genetic and epigenetic alterations in colon cancer," *Genes Chromosomes and Cancer*, vol. 47, no. 10, pp. 835–844, 2008.

[64] H. G. Linhart, H. Lin, Y. Yamada et al., "Dnmt3b promotes tumorigenesis in vivo by gene-specific de novo methylation and transcriptional silencing," *Genes and Development*, vol. 21, no. 23, pp. 3110–3122, 2007.

[65] K. Nosho, K. Shima, N. Irahara et al., "DNMT3B expression might contribute to CpG island methylator phenotype in colorectal cancer," *Clinical Cancer Research*, vol. 15, no. 11, pp. 3663–3671, 2009.

[66] A. E. Ibrahim, M. J. Arends, A. L. Silva et al., "Sequential DNA methylation changes are associated with DNMT3B overexpression in colorectal neoplastic progression," *Gut*, vol. 60, no. 4, pp. 444–508, 2011.

[67] S. Ogino, A. T. Chan, C. S. Fuchs, and E. Giovannucci, "Molecular pathological epidemiology of colorectal neoplasia: an emerging transdisciplinary and interdisciplinary field," *Gut*, vol. 60, no. 3, pp. 397–411, 2011.

[68] L. A. E. Hughes, P. A. van den Brandt, A. P. de Bruïne et al., "Early life exposure to famine and colorectal cancer risk: a role for epigenetic mechanisms," *PLoS ONE*, vol. 4, no. 11, article e7951, 2009.

[69] M. L. Slattery, A. Lundgreen, J. S. Herrick, and R. K. Wolff, "Genetic variation in RPS6KA1, RPS6KA2, RPS6KB1, RPS6KB2, and PDK1 and risk of colon or rectal cancer," *Mutation Research*, vol. 706, no. 1-2, pp. 13–20, 2011.

[70] M. L. Slattery, K. Curtin, E. M. Poole et al., "Genetic variation in C-reactive protein (CRP) in relation to colon and rectal cancer risk and survival," *International Journal of Cancer*, vol. 128, no. 11, pp. 2726–2734, 2011.

[71] A. Portela and M. Esteller, "Epigenetic modifications and human disease," *Nature Biotechnology*, vol. 28, no. 10, pp. 1057–1068, 2010.

[72] M. Esteller, "Epigenetic gene silencing in cancer: the DNA hypermethylome," *Human Molecular Genetics*, vol. 16, no. 1, pp. R50–R59, 2007.

[73] J. Frigola, J. Song, C. Stirzaker, R. A. Hinshelwood, M. A. Peinado, and S. J. Clark, "Epigenetic remodeling in colorectal cancer results in coordinate gene suppression across an entire chromosome band," *Nature Genetics*, vol. 38, no. 5, pp. 540–549, 2006.

[74] J. J. Wong, N. J. Hawkins, R. L. Ward, and M. P. Hitchins, "Methylation of the 3p22 region encompassing MLH1 is representative of the CpG island methylator phenotype in colorectal cancer," *Modern Pathology*, vol. 24, pp. 396–411, 2011.

[75] R. A. Irizarry, C. Ladd-Acosta, B. Wen et al., "The human colon cancer methylome shows similar hypo- and hypermethylation at conserved tissue-specific CpG island shores," *Nature Genetics*, vol. 41, no. 2, pp. 178–186, 2009.

[76] P. Malik, N. Zuleger, and E. C. Schirmer, "Nuclear envelope influences on genome organization," *Biochemical Society Transactions*, vol. 38, no. 1, pp. 268–272, 2010.

[77] H. P. Easwaran, L. Van Neste, L. Cope et al., "Aberrant silencing of cancer-related genes by CpG hypermethylation occurs independently of their spatial organization in the nucleus," *Cancer Research*, vol. 70, no. 20, pp. 8015–8024, 2010.

[78] J. E. Ohm, K. M. McGarvey, X. Yu et al., "A stem cell-like chromatin pattern may predispose tumor suppressor genes to DNA hypermethylation and heritable silencing," *Nature Genetics*, vol. 39, no. 2, pp. 237–242, 2007.

[79] Y. Schlesinger, R. Straussman, I. Keshet et al., "Polycomb-mediated methylation on Lys27 of histone H3 pre-marks genes for de novo methylation in cancer," *Nature Genetics*, vol. 39, no. 2, pp. 232–236, 2007.

[80] H. P. Mohammad, Y. Cai, K. M. McGarvey et al., "Polycomb CBX7 promotes initiation of heritable repression of genes frequently silenced with cancer-specific DNA hypermethylation," *Cancer Research*, vol. 69, no. 15, pp. 6322–6330, 2009.

[81] J. P. J. Issa and H. M. Kantarjian, "Targeting DNA methylation," *Clinical Cancer Research*, vol. 15, no. 12, pp. 3938–3946, 2009.

[82] J. Kaiser, "Epigenetic drugs take on cancer," *Science*, vol. 330, no. 6004, pp. 576–578, 2010.

[83] J. E. Ohm, P. Mali, L. Van Neste et al., "Cancer-related epigenome changes associated with reprogramming to induced pluripotent stem cells," *Cancer Research*, vol. 70, no. 19, pp. 7662–7673, 2010.

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Review Article

Molecular Pathology of Hepatic Neoplasms: Classification and Clinical Significance

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Recent technological advances have enabled investigators to characterize the molecular genetics and genomics of hepatic neoplasia in remarkable detail. From these studies, an increasing number of molecular markers are being identified that correlate with clinically important tumor phenotypes. This paper discusses current knowledge relevant to the molecular classification of epithelial primary hepatic tumors that arise in adults, including focal nodular hyperplasia (FNH), hepatocellular adenoma (HCA), hepatocellular carcinoma (HCC), cholangiocarcinoma (CC), and combined HCC-CC. Genetic analysis has defined molecular subtypes of HCA that are clinicopathologically distinct and can be distinguished through immunohistochemistry. Gene expression studies have identified molecular signatures of progression from dysplastic nodules (DNs) to early HCC in cirrhosis. Analyses of the mutational spectra, chromosomal aberrations and instability, transcriptomics, and microRNA profiles of HCC have revealed the existence of biologically distinct subtypes of this common malignancy, with prognostic implications. Molecular characterization of biliary and hepatic progenitor cell phenotypes in liver cancer has shed new light on the histogenesis of these tumors and has focused attention on novel therapeutic targets. In coming years, the molecular classification of hepatic neoplasms will be increasingly valuable for guiding patient care, as targeted therapies for liver cancer are developed and brought into clinical practice.

1. Introduction

Over the past decade, tremendous advances have been made in the technologies available for characterizing the molecular genetics, genomics and epigenetics of neoplasia. These advances have greatly accelerated basic research aimed at elucidating the molecular mechanisms underlying tumorigenesis. In addition, they have led to the identification of molecular markers that correlate with important biological characteristics of tumors. Such markers are increasingly valuable in clinical practice as tools for facilitating the diagnosis and categorization of tumors, determining their aggressiveness and, in some cases, predicting their responses to particular forms of therapy. The ideal classification system for any group of neoplasms would be based on our understanding of their ontogeny and would integrate histologic features with molecular data in a clinically meaningful way. In this paper, we will discuss the molecular biology of primary hepatic tumors, with particular emphasis on the roles of molecular characterization in clinical practice. We will focus on primary hepatic neoplasms that arise in adults, including hepatocellular adenoma (HCA), hepatocellular carcinoma (HCC), cholangiocarcinoma (CC), and the rare entity, combined hepatocellular and cholangiocarcinoma (HCC-CC). Focal nodular hyperplasia (FNH), although thought to be a nonneoplastic lesion, is often considered in the differential diagnosis of other hepatocytic tumors and, therefore, has also been included in this discussion. However, consideration of mesenchymal, hematopoietic and childhood liver tumors is beyond the scope of this review.

FNH is the most common of the benign hepatocellular tumors, arising in approximately 3% of adults [1, 2]. Although the other major type of benign hepatocellular tumor, HCA occurs much less frequently, with an estimated incidence in Europe and North America of 0.003% [3], its association with hemorrhage, rupture, and risk of malignant transformation necessitates its accurate diagnostic distinction from FNH. This is an area in which molecular

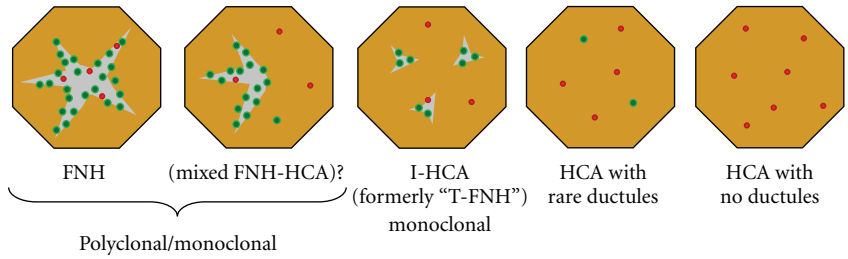


Figure 1: Schematic illustration of the histologic spectrum of benign hepatocellular proliferations. Polyclonal FNH is at one end of the spectrum and nontelangiectatic HCA is at the other; inflammatory HCA (I-HCA), formerly classified as telangiectatic FNH (T-FNH), has overlapping features and is shown in the middle. Gray areas indicate fibrosis; green and red dots represent bile ductules and arterioles, respectively.

characterization has become very informative and clinically useful. The most common primary hepatic malignancy is HCC, which constitutes 80–85% of all malignant epithelial neoplasms originating in the liver. HCC often arises in a background of cirrhosis and presents a global public health problem far greater than that of HCA. In the United States, the annual incidence of HCC has been rising over the past three decades [4]. The second most common primary hepatic malignancy is CC, constituting 15–20% of the total, and its incidence has also been rising. There has been an explosion of basic research in the field of primary hepatic epithelial cancers in recent years, and we are just beginning to understand their molecular pathogenesis. We anticipate rapid advancements over the next several years in personalized medical approaches to the treatment of liver cancer, as the molecular mechanisms of hepatic tumorigenesis are elucidated and molecular classification of these lesions is integrated into clinical study design [5].

2. Focal Nodular Hyperplasia

Focal nodular hyperplasia (FNH) is a common, benign hepatocellular lesion that is believed to arise in response to localized hyperperfusion of the liver parenchyma, typically in association with a microscopic arterial malformation [6, 7]. FNH frequently presents as an incidental hepatic mass noted in imaging studies, and it can often be diagnosed on the basis of its radiographic appearance alone. However, sometimes the diagnosis requires histologic confirmation. Although the gross morphology and microscopic appearance of FNH in resection specimens are usually characteristic and easily recognized, distinguishing FNH from HCA in needle biopsies can be difficult. The distinction is, however, of great importance because of the differing clinical management of these entities.

Traditionally, FNH has been considered nonneoplastic and, accordingly, it is thought to have no malignant potential. However, molecular analyses of the clonality of FNH have yielded conflicting results, with some studies finding that up to 50% show X chromosome inactivation patterns suggestive of monoclonality [7, 8]. In general, polyclonality argues strongly against neoplasia, but the implications of monoclonality are less clear, since it may be influenced by factors such as variability in X-inactivation patterns, tissue architecture and turnover rate. Thus, the question of whether classic FNH is sometimes neoplastic remains controversial. Atypical forms of FNH have been described, including mixed lesions with both FNH-like and HCA-like areas, as well as lesions with histologic features intermediate between FNH and HCA [9]. The latter tumors were originally designated "telangiectatic FNH" (T-FNH), but subsequent molecular analyses have led to their reclassification as a variant of HCA (see below). Mixed FNH-HCA have not been subjected to molecular analysis and, indeed, have not been described in recent publications. Therefore, their existence as a distinct entity is uncertain. Although FNH and variants of HCA comprise a histologic spectrum (Figure 1), there is no compelling evidence that they are biologically related in the sense of one being the precursor of another.

Given the uncertainty surrounding the issue of neoplasia in FNH, several investigators have searched for genetic alterations that might define these lesions. If a genetic abnormality characteristic of FNH were found, it would greatly support its neoplastic nature, and the finding could be diagnostically useful in clinical practice. However, the results of such studies thus far have been uniformly negative, with no mutations detected in the APC, axin, β-catenin, HNF1α, or p53 genes in classic FNH [8, 10–12].

Both FNH and HCA consist predominantly of well-differentiated hepatocytes. In FNH, there are intervening fibrous bands that radiate from a central scar and contain abundant, proliferating bile ductules. In contrast, HCA have only rare bile ductules, if any, and typically show much less fibrosis. However, the regions of fibrous scar in FNH are variable in abundance and are not always adequately sampled in needle biopsies, sometimes making the histologic distinction from HCA difficult. We have found that the expression patterns of biliary markers, as determined by immunohistochemistry, are frequently different in FNH and HCA, not only within regions containing bile ductules, but also in hepatocellular areas [13]. Antibodies against cytokeratin 19 (CK19) and neuronal cell adhesion molecule (NCAM, also known as CD56), which are markers of hepatic progenitor cells as well as biliary epithelium, clearly stain the proliferating ductules within fibrous tracts in FNH and rare, isolated ductules in HCA. Cytokeratin 7 (CK7), which is known to be a marker of immature hepatocytes

as well as progenitor cells and biliary epithelium [14], shows a distinct pattern of expression in HCA [15] that distinguishes the latter from FNH in most cases (Figure 2). In our experience, although occasional needle biopsies remain diagnostically challenging even after the combined use of immunohistochemical stains for CK7 and CK19, this analysis is usually quite helpful in the differential diagnosis of FNH and HCA [13, 16].

Immunohistochemical staining for the cytoplasmic enzyme, glutamine synthetase (GS), has also been proposed as a technique for differentiating between FNH and HCA [2]. GS is an enzyme that combines glutamate with ammonia to produce the amino acid glutamine. In normal liver, GS expression is limited to the centrilobular hepatocytes that directly border central veins, and its most important function is to aid in ammonia detoxification [17]. In recent years, the Wnt signaling pathway has been identified as a critical regulator of zonation in the liver [18]. Beta-catenin activation in perivenular hepatocytes, presumably the result of Wnt signaling from central veins, drives the expression of GS. In FNH, the centrilobular zones of β-catenin activation are expanded [12], and this leads to an irregular, geographic distribution of GS overexpression [2]. In HCA and HCC, several different patterns of GS expression have been observed, all distinct from that seen in FNH. As discussed later, activating mutations of the β-catenin gene occur in a subset of HCA. In adenomas with β-catenin mutation, GS is diffusely expressed, whereas in adenomas with normal β-catenin, GS is absent in large areas of the lesion. Unfortunately, however, in the latter type of HCA, irregular and/or centrizonal expression of GS can be present focally, particularly at the periphery of the tumor [2]. Therefore, although GS expression appears to robustly differentiate FNH from HCA in full resection specimens, it is less reliable for making this distinction in needle biopsies.

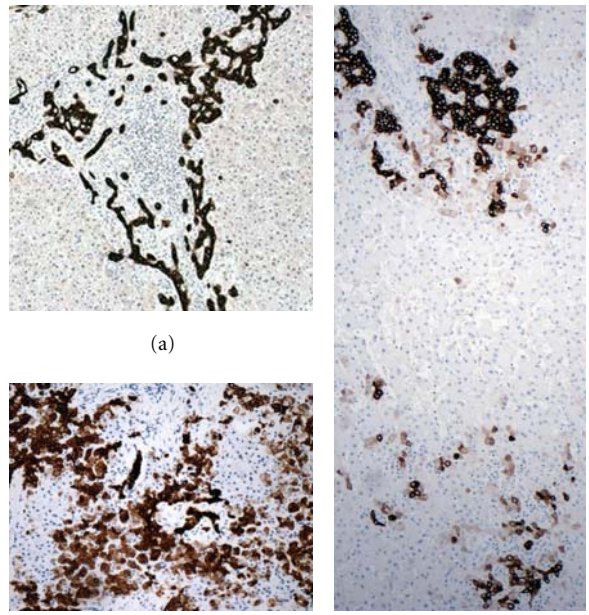


Figure 2: Patterns of immunohistochemical positivity for CK7 in benign hepatocellular lesions. (a) FNH displays strong CK7 staining of proliferating ductules but virtually no positivity within hepatocytes. (b) An example of HCA (nonmutated, noninflammatory type) showing focal, moderate CK7 staining within hepatocytes as well as heavy staining of a few bile ductules. (c) An I-HCA containing areas of bile ductular proliferation (arrow) with strong CK7 expression, resembling a ductular reaction in FNH, as well as other areas (e.g., bottom of image) in which there is focal hepatocellular staining for CK7, as is typical of HCA.

3. Hepatocellular Adenoma (HCA)

First described a half-century ago, HCA is a rare, benign liver tumor that most commonly occurs in women and has been associated with oral contraceptive use [19]. It is a neoplasm of demonstrated clonal origin [20, 21] and has a small but nonnegligible risk of malignant transformation [22]. Hemorrhage, pain, and rupture are other, more frequent complications of HCA, and their likelihood is proportional to the size of the tumor [1]. Histologically, HCA is a proliferation of mature-appearing hepatocytes arranged in cords one to two cells thick and lacking portal tracts. Isolated arterioles surrounded by hepatocytes and lacking a fibrous sheath ("naked" or "unpaired" arteries) can be seen scattered throughout the lesion. Arterioles accompanied by portal venules may be observed in a few portal-like structures, but these lack bile ducts. In some HCA, occasional isolated bile ductules may be seen, especially when immunohistochemical stains for CK7 or CK19 are applied.

In 2002, Bluteau et al. published the results of a genome-wide search for tumor suppressor genes in HCA [23]. Microsatellite analysis revealed a loss of heterozygosity (LOH) for markers at chromosome 12q caused by a small deletion of this region in five out of ten HCA. The TCF1 gene, encoding hepatocyte nuclear factor 1 (HNF1), was found to reside within the deletion. Because the function of HNF1 as a liver-specific transcription factor was already well established, this gene was pursued as the most likely candidate tumor suppressor affected by LOH in these adenomas. Further investigation confirmed that in ten out of sixteen HCA analyzed, HNF1 was somatically inactivated, either through a combination of gene deletion and mutation or via bi-allelic mutation. Interestingly, germline mutation of the HNF1 gene had been previously discovered to underlie a rare form of familial noninsulin dependent diabetes, MODY3 (maturity-onset diabetes of the young, type 3) [24]. This form of diabetes is associated with an increased risk of hepatic adenomatosis, due to random somatic inactivation of the second, nongermline-mutant HNF1 allele in the liver [25].

Shortly after genetic alterations of HNF1 in HCA were reported, a group in Taiwan published their discovery of activating mutations of the β-catenin gene in a fraction of these tumors. Earlier work by many independent groups had implicated Wnt pathway activation in liver carcinogenesis, and β-catenin mutations have been found in a significant percentage of HCC. To investigate the possibility that aberrant β-catenin signaling plays a role in very early hepatic

neoplasia, Chen et al. performed directed LOH analysis and genomic sequencing of several Wnt pathway genes [10]. Three of ten HCA were found to bear small, in-frame deletions in one allele of the β-catenin gene, and to express truncated forms of the protein that are predicted to be constitutively activated.

The French group that had originally identified HNF1 inactivation in HCA next expanded their study to include assessment of β-catenin mutation status as well as genotype-phenotype correlations in these tumors [26]. In a collection of 96 HCA, 44 were found to have HNF1 gene inactivation and to display a characteristic histology, including marked hepatocellular steatosis, a lack of cytologic atypia, and an absence of inflammatory infiltrates in the lesion. Beta-catenin mutation was identified in 12 of the 96 tumors and was frequently associated with histologic features suggestive of malignancy, including nuclear atypia and pseudoacinar formation. Importantly, β-catenin mutation and HNF1 inactivation were mutually exclusive in these tumors, indicating the existence of at least two distinct, nonoverlapping molecular subtypes of HCA.

In the 1990s, a class of histologically unusual hepatic tumors was described and termed "telangiectatic FNH" (T-FNH) [9]. These lesions lacked a central scar, but because they displayed focal ductular reaction and a nodular architecture, they were categorized as an atypical variant of FNH. However, the sinusoidal dilatation, naked arterioles, and frequent intralesional hemorrhage that typified T-FNH were recognized as being similar to HCA (Figure 1). These peculiar features of T-FNH invited molecular characterization and comparison with both classic FNH and HCA. Two groups performed clonality assays and found a much higher rate of monoclonality in T-FNH than in classic FNH [11, 20]. The angiopoietin mRNA expression pattern, which had been shown to differ between FNH and HCA, was found in T-FNH to resemble that of HCA, and a global proteomic profile of T-FNH matched that of HCA while distinguishing both T-FNH and HCA from classic FNH [20]. Together, these findings strongly suggest that so-called telangiectatic FNH is actually a variant of HCA.

In the genotype-phenotype study described above, HCA that were found not to harbor genetic alterations in either HNF1 or β-catenin were further subdivided into two groups on the basis of the presence or absence of inflammatory infiltrates. The subgroup showing inflammation, named inflammatory HCA (I-HCA), was found to include all lesions formerly classified as T-FNH. Thus, four subtypes of HCA were proposed: (1) HNF1-inactivated HCA (H-HCA), (2) β-catenin-mutated HCA (β-HCA), (3) I-HCA, and (4) nonmutated, noninflammatory HCA [26]. A subsequent study of 93 HCA (47 of which had been previously analyzed) supported this classification system in general, except that β-catenin mutation was discovered to occur in a fraction of I-HCA as well as in noninflammatory HCA [27].

The hypothesis that I-HCA represents a biologically distinct entity was greatly strengthened with the finding of a marked overexpression of acute-phase reactants by the hepatocytes of these lesions [27]. At both the mRNA and the protein level, serum amyloid A (SAA) and C-reactive protein (CRP) are significantly overexpressed in I-HCA relative to nonneoplastic liver. When all types of hepatocellular adenoma are examined, the sensitivity and specificity of SAA overexpression by immunohistochemistry for identifying I-HCA was found to be 94%. The degree of SAA overexpression did not correlate with the extent of infiltration by inflammatory cells, which suggested that acute-phase protein expression might be an intrinsic feature of the neoplastic hepatocytes, whereas inflammatory infiltration might be secondary. To explore this possibility, Rebouissou et al. undertook a genome-wide mRNA expression study, comparing I-HCA to normal liver, and identified a pronounced activation of acute-phase inflammatory signaling in I-HCA [28]. Further inquiry into the potential causes of this inflammatory gene expression signature led to the discovery of mutations in the IL6ST gene, which encodes gp130, a component of the IL-6 receptor, in 60% of I-HCA [28]. These IL6ST mutations lead to ligand-independent activation of the IL-6 receptor, which promotes STAT3 signaling and induces the acute-phase inflammatory response within hepatocytes. The recruitment of inflammatory cells into I-HCA appears to be secondary to gp130-mediated hepatocellular production of the chemokine, CCL20, which attracts immune cells. In this study, all 43 I-HCA were found to show expression signatures indicative of IL-6 pathway activation, even those tumors in which no IL6ST mutation was identified. In I-HCA lacking IL6ST mutation, no other genetic alterations could be identified in various components of the STAT3 signaling pathway. However, gp130 protein levels were elevated in most of these lesions, suggesting that posttranslational control of gp130 was aberrant, perhaps due to occult mutation of a gene that normally regulates gp130's protein abundance.

In summary, HCA can be assigned to one of four categories [3]. The first is H-HCA and is defined by HNF1 inactivation. This variety accounts for 35–40% of all HCA and occurs almost exclusively in women. Rarely, these tumors arise in the context of familial diabetes (MODY3) and can be multiple. H-HCA can be identified through immunohistochemical staining for liver fatty acid binding protein (LFABP), which is a transcriptional target of HNF1 and is completely absent in the hepatocytes of these lesions. Histologically, H-HCA usually displays marked steatosis but no inflammation, and it lacks cytologic atypia. The second category, I-HCA, comprises over 50% of all HCA. It is characterized by the presence of inflammatory infiltrates, focal ductular reaction, sinusoidal dilation, and dystrophic arterioles. I-HCA almost invariably shows dramatic overexpression of acute-phase reactants, including SAA and CRP, which can be demonstrated by immunohistochemistry (Figure 3). Although the HNF1 gene is never inactivated in I-HCA, β-catenin mutations are sometimes found [29]. This type of HCA is associated with obesity, smoking, and alcohol consumption and is the most common variety of HCA in men.

The third category of HCA is somewhat controversial and consists of lesions that harbor activating mutations of β-catenin but are noninflammatory; it has been reported

that approximately 10% of adenomas belong to this β-HCA subtype. Beta-catenin activation is most easily assessed through immunostaining for glutamine synthetase, which is diffusely overexpressed in these lesions. Alternatively, immunohistochemical staining for β-catenin itself may be used to distinguish between tumors without mutation, in which β-catenin has a membranous localization, and those with mutation, in which an aberrant, activated form of β-catenin accumulates within nuclei. In both I-HCA and β-HCA, β-catenin mutation has been associated with a high risk of malignancy. These tumors are common in men, and they often show features, such as pseudoacini and hepatocellular dysplasia, that are frequent in HCC. In fact, because β-HCA may be histologically indistinguishable from well-differentiated HCC [28], their existence as a biologically distinct entity has been called into question. A group in the United States has recently reported their failure to find any examples of β-HCA in a collection of 41 adenomas subjected to molecular analysis [30]. We feel that in well-differentiated hepatocellular tumors, the presence of β-catenin mutation in association with morphologic features suspicious for malignancy should warrant a pathologic diagnosis of either outright or suspected hepatocellular carcinoma, rather than HCA. This is an area in need of further research to determine the molecular differences, if any exist, between β-HCA and HCC. Such findings would help to elucidate the pathogenesis of HCC and might serve as the basis for a diagnostic test of malignancy in histologically equivocal cases.

The fourth and final category of HCA are those that are noninflammatory (negative for acute phase markers) and do not harbor mutations in HNF1, β-catenin, or gp130. This is the smallest group of HCA, accounting for only about 5% of the total. Lesions that cannot be fully evaluated due to extensive necrosis and/or hemorrhage are also classified in this group. Whether these noninflammatory, nonmutant tumors represent a truly distinct subtype of HCA, or whether they are biologically related to one or more of the other three subtypes, is an open question that awaits further investigation.

4. Hepatocellular Carcinoma (HCC)

HCC almost always arises in the context of chronic liver disease, usually in patients with cirrhosis. Cirrhosis of any etiology constitutes the predominant risk factor for HCC. Worldwide, infection with hepatitis B virus (HBV) accounts for the majority of cases, whereas in the United States, chronic hepatitis C is the most common predisposing factor. HCC is often diagnosed late in the course of disease, but in developed countries, surveillance programs that use improved radiologic techniques for monitoring liver lesions in patients with cirrhosis are leading to increased rates of early HCC detection.

4.1. Dysplastic Nodules and Early Hepatocellular Carcinoma in Cirrhosis. It is now well accepted that in cirrhotic livers, the vast majority of HCC arise from benign precursor lesions called dysplastic nodules (DN) [31]. Although fully developed HCCs typically show histologic features that are easily recognizable to pathologists, the morphologic distinction between advanced DN and early HCCs is often more subtle and can be very difficult to identify, especially in needle biopsies [32]. As the molecular genetic events that drive the early steps of hepatic carcinogenesis are more fully elucidated, it is anticipated that molecular markers will be found that can help pathologists in making these clinically important distinctions. Indeed, in recent years, molecular analyses of the dysplasia-to-carcinoma pathway in cirrhosis have begun to yield valuable information for the recognition and classification of early hepatic neoplasia.

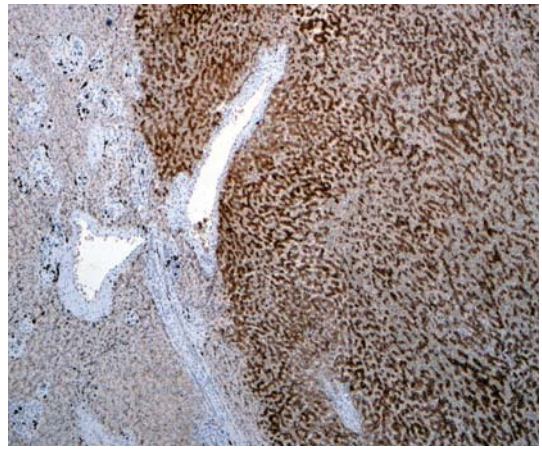
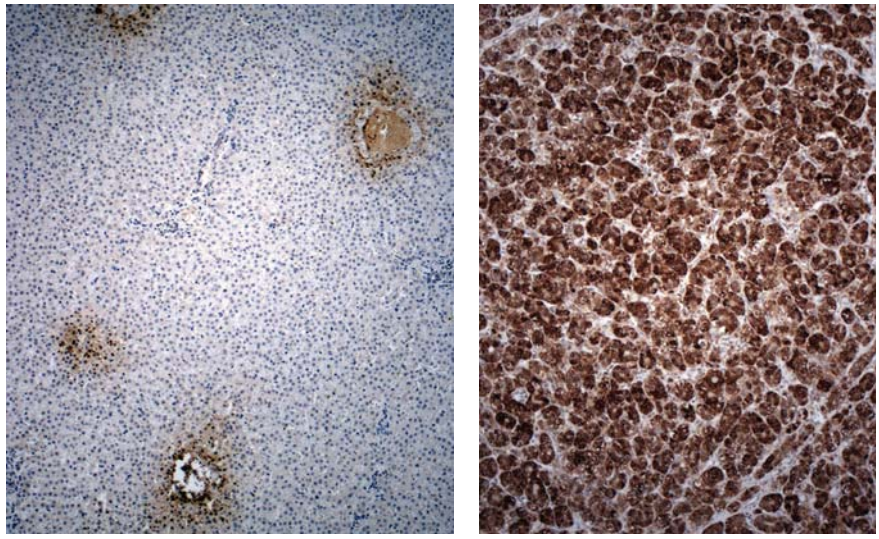


Figure 3: Immunohistochemical staining for serum amyloid A (SAA) in an inflammatory HCA shows strong, diffuse positivity in the tumor (right) but virtually no staining in adjacent normal liver (left).

Several groups have used gene expression profiling to identify "molecular signatures" that can accurately distinguish between DN and early HCC [33–37]. Perhaps the most promising of these studies is that of Llovet et al., which analyzed the expression patterns of 55 hepato carcinogenesis-related genes in dysplastic nodules and early HCCs of hepatitis C-infected patients. These authors identified a panel of only three genes (GPC3, LYVE1 and survivin) whose mRNA expression levels, as assessed by quantitative reverse transcription-PCR (qRT-PCR), were shown to correctly predict malignancy in 19 out of 20 early HCCs and nonmalignancy in 16 out of 17 DNs. As a test for HCC in this sample set, the sensitivity of this 3-gene expression panel was 95% and its specificity was 94%. Two of the genes in the panel, GPC3 (which encodes glypican-3) and survivin, are expressed by hepatocytes and are upregulated in HCCs, as compared to DN. The third gene, LYVE1, is expressed by endothelial cells and is downregulated in malignancy. All three genes had been previously implicated in hepatic carcinogenesis, and their differential expression levels in DNs and HCCs is most likely independent of the underlying cause of cirrhosis. The advantage of using qRT-PCR to identify this sort of molecular signature is that the method is highly quantitative, much more so than conventional immunohistochemistry, and, therefore, more objective. However, a disadvantage is that it must be performed on pure lesional tissue,



(a) (b)

Figure 4: Glutamine synthase expression is (a) restricted to perivenular (zone 3) hepatocytes in normal liver but (b) diffuse and strong in HCC.

which can be scarce in needle biopsies. Furthermore, the method requires the sacrifice of a small amount of lesional tissue for RNA extraction and thus necessitates the destruction of some potentially valuable histologic information. Nevertheless, in cases with an adequate amount of tissue, this qRT-PCR-based approach has the potential for considerable practical utility. If its predictive accuracy can be shown to extend to lesions that arise in cirrhosis of all etiologies, and if it can be successfully employed for the analysis of formalin-fixed needle biopsies, it may become a valuable adjunct to histology for differentiating between DN and early HCCs.

The gold standard for diagnosis of DN and early HCCs is still histology; in fact, internationally agreed upon histologic definitions of these lesions and a standardized nomenclature have only recently been formulated [32]. This morphologic classification system was developed and refined on the basis of meticulous histologic analyses of entire lesions within surgical resection specimens. It is recognized that needle biopsies of such lesions will often lack areas that are critical for making a histologic diagnosis of malignancy. In particular, the diagnosis of early HCCs may require identifying the invasion of lesional hepatocytes into portal tracts within the surrounding, benign parenchyma. Because biopsies frequently fail to sample such areas, molecular markers capable of distinguishing between dysplastic and malignant hepatocytes are urgently needed. However, because histology is still the gold standard for diagnosis, the immunohistochemical assessment of molecular markers, as an adjunct to standard H&E histology, is generally preferred over nucleic acid analysis in clinical practice.

Many immunohistochemical markers have been assessed individually, but what has emerged as most useful currently is a panel of three markers: GPC3, GS, and HSP-70. Glypican-3 was first identified as a gene whose mRNA is frequently expressed in HCC but not in benign, adult liver, HCA or CC [38]. Numerous laboratories have since confirmed the overexpression of GPC3 in HCC at the mRNA and protein levels. Its utility as an immunohistochemical marker for HCC was first demonstrated by Yamauchi et al., who found diffusely positive GPC3 staining of malignant hepatocytes in 84% of HCCs and only focal, weak staining in a small set of DN [39]. Similar results were obtained by Llovet et al. in the study cited above.

In an effort to augment the sensitivity of GPC3 immunostaining as a molecular test for HCC, and to retain its specificity in distinguishing DN from malignant lesions, Di Tommaso et al. examined the value of adding other tumor markers to the analysis [40]. Heat-shock protein 70 (HSP70) is an antiapoptotic, "stress response" gene that had been found through mRNA expression profiling to be markedly upregulated in early HCC, as compared with adjacent benign liver tissue [36]. Glutamine synthetase (GS) is a metabolic enzyme (now known to be upregulated by β-catenin signaling [41], as discussed above) that had also been shown to be overexpressed in primary liver cancer (Figure 4) [42]. When these three tumor markers, GPC3, HSP70, and GS, were applied as an immunohistochemical panel to a set of benign and malignant nodules that had been resected from cirrhotic livers, positivity for any two of the three markers was found to indicate malignancy with 72% sensitivity and 100% specificity. In a recent study, these authors used the same panel of immunostains to retrospectively analyze needle biopsies of a similar set of lesions and found that for distinguishing between high grade dysplastic nodules and well- or very well-differentiated HCC, the sensitivity of two-marker positivity as an indicator of malignancy decreased from 72% to 49%, while its specificity remained 100% [43]. This reduction in sensitivity may be attributable to sampling error and the somewhat heterogeneous expression patterns of HSP70 and GPC3 proteins within HCC. It would be

interesting to know whether the mRNA expression patterns of GPC3, LYVE1, and survivin suffer from the same sort of intratumoral heterogeneity, which might complicate their use as molecular markers for HCC in needle biopsies.

Although immunohistochemical staining for the GPC3/HSP70/GS panel is currently recommended as the best ancillary technique to aid in the diagnosis of early HCC in difficult needle biopsy specimens [44], the search continues for biomarkers that can increase the sensitivity of this panel and that are easily and reproducibly stainable in tissue sections. Most candidate markers have been discovered through gene expression studies, and beyond their practical diagnostic utility, investigation into their functions in human liver and in model systems has yielded important insights into the mechanisms of hepatic carcinogenesis. A recent example of gene expression profiling leading to this kind of basic insight is provided by Kaposi-Novak et al. in their study of dysplastic nodules and early HCC in cirrhotic liver explants [37]. In addition to identifying molecular signatures able to discriminate between regenerative, dysplastic, and malignant nodules as well as confirming the upregulation of HSP70, GPC3, and several other putative tumor markers in early HCC, these investigators performed a comparative functional analysis of DN and HCCs gene expression signatures. Their results show that the gene expression profile of early HCCs differs from that of DNs in a pattern strongly suggestive of MYC oncogene activation. Interestingly, no mutations in the MYC gene were detected by sequencing of HCC genomic DNA, and MYC itself was not found to be overexpressed. However, a protein called CSN5 (also known as Jab1) that had been previously shown to posttranscriptionally promote MYC activation in breast epithelium [45] was found to be overexpressed in early HCC in parallel with induction of the MYC-regulated gene expression signature. This suggests a mechanistic model of early hepatocarcinogenesis in which an increase in the expression of CSN5 within DN leads to aberrant MYC activation, and thereby drives the progression of these lesions to HCC. If this model proves true, it may have profound implications for the molecular classification, diagnosis, and treatment of early HCC.

4.2. Hepatocellular Carcinoma Arising in Noncirrhotic Liver. The overall incidence of HCCs in patients without cirrhosis is difficult to estimate and varies with geography and the prevalence of risk factors in the population [46, 47]. Most cases occur in association with chronic liver disease, sometimes in a background of hepatic fibrosis that falls short of full-blown cirrhosis. Consequently, the risk factors for HCC development are the same in the absence of cirrhosis as in its presence; they include chronic HBV or HCV infection, chronic alcohol- or toxin-induced liver injury, and nonalcoholic steatohepatitis [46, 48]. Noncirrhotic patients with HCC are more likely than those with cirrhosis to have multiple risk factors. Although the molecular pathogenesis of hepatic malignancy is likely variable, with some genetic alterations more common in lesions that arise in association with particular risk factors than others, there is no strict correlation between the etiology of underlying chronic liver disease and histologic or molecular subtype of HCCs (see below).

Hepatocellular carcinomas that arise in the absence of chronic liver disease or known risk factors for malignancy are almost always a histologic variant known as fibrolamellar HCC (FL-HCC). The great majority of these tumors occur in patients under the age of 35, and they display a characteristic histology, with nests of large, oncocytic, malignant hepatocytes surrounded by thick bands of layered fibrosis. Although the rarity of these tumors has hampered efforts to elucidate their molecular pathogenesis, FL-HCCs have been found to contain unique molecular alterations that distinguish them from the more common forms of HCCs (reviewed in [49]). The diagnosis of FL-HCCs depends mostly on histology, with the application of immunohistochemical markers when needed [50], and the recognition of clinical characteristics typical of this distinct clinicopathologic entity.

4.3. Hepatocellular Carcinoma Heterogeneity. Hepatocellular carcinoma has long been known to display extraordinary genetic complexity and molecular heterogeneity. However, it is not clear which of the many genomic, genetic, and epigenetic alterations found in HCCs are the most critical in driving its molecular evolution and/or defining its biological behavior. For this reason, there is currently no molecular subclassification of HCCs that is widely accepted and routinely implemented in clinical practice. Nonetheless, a host of molecular genetic markers have been found to correlate with clinical parameters, and in some instances, to have independent prognostic value in particular circumstances.

Although in recent years, surveillance of those at high risk of hepatic malignancy has initiated a trend toward earlier diagnosis of liver cancer, a substantial majority of HCC patients still present with locally advanced or metastatic disease [51]. Even among those whose cancer is diagnosed at a stage early enough to allow them to undergo potentially curative therapy (such as resection, percutaneous ablation, or liver transplantation), the rate of tumor recurrence remains significant. Overall long-term survival of patients with HCCs is poor, in part because most suffer from concomitant, underlying chronic liver disease. However, rates of recurrence and metastasis are not uniform, even within stage-matched patient groups, and there is evidence that from their beginnings, HCC tumors show individual variability in their degree of intrinsic biological aggressiveness [52]. In addition, the number of therapeutic options for HCCs is growing rapidly, due to advances in fields such as interventional radiology and transplantation as well as the emergence of a large armamentarium of molecularly targeted antineoplastic drugs [53, 54]. Thus, for HCC patients at all stages, there is a need for prognostic and predictive biomarkers that can aid in the subclassification of these tumors and help to identify patients who are most likely to respond to each available treatment modality.

4.4. Chromosomal Abnormalities and Genetic Mutations in HCC. HCCs generally show a high level of chromosomal instability, and this is a phenotype that is acquired early

in the process of carcinogenesis [55]. Thus, chromosomal aberrations are common in this type of cancer. However, there is an enormous variety of alterations that can occur, and they have been found throughout the genome. Many of these chromosomal alterations are undoubtedly "passenger" changes, rather than the "drivers" of tumor progression. In the past decade, advances in genomics technologies such as the development of array-based comparative genomic hybridization (array CGH) have allowed investigators to map HCC-associated chromosomal alterations at a much higher resolution than was previously possible. A number of chromosomal regions have now been identified in which gains or losses occur frequently in HCCs (reviewed in [56]), and progress is being made in determining which genes are the targets of these recurrent changes. For example, by comparing array CGH data with global gene expression patterns (determined using DNA microarrays) in 49 HCC samples, Patil et al. were able to correlate the recurrent gain in chromosome 8q with Jab1 overexpression [57]. Interestingly, this is the same gene (also known as CSN5) whose overexpression was recently implicated in the activation of MYC that appears to drive the progression of DN to early HCC [37]. Thus, 8q gain may be a common mechanism promoting early hepatocarcinogenesis.

Although high-resolution mapping of chromosomal alterations in cancer is valuable mostly as a research tool, a group in Japan has demonstrated that HCCs can be subclassified into distinct, clinically meaningful groups based solely on their patterns of chromosomal alterations [58]. In this study, hierarchical cluster analysis was performed on the genomic gain/loss profiles of 87 HCCs, and the tumors were found to partition into two classes, termed A and B. Chromosomal alterations were more numerous in cluster A and typically included gains of 1q, 6p, and 8q as well as 8p losses; this group of tumors was associated with poor patient survival. Cluster A was further subdivided into three subgroups, each characterized by specific high-level amplifications (1q and 6p, 8q, and 17q). Cluster B contained two important subgroups, one without frequent chromosomal alterations and another with amplification of 17q. The authors reasoned that by analogy with known gene amplifications in other cancer types, the recurrent amplifications they discovered in HCC subgroups might indicate the presence of important oncogenes and that these oncogenes might be suitable therapeutic targets. Accordingly, the vascular endothelial growth factor A gene (VEGFA) was found to be contained within the amplified region of chromosome 6p in a subgroup of cluster A tumors, and a gene encoding an effector molecule of the mTOR signaling pathway was found to reside within the amplified region of 17q. The frequent high-level copy number gain of the 6p region encompassing VEGFA has been confirmed in a larger set of HCC tumors and correlated with VEGFA overexpression [59]. Both VEGF and the mTOR pathway are promising therapeutic targets in liver cancer. Therefore, the predictive value of amplifications at 6p and 17q in HCCs as molecular markers of response to VEGF- and mTOR-targeted therapies should be further explored in clinical studies.

The quantitative assessment of genomic alterations as an indicator of tumor aggressiveness has proven useful in predicting the risk of HCC recurrence after liver transplantation. In an effort to identify molecular markers of recurrence risk in HCC patients who had undergone transplantation, Marsh et al. analyzed explanted tumors for loss of heterozygosity (LOH) of microsatellite markers at nine tumor suppressor gene loci [60]. They found that the amount of allelic loss demonstrated within a particular tumor, when combined with other parameters such as tumor size and patient gender, correlated significantly with risk of tumor recurrence. In a subsequent study, the panel of microsatellite markers used in this analysis was refined, and a simple measure of the amount of LOH detected in each tumor was developed, called the fractional allelic imbalance (FAI) [61]. The measurement of FAI and determination of the presence or absence of macrovascular tumor invasion form the basis of the Pittsburgh staging system [62], which is reported to more accurately predict the risk of HCC recurrence after transplantation than staging systems based on radiologic and clinical parameters [63].

HCCs have been extensively analyzed for the detection of somatic mutations affecting known oncogenes and tumor suppressors (reviewed in [64]). The gene encoding β-catenin (CTNNB1), which is mutated in approximately 30% of HCC, is the oncogene most frequently activated in this cancer. HCC bearing β-catenin mutations are more likely than their nonmutant counterparts to show chromosome stability, an absence of HBV infection [65], a well-differentiated histology, and cholestasis [66]. However, there are conflicting data in the literature on the question of whether β-catenin mutation in HCC is associated with favorable or unfavorable prognosis [67, 68]. In general, tumor suppressor genes are more often mutated in HCC than oncogenes. The tumor suppressor most commonly mutated in HCC, overall, is TP53 (encoding the well-known cell cycle regulator, p53), but its frequency of mutation varies with geographical location. TP53 mutation in HCC occurs most commonly in Asia and Africa, where the combination of widespread dietary aflatoxin exposure and endemic hepatitis B fosters a high rate of mutagenesis in the liver [69]. In the West, TP53 mutations are considerably less frequent, affecting about 20% of HCCs. TP53 mutation in these tumors is associated with poor prognosis, chromosomal instability, and HBV infection [64].

4.5. Gene Expression Profiling and Molecular Subtypes of HCCs. A large number of studies have examined global gene expression patterns in HCCs using DNA microarray technology (reviewed in [70, 71]). The advantage of this approach is that it provides information about which signaling pathways and cellular processes are activated or suppressed in these tumors, regardless of the mechanisms (e.g., mutational or epigenetic) underlying the aberrant regulation. Indeed, transcriptomic analysis has led to the identification of reproducible HCC subtypes with differing cellular differentiation and biological behavior that correlate well with prognosis

and may soon allow better patient stratification for treatment than current clinical HCC staging systems.

In 2004, a group led by Dr. Thorgeirsson at the National Cancer Institute published the results of a genome-wide expression study of 91 HCCs showing that the tumors fell into two subclasses with distinct mRNA expression profiles and dramatically different patient survival [72]. The poor survival group, designated A, displayed gene expression signatures of high cellular proliferation and low apoptotic rate. A subsequent study, in which human HCC expression profiles were compared with those of rodent hepatocytes (fetal hepatoblasts, adult hepatocytes, and genetically engineered murine HCC), led to further subdivision of group A HCCs into hepatoblast-like (HB) and mature hepatocyte-like (HC) subgroups [73]. The hepatoblast gene signature was associated with earlier recurrence and worse survival than the hepatocyte signature, and this association was independent of other pathologic variables. The authors speculated that the HB phenotype may reflect the origin of this subset of tumors from adult hepatic progenitor cells (HPCs), which, like fetal hepatoblasts, are able to differentiate into both cholangiocytes and hepatocytes. Consistent with this hypothesis, several well-known HPC markers such as cytokeratins 7 and 19 (CK7 and CK19) were found to be included in the HB gene signature.

The existence of two broad categories of HCCs, one with and the other without a "high-proliferation" gene expression signature, has been confirmed in multiple, independent studies [71]. Most have found that tumors of the high-proliferation group are more aggressive and less histologically differentiated than the group lacking this expression signature. Boyault et al. presented a remarkably detailed refinement of this molecular classification system, in which three subgroups of each major category of HCC were identified (yielding a total of six HCC subtypes) through expression analysis followed by correlation with various genetic, genomic, and clinical factors [74]. Although not yet clinically applicable, this sort of analysis helps to elucidate the biological basis of HCC heterogeneity and provides a foundation for future prospective studies aimed at correlating treatment responses with molecular subtype classification.

The epithelial cell adhesion molecule, EpCAM, is a known marker of fetal hepatoblasts and adult hepatic progenitor cells as well as biliary epithelium. In 2004, Kim et al. performed microarray studies of cirrhotic liver and found that EpCAM is dramatically overexpressed in premalignant lesions as well as in a subset of HCC [75]. Molecular characterization of the EpCAM-expressing HCC subgroup demonstrated a signature of coexpressed genes that included other HPC markers, such as c-kit and CK19 [76]. The further stratification of tumors into four groups according to EpCAM expression and the patients' α-fetoprotein (AFP) status (positive or negative for elevated serum AFP) was shown to define four prognostic categories, each with a characteristic gene expression pattern. Importantly, this result was replicated in three independent cohorts of HCC (all HBV-related), in one of which the EpCAM analysis was performed by immunohistochemistry, rather than mRNA hybridization. Thus, the EpCAM-AFP classification system has the potential for easy application in clinical practice. It is not yet clear whether the stratification of AFP-positive cases according to EpCAM expression will be clinically useful as a prognostic indicator, since AFP positivity is already well-known to be associated with aggressiveness in HCC [77]. However, there is evidence that EpCAM expression correlates with biological variables that can be targeted therapeutically, and, therefore, it may prove valuable as a predictive marker of responsiveness to antiangiogenic therapies [78], Wnt/β-catenin pathway inhibitors, and/or stem-cell targeted agents such as anti-EpCAM antibodies [79].

Gene expression profiling has yielded novel insights into the biological heterogeneity of HCCs and has particularly highlighted the importance of hepatic progenitor cell/stem-cell characteristics in defining its aggressiveness. In addition, these studies have focused attention on the roles of "cancer stem-cells" in HCCs [79]. However, much remains to be learned about the origins and behavior of hepatic progenitor cell lineages in benign and premalignant conditions, as well as their relationships to cancer stem-cells, cell-population dynamics within tumors, and the clinicopathologic ramifications of stem-cell marker expression in different HCC molecular subtypes (Figure 5). Although it is not yet clear which gene signatures and molecular markers will have the broadest applicability, recent rapid progress in this field implies that the incorporation of molecular data into HCC classification and staging systems in the near future will lead to continued improvements in the clinical management of HCC.

4.6. MicroRNA Profiling of HCCs. MicroRNAs (miRNAs) are short, noncoding RNA molecules that regulate gene expression by binding to specific messenger RNAs and preventing their translation into protein. Because each type of miRNA is able to downregulate hundreds of genes at a time, miRNAs often control entire transcriptional programs that determine fundamental cellular properties and behavior. Accordingly, miRNA profiling has emerged as an extremely valuable method for phenotyping and subclassifying tumors [80]. Compared to conventional gene expression profiling (in which protein-coding, messenger RNAs are examined), miRNA analysis has several advantages. Due to the stability of miRNAs, formalin-fixed samples (rather than frozen tissue) can be used. Furthermore, the interrogation of hundreds of miRNAs (and often significantly fewer) yields as much information as might be gleaned from examining thousands of messenger RNAs.

Many independent groups have conducted comprehensive analyses of miRNAs in HCC, and a plethora of informative miRNA markers have been identified. Many of these miRNA signatures correlate with important biological parameters, such as metastasis [81–83], differentiation [83–85], HBV or HCV infection [86, 87], tumor recurrence [88], and patient survival [89–91]. In addition to providing new candidates for investigation as possible diagnostic, prognostic, and/or predictive molecular markers in HCCs, these studies are opening new avenues in basic research on the mechanisms of hepatocarcinogenesis. For example,

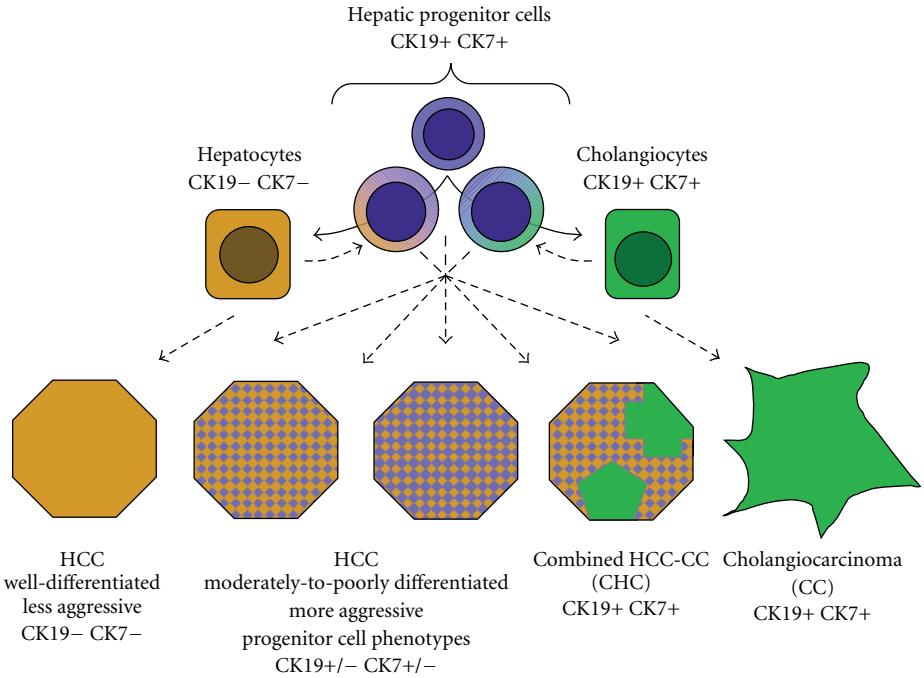


Figure 5: Hypothetical classification of primary hepatic malignancies according to corresponding patterns of hepatic progenitor cell differentiation. Dashed lines illustrate possible lineages of tumor origin and evolution. At left is well-differentiated HCC, with a mature hepatocytic phenotype (orange), and at right is cholangiocarcinoma, showing complete biliary differentiation (green). Varying degrees and patterns of expression of progenitor cell markers (purple) and biliary-type cytokeratins (CK7, CK19) correlate with increased tumor aggressiveness.

Ji et al. [92] have discovered that miRNA-181 is overexpressed in EpCAM-positive HCC cells and is a critical, functional determinant of the progenitor phenotype in these cells. MiRNA-181 appears to promote a HPC phenotype by down regulating the expression of key transcription factors that mediate the differentiation and maturation of hepatocytes.

It has been shown recently that the reduced expression (silencing) of miRNA-26 in HCC tumors of male patients is associated both with poor survival and response to interferon-alpha adjuvant therapy [89]. Interestingly, miRNA-26 is differentially expressed according to gender in benign liver, with significantly higher expression in women than in men. Among individuals with HCC, those whose benign liver tissue showed low miRNA-26 expression were found to have tumors in which miRNA-26 was even further downregulated, whereas in patients whose benign liver miRNA-26 expression levels were high, tumor levels of this miRNA were unchanged. These findings suggested that miRNA-26 acts as a tumor suppressor in the liver and that its higher expression in females may be a protective factor against HCC and may contribute to the marked gender bias in risk for this malignancy. Intriguingly, an analysis of gene expression in tumors that had undergone miRNA-26 silencing revealed the activation of interleukin-6 (IL-6) signaling networks. This is interesting in light of recent evidence that estrogen-related inhibition of IL-6 signaling underlies the observed female-specific resistance to hepatocarcinogenesis in mice [93]. An even more exciting finding with regard to miRNA-26 in human HCC was that miRNA-26 silencing within tumors identified the subgroup of patients who benefited from postoperative treatment with interferon-alpha after HCC resection in randomized trials [89]. The idea that the tumors most likely to respond to interferon treatment are those in which miRNA-26 silencing has led to increased proinflammatory and IL-6 signaling is intuitively appealing. Future prospective studies will undoubtedly test the efficacy of interferon-alpha in preventing recurrence of miRNA-26-silenced HCC in broader patient populations, as well as its value in combination with other therapeutic regimens. Thus, miRNA-26 promises to become the first predictive molecular marker in HCC that can be used to match individual patients with the treatments most likely to benefit them.

5. Intrahepatic Cholangiocarcinoma and Combined HCC-CC

Cholangiocarcinoma (CC), defined as carcinoma of biliary-type epithelium, can arise in the liver parenchyma or anywhere along the extrahepatic biliary tract. The extrahepatic type (which includes "Klatskin tumors" of the liver hilum) is much more frequent, accounting for up to 90% of CC cases [94]. The histologic appearance of intrahepatic CC (ICC) is the same as that of extrahepatic forms, typically showing malignant glands embedded in a prominent desmoplastic stroma. Although these morphologic features usually make ICC clearly distinguishable from HCC (a distinction that can

be confirmed through staining for the hepatocytic marker, HepPar-1, and the biliary markers, CK7 and CK19), it can be difficult to differentiate between ICC and metastasis to the liver from a nonhepatic primary tumor. A few new molecular markers of biliary differentiation have recently been described [95], but these have not yet been tested for their ability to distinguish ICC from hepatic metastasis.

CC generally presents at a late stage, when curative resection is no longer possible. Unfortunately, this malignancy is highly aggressive and resistant to standard chemotherapeutic regimens. Molecular characterization of CC is in the early investigative stages (reviewed in [96]), and useful prognostic molecular markers have not yet been reported. The need for more research in this area, and for the development of targeted therapies and predictive molecular markers, is well recognized.

Combined HCC-CC is a rare primary liver tumor characterized by the intimate intermingling of histologically unequivocal HCC and CC components [97]. Most cases show, in addition to typical HCC and CC areas, transitional regions containing immature-appearing cells that express hepatic progenitor markers such as EpCAM. When such transitional regions predominate, the tumor is classified as a subtype of HCC-CC, "with stem-cell features" [98]. Three histologic patterns of intermediate/stem-cell distribution have been described in lesions of this category, including a pattern sometimes termed cholangiolocarcinoma, formerly classified as a variant of CC. Although several immunohistochemical studies of HCC-CC have confirmed the expression of various hepatic progenitor cell markers in these lesions [99, 100], their classification is based on histomorphology, rather than positivity for stem-cell markers *per se*. The fact that many HPC markers are also expressed by biliary epithelium and the recent findings on progenitor cell marker expression by HCC greatly complicate this field. Thus, although there is evidence that HCC-CC arises from a bipotential hepatic progenitor cell, the histogenesis of this rare neoplasm is still debated (Figure 5).

Only a few studies have examined the molecular characteristics of HCC-CC. Zucman-Rossi's group in France used microsatellite-based LOH analysis to assess chromosome stability in 9 CC, 15 HCC-CC, and 3 HCC/CC collision tumors [101]. Combined HCC-CC was found to show a high degree of chromosomal instability, similar to CC and unlike HCC. Investigators in Germany recently reported the results of CGH analysis of 49 HCC, 22 hepatic CC, and 7 HCC-CC cases [102]. In this study, combined HCC-CC was found to resemble both HCC and CC, showing several of the specific chromosomal gains and losses typical of HCC, while also displaying a high total number of chromosomal imbalances, similar to CC. Woo et al. recently performed gene expression profiling of 70 HCC, 13 CC, and 7 HCC-CC [103]. Results showed that the gene expression pattern of HCC was markedly different from that of CC, and combined HCC-CC clearly clustered with CC. Further analysis led to the identification of a CC gene signature containing many known biliary and hepatic progenitor cell markers. Interestingly, closer examination of the HCC data revealed that a subset of HCC also expressed the CC gene signature; this subset was designated CC-like HCC (CLHCC). Expression of the CC gene signature by HCCs was associated with poor prognosis. However, its prognostic significance was found to be independent of previously described poor-prognostic gene signatures such as that of the hepatoblast subtype of HCC. Thus, there are many layers of complexity regarding hepatic progenitor cell and biliary phenotypes in primary liver cancers. Overall, stem-cell and biliary characteristics seem to correlate with increased tumor aggressiveness, resistance to chemotherapy, and poor prognosis.

6. Conclusions

In recent years, remarkable progress has been made in elucidating the molecular pathology of hepatic tumors. In the area of benign hepatocellular lesions, advances in our understanding of molecular subtypes have led to a new classification system of HCA, with important genotype-phenotype correlations and easy application to routine clinical practice. In the field of early hepatic neoplasia, microarray studies of gene expression have identified simple molecular signatures that can distinguish dysplastic nodules from early HCCs in a background of cirrhosis. Although such expression signatures can be determined by quantitative RT-PCR, the use of immunohistochemistry, which allows the simultaneous assessment of histology and protein expression, is preferred in needle biopsies. Molecular measures of prognosis in patients with HCCs and CC are emerging; in retrospective studies, a variety of molecular techniques have successfully identified subgroups of HCCs with distinct clinical associations, such as prognosis, risk of recurrence after attempted curative treatment, and response to adjuvant interferon therapy. Although these assays are not yet developed for widespread clinical application, their refinement and use for the stratification of patients undergoing treatment in clinical trials will lead to further advances in therapeutic options for liver cancer. The current system of classifying hepatic tumors based on histologic architecture and patterns of differentiation has many limitations. Molecular studies have supported a model of hepatic tumorigenesis in which neoplasms recapitulate various lineages and stages of hepatic progenitor cell differentiation. These discoveries are likely to have an important impact on liver tumor classification and to lead to a newer classification system, based on histologic and molecular features, with improved prognostic and therapeutic significance.

References

[1] J. F. Buell, H. Tranchart, R. Cannon, and I. Dagher, "Management of benign hepatic tumors," *Surgical Clinics of North America*, vol. 90, no. 4, pp. 719–735, 2010.

[2] P. Bioulac-Sage, H. Laumonier, A. Rullier et al., "Over-expression of glutamine synthetase in focal nodular hyperplasia: a novel easy diagnostic tool in surgical pathology," *Liver International*, vol. 29, no. 3, pp. 459–465, 2009.

[3] P. Bioulac-Sage, C. Balabaud, and J. Zucman-Rossi, "Subtype classification of hepatocellular adenoma," *Digestive Surgery*, vol. 27, no. 1, pp. 39–45, 2010.

[4] S. F. Altekruse, K. A. McGlynn, and M. E. Reichman, "Hepatocellular carcinoma incidence, mortality, and survival trends in the United States from 1975 to 2005," *Journal of Clinical Oncology*, vol. 27, no. 9, pp. 1485–1491, 2009.

[5] M. B. Thomas, D. Jaffe, M. M. Choti et al., "Hepatocellular carcinoma: consensus recommendations of the national cancer institute clinical trials planning meeting," *Journal of Clinical Oncology*, vol. 28, no. 25, pp. 3994–4005, 2010.

[6] I. R. Wanless, C. Mawdsley, and R. Adams, "On the pathogenesis of focal nodular hyperplasia of the liver," *Hepatology*, vol. 5, no. 6, pp. 1194–1200, 1985.

[7] S. Rebouissou, P. Bioulac-Sage, and J. Zucman-Rossi, "Molecular pathogenesis of focal nodular hyperplasia and hepatocellular adenoma," *Journal of Hepatology*, vol. 48, no. 1, pp. 163–170, 2008.

[8] S. Zheng, O. W. Cummings, R. Saxena et al., "Clonality and TP53 mutation analysis of focal nodular hyperplasia of the liver," *American Journal of Clinical Pathology*, vol. 134, no. 1, pp. 65–70, 2010.

[9] B. N. Nguyen, J. F. Fléjou, B. Terris, J. Belghiti, and C. Degott, "Focal nodular hyperplasia of the liver: a comprehensive pathologic study of 305 lesions and recognition of new histologic forms," *American Journal of Surgical Pathology*, vol. 23, no. 12, pp. 1441–1454, 1999.

[10] Y. W. Chen, Y. M. Jeng, S. H. Yeh, and P. J. Chen, "p53 gene and Wnt signaling in benign neoplasms: β-catenin mutations in hepatic adenoma but not in focal nodular hyperplasia," *Hepatology*, vol. 36, no. 4, pp. 927–935, 2002.

[11] P. Bioulac-Sage, S. Rebouissou, A. Sa Cunha et al., "Clinical, morphologic, and molecular features defining so-called telangiectatic focal nodular hyperplasias of the liver," *Gastroenterology*, vol. 128, no. 5, pp. 1211–1218, 2005.

[12] S. Rebouissou, G. Couchy, L. Libbrecht et al., "The β-catenin pathway is activated in focal nodular hyperplasia but not in cirrhotic FNH-like nodules," *Journal of Hepatology*, vol. 49, no. 1, pp. 61–71, 2008.

[13] A. Iyer, M. E. Robert, C. B. Bifulco, R. R. Salem, and D. Jain, "Different cytokeratin and neuronal cell adhesion molecule staining patterns in focal nodular hyperplasia and hepatic adenoma and their significance," *Human Pathology*, vol. 39, no. 9, pp. 1370–1377, 2008.

[14] B. Spee, G. Carpino, B. A. Schotanus et al., "Characterisation of the liver progenitor cell niche in liver diseases: potential involvement of Wnt and Notch signalling," *Gut*, vol. 59, no. 2, pp. 247–257, 2010.

[15] L. Libbrecht, R. De Vos, D. Cassiman, V. Desmet, R. Aerts, and T. Roskams, "Hepatic progenitor cells in hepatocellular adenomas," *American Journal of Surgical Pathology*, vol. 25, no. 11, pp. 1388–1396, 2001.

[16] K. A. Mitchell and D. Jain, "Diagnostic approach to needle biopsies of hepatic mass lesions," *Diagnostic Histopathology*, vol. 14, no. 12, pp. 598–608, 2008.

[17] D. Häussinger, "Liver glutamine metabolism," *Journal of Parenteral and Enteral Nutrition*, vol. 14, no. 4, pp. 56S–62S, 1990.

[18] Z. D. Burke and D. Tosh, "The Wnt/β-catenin pathway: master regulator of liver zonation?" *BioEssays*, vol. 28, no. 11, pp. 1072–1077, 2006.

[19] L. Barthelmes and I. S. Tait, "Liver cell adenoma and liver cell adenomatosis," *HPB*, vol. 7, no. 3, pp. 186–196, 2005.

[20] V. Paradis, A. Benzekri, D. Dargére et al., "Telangiectatic focal nodular hyperplasia: a variant of hepatocellular adenoma," *Gastroenterology*, vol. 126, no. 5, pp. 1323–1329, 2004.

[21] Y. R. Cai, L. Gong, X. Y. Teng et al., "Clonality and allelotype analyses of focal nodular hyperplasia compared with hepatocellular adenoma and carcinoma," *World Journal of Gastroenterology*, vol. 15, no. 37, pp. 4695–4708, 2009.

[22] O. Farges and S. Dokmak, "Malignant transformation of liver adenoma: an analysis of the literature," *Digestive Surgery*, vol. 27, no. 1, pp. 32–38, 2010.

[23] O. Bluteau, E. Jeannot, P. Bioulac-Sage et al., "Bi-allelic inactivation of TCF1 in hepatic adenomas," *Nature Genetics*, vol. 32, no. 2, pp. 312–315, 2002.

[24] K. Yamagata, N. Oda, P. J. Kaisaki et al., "Mutations in the hepatocyte nuclear factor-1α gene in maturity-onset diabetes of the young (MODY3)," *Nature*, vol. 384, no. 6608, pp. 455–458, 1996.

[25] Y. Reznik, T. Dao, R. Coutant et al., "Hepatocyte nuclear factor-1α gene inactivation: cosegregation between liver adenomatosis and diabetes phenotypes in two maturity-onset diabetes of the young (MODY)3 families," *Journal of Clinical Endocrinology and Metabolism*, vol. 89, no. 3, pp. 1476–1480, 2004.

[26] J. Zucman-Rossi, E. Jeannot, J. T. Van Nhieu et al., "Genotype-phenotype correlation in hepatocellular adenoma: new classification and relationship with HCC," *Hepatology*, vol. 43, no. 3, pp. 515–524, 2006.

[27] P. Bioulac-Sage, S. Rebouissou, C. Thomas et al., "Hepatocellular adenoma subtype classification using molecular markers and immunohistochemistry," *Hepatology*, vol. 46, no. 3, pp. 740–748, 2007.

[28] S. Rebouissou, M. Amessou, G. Couchy et al., "Frequent in-frame somatic deletions activate gp130 in inflammatory hepatocellular tumours," *Nature*, vol. 457, no. 7226, pp. 200–204, 2009.

[29] P. Bioulac-Sage, H. Laumonier, G. Couchy et al., "Hepatocellular adenoma management and phenotypic classification: the Bordeaux experience," *Hepatology*, vol. 50, no. 2, pp. 481–489, 2009.

[30] A. Gru, R. K. Pai, and E. M. Brunt, "Clinicopathologic and molecularcharacterization of hepatocellular adenomas (HCA) and focal nodular hyperplasias (FNH)," *Modern Pathology*. In press.

[31] P. Hytiroglou, Y. N. Park, G. Krinsky, and N. D. Theise, "Hepatic precancerous lesions and small hepatocellular carcinoma," *Gastroenterology Clinics of North America*, vol. 36, no. 4, pp. 867–887, 2007.

[32] M. Kojiro, I. R. Wanless, V. Alves et al., "Pathologic diagnosis of early hepatocellular carcinoma: a report of the International Consensus Group for Hepatocellular Neoplasia," *Hepatology*, vol. 49, no. 2, pp. 658–664, 2009.

[33] V. Paradis, I. Bièche, D. Dargère et al., "Molecular profiling of hepatocellular carcinomas (HCC) using a large-scale real-time RT-PCR approach: determination of a molecular diagnostic index," *American Journal of Pathology*, vol. 163, no. 2, pp. 733–741, 2003.

[34] S. W. Nam, J. Y. Park, A. Ramasamy et al., "Molecular changes from dysplastic nodule to hepatocellular carcinoma through gene expression profiling," *Hepatology*, vol. 42, no. 4, pp. 809–818, 2005.

[35] J. M. Llovet, Y. Chen, E. Wurmbach et al., "A molecular signature to discriminate dysplastic nodules from early hepatocellular carcinoma in HCV cirrhosis," *Gastroenterology*, vol. 131, no. 6, pp. 1758–1767, 2006.

[36] M. Chuma, M. Sakamoto, K. Yamazaki et al., "Expression profiling in multistage hepatocarcinogenesis: identification of HSP70 as a molecular marker of early hepatocellular carcinoma," *Hepatology*, vol. 37, no. 1, pp. 198–207, 2003.

[37] P. Kaposi-Novak, I. Libbrecht, HG. Woo et al., "Central role of c-Myc during malignant conversion in human hepatoeareinogenesis," *Cancer Research*, vol. 69, no. 8, pp. 2775–2782, 2009.

[38] H. C. Hsu, W. Cheng, and PO. L. Lai, "Cloning and expression of a developmentally regulated transcript MXR7 in hepatocellular carcinoma: biological significance and temporospatial distribution," *Cancer Research*, vol. 57, no. 22, pp. 5179–5184, 1997.

[39] N. Yamauchi, A. Watanabe, M. Hishinuma et al., "The glypican 3 oncofetal protein is a promising diagnostic marker for hepatocellular carcinoma," *Modern Pathology*, vol. 18, no. 12, pp. 1591–1598, 2005.

[40] L. Di Tommaso, G. Franchi, N. P. Young et al., "Diagnostic value of HSP70, glypican 3, and glutamine synthetase in hepatocellular nodules in cirrhosis," *Hepatology*, vol. 45, no. 3, pp. 725–734, 2007.

[41] A. Cadoret, C. Ovejero, B. Terris et al., "New targets of β-catenin signaling in the liver are involved in the glutamine metabolism," *Oncogene*, vol. 21, no. 54, pp. 8293–8301, 2002.

[42] L. Christa, M. T. Simon, J. P. Flinois, R. Gebhardt, C. Brechot, and C. Lasserre, "Overexpression of glutamine synthetase in human primary liver cancer," *Gastroenterology*, vol. 106, no. 5, pp. 1312–1320, 1994.

[43] L. Di Tommaso, A. Destro, J. Y. Seok et al., "The application of markers (HSP70 GPC3 and GS) in liver biopsies is useful for detection of hepatocellular carcinoma," *Journal of Hepatology*, vol. 50, no. 4, pp. 746–754, 2009.

[44] T. Roskams and M. Kojiro, "Pathology of early hepatocellular carcinoma: conventional and molecular diagnosis," *Seminars in Liver Disease*, vol. 30, no. 1, pp. 17–25, 2010.

[45] A. S. Adler, M. Lin, H. Horlings, D. S. A. Nuyten, M. J. Van De Vijver, and H. Y. Chang, "Genetic regulators of large-scale transcriptional signatures in cancer," *Nature Genetics*, vol. 38, no. 4, pp. 421–430, 2006.

[46] F. Trevisani, M. Frigerio, V. Santi, A. Grignaschi, and M. Bernardi, "Hepatocellular carcinoma in non-cirrhotic liver: a reappraisal," *Digestive and Liver Disease*, vol. 42, no. 5, pp. 341–347, 2010.

[47] M. F. Madhoun, J. Fazili, B. C. Bright, T. Bader, D. N. Roberts, and M. S. Bronze, "Hepatitis C prevalence in patients with hepatocellular carcinoma without cirrhosis," *American Journal of the Medical Sciences*, vol. 339, no. 2, pp. 169–173, 2010.

[48] Y. Takuma and K. Nouso, "Nonalcoholic steatohepatitis-associated hepatocellular carcinoma: our case series and literature review," *World Journal of Gastroenterology*, vol. 16, no. 12, pp. 1436–1441, 2010.

[49] S. Liu, K. W. Chan, B. Wang, and L. Qiao, "Fibrolamellar hepatocellular carcinoma," *American Journal of Gastroenterology*, vol. 104, no. 10, pp. 2617–2624, 2009.

[50] H. M. Ross, H. D.J. Daniel, P. Vivekanandan et al., "Fibrolamellar carcinomas are positive for CD68," *Modern Pathology*, vol. 24, no. 3, pp. 390–395, 2011.

[51] J. M. Llovet, A. M. Di Bisceglie, J. Bruix et al., "Design and endpoints of clinical trials in hepatocellular carcinoma," *Journal of the National Cancer Institute*, vol. 100, no. 10, pp. 698–711, 2008.

[52] Q. H. Ye, L. X. Qin, M. Forgues et al., "Predicting hepatitis B virus-positive metastatic hepatocellular carcinomas using gene expression profiling and supervised machine learning," *Nature Medicine*, vol. 9, no. 4, pp. 416–423, 2003.

[53] A. Forner, M. E. Reig, C. Rodriguez De Lope, and J. Bruix, "Current strategy for staging and treatment: the BCLC update and future prospects," *Seminars in Liver Disease*, vol. 30, no. 1, pp. 61–74, 2010.

[54] H. Huynh, "Molecularly targeted therapy in hepatocellular carcinoma," *Biochemical Pharmacology*, vol. 80, no. 5, pp. 550–560, 2010.

[55] N. Nishida, T. Nishimura, T. Ito, T. Komeda, Y. Fukuda, and K. Nakao, "Chromosomal instability and human hepatocarcinogenesis," *Histology and Histopathology*, vol. 18, no. 3, pp. 897–909, 2003.

[56] Y. Midorikawa, W. Tang, and Y. Sugiyama, "High-resolution mapping of copy number aberrations and identification of target genes in hepatocellular carcinoma," *Bioscience Trends*, vol. 1, no. 1, pp. 26–32, 2007.

[57] M. A. Patil, I. Gütgemann, J. Zhang et al., "Array-based comparative genomic hybridization reveals recurrent chromosomal aberrations and Jab1 as a potential target for 8q gain in hepatocellular carcinoma," *Carcinogenesis*, vol. 26, no. 12, pp. 2050–2057, 2005.

[58] H. Katoh, H. Ojima, A. Kokubu et al., "Genetically distinct and clinically relevant classification of hepatocellular carcinoma: putative therapeutic targets," *Gastroenterology*, vol. 133, no. 5, pp. 1475–1486, 2007.

[59] D. Y. Chiang, A. Villanueva, Y. Hoshida et al., "Focal gains of VEGFA and molecular classification of hepatocellular carcinoma," *Cancer Research*, vol. 68, no. 16, pp. 6779–6788, 2008.

[60] J. W. Marsh, S. D. Finkelstein, A. J. Demetris et al., "Genotyping of hepatocellular carcinoma in liver transplant recipients adds predictive power for determining recurrence-free survival," *Liver Transplantation*, vol. 9, no. 7, pp. 664–671, 2003.

[61] I. Dvorchik, M. Schwartz, M. I. Fiel, S. D. Finkelstein, and J. W. Marsh, "Fractional allelic imbalance could allow for the development of an equitable transplant selection policy for patients with hepatocellular carcinoma," *Liver Transplantation*, vol. 14, no. 4, pp. 443–450, 2008.

[62] C. Schmidt and J. W. Marsh, "Molecular signature for HCC: role in predicting outcomes after liver transplant and selection for potential adjuvant treatment," *Current Opinion in Organ Transplantation*, vol. 15, no. 3, pp. 277–282, 2010.

[63] M. Schwartz, I. Dvorchik, S. Roayaie et al., "Liver transplantation for hepatocellular carcinoma: extension of indications based on molecular markers," *Journal of Hepatology*, vol. 49, no. 4, pp. 581–588, 2008.

[64] S. Imbeaud, Y. Ladeiro, and J. Zucman-Rossi, "Identification of novel oncogenes and tumor suppressors in hepatocellular carcinoma," *Seminars in Liver Disease*, vol. 30, no. 1, pp. 75–86, 2010.

[65] P. Laurent-Puig, P. Legoix, O. Bluteau et al., "Genetic alterations associated with hepatocellular carcinomas define distinct pathways of hepatocarcinogenesis," *Gastroenterology*, vol. 120, no. 7, pp. 1763–1773, 2001.

[66] V. Audard, G. Grimber, C. Elie et al., "Cholestasis is a marker for hepatocellular carcinomas displaying β-catenin mutations," *Journal of Pathology*, vol. 212, no. 3, pp. 345–352, 2007.

[67] H. C. Hsu, Y. M. Jeng, T. L. Mao, J. S. Chu, P. L. Lai, and S. Y. Peng, "β-catenin mutations are associated with a subset of low-stage hepatocellular carcinoma negative for hepatitis B virus and with favorable prognosis," *American Journal of Pathology*, vol. 157, no. 3, pp. 763–770, 2000.

[68] B. Cieply, G. Zeng, T. Proverbs-Singh, D. A. Geller, and S. P. S. Monga, "Unique phenotype of hepatocellular cancers with exon-3 mutations in beta-catenin gene," *Hepatology*, vol. 49, no. 3, pp. 821–831, 2009.

[69] D. Gouas, H. Shi, and P. Hainaut, "The aflatoxin-induced TP53 mutation at codon 249 (R249S): biomarker of exposure, early detection and target for therapy," *Cancer Letters*, vol. 286, no. 1, pp. 29–37, 2009.

[70] Y. Hoshida, S. Toffanin, A. Lachenmayer, A. Villanueva, B. Minguez, and J. M. Llovet, "Molecular classification and novel targets in hepatocellular carcinoma: recent advancements," *Seminars in Liver Disease*, vol. 30, no. 1, pp. 35–51, 2010.

[71] O. M. Andrisani, L. Studach, and P. Merle, "Gene signatures in hepatocellular carcinoma (HCC)," *Seminars in Cancer Biology*, vol. 21, no. 1, pp. 4–9, 2011.

[72] J. S. Lee, I. S. Chu, J. Heo et al., "Classification and prediction of survival in hepatocellular carcinoma by gene expression profiling," *Hepatology*, vol. 40, no. 3, pp. 667–676, 2004.

[73] J. S. Lee, J. Heo, L. Libbrecht et al., "A novel prognostic subtype of human hepatocellular carcinoma derived from hepatic progenitor cells," *Nature Medicine*, vol. 12, no. 4, pp. 410–416, 2006.

[74] S. Boyault, D. S. Rickman, A. De Reyniès et al., "Transcriptome classification of HCC is related to gene alterations and to new therapeutic targets," *Hepatology*, vol. 45, no. 1, pp. 42–52, 2007.

[75] J. W. Kim, Q. Ye, M. Forgues et al., "Cancer-associated molecular signature in the tissue samples of patients with cirrhosis," *Hepatology*, vol. 39, no. 2, pp. 518–527, 2004.

[76] T. Yamashita, M. Forgues, W. Wang et al., "EpCAM and α-fetoprotein expression defines novel prognostic subtypes of hepatocellular carcinoma," *Cancer Research*, vol. 68, no. 5, pp. 1451–1461, 2008.

[77] L. X. Qin and Z. Y. Tang, "The prognostic significance of clinical and pathological features in hepatocellular carcinoma," *World Journal of Gastroenterology*, vol. 8, no. 2, pp. 193–199, 2002.

[78] Y. F. Shan, Y. L. Huang, Y. K. Xie et al., "Angiogenesis and clinicopathologic characteristics in different hepatocellular carcinoma subtypes defined by EpCAM and alpha-fetoprotein expression status," *Medical Oncology*. In press.

[79] T. Yamashita, J. Ji, A. Budhu et al., "EpCAM-positive hepatocellular carcinoma cells are tumor-initiating cells with stem/progenitor cell features," *Gastroenterology*, vol. 136, no. 3, pp. 1012–e4, 2009.

[80] M. Ferracin, A. Veronese, and M. Negrini, "Micromarkers: MiRNAs in cancer diagnosis and prognosis," *Expert Review of Molecular Diagnostics*, vol. 10, no. 3, pp. 297–308, 2010.

[81] A. Budhu, H. L. Jia, M. Forgues et al., "Identification of metastasis-related microRNAs in hepatocellular carcinoma," *Hepatology*, vol. 47, no. 3, pp. 897–907, 2008.

[82] J. Yao, L. Liang, S. Huang et al., "Microrna-30d promotes tumor invasion and metastasis by targeting galphai2 in hepatocellular carcinoma," *Hepatology*, vol. 51, no. 3, pp. 846–856, 2010.

[83] C. C. Wong, C. Wong, E. K. Tung et al., "The MicroRNA miR-139 suppresses metastasis and progression of hepatocellular carcinoma by down-regulating rho-kinase 2," *Gastroenterology*, vol. 140, no. 1, pp. 322–331, 2011.

[84] Y. Murakami, T. Yasuda, K. Saigo et al., "Comprehensive analysis of microRNA expression patterns in hepatocellular carcinoma and non-tumorous tissues," *Oncogene*, vol. 25, no. 17, pp. 2537–2545, 2006.

[85] C. Coulouarn, V. M. Factor, J. B. Andersen, M. E. Durkin, and S. S. Thorgeirsson, "Loss of miR-122 expression in liver cancer correlates with suppression of the hepatic phenotype and gain of metastatic properties," *Oncogene*, vol. 28, no. 40, pp. 3526–3536, 2009.

[86] Y. Ladeiro, G. Couchy, C. Balabaud et al., "MicroRNA profiling in hepatocellular tumors is associated with clinical features and oncogene/tumor suppressor gene mutations," *Hepatology*, vol. 47, no. 6, pp. 1955–1963, 2008.

[87] S. Ura, M. Honda, T. Yamashita et al., "Differential microRNA expression between hepatitis B and hepatitis C leading disease progression to hepatocellular carcinoma," *Hepatology*, vol. 49, no. 4, pp. 1098–1112, 2009.

[88] G. E. Chung, J. H. Yoon, S. J. Myung et al., "High expression of microRNA-15b predicts a low risk of tumor recurrence following curative resection of hepatocellular carcinoma," *Oncology Reports*, vol. 23, no. 1, pp. 113–119, 2010.

[89] J. Ji, J. Shi, A. Budhu et al., "MicroRNA expression, survival, and response to interferon in liver cancer," *New England Journal of Medicine*, vol. 361, no. 15, pp. 1437–1447, 2009.

[90] Y. Xiong, J. H. Fang, J. P. Yun et al., "Effects of microrna-29 on apoptosis, tumorigenicity, and prognosis of hepatocellular carcinoma," *Hepatology*, vol. 51, no. 3, pp. 836–845, 2010.

[91] J. Zhang, Y. Yang, T. Yang et al., "MicroRNA-22, down-regulated in hepatocellular carcinoma and correlated with prognosis, suppresses cell proliferation and tumourigenicity," *British Journal of Cancer*, vol. 103, no. 8, pp. 1215–1220, 2010.

[92] J. Ji, T. Yamashita, A. Budhu et al., "Identification of MicroRNA-181 by genome-wide screening as a critical player in EpCAM—positive hepatic cancer stem cells," *Hepatology*, vol. 50, no. 2, pp. 472–480, 2009.

[93] W. E. Naugler, T. Sakurai, S. Kim et al., "Gender disparity in liver cancer due to sex differences in MyD88-dependent IL-6 production," *Science*, vol. 317, no. 5834, pp. 121–124, 2007.

[94] B. Blechacz and G. J. Gores, "Cholangiocarcinoma: advances in pathogenesis, diagnosis, and treatment," *Hepatology*, vol. 48, no. 1, pp. 308–321, 2008.

[95] R. Nishino, M. Honda, T. Yamashita et al., "Identification of novel candidate tumour marker genes for intrahepatic cholangiocarcinoma," *Journal of Hepatology*, vol. 49, no. 2, pp. 207–216, 2008.

[96] H. Francis, G. Alpini, and S. DeMorrow, "Recent advances in the regulation of cholangiocarcinoma growth," *American Journal of Physiology*, vol. 299, no. 1, pp. G1–G9, 2010.

[97] M. M. Yeh, "Pathology of combined hepatocellular-cholangiocarcinoma," *Journal of Gastroenterology and Hepatology*, vol. 25, pp. 1485–1492, 2010.

[98] N. D. Theise, O. Nakashima, Y. N. Park, and Y. Nakanuma, "Combined hepatocellular-cholangiocarcinoma," in *WHO Classification of Tumours of the Digestive System*, F. T. Bosman, F. Carneiro, R. H. Hruban, and N. D. Theise, Eds., pp. 225–227, IARC, Lyon, France, 4th edition, 2010.

[99] S. Tanaka, T. Yamamoto, H. Tanaka et al., "Potentiality of combined hepatocellular and intrahepatic cholangiocellular carcinoma originating from a hepatic precursor

cell: immunohistochemical evidence," *Hepatology Research*, vol. 32, no. 1, pp. 52–57, 2005.

[100] F. Zhang, X. P. Chen, W. Zhang et al., "Combined hepatocellular cholangiocarcinoma originating from hepatic progenitor cells: immunohistochemical and double-fluorescence immunostaining evidence," *Histopathology*, vol. 52, no. 2, pp. 224–232, 2008.

[101] D. Cazals-Hatem, S. Rebouissou, P. Bioulac-Sage et al., "Clinical and molecular analysis of combined hepatocellular-cholangiocarcinomas," *Journal of Hepatology*, vol. 41, no. 2, pp. 292–298, 2004.

[102] K. Homayounfar, B. Gunawan, S. Cameron et al., "Pattern of chromosomal aberrations in primary liver cancers identified by comparative genomic hybridization," *Human Pathology*, vol. 40, no. 6, pp. 834–842, 2009.

[103] H. G. Woo, J. H. Lee, J. H. Yoon et al., "Identification of a cholangiocarcinoma-like gene expression trait in hepatocellular carcinoma," *Cancer Research*, vol. 70, no. 8, pp. 3034–3041, 2010.

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Review Article

Molecular Characteristics of Pancreatic Ductal Adenocarcinoma

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Pancreatic cancer is an almost universally lethal disease and despite extensive research over the last decades, this has not changed significantly. Nevertheless, much progress has been made in understanding the pathogenesis of pancreatic ductal adenocarcinoma (PDAC) suggesting that different therapeutic strategies based on these new insights are forthcoming. Increasing focus exists on designing the so-called targeted treatment strategies in which the genetic characteristics of a tumor guide therapy. In the past, the focus of research was on identifying the most frequently affected genes in PDAC, but with the complete sequencing of the pancreatic cancer genome the focus has shifted to defining the biological function that the altered genes play. In this paper we aimed to put the genetic alterations present in pancreatic cancer in the context of their role in signaling pathways. In addition, this paper provides an update of the recent advances made in the development of the targeted treatment approach in PDAC.

1. Pancreatic Ductal Adenocarcinoma

Annually, approximately 43,140 people are diagnosed (incidence 10–12: 100,000) with pancreatic ductal adenocarcinoma (PDAC) in the Unites States and the mortality rate of 36,800, almost equals this number [1]. PDAC ranks fourth on the list of cancer-related causes of death and despite extensive clinical and scientific effort, the prognosis of this exceptionally lethal disease has not improved significantly over the past decades. Surgical resection, for which only a minority (<20%) of patients qualify due to advanced stage of disease at time of diagnosis, is currently the only chance for cure, improving five-year survival rates from <4% if left untreated to 25–30% after resection [2–4]. Though of marginal impact, chemo(radiation) therapy administered in (neo)adjuvant setting has been shown to increase short-term survival rates in resectable and advanced stage disease [5–7]. Despite subtle progress over the years in terms of therapeutic strategies, no major new treatment options have come forward from numerous clinical trails. Nevertheless, much progress has been made in understanding the pathogenesis of PDAC during the past decades, suggesting that different therapeutic strategies based on these new insights are on the horizon [8–10].

PDAC, like all cancers, is fundamentally a genetic disease caused by alterations in cancer-associated genes. The identification of such specific mutated genes is critical for understanding the pathogenesis of PDAC. Nevertheless, one cannot achieve a reasonable overview by considering only individual genes in a cancer cell because the neoplastic potential of this cell is the end product of mutations in multiple genes and changes in multiple pathways that interact and reinforce each other. The rapidly expanding knowledge of genetic and molecular alterations and their role in pancreatic carcinogenesis has led to the question whether it is possible to design a patient-specific therapy based on the genetic fingerprint of an individual tumor. Since an increasing focus exists on designing these so-called targeted treatment strategies, this paper is aimed to put genetic alterations pancreatic cells undergo during malignant transformation in the context of their role in signaling pathways. In addition, this paper provides an update of

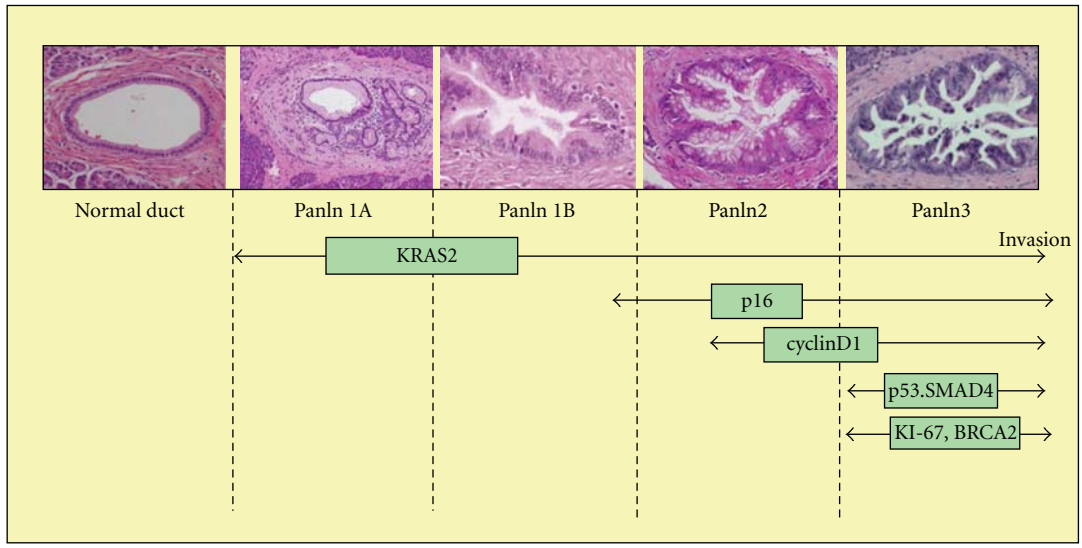

Figure 1: Progression model of pancreatic ductal adenocarcinoma from normal epithelium to invasively growing tumor. The progression is associated with the stepwise accumulation of specific genetic alterations depicted below the pictures.

the most recent advances made in the development of the targeted treatment approach in PDAC.

2. Precursors of PDAC

The development of invasive carcinoma in the pancreas is a stepwise process. Similar to colon cancer, noninvasive stages have been identified in PDAC preceding invasive carcinoma [11]. In recently published research, the clonal evolution of the earliest genetic alterations in tumor initiating cells towards frankly invasive and metastasized PDAC was followed and these studies indicated that such tumor progression takes at least more than a decade [12, 13]. This creates an important window of opportunity for early detection and much effort is put into attempts to map the genetic changes that take place in the pancreatic ductal cells of precursor lesions before they become invasive.

Since 2004, there have been clear guidelines for classifying these precursor lesions of PDAC and three different types have been identified: pancreatic intraepithelial neoplasia (PanIN), mucinous cystic neoplasia (MCN), and intraductal pancreatic mucinous neoplasia (IPMN) [14]. MCN and IPMN are considered separate and specific entities that fall beyond the scope of this review [15, 16]. By far, the most common and also the generic precursor lesion of PDAC is the PanIN lesion. PanINs are found in the smaller pancreatic ducts and based on the degree of dysplasia reflected in the cytonuclear atypia and architectural change can be classified in four grades: PanIN-1A, PanIN-1B, PanIN-2, and PanIN-3. The least severe abnormalities are seen in PanIN-1 lesions; minimal cytonuclear atypia is present and cell polarity is retained with a basally located nucleus. The difference between PanIN-1A and -1B is that the cells in PanIN-1A lesions are flat, whereas the cells in PanIN-1B lesions are arranged in a micropapillary architecture. PanIN-2 lesions are characterized by evident cytonuclear atypia and infrequent mitoses. PanIN-3 lesions, also called carcinoma-*in-situ*, demonstrate all of the hallmarks of cancer including loss of polarity, nuclear atypia, frequent mitoses, and budding of groups of cells in the lumen. Yet, the lesion is confined within the basement membrane and no invasive growth is present [14]. The increasing grades of dysplasia in the various PanIN lesions manifest the morphological steps of tumor progression that precede invasive PDAC. These consecutive steps of tumor progression are genetically accompanied by a cumulative occurrence of specific and generalized molecular genetic alterations. Typically, an interplay between mutations in tumor-suppressor genes, oncogenes, and genome maintenance genes ultimately results in the development of PDAC.

Telomere shortening is considered the initiating event in pancreatic tumorigenesis by inducing genetic instability and is discussed separately below. Another early event in PDAC development is mutation of the oncogene *KRAS2,* which is found altered in 20% of PanIN-1 lesions and this percentage increases with progression to invasive carcinoma. The tumor suppressor gene most commonly found mutated in PDAC is *CDKN2A*. This gene is found mutated in, respectively, 30%, 50%, and 70% of PanIN-1, PanIN-2, and PanIN-3 lesions [17]. Two additional important tumor suppressor genes in PDAC are *TP53* and *SMAD4*. In precursor lesions, mutations in these genes are mainly observed in PanIN-3 lesions in transition to invasive growth warranting *TP53/SMAD4* defects as a late event in PDAC development [18, 19]. In Figure 1 and Table 1, the most commonly observed specific genetic alterations in preinvasive lesions of PDAC are mentioned, but many additional alterations exist. In order to understand pancreatic carcinogenesis, the whole known spectrum of alterations has to be considered as well as the cellular interactions.

Table 1: Most commonly affected genes in PDAC.

Type	Gene	Cellular function	Affected in PDAC
Tumor suppressor genes	*CDKN2A/p16*	G1-S phase cell cycle inhibition	95%
	SMAD4	TGFβ	55%
	TP53	Cell-cycle arrest	75%
Oncogenes	*KRAS2*	ERK-MAPkinase signaling	>90%
	CyclinD	Cell cycle progression	65%
	BRAF	ERK-MAPkinase signaling	5%
Genome maintenance genes	*MLH1/MSH2*	DNA damage (mismatch) repair	4%
	BRCA2	DNA damage repair	7–10%

3. Molecular Characteristics and Regulatory Pathways in PDAC

In 2008, Jones et al. used global genomic sequencing to identify the genetic alterations in pancreatic cancer cells. Over 21,000 genes were screened in 24 different PDAC samples. On average, 63 relevant genetic alterations were found per sample, emphasizing the extreme complexity of this disease. These genetic alterations mostly affected 12, partially overlapping, signaling pathways that consequently contained abnormalities in the majority of cases [20, 21]. The identification of these pathways intelligently created a comprehensible view of pancreatic carcinogenesis without simplifying too much [21]. All previously known genetic alterations were included and put into the context of the pathways in which they function. Five of the pathways describe specific cellular functions; apoptosis, DNA damage repair, G1/S phase cell cycle progression, cell-cell adhesion and invasion (Figure 2). The other pathways are signaling cascades and can be divided into three groups: embryonic signaling pathways, the MAPkinase signaling pathways, and TGF-β signaling. The molecular characteristics of PDAC are described within the context of these various specific pathways in the subsequent paragraphs. Table 2 gives an overview of the various affected pathways and their most commonly mutated genes in PDAC.

For the acquisition of an accumulation of genetic alterations by the neoplastic cells, genetic instability is a precondition [22]. Telomere shortening is considered as the initial neoplastic event that provides pancreatic epithelial cells the genetic instability that leads to the subsequently specific and generalized molecular alterations [23, 24].

3.1. Telomere Shortening. Telomere shortening is encountered in virtually all precursor lesions and invasive carcinomas [23, 25]. Telomeres are repeat sequences at the end of linear chromosomes that prevent fusion between the ends of these chromosomes. Pathologically short telomeres can result in ring and dicentric chromosomes that form so-called anaphase bridges during mitosis. Breakage of these anaphase bridges generates highly recombinogenic-free DNA ends, which in turn can result in chromosomal rearrangement. These cycles of chromosome bridging and breakage, called anaphase bridge-breakage-fusion cycles, repeat and thereby create the genetic instability that facilitates tumor development [26]. Telomerase, the gene that maintains telomere length, shows low expression during early pancreatic tumorigenesis before markedly increasing in the invasive tumor. The re-expression of telomerase probably restores genomic stability, enabling tumor progression by preventing further, possibly lethal, chromosomal damage [25, 27].

Table 2: The 12 commonly affected signaling pathways in PDAC accompanied by the most commonly affected genes from these pathways.

Regulatory pathway	Affected genes
Apoptosis	*TP53*
DNA damage repair	*TP53*
G1/S transition	*CDKN2A/p16, CyclinD*
Cell-cell adhesion	
Regulation of invasion	
Integrin signaling	
Homophilic cell adhesion	*CDH1*
Embryonic signaling	
Notch pathway	
Hedgehog pathway	
Wnt pathway	
MAPK signaling	
c-Jun N-terminal kinase	
ERK	*KRAS2*
TGF-β signaling	*SMAD4*

3.2. Apoptosis. Apoptosis, or programmed cell death, plays an essential role in cancer development since resistance to apoptosis is a key factor in the survival of a cancer cell (Figure 3). Apoptosis is induced by executioner caspases upon activation by the apoptosome complex. This complex consists, among others, of cytochrome C, Caspase-9, and Apaf1 and is released from the mitochondria when pro-apoptotic signaling by Bak/Bax outweighs antiapoptotic signaling by Bcl2, Bcl-X(L) or Mcl-1. Inhibitors of apoptosis proteins (IAPs) can inhibit apoptosis at the end of the signaling cascade by direct inhibition of the executioner caspases. In PDAC, genes implicated in the apoptosis pathway were found altered in all tumors studied [21]. Also, previous reports document impaired apoptotic signaling

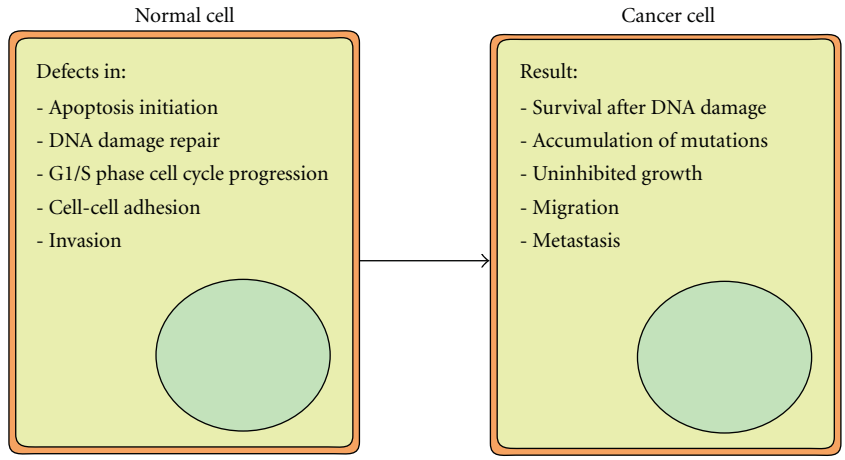

Figure 2: Cellular functions affected in pancreatic cancer.

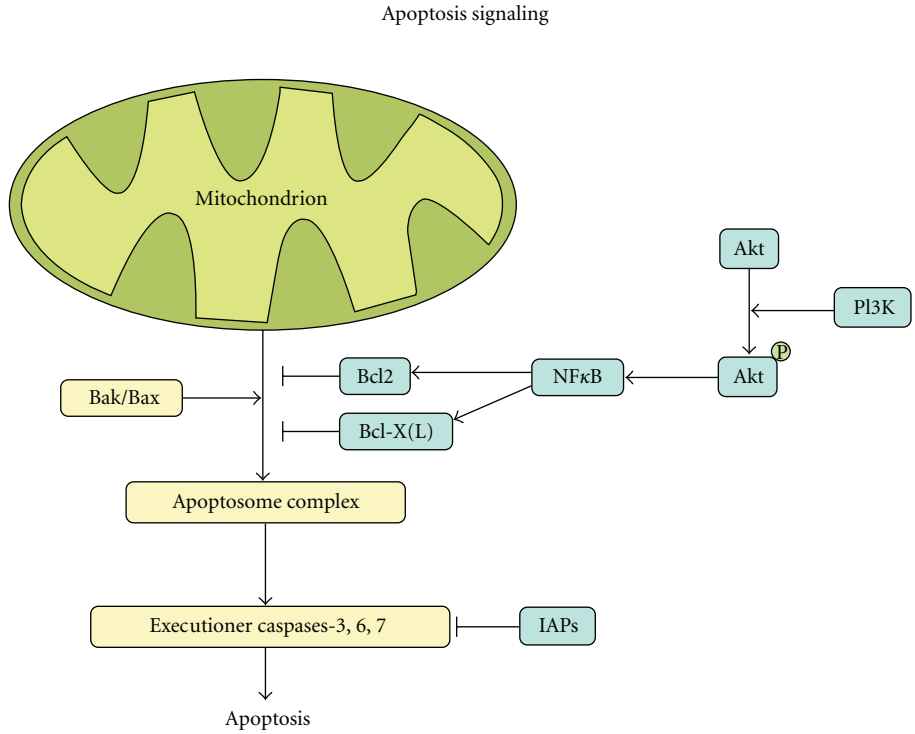

Figure 3: Apoptosis. Apoptosis is induced by executioner caspases upon activation by the apoptosome complex. This complex consists, among others, of cytochrome C, Caspase-9, and Apaf1 and is released from the mitochondria when proapoptotic signaling by Bak/Bax outweighs antiapoptotic signaling by Bcl2/Bcl-X(L). PI3K activates Akt through phosphorylation which subsequently activates NF-κB. NF-κB stimulates antiapoptotic signaling by Bcl2 and Bcl-X(L). Inhibitors of apoptosis proteins (IAPs) can inhibit apoptosis at the end of the signaling cascade by direct inhibition of the executioner caspases.

in this disease. For example, a high fraction of apoptotic cells has been correlated with longer overall survival as well as absence of nodal involvement [28]. Moreover, most chemotherapeutics act through apoptosis induction whereby therapy resistance often is the result of defective apoptosis pathways. Antiapoptotic genes *BCL-2*, *BCL-X(L)*, and *MCL-1* are expressed in, respectively, 13%, 54%, and 86% of PDAC samples as shown by immunohistochemistry, and repression of *BCL-2* and *MCL-1* was shown to enhance apoptosis in PDAC [29, 30]. The observed apoptotic effect was even more pronounced when treatment was combined with gemcitabine [31].

NF-κB is a transcription factor that regulates several different cellular mechanisms, of which most importantly apoptosis. NF-κB stimulates antiapoptotic signaling by targeting *BCL-2 and BCL-X(L)* [32–34]. The NF-κB signaling pathway is activated by a variety of different mechanisms in PDAC amongst others oncogenic K-ras signaling [35].

Oncogenic K-ras signaling also activates phosphatidylinositol 3-kinase (PI3K), another important protein in apoptosis. PI3K activates Akt through phosphorylation which subsequently activates NF-κB. The *AKT2* gene located on chromosome 19q is amplified in 10–20% of pancreatic cancers [36, 37], whereas PI3K/Akt signaling is activated in approximately 60% of PDACs [38]. PI3K signaling also involves mammalian target of rapamycin (mTOR), a downstream target of Akt. Activation of mTOR has been observed in approximately 75% of PDACs [39]. Therefore, inhibition of mTOR is an interesting target for therapy for which there are currently FDA approved inhibitors on the market. Although the exact role of the PI3K/Akt/mTOR pathway in pancreatic cancer remains to be elucidated, signaling of this pathway was shown to inhibit apoptosis, and inhibition of the pathway increased cellular sensitivity to gemcitabine [40, 41].

3.3. DNA Damage Repair. DNA damage control genes are responsible for safeguarding the integrity of DNA as they code for proteins that repair any damage that occurs in the cell during its lifespan. An important DNA damage repair gene is *TP53*, a tumor suppressor gene located on chromosome 17p that is frequently disrupted in many different human malignancies. *TP53* expression is lost in 50–75% of PDACs [42, 43]. P53 is involved in the cellular response to genotoxic stress where it mediates cell cycle arrest and apoptosis upon DNA damage. Therefore, loss of *TP53* signaling results in a decrease in apoptosis and increases the opportunity for genetic alterations to accumulate in the cells.

Germline *BRCA2* gene mutations are responsible for ~10% of familial pancreatic cancer but mutations in this gene are also observed in approximately 7–10% of sporadic PDAC. The BRCA2 protein is involved in DNA damage repair, especially interstrand cross-linking repair [44–46]. The BRCA2 gene will be further discussed in the paragraph on hereditary PDAC.

A third group of DNA damage repair genes involved in the development of PDAC is the mismatch repair family (MMR) of genes. The MMR proteins target base substitution mismatches and insertion-deletion mismatches that arise as a result of errors occurring during replication. Alterations in the mismatch repair genes *MLH1, MSH2, MSH6,* and *PMS2* eventually lead to microsatellite instability (MSI) and this genetic instability makes the genome vulnerable for the accumulation of other, more specific genetic alterations. Tumors of the pancreas with MSI are relatively rare compared to other malignancies of the digestive tract and are found in only 5% of pancreatic carcinomas. Pancreatic cancers with MSI have a distinct microscopic morphology that resembles their counterpart in the colon and are similarly called medullary type carcinomas [47–49]. Remarkably, microsatellite instable tumors have a significantly better prognosis compared to their microsatellite-stable counterparts [47, 48]. PDACs with MSI exhibit a higher antitumor reaction by T-lymphocytes and this could possibly be the reason for a better outcome [49].

3.4. G1/S Phase Cell-Cycle Progression. Cell-cycle progression and regulation is affected in virtually all cancers as is the case for PDAC. Alterations in genes regulating G1/S-phase transition play an important role in facilitating the uncontrolled growth rate of cancer cells. The most commonly affected tumor suppressor gene in PDAC involved in G1/S phase transition is the *CDKN2A* gene [50, 51]. This gene is located on the short arm of chromosome 9 (9p21) and is known for its involvement in hereditary melanoma when mutated in the germline [52]. The gene is inactivated in >90% of all PDACs, either by homozygous deletion (40%) or an intragenic mutation combined with loss of heterogeneity of the remaining wild type allele (40%) [50, 53]. Promoter hypermethylation is the cause for loss of *CDKN2A* function in 15% of the cases [50]. P16, the protein product of CDKN2A inhibits phosphorylation of Rb-1, thereby preventing G1/S transition and acting as an inhibitory cell-cycle regulator [54]. Loss of p16 expression therefore leads to uncontrolled G1/S transition and unregulated cell division, which facilitates tumor progression [55].

Other genes involved in cell cycle progression that occasionally show alterations in PDAC are *FBXW7, CHD1* and *APC2*, although much less frequently than *CDKN2A* [21, 56].

3.5. Cell Adhesion and Invasion. In normal pancreatic tissue, cells are anchored to each other and their surroundings via multiple connections. A decrease in these interactions can allow cells to detach from their surrounding and migrate/metastasize. As such, cell to cell adhesion and interaction play an important role in carcinogenesis. The connection between epithelial cells is mostly mediated by the adherent junctions composed of E-cadherin and catenins. E-cadherin proteins interlock with each other in the extracellular space, while intracellularly the E-cadherin protein is bound to actin filaments through catenins. Reduced expression of E-cadherin and α- and ß-catenins was demonstrated in approximately 60%, 40%, and 60% of pancreatic cancer samples, respectively [57, 58]. Reduced expression of E-cadherin is correlated with tumor dedifferentiation and correlates with tumor stage and lymph node involvement [57, 59]. Not only is the interaction between epithelial cells important in the preservation of the integrity of the tissue, the interactions between the neoplastic cells and extracellular matrix also play an important role, especially in PDAC, because stromal tissue surrounds most tumor cells. Integrins comprise a large family of cell surface receptors and they act as a bridge between the extracellular matrix (ECM) and the cytoskeleton [60]. These integrins direct cell migration and play an important role in invasion. In addition to this, integrins also regulate cell proliferation and apoptosis. Integrin-ECM interactions are vital for cell survival but since apoptosis pathways are often affected in PDAC, loss of the interaction does not necessarily lead to apoptosis in cancer cells. Integrin signaling can activate the ERK, JNK MAPK pathway and the PI3K pathway, important pathways in pancreatic tumorigenesis. In approximately two-thirds of the PDAC cases, a defect in integrin signaling can be identified

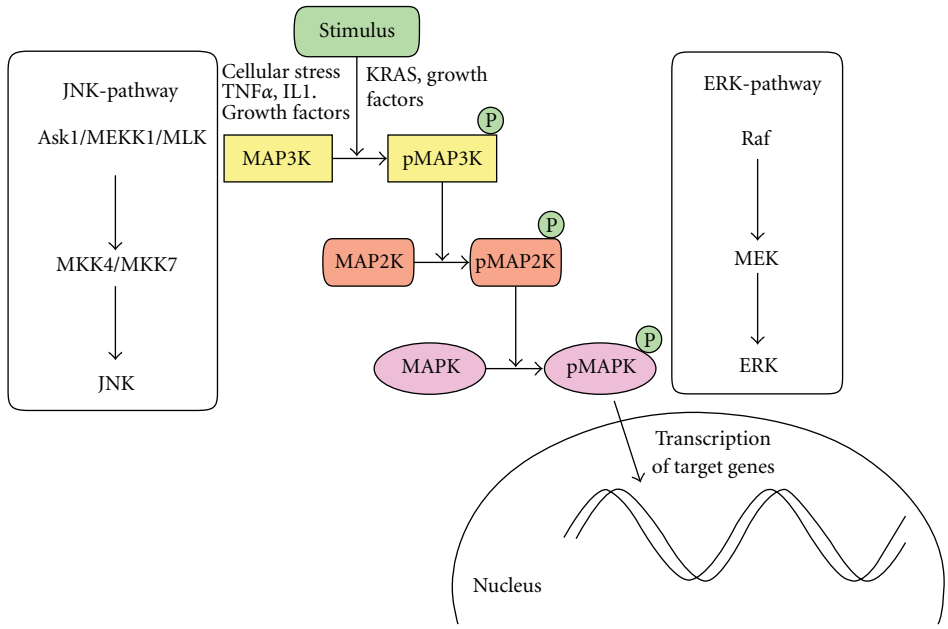

Figure 4: MAPkinase signalling. Mitogen-activated phosphorylated kinase signaling occurs through a common pathway. A cellular stimulus results in phosphorylation of MAP3K, which in turn phosphorylates MAP2K. MAP2K subsequently phosphorylates MAPK, resulting in altered transcription of the MAPK target genes. The different components of the two MAPK signaling pathways often affected in PDAC, the ERK pathway and the JNK pathway, are depicted in the boxes on each side of the signaling cascade.

[21]. Different components of integrin signaling can be deregulated; for example, Integrin $\alpha 6\beta 1$ expression has been correlated with metastatic behavior in pancreatic cancer cell lines [61]. Furthermore, Niu et al. investigated the role of $\alpha v\beta 6$ integrin in PDAC and found that $\alpha v\beta 6$ inhibition resulted in a significant reduction in cell proliferation and invasion. Apoptosis was induced and more remarkably, $\alpha v\beta 6$ integrin knockdown increased gemcitabine sensitivity [62].

Another group of proteins involved in cell adhesion is the a-disintegrin and metalloproteinase (ADAM) protein family. ADAM proteins are cell surface proteins that activate MAPK pathways and integrin signaling through the release of growth factors. ADAM proteins have the ability to cleave ECM components and influence integrin/ECM interactions. ADAMs have only recently caught attention, thus not much is known about the specific role these proteins play in pancreatic carcinogenesis, though upregulation of different ADAM proteins has been reported in PDAC [63, 64]. Jones et al. found genetic alterations in various different ADAM proteins [21]. Because ADAM proteins influence many different substrates through autocrine and paracrine signaling, they may comprise promising new targets for therapy development.

3.6. MAPK Signaling Pathways. There are three major mitogen activated phosphorylated kinases (MAPK): extracellular signal-regulated kinase (ERK), c-Jun N-terminal kinase (JNK), and p38. All MAPK signaling pathways consist of the same basic kinase components. Stimulation of an upstream MAP2K kinase (MAP3K) by growth factors, stress, or other extracellular signals leads to phosphorylation of a MAPK kinase (MAP2K), culminating in the phosphorylation of a terminal MAPK (Figure 4).

The most influential of the three MAPK pathways in PDAC is the ERK pathway. It consists of the Raf protein (MAP3K) that phosphorylates MEK (MAP2K), which in turn phosphorylates ERK (MAPK), the latter influencing transcription of different target genes. This signaling cascade results in the activation of multiple oncogenic cellular functions. The most commonly mutated oncogene in PDAC is the *KRAS2* gene, of which the protein product Ras is an upstream activator of ERK signaling. *KRAS2* is located on chromosome 12p and the protein has an intrinsic GTP-ase activity. In PDAC, the gene is virtually always activated by a point mutation in codon 12, the GTP binding domain, leading to a constitutively active Ras protein [65, 66]. Therefore, it can be considered a molecular switch that in this fashion remains in the "on" position firing its oncogenic stimuli. As said before, *KRAS2* gene mutations are an early phenomenon in the development of PDAC, and the *KRAS2* gene is mutated in ~95% of PDACs. Interestingly, the few tumors that contain wild-type *KRAS2* often have a mutation in the *BRAF* gene, an oncogene located on chromosome 7q. The *BRAF* gene is

mutated in approximately 5% of the PDACs and Raf, the protein product of *BRAF*, is a downstream target in the Ras signaling pathway. This explains the mutually exclusive nature of *KRAS2* and *BRAF* mutations in PDAC [56]. The high frequency and early nature of *KRAS2* mutations suggest an initiating role in PDAC development as confirmed in several studies on genetically engineered mice [67, 68]. Besides the effects Ras has on the ERK-pathway, Ras also influences multiple other genes among which NF-κB and PI3K/Akt as discussed above.

The second MAPK pathway often affected in PDAC is the JNK pathway. In the whole genome sequence analysis study by Jones et al. mentioned earlier, in all but one of the sequenced samples a genetic alteration in the JNK pathway was identified [21]. In this signaling cascade the MAP3Ks, Ask1, MEKK1, and MLK phosphorylate the MAP2Ks, MKK4, and MKK7 which in turn phosphorylate JNK. The JNK pathway becomes activated upon cellular stress but more importantly, the pathway is activated by proinflammatory cytokines such as tumor necrosis factor α (TNF-α) or interleukin 1 (IL1) [69]. *MKK4* expression is lost in approximately 4–15% of PDACs [70, 71]. Remarkably, the JNK pathway has both tumor suppressor and oncogenic functions that have to be further investigated. Also it should be noted that the Kras and the JNK pathways interact; phosphorylation of JNK is partly responsible for induction of angiogenesis through Kras [72]. Recent studies have also connected MKK4 and its downstream targets (JNK and p38) to the TGF-β pathway.

3.7. TGF-β Pathway. The transforming growth factor β (TGF-β) pathway has been linked to PDAC for many years. TGF-β signaling is involved in a wide range of cellular processes [73]: It is one of the most potent cell proliferation inhibitors and has many other cellular responsibilities including differentiation, apoptosis, and angiogenesis [74]. Binding of a TGF-β family ligand to the TGF-βII receptor leads to phosphorylation of the TGF-βI receptor and thereby activation of the TGF-β receptor substrates capable of signal transduction, that is, the Smad family proteins. Eight different *SMAD* genes have been described. Once activated, the receptor subsequently phosphorylates a regulatory Smad (Smad1–3, 5, 8), allowing this protein to associate with Smad4. The latter aids the regulatory Smad complex in its transfer to the nucleus where subsequent transcription of the target genes is induced. Inhibitory Smads regulate Smad-signaling through inhibition of the TGF-β receptor phosphorylation. Thus far, Smad7 is the only characterized inhibitory Smad (Figure 5) [73].

TGF-β influences cellular proliferation through inhibition of G1/S-transition. This is accomplished though expression of cyclin kinase inhibitors such as p15, p21, and p27 [75]. Also, TGF-β signaling represses c-Myc expression, an ubiquitous promoter of cell cycle progression. Jones et al. found altered TGF-β pathway expression in all their PDAC samples [21]. The most commonly affected protein in the TGF-β pathway is Smad4. Smad4 is inactivated in ~55% of PDACs [76, 77]. Patients with preserved Smad4 signaling have a significantly longer survival than patients with Smad4 loss [77–79]. Also, Iacobuzio-Donahue et al. found a significantly higher percentage of Smad4 loss in patients who had died from PDAC with widespread metastatic disease compared to patients who died of locally advanced tumors [79]. Loss of Smad4 expression is not only a prognosticator but it can also serve as a diagnostic biomarker since sensitive and specific antibodies are available that can be used to characterize Smad4 protein expression by immunohistochemistry (Figure 6) [78].

Other proteins in the TGF-β pathway that are occasionally found altered in PDAC are the TGF-βRII (4%) and TGF-βRI (1%) [80]. Apart from binding to the TGF-βR, TGF-β ligands can also activate other signaling pathways including the MAPK pathways ERK and JNK [81–83]. This depicts the fact that although TGF-β signaling has a tumor suppressive function in the normal epithelium, it can promote tumor progression in late disease stages. Further research has to be conducted to determine the true potential of this pathway for the development of targeting agents.

3.8. Embryonic Pathways. Not surprisingly, since embryogenesis shares many characteristics with carcinogenesis, different embryonic pathways are involved in tumor development. There are three embryonic pathways involved in pancreatic carcinogenesis: Notch, Hedgehog, and Wnt (Figure 7).

The Notch pathway plays an important role in pancreatic organogenesis, but after formation of the pancreas, signaling is largely restricted to a putative progenitor population known as centroacinar cells [84–87]. Several studies have shown upregulation of Notch pathway activity in PDAC and inhibition of this pathway resulted in decreased tumor proliferation and increased apoptosis [87–90]. Somatic point mutations in one of the four Notch-receptor genes do not seem to be the driving force behind altered Notch signaling in pancreatic cancer [90]. Still, 100% of the PDAC samples in a genome-wide sequencing study revealed alterations in the Notch pathway [21]. Notch signaling interacts with many other oncogenic pathways including the Hedgehog pathway, KRAS signaling and the NF-κB pathway.

The second embryonic pathway often affected in PDAC is the Hedgehog (Hh) pathway. This signaling cascade plays an important role in the organogenesis of the gastrointestinal tract. Surprisingly, Hh signaling is absent in the developing pancreas [91, 92] but the pathway is activated in 70% of PDACs [93]. Some of the Hh signaling targets are components of other signaling pathways involved in PDAC such as Wnt proteins, TGF-β, and CyclinD [94–96]. Although it has long been common knowledge that Hh signaling is active in PDAC, its exact role in tumorigenesis is unclear. It seems that neoplastic epithelial cells do not have the ability to react to Hh signaling. Instead, Hh ligands are expressed in the epithelial cells and it has been suggested that these affect the stromal compartment of a tumor through paracrine signaling. In one particular study, the strong desmoplastic reaction characteristic for PDAC was shown to be further enhanced when Hh signaling was activated [96]. In addition, inhibition of the Hh pathway decreased the total

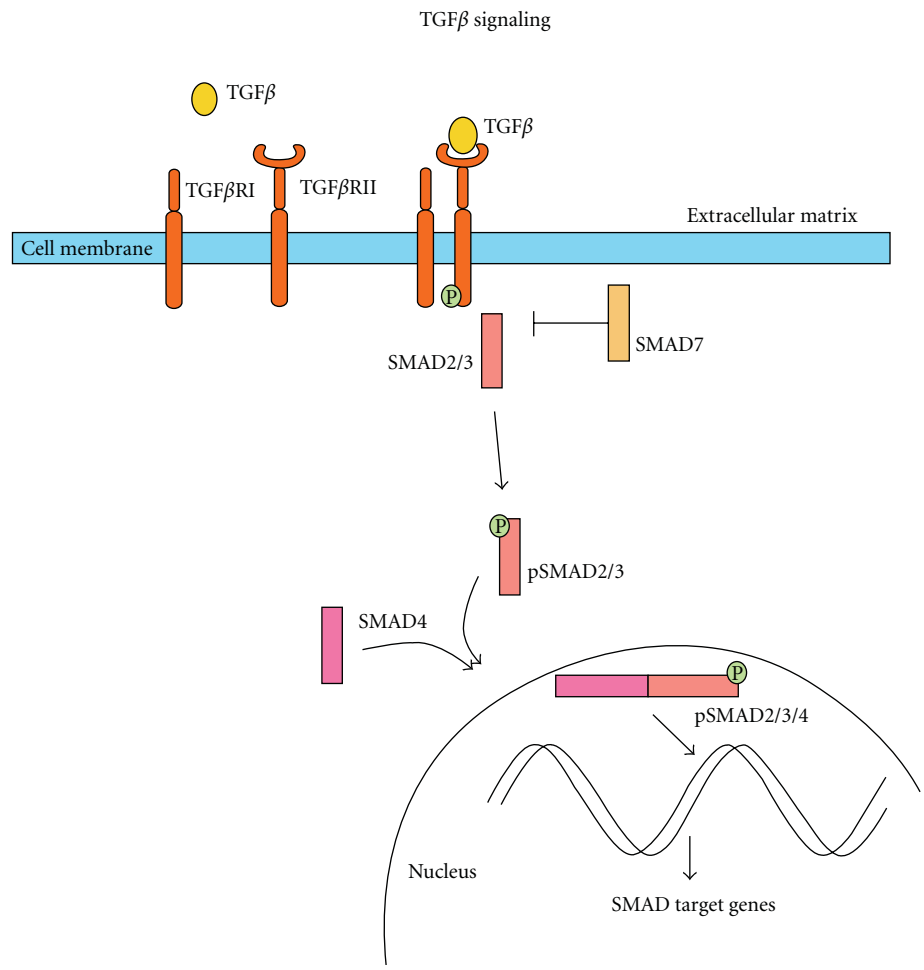

Figure 5: The TGF-β signaling pathway. The TGF-β signaling pathway is activated by binding of TGF-β to a type II receptor, which facilitates the recruitment and phosphorylation of the type I receptor. This pTGF-βRI activates either SMAD2 or SMAD3 by phosphorylation. The phosphorylated SMAD2/3 forms a complex with SMAD4 and transports to the nucleus where it influences SMAD target gene transcription. SMAD7 can inhibit TGF-β signaling through inhibition of TGF-βRI phosphorylation.

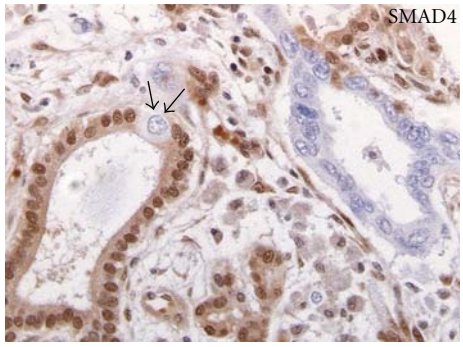

Figure 6: SMAD4 immunohistochemistry. Loss of *SMAD4* expression is clearly depicted in the PDAC cells. Arrows: single cell with clear histological changes exhibiting SMAD4 loss, surrounded by *SMAD4* wild-type cells.

volume of orthotopically implanted tumors by inhibiting the stromal component in mice [97]. Another study showed that treatment with an Hh pathway inhibitor produced a clear decrease in tumor growth primarily through a decrease in number of stromal cells [98]. Similarly, disruption of Hh signaling in a transgenic mouse model increased response to chemotherapy [99]. This improved response was due to a diminished desmoplastic reaction and better accessibility of the tumor cells for the chemotherapeutic agent. In short, there seems to be an important role for Hh signaling in the stromal component of PDAC. Moreover, since the desmoplastic reaction has been related to resistance to therapy it warrants further investigation of Hh and its role in the development of PDAC. Clinical trials with inhibitors of hedgehog signaling are in progress.

The third embryonic pathway, Wnt, shows increased activity in approximately 30–65% of PDACs, and an increase in Wnt-target expression correlates with poorer differentiation and poor prognosis [100–102]. Active Wnt expression results in the transcription of different target genes including *CyclinD*, matrix metalloproteinase 7 (*MMP7*), and *c-MYC*. CyclinD is overexpressed in 65% of PDACs and stimulates

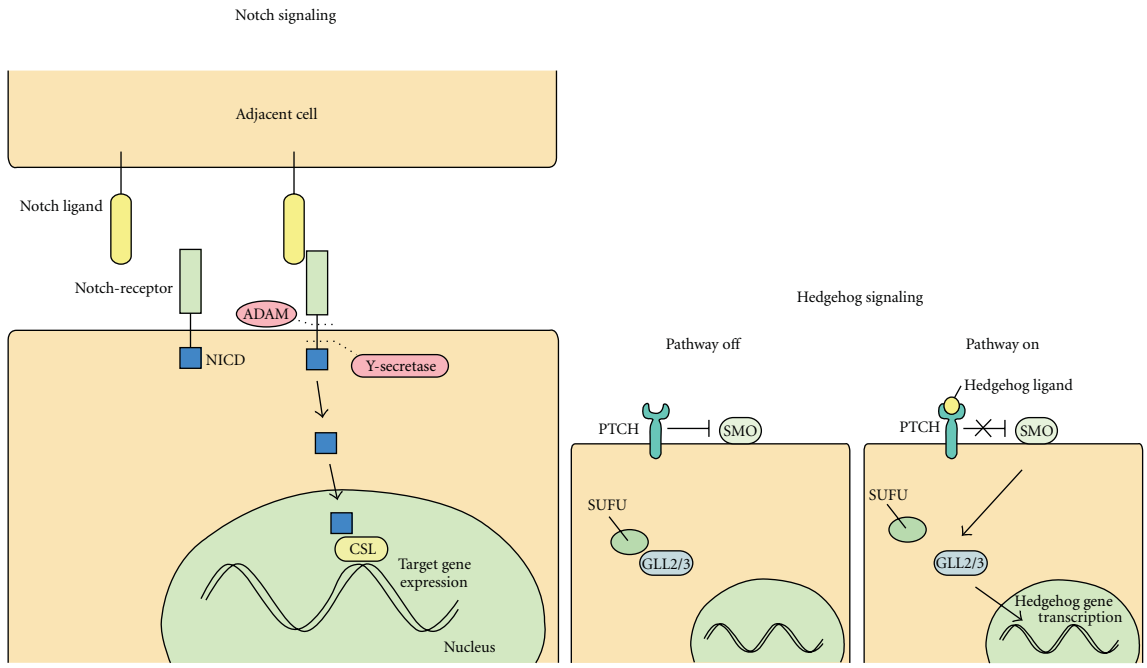

Figure 7: Left Notch signaling. Upon Notch ligand binding, ADAM performs the first cleavage quickly followed by the second cleavage performed by γ-secretase. This second cleavage releases the Notch intracellular domain (NICD) into the cellular lumen. NICD transports to the nucleus where it interacts with a transcription domain resulting in transcription of the Notch target genes. Right Hedgehog signaling. In normal pancreatic cells, Hedgehog signaling is repressed; the patched (PTCH) receptor represses the smoothened (SMO) receptor. Intracellularly, the hedgehog inhibitor suppressor of fused (SUFU) binds the GLI family zink finger transcription factors GLI2/3 thereby inducing proteasomal cleavage resulting in repressor forms of GLI2/3. When one of the Hedgehog ligands attaches to Patched, this abrogates the inhibition on Smoothened. Smoothened inhibits proteasomal cleavage of GLI2/3 and thereby facilitates transcriptional activity.

G1/S transition. Expression of this protein is associated with poor prognosis [103]. MMP7, a member of the matrix metalloproteinase family, degrades extracellular matrix proteins, and MMP7 expression is implicated in metastases. Overexpression of this protein is found in practically all PDACs [104]. C-Myc is a transcription factor that regulates thousands of genes involving a large spectrum of cellular functions including cell proliferation, differentiation, death, and tissue reorganization. Amplification of *CMYC* is identified in 20–50% of all PDACs [105]. The Wnt signaling cascade can be activated through interactions with the Hh, NF-κB, TGF-β, and Notch pathways [106–108].

4. Genetic Susceptibility

Approximately 5–10% of patients with pancreatic cancer have a positive family history for the disease. Having a first-degree relative with PDAC doubles the chance of developing pancreatic cancer compared to individuals without such a history, and the risk increases with increasing number of affected relatives suggesting a hereditary component. Some of these PDACs arise in the setting of a known familial cancer syndrome; however, in most instances the genetic basis for the familial aggregation is not known [109].

To date, at least 5 hereditary disorders that significantly increase the chance of pancreatic cancer development have been described. These include familial atypical multiple melanoma and mole (FAMMM) syndrome, Peutz-Jeghers syndrome, hereditary pancreatitis, familial breast cancer, and other syndromes related to alterations in Fanconi anemia genes, and the Lynch syndrome (Table 3).

The FAMMM syndrome is caused by germline mutations in the *CDKN2A* gene. As stated before, this gene is often somatically mutated in sporadic pancreatic cancer. Patients suffering from this syndrome have a 20–34-fold risk of developing PDAC [110]. This risk is especially high when the mutation is a specific 19-base-pair deletion in *CDKN2A*: the p16-Leiden deletion [111].

Peutz-Jeghers syndrome is caused by mutations in the *STK11/LKB1* gene, a serine threonine kinase involved in a large number of cellular functions, from control of cell polarity to metabolism. Patients suffering from this syndrome have a 132-fold increased risk of developing PDAC with a 30–60% lifetime risk of PDAC at age 70 [112–114].

Patients with hereditary pancreatitis develop recurrent episodes of pancreatitis, starting at a young age. The syndrome is most commonly caused by mutations in the cationic trypsinogen gene *PRSS1* [115]. Another gene that is occasionally found altered in patients with hereditary pancreatitis is the serine peptidase inhibitor *SPINK1* [116]. Carriers of either of these mutations have a highly increased risk of developing pancreatic cancer with a lifetime risk of 25–40% at age 60.

Table 3: Hereditary syndromes associated with an increased risk of PDAC development.

Syndrome	Affected gene(s)	Relative risk of PDAC
Familial atypical multiple melanoma and mole syndrome	*CDKN2A*	20–34
Peutz-Jeghers syndrome	*LKB1*	>100
Hereditary pancreatitis	*PRSS1/SPINK1*	~90
Familial breast cancer	*BRCA1/2*	3–10
Lynch syndrome	*mismatch repair genes*	unknown

The two *BRCA* genes are best known for their role in familial breast and ovarian cancers but *BRCA2* also plays a role in pancreatic cancer development. Carriers of germline *BRCA2* gene mutations have a 3–10-fold increased risk of developing PDAC. A specific interest goes to the Ashkenazi Jewish population as approximately 1% of Ashkenazi Jews are carriers of a founder *BRCA2* mutation, 6174delT [117].

Fanconi anemia is a hereditary cancer susceptibility disorder, with occurrence of multiple haematological malignancies. The Brca2 protein interacts with different Fanconi anemia pathway components, and the corresponding encoding genes, especially *FANCC* and *FANCG,* have also been reported to increase the chance of PDAC development when mutated. Recently, *PALB2*, yet another FANC gene,was reported to be responsible for ~3% of the cases of familial pancreatic cancer [118, 119]. *PALB2* encodes a protein that enables the localization and binding of Brca2 to sites of double-strand DNA breaks.

Lynch syndrome is caused by germline mutations in a number of DNA mismatch repair genes. Patients suffering from the syndrome have a slightly increased chance of developing pancreatic cancer although there is still some debate about the exact role in PDAC development [120].

Identification of germline mutations in the previously discussed genes is of great importance, not only for screening purposes but also because they could potentially hold therapeutic consequences. Furthermore, no genetic basis for cancer susceptibility is identified in most cases of families exhibiting high numbers of PDAC affected individuals. More research on genetic susceptibility for PDAC will have to be conducted to explain the genetic basis for disease development.

5. Treatment of PDAC

Adjuvant therapy after resection of the tumor consisting of gemcitabine has been the treatment of choice since 1997 when it was shown to improve both disease-free and overall survival [5, 6]. Several studies examining the effect of adding other therapeutic agents to gemcitabine have been conducted over the past years with disappointing results [121, 122]. Only the addition of erlotinib showed slight improvement of overall survival [123]. A recently published report confirmed the earlier observed limited beneficial effect of adding erlotinib; however, the authors concluded that this was no justification for a phase III trail [124]. Reports comparing single-agent gemcitabine to adjuvant chemoradiation therapy have been inconclusive. Chemoradiation therapy has been implicated in the USA since the Gastro-Intestinal Tumor Study Group trial was published which showed longer overall survival in patients treated with adjuvant chemoradiation [125, 126]. In Europe, however, a similar study failed to find a significant survival advantage for the group receiving additional radiotherapy thus chemoradiation therapy did not become the standard treatment [127]. Although the most recent reports on this subject suggest a significant advantage for the addition of radiotherapy, there is still controversy about this subject and more research needs to be done before radiation therapy can be included as standard first-line of treatment for PDAC in Europe [125]. It has been suggested that neoadjuvant treatment with chemotherapy, radiation therapy, or chemoradiation therapy, could downstage borderline resectable tumors. Several recent studies have shown promising results for treating borderline resectable tumors with chemoradiation, enabling resection and approaching similar survival rates as truly resectable tumors [128–131]. This is still under investigation and future studies will have to be conducted to justify the use of neoadjuvant treatment.

Approximately 80% of patients present with locally or systemically advanced disease-making resection redundant. For these patients, only palliative treatment options remain. Single-agent gemcitabine is currently recommended as standard first-line chemotherapy for patients with advanced disease [5].

Since the arrival of whole genome sequencing, it has become possible to identify all the genomic alterations that lead to the development of pancreatic cancer. The next logical step is to translate this knowledge into better treatment options. Until recently, no targeted agents were found to improve outcome in the clinical setting although many studies have shown promise in the *in vitro* setting. In the past year, a group used mutation analysis to guide their treatment strategy for the first time [132]. Earlier studies had shown that PDAC cell lines harboring mutations in the above-mentioned *BRCA2* gene, but also other genes related to the Fanconi Anemia syndrome (*FANCC, FANCG*) responded better to treatment with interstrand cross linking (ICL) agents than *FANC/BRCA* wild-type tumors [133]. The *FANC/BRCA* pathway is involved in the repair of double stranded DNA-breaks. As ICL-forming agents induce this type of DNA damage, susceptibility of *FANC/BRCA* mutated tumors to the ICL-forming agents seemed reasonable. Showalter et al. performed mutation analysis for *BRCA2* and one patient harboring a mutation in this gene was treated

with cisplatin, an ICL agent, in addition to gemcitabine showing favorable results (the patient is still alive after 32 months). In theory, PDACs carrying *PALB2* mutations should be sensitive to the same targeted therapeutic as *PALB2* is a binding partner of *BRCA2*. Trials justifying use of ICL agents in *PALB2* mutated PDAC still have to be conducted.

We have recently seen a Peutz-Jeghers syndrome patient with pancreatic cancer whose tumor showed complete loss of *LKB1*, an inhibitor of mTOR. This patient responded to treatment with everolimus, one of the known mTOR inhibitors used in clinical setting. Specifically, the tumor diminished in size by more than 50% within 6 months but became resistant thereafter [134].

Inhibition of Kras signaling with farnesyl transferase inhibitors used in the past did not have a beneficial effect (reviewed by [135]). In 2010, a new therapeutic agent was identified targeting the Kras pathway. Protein Kinase C iota (PKC iota) was shown to drive transformed growth in pancreatic cancer cell lines via inhibition of oncogenic Kras activity, and inhibition of PKC iota resulted in a significant reduction of metastases and invasion in preclinical models [136]. Further research has to be done to map the effectiveness of inhibiting PKC iota *in vivo*.

MTAP, a gene located near *CDKN2A*, is codeleted with the *CDKN2A* gene in 30% of the pancreatic cancers. *MTAP* might be a possible therapeutic target as approaches to selectively target cells with *MTAP* defects have already been developed [137, 138]. However, these have not been tested in a clinical setting yet.

It seems logical that over the next few years multiple small steps, hopefully adding up to significant progress, will be taken on the road to targeted treatment of PDAC.

6. Conclusion

The aim of this review was to emphasize the complexity of tumorigenesis in pancreatic ductal adenocarcinoma and to provide an introductory overview of the pathways affected in PDAC. As the knowledge on tumorigenesis of PDAC expands rapidly, so do the possibilities to design more effective treatment. The arrival of genome sequencing has offered the opportunity to establish an overview of the genetic alterations that lead to tumor development and this could subsequently play an important role in our search for new therapeutic targets. The complexity of the genetics accompanying PDAC indicates that it is impossible to design a treatment that fits all. From this can be deducted that personalized treatment based on tumor genotyping will probably be most effective and feasible. The use of ICL agents for tumors harboring *BRCA2* mutations is the first step in that direction.

Based on the data presented in this review, it seems advisable to shift the focus of research from most commonly affected genes to the most commonly affected pathways as some important yet rare alterations could be missed. The interactions all these pathways undergo are extensive and complex as mentioned earlier. When considering personalizing treatment, designing workable and quick tumor characterizing assays and targeting pathways rather than individual genes seems to hold the future of cancer therapy in PDAC.

Abbreviations

PDAC:	Pancreatic ductal adenocarcinoma
PanIN:	Pancreatic intraepithelial neoplasia
MCN:	Mucinous cystic neoplasia
IPMN:	Intraductal pancreatic mucinous neoplasia
PI3K:	Phosphatidylinositol 3-kinase
mTOR:	Mammalian target of rapamycin
MMR:	Mismatch repair
MSI:	Microsatellite instability
ECM:	Extracellular matrix
ADAM:	A-disintegrin and metalloproteinase
ERK:	Extracellular signal-regulated kinase
JNK:	C-Jun N-terminal kinase
MAPK:	Mitogen activated phosphorylated kinases
Hh:	Hedgehog
TGF-β:	Transforming growth factor β
TNF-α:	Tumor necrosis factor α
IL1:	Interleukin 1
MMP7:	Matrix metalloproteinase 7
FAMMM:	Familial atypical multiple melanoma and mole
ICL:	Interstrand crosslink
PKC iota:	Protein kinase c iota.

Conflict of Interests

Dr. Hruban has received royalties from Myriad genetics for the PALB2 invention.

References

[1] A. Jemal, R. Siegel, E. Ward, Y. Hao, J. Xu, and M. J. Thun, "Cancer statistics, 2009," *CA: A Cancer Journal for Clinicians*, vol. 59, no. 4, pp. 225–249, 2009.

[2] T. P. Yeo et al., "Pancreatic cancer," *Current Problems in Cancer*, vol. 26, no. 4, pp. 176–275, 2002.

[3] T. S. Riall, J. L. Cameron, K. D. Lillemoe et al., "Pancreaticoduodenectomy with or without distal gastrectomy and extended retroperitoneal lymphadenectomy for periampullary adenocarcinoma—part 3: update on 5-year survival," *Journal of Gastrointestinal Surgery*, vol. 9, no. 9, pp. 1191–1206, 2005.

[4] A. Maitra, S. E. Kern, and R. H. Hruban, "Molecular pathogenesis of pancreatic cancer," *Best Practice and Research: Clinical Gastroenterology*, vol. 20, no. 2, pp. 211–226, 2006.

[5] H. A. Burris III, M. J. Moore, J. Andersen et al., "Improvements in survival and clinical benefit with gemcitabine as first-line therapy for patients with advanced pancreas cancer: a randomized trial," *Journal of Clinical Oncology*, vol. 15, no. 6, pp. 2403–2413, 1997.

[6] H. Oettle, S. Post, P. Neuhaus et al., "Adjuvant chemotherapy with gemcitabine vs observation in patients undergoing curative-intent resection of pancreatic cancer: a randomized

controlled trial," *Journal of the American Medical Association*, vol. 297, no. 3, pp. 267–277, 2007.

[7] D. E. Abbott, M. S. Baker, and M. S. Talamonti, "Neoadjuvant therapy for pancreatic cancer: current review," *Journal of Surgical Oncology*, vol. 101, no. 4, pp. 315–320, 2010.

[8] S. R. Bramhall, J. Schulz, J. Nemunaitis, P. D. Brown, M. Baillet, and J. A. C. Buckels, "A double-blind placebo-controlled, randomised study comparing gemcitabine and marimastat with gemcitabine and placebo as first line therapy in patients with advanced pancreatic cancer," *British Journal of Cancer*, vol. 87, no. 2, pp. 161–167, 2002.

[9] E. Van Cutsem, H. Van De Velde, P. Karasek et al., "Phase III trial of gemcitabine plus tipifarnib compared with gemcitabine plus placebo in advanced pancreatic cancer," *Journal of Clinical Oncology*, vol. 22, no. 8, pp. 1430–1438, 2004.

[10] E. Van Cutsem, W. L. Vervenne, J. Bennouna et al., "Phase III trial of bevacizumab in combination with gemcitabine and erlotinib in patients with metastatic pancreatic cancer," *Journal of Clinical Oncology*, vol. 27, no. 13, pp. 2231–2237, 2009.

[11] M. Singh and A. Maitra, "Precursor lesions of pancreatic cancer: molecular pathology and clinical implications," *Pancreatology*, vol. 7, no. 1, pp. 9–19, 2007.

[12] P. J. Campbell, S. Yachida, L. J. Mudie et al., "The patterns and dynamics of genomic instability in metastatic pancreatic cancer," *Nature*, vol. 467, no. 7319, pp. 1109–1113, 2010.

[13] S. Yachida, S. Jones, I. Bozic et al., "Distant metastasis occurs late during the genetic evolution of pancreatic cancer," *Nature*, vol. 467, no. 7319, pp. 1114–1117, 2010.

[14] R. H. Hruban, K. Takaori, D. S. Klimstra et al., "An illustrated consensus on the classification of pancreatic intraepithelial neoplasia and intraductal papillary mucinous neoplasms," *American Journal of Surgical Pathology*, vol. 28, no. 8, pp. 977–987, 2004.

[15] H. Kargozaran, V. Vu, P. Ray et al., "Invasive IPMN and MCN: same organ, sifferent outcomes?" *Annals of Surgical Oncology*, vol. 18, no. 2, pp. 345–351, 2011.

[16] A. V. Biankin, J. G. Kench, S. A. Biankin et al., "Pancreatic intraepithelial neoplasla in association with intraductal papillary mucinous neoplasms of the pancreas: implications for disease progression and recurrence," *American Journal of Surgical Pathology*, vol. 28, no. 9, pp. 1184–1192, 2004.

[17] R. E. Wilenz, J. Geradts, R. Maynard et al., "Inactivation of the p16 (INK4A) tumor-suppressor gene in pancreatic duct lesions: loss of intranuclear expression," *Cancer Research*, vol. 58, no. 20, pp. 4740–4744, 1998.

[18] J. B. M. Koorstra, S. M. Hong, C. Shi et al., "Widespread activation of the DNA damage response in human pancreatic intraepithelial neoplasia," *Modern Pathology*, vol. 22, no. 11, pp. 1439–1445, 2009.

[19] R. E. Wilentz, C. A. Iacobuzio-Donahue, P. Argani et al., "Loss of expression of Dpc4 in pancreatic intraepithelial neoplasia: evidence that DPC4 inactivation occurs late in neoplastic progression," *Cancer Research*, vol. 60, no. 7, pp. 2002–2006, 2000.

[20] H. Carter, J. Samayoa, R. H. Hruban, and R. Karchin, "Prioritization of driver mutations in pancreatic cancer using cancer-specific high-throughput annotation of somatic mutations (CHASM)," *Cancer Biology and Therapy*, vol. 10, no. 6, pp. 582–587, 2010.

[21] S. Jones, X. Zhang, D. W. Parsons et al., "Core signaling pathways in human pancreatic cancers revealed by global genomic analyses," *Science*, vol. 321, no. 5897, pp. 1801–1806, 2008.

[22] C. Lengauer, K. W. Kinzler, and B. Vogelstein, "Genetic instabilities in human cancers," *Nature*, vol. 396, no. 6712, pp. 643–649, 1998.

[23] N. T. Van Heek, A. K. Meeker, S. E. Kern et al., "Telomere shortening is nearly universal in pancreatic intraepithelial neoplasia," *American Journal of Pathology*, vol. 161, no. 5, pp. 1541–1547, 2002.

[24] S. E. Artandi and R. A. DePinho, "Telomeres and telomerase in cancer," *Carcinogenesis*, vol. 31, no. 1, pp. 9–18, 2010.

[25] D. Gisselsson, T. Jonson, P. D. Cin et al., "Telomere dysfunction triggers extensive DNA fragmentation and evolution of complex chromosome abnormalities in human malignant tumors," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 98, no. 22, pp. 12683–12688, 2001.

[26] S. E. Artandi, S. Chang, S. L. Lee et al., "Telomere dysfunction promotes non-reciprocal translocations and epithelial cancers in mice," *Nature*, vol. 406, no. 6796, pp. 641–645, 2000.

[27] J. A. Hackett and C. W. Greider, "Balancing instability: dual roles for telomerase and telomere dysfunction in tumorigenesis," *Oncogene*, vol. 21, no. 4, pp. 619–626, 2002.

[28] P. Magistrelli, R. Coppola, G. Tonini et al., "Apoptotic index or a combination of Bax/Bcl-2 expression correlate with survival after resection of pancreatic adenocarcinoma," *Journal of Cellular Biochemistry*, vol. 97, no. 1, pp. 98–108, 2006.

[29] N. Virkajärvi, P. Pääkkö, and Y. Soini, "Apoptotic index and apoptosis influencing proteins bcl-2, mcl-1, bax and caspases 3, 6 and 8 in pancreatic carcinoma," *Histopathology*, vol. 33, no. 5, pp. 432–439, 1998.

[30] J. D. Evans, P. A. Cornford, A. Dodson, W. Greenhalf, C. S. Foster, and J. P. Neoptolemos, "Detailed tissue expression of bcl-2, bax, bak and bcl-x in the normal human pancreas and in chronic pancreatitis, ampullary and pancreatic ductal adenocarcinomas," *Pancreatology*, vol. 1, no. 3, pp. 254–262, 2001.

[31] S. Banerjee, M. Choi, A. Aboukameel et al., "Preclinical studies of apogossypolone, a novel pan inhibitor of Bcl-2 and Mcl-1, synergistically potentiates cytotoxic effect of gemcitabine in pancreatic cancer cells," *Pancreas*, vol. 39, no. 3, pp. 323–331, 2010.

[32] C. Y. Wang, M. W. Mayo, R. G. Korneluk, D. V. Goeddel, and A. S. Baldwin, "NF-κB antiapoptosis: induction of TRAF1 and TRAF2 and c-IAP1 and c-IAP2 to suppress caspase-8 activation," *Science*, vol. 281, no. 5383, pp. 1680–1683, 1998.

[33] C. Y. Wang, D. C. Guttridge, M. W. Mayo, and A. S. Baldwin, "NF-κB induces expression of the Bcl-2 homologue A1/Bfl-1 to preferentially suppress chemotherapy-induced apoptosis," *Molecular and Cellular Biology*, vol. 19, no. 9, pp. 5923–5929, 1999.

[34] S. Kreuz, D. Siegmund, P. Scheurich, and H. Wajant, "NF-κB inducers upregulate cFLIP, a cycloheximide-sensitive inhibitor of death receptor signaling," *Molecular and Cellular Biology*, vol. 21, no. 12, pp. 3964–3973, 2001.

[35] B. Holcomb, M. Yip-Schneider, and C. M. Schmidt, "The role of nuclear factor κB in pancreatic cancer and the clinical applications of targeted therapy," *Pancreas*, vol. 36, no. 3, pp. 225–235, 2008.

[36] J. Q. Cheng, B. Ruggeri, W. M. Klein et al., "Amplification of AKT2 in human pancreatic cancer cells and inhibition of AKT2 expression and tumorigenicity by antisense RNA,"

Proceedings of the National Academy of Sciences of the United States of America, vol. 93, no. 8, pp. 3636–3641, 1996.

[37] B. A. Ruggeri, L. Huang, M. Wood, J. Q. Cheng, and J. R. Testa, "Amplification and overexpression of the AKT2 oncogene in a subset of human pancreatic ductal adenocarcinomas," *Molecular Carcinogenesis*, vol. 21, no. 2, pp. 81–86, 1998.

[38] M. G. Schlieman, B. N. Fahy, R. Ramsamooj, L. Beckett, and R. J. Bold, "Incidence, mechanism and prognostic value of activated AKT in pancreas cancer," *British Journal of Cancer*, vol. 89, no. 11, pp. 2110–2115, 2003.

[39] A. M. Bellizzi, M. Bloomston, X. P. Zhou, O. H. Iwenofu, and W. L. Frankel, "The mTOR pathway is frequently activated in pancreatic ductal adenocarcinoma and chronic pancreatitis," *Applied Immunohistochemistry and Molecular Morphology*, vol. 18, no. 5, pp. 442–447, 2010.

[40] A. Arlt, A. Gehrz, S. Müerköster et al., "Role of NF-κB and Akt/PI3K in the resistance of pancreatic carcinoma cell lines against gemcitabine-induced cell death," *Oncogene*, vol. 22, no. 21, pp. 3243–3251, 2003.

[41] S. K. Roy, R. K. Srivastava, and S. Shankar, "Inhibition of PI3K/AKT and MAPK/ERK pathways causes activation of FOXO transcription factor, leading to cell cycle arrest and apoptosis in pancreatic cancer," *Journal of Molecular Signaling*, vol. 5, article 10, 2010.

[42] M. S. Redston, C. Caldas, A. B. Seymour et al., "p53 mutations in pancreatic carcinoma and evidence of common involvement of homocopolymer tracts in DNA microdeletions," *Cancer Research*, vol. 54, no. 11, pp. 3025–3033, 1994.

[43] C. M. Barton, S. L. Staddon, C. M. Hughes et al., "Abnormalities of the p53 tumour suppressor gene in human pancreatic cancer," *British Journal of Cancer*, vol. 64, no. 6, pp. 1076–1082, 1991.

[44] M. Schutte, L. T. Da Costa, S. A. Hahn et al., "Identification by representational difference analysis of a homozygous deletion in pancreatic carcinoma that lies within the BRCA2 region," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 92, no. 13, pp. 5950–5954, 1995.

[45] M. S. Van Der Heijden, J. R. Brody, E. Gallmeier et al., "Functional defects in the Fanconi anemia pathway in pancreatic cancer cells," *American Journal of Pathology*, vol. 165, no. 2, pp. 651–657, 2004.

[46] M. Goggins, M. Sehutte, J. Lu et al., "Germline BRCA2 gene mutations in patients with apparently sporadic pancreatic carcinomas," *Cancer Research*, vol. 56, no. 23, pp. 5360–5364, 1996.

[47] B. Nakata, Y. Q. Wang, M. Yashiro et al., "Prognostic value of microsatellite instability in resectable pancreatic cancer," *Clinical Cancer Research*, vol. 8, no. 8, pp. 2536–2540, 2002.

[48] M. Goggins, G. J. A. Offerhaus, W. Hilgers et al., "Pancreatic adenocarcinomas with DNA replication errors (RER+) are associated with wild-type K-ras and characteristic histopathology. Poor differentiation, a syncytial growth pattern, and pushing borders suggest RER+," *American Journal of Pathology*, vol. 152, no. 6, pp. 1501–1507, 1998.

[49] H. Yamamoto, F. Itoh, H. Nakamura et al., "Genetic and clinical features of human pancreatic ductal adenocarcinomas with widespread microsatellite instability," *Cancer Research*, vol. 61, no. 7, pp. 3139–3144, 2001.

[50] M. Schutte, R. H. Hruban, J. Geradts et al., "Abrogation of the Rb/p16 tumor-suppressive pathway in virtually all pancreatic carcinomas," *Cancer Research*, vol. 57, no. 15, pp. 3126–3130, 1997.

[51] R. E. Wilentz, J. Geradts, R. Maynard et al., "Inactivation of the p16 (INK4A) tumor-suppressor gene in pancreatic duct lesions: loss of intranuclear expression," *Cancer Research*, vol. 58, no. 20, pp. 4740–4744, 1998.

[52] C. B. Begg, I. Orlow, A. J. Hummer et al., "Lifetime risk of melanoma in CDKN2A mutation carriers in a population-based sample," *Journal of the National Cancer Institute*, vol. 97, no. 20, pp. 1507–1515, 2005.

[53] C. Caldas, S. A. Hahn, L. T. Da Costa et al., "Frequent somatic mutations and homozygous deletions of the p16 (MTS1) gene in pancreatic adenocarcinoma," *Nature Genetics*, vol. 8, no. 1, pp. 27–32, 1994.

[54] M. Serrano, G. J. Hannon, and D. Beach, "A new regulatory motif in cell-cycle control causing specific inhibition of cyclin D/CDK4," *Nature*, vol. 366, no. 6456, pp. 704–707, 1993.

[55] A. Maitra and R. H. Hruban, "Pancreatic cancer," *Annual Review of Pathology*, vol. 3, pp. 157–188, 2008.

[56] E. S. Calhoun, J. B. Jones, R. Ashfaq et al., "BRAF and FBXW7 (CDC4, FBW7, AGO, SEL10) mutations in distinct subsets of pancreatic cancer: potential therapeutic targets," *American Journal of Pathology*, vol. 163, no. 4, pp. 1255–1260, 2003.

[57] Y. E. Joo, J. S. Rew, C. S. Park, and S. J. Kim, "Expression of E-cadherin, alpha-and beta-catenins in patients with pancreatic adenocarcinoma," *Pancreatology*, vol. 2, no. 2, pp. 129–137, 2002.

[58] Y. J. Li and X. R. Ji, "Relationship between expression of E-cadherin-catenin complex and clinicopathologic characteristics of pancreatic cancer," *World Journal of Gastroenterology*, vol. 9, no. 2, pp. 368–372, 2003.

[59] J. M. Winter, A. H. Ting, F. Vilardell et al., "Absence of E-cadherin expression distinguishes noncohesive from cohesive pancreatic cancer," *Clinical Cancer Research*, vol. 14, no. 2, pp. 412–418, 2008.

[60] F. G. Giancotti and E. Ruoslahti, "Integrin signaling," *Science*, vol. 285, no. 5430, pp. 1028–1032, 1999.

[61] H. Sawai, H. Takeyama, M. Yamamoto et al., "Enhancement of integrins by interleukin-1α, and their relationship with metastatic and invasive behavior of human pancreatic ductal adenocarcinoma cells," *Journal of Surgical Oncology*, vol. 82, no. 1, pp. 51–56, 2003.

[62] W. Niu, X. Liu, Z. Zhang et al., "Effects of $\alpha v\beta 6$ gene silencing by RNA interference in PANC-1 pancreatic carcinoma cells," *Anticancer Research*, vol. 30, no. 1, pp. 135–142, 2010.

[63] D. Yamada, K. Ohuchida, K. Mizumoto et al., "Increased expression of ADAM 9 and ADAM 15 mRNA in pancreatic cancer," *Anticancer Research*, vol. 27, no. 2, pp. 793–799, 2007.

[64] R. Grützmann, J. Lüttges, B. Sipos et al., "ADAM9 expression in pancreatic cancer is associated with tumour type and is a prognostic factor in ductal adenocarcinoma," *British Journal of Cancer*, vol. 90, no. 5, pp. 1053–1058, 2004.

[65] R. H. Hruban, A. D. M. Van Mansfeld, G. J. A. Offerhaus et al., "K-ras oncogene activation in adenocarcinoma of the human pancreas: a study of 82 carcinomas using a combination of mutant-enriched polymerase chain reaction analysis and allele-specific oligonucleotide hybridization," *American Journal of Pathology*, vol. 143, no. 2, pp. 545–554, 1993.

[66] C. Almoguera, D. Shibata, K. Forrester, J. Martin, N. Arnheim, and M. Perucho, "Most human carcinomas of the exocrine pancreas contain mutant c-K-ras genes," *Cell*, vol. 53, no. 4, pp. 549–554, 1988.

[67] A. J. Aguirre, N. Bardeesy, M. Sinha et al., "Activated Kras and Ink4a/Arf deficiency cooperate to produce metastatic

pancreatic ductal adenocarcinoma," *Genes and Development*, vol. 17, no. 24, pp. 3112–3126, 2003.

[68] S. R. Hingorani, E. F. Petricoin, A. Maitra et al., "Preinvasive and invasive ductal pancreatic cancer and its early detection in the mouse," *Cancer Cell*, vol. 4, no. 6, pp. 437–450, 2003.

[69] R. J. Davis, "Signal transduction by the JNK group of MAP kinases," *Cell*, vol. 103, no. 2, pp. 239–252, 2000.

[70] W. Xin, K. J. Yun, F. Ricci et al., "MAP2K4/MKK4 expression in pancreatic cancer: genetic validation of immunohistochemistry and relationship to disease course," *Clinical Cancer Research*, vol. 10, no. 24, pp. 8516–8520, 2004.

[71] D. H. F. Teng, W. L. Perry, J. K. Hogan et al., "Human mitogen-activated protein kinase kinase 4 as a candidate tumor suppressor," *Cancer Research*, vol. 57, no. 19, pp. 4177–4182, 1997.

[72] Y. Matsuo, P. M. Campbell, R. A. Brekken et al., "K-Ras promotes angiogenesis mediated by immortalized human pancreatic epithelial cells through mitogen-activated protein kinase signaling pathways," *Molecular Cancer Research*, vol. 7, no. 6, pp. 799–808, 2009.

[73] J. Massagué, "TGF-β signal transduction," *Annual Review of Biochemistry*, vol. 67, pp. 753–791, 1998.

[74] K. Miyazono, P. Ten Dijke, and C. H. Heldin, "TGF-β signaling by Smad proteins," *Advances in Immunology*, vol. 75, pp. 115–157, 2000.

[75] B. Peng, J. B. Fleming, T. Breslin et al., "Suppression of tumorigenesis and induction of p15 by Smad4/DPC4 in human pancreatic cancer cells," *Clinical Cancer Research*, vol. 8, no. 11, pp. 3628–3638, 2002.

[76] S. A. Hahn, M. Schutte, A. T. M. Shamsul Hoque et al., "DPC4, a candidate tumor suppressor gene at human chromosome 18q21.1," *Science*, vol. 271, no. 5247, pp. 350–353, 1996.

[77] A. Blackford, O. K. Serrano, C. L. Wolfgang et al., "SMAD4 gene mutations are associated with poor prognosis in pancreatic cancer," *Clinical Cancer Research*, vol. 15, no. 14, pp. 4674–4679, 2009.

[78] M. Tascilar, H. G. Skinner, C. Rosty et al., "The SMAD4 protein and prognosis of pancreatic ductal adenocarcinoma," *Clinical Cancer Research*, vol. 7, no. 12, pp. 4115–4121, 2001.

[79] C. A. Lacobuzio-Donahue, B. Fu, S. Yachida et al., "DPC4 gene status of the primary carcinoma correlates with patterns of failure in patients with pancreatic cancer," *Journal of Clinical Oncology*, vol. 27, no. 11, pp. 1806–1813, 2009.

[80] M. Goggins, M. Shekher, K. Turnacioglu, C. J. Yeo, R. H. Hruban, and S. E. Kern, "Genetic alterations of the transforming growth factor β receptor genes in pancreatic and biliary adenocarcinomas," *Cancer Research*, vol. 58, no. 23, pp. 5329–5332, 1998.

[81] L. Yu, M. C. Hébert, and Y. E. Zhang, "TGF-β receptor-activated p38 MAP kinase mediates smad-independent TGF-β responses," *EMBO Journal*, vol. 21, no. 14, pp. 3749–3759, 2002.

[82] E. Janda, K. Lehmann, I. Killisch et al., "Ras and TGFβ cooperatively regulate epithelial cell plasticity and metastasis: dissection of Ras signaling pathways," *Journal of Cell Biology*, vol. 156, no. 2, pp. 299–313, 2002.

[83] J. D. Brown, M. R. DiChiara, K. R. Anderson, M. A. Gimbrone, and J. N. Topper, "MEKK-1, a component of the stress (stress-activated protein kinase/c-jun N-terminal kinase) pathway, can selectively activate Smad2-mediated transcriptional activation in endothelial cells," *Journal of Biological Chemistry*, vol. 274, no. 13, pp. 8797–8805, 1999.

[84] A. Apelqvist, H. Li, L. Sommer et al., "Notch signalling controls pancreatic cell differentiation," *Nature*, vol. 400, no. 6747, pp. 877–881, 1999.

[85] J. T. Siveke, C. Lubeseder-Martellato, M. Lee et al., "Notch signaling is required for exocrine regeneration after acute pancreatitis," *Gastroenterology*, vol. 134, no. 2, pp. 544–555, 2008.

[86] J. Jensen, R. S. Heller, T. Funder-Nielsen et al., "Independent development of pancreatic and β-cells from Neurogenin3-expressing precursors: a role for the notch pathway in repression of premature differentiation," *Diabetes*, vol. 49, no. 2, pp. 163–176, 2000.

[87] Y. Miyamoto, A. Maitra, B. Ghosh et al., "Notch mediates TGFα-induced changes in epithelial differentiation during pancreatic tumorigenesis," *Cancer Cell*, vol. 3, no. 6, pp. 565–576, 2003.

[88] Z. Wang, S. Banerjee, Y. Li, K. M. W. Rahman, Y. Zhang, and F. H. Sarkar, "Down-regulation of Notch-1 inhibits invasion by inactivation of nuclear factor-κB, vascular endothelial growth factor, and matrix metalloproteinase-9 in pancreatic cancer cells," *Cancer Research*, vol. 66, no. 5, pp. 2778–2784, 2006.

[89] Z. Wang, Y. Zhang, Y. Li, S. Banerjee, J. Liao, and F. H. Sarkar, "Down-regulation of Notch-1 contributes to cell growth inhibition and apoptosis in pancreatic cancer cells," *Molecular Cancer Therapeutics*, vol. 5, no. 3, pp. 483–493, 2006.

[90] M. E. Mullendore, J. B. Koorstra, Y. M. Li et al., "Ligand-dependent notch signaling is involved in tumor initiation and tumor maintenance in pancreatic cancer," *Clinical Cancer Research*, vol. 15, no. 7, pp. 2291–2301, 2009.

[91] M. Hebrok, S. K. Kim, and D. A. Melton, "Notochord repression of endodermal sonic hedgehog permits pancreas development," *Genes and Development*, vol. 12, no. 11, pp. 1705–1713, 1998.

[92] A. Apelqvist, U. Ahlgren, and H. Edlund, "Sonic hedgehog directs specialised mesoderm differentiation in the intestine and pancreas," *Current Biology*, vol. 7, no. 10, pp. 801–804, 1997.

[93] D. M. Berman, S. S. Karhadkar, A. Maitra et al., "Widespread requirement for Hedgehog ligand stimulation in growth of digestive tract tumours," *Nature*, vol. 425, no. 6960, pp. 846–851, 2003.

[94] L. L. Rubin and F. J. de Sauvage, "Targeting the Hedgehog pathway in cancer," *Nature Reviews Drug Discovery*, vol. 5, no. 12, pp. 1026–1033, 2006.

[95] M. Hebrok, S. K. Kim, B. St-Jacques, A. P. McMahon, and D. A. Melton, "Regulation of pancreas development by hedgehog signaling," *Development*, vol. 127, no. 22, pp. 4905–4913, 2000.

[96] G. Feldmann, N. Habbe, S. Dhara et al., "Hedgehog inhibition prolongs survival in a genetically engineered mouse model of pancreatic cancer," *Gut*, vol. 57, no. 10, pp. 1420–1430, 2008.

[97] J. M. Bailey, B. J. Swanson, T. Hamada et al., "Sonic hedgehog promotes desmoplasia in pancreatic cancer," *Clinical Cancer Research*, vol. 14, no. 19, pp. 5995–6004, 2008.

[98] R. L. Yauch, S. E. Gould, S. J. Scales et al., "A paracrine requirement for hedgehog signalling in cancer," *Nature*, vol. 455, no. 7211, pp. 406–410, 2008.

[99] K. P. Olive, M. A. Jacobetz, C. J. Davidson et al., "Inhibition of Hedgehog signaling enhances delivery of chemotherapy in a mouse model of pancreatic cancer," *Science*, vol. 324, no. 5933, pp. 1457–1461, 2009.

[100] A. M. Lowy, C. Fenoglio-Preiser, O. J. Kim et al., "Dysregulation of β-catenin expression correlates with tumor differentiation in pancreatic duct adenocarcinoma," *Annals of Surgical Oncology*, vol. 10, no. 3, pp. 284–290, 2003.

[101] G. Zeng, M. Germinaro, A. Micsenyi et al., "Aberrant Wnt/β-catenin signaling in pancreatic adenocarcinoma," *Neoplasia*, vol. 8, no. 4, pp. 279–289, 2006.

[102] I. Watanabe, T. Hasebe, S. Sasaki et al., "Advanced pancreatic ductal cancer: fibrotic focus and β-catenin expression correlate with outcome," *Pancreas*, vol. 26, no. 4, pp. 326–333, 2003.

[103] S. Gansauge, F. Gansauge, M. Ramadani et al., "Overexpression of cyclin D1 in human pancreatic carcinoma is associated with poor prognosis," *Cancer Research*, vol. 57, no. 9, pp. 1634–1637, 1997.

[104] H. C. Crawford, C. R. Scoggins, M. Kay Washington, L. M. Matrisian, and S. D. Leach, "Matrix metalloproteinase-7 is expressed by pancreatic cancer precursors and regulates acinar-to-ductal metaplasia in exocrine pancreas," *Journal of Clinical Investigation*, vol. 109, no. 11, pp. 1437–1444, 2002.

[105] C. Schleger, C. Verbeke, R. Hildenbrand, H. Zentgraf, and U. Bleyl, "c-MYC activation in primary and metastatic ductal adenocarcinoma of the pancreas: incidence, mechanisms, and clinical significance," *Modern Pathology*, vol. 15, no. 4, pp. 462–469, 2002.

[106] M. Katoh and M. Katoh, "Transcriptional mechanisms of WNT5A based on NF-κB, Hedgehog, TGFβ, and Notch signaling cascades," *International Journal of Molecular Medicine*, vol. 23, no. 6, pp. 763–769, 2009.

[107] M. Katoh and M. Katoh, "Transcriptional regulation of WNT2B based on the balance of Hedgehog, Notch, BMP and WNT signals," *International Journal of Oncology*, vol. 34, no. 5, pp. 1411–1415, 2009.

[108] D. Romero, M. Iglesias, C. P. H. Vary, and M. Quintanilla, "Functional blockade of Smad4 leads to a decrease in β-catenin levels and signaling activity in human pancreatic carcinoma cells," *Carcinogenesis*, vol. 29, no. 5, pp. 1070–1076, 2008.

[109] B. H. Shirts, R. W. Burt, S. J. Mulvihill, and L. A. Cannon-Albright, "A population-based description of familial clustering of pancreatic cancer," *Clinical Gastroenterology and Hepatology*, vol. 8, no. 9, pp. 812–816, 2010.

[110] D. K. Bartsch, M. Sina-Frey, S. Lang et al., "CDKN2A germline mutations in familial pancreatic cancer," *Annals of Surgery*, vol. 236, no. 6, pp. 730–737, 2002.

[111] W. H. De Vos tot Nederveen Cappel, G. J. A. Offerhaus, M. Van Puijenbroek et al., "Pancreatic carcinoma in carriers of a specific 19 base pair deletion of CDKN2A/p16 (p16-Leiden)," *Clinical Cancer Research*, vol. 9, no. 10, part 1, pp. 3598–3605, 2003.

[112] F. M. Giardiello, J. D. Brensinger, A. C. Tersmette et al., "Very high risk of cancer in familial Peutz-Jeghers syndrome," *Gastroenterology*, vol. 119, no. 6, pp. 1447–1453, 2000.

[113] C. D. Rogers, M. S. Van Der Heijden, K. Brune et al., "The genetics of FANCC and FANCG in familial pancreatic cancer," *Cancer Biology and Therapy*, vol. 3, no. 2, pp. 167–169, 2004.

[114] F. M. Giardiello, S. B. Welsh, S. R. Hamilton et al., "Increased risk of cancer in the Peutz-Jeghers syndrome," *New England Journal of Medicine*, vol. 316, no. 24, pp. 1511–1514, 1987.

[115] N. Howes, M. M. Lerch, W. Greenhalf et al., "Clinical and genetic characteristics of hereditary pancreatitis in Europe," *Clinical Gastroenterology and Hepatology*, vol. 2, no. 3, pp. 252–261, 2004.

[116] J. Threadgold, W. Greenhalf, I. Ellis et al., "The N34S mutation of SPINK1 (PSTI) is associated with a familial pattern of idiopathic chronic pancreatitis but does not cause the disease," *Gut*, vol. 50, no. 5, pp. 675–681, 2002.

[117] H. Ozçelik, B. Schmocker, N. Di Nicola et al., "Germline BRCA2 6174delT mutations in Ashkenazi Jewish pancreatic cancer patients," *Nature Genetics*, vol. 16, no. 1, pp. 17–18, 1997.

[118] E. P. Slater, P. Langer, E. Niemczyk et al., "PALB2 mutations in European familial pancreatic cancer families," *Clinical Genetics*, vol. 78, no. 5, pp. 490–494, 2010.

[119] S. Jones, R. H. Hruban, M. Kamiyama et al., "Exomic sequencing identifies PALB2 as a pancreatic cancer susceptibility gene," *Science*, vol. 324, no. 5924, p. 217, 2009.

[120] F. Kastrinos, B. Mukherjee, N. Tayob et al., "Risk of pancreatic cancer in families with Lynch syndrome," *Journal of the American Medical Association*, vol. 302, no. 16, pp. 1790–1795, 2009.

[121] H. L. Kindler, D. Niedzwiecki, D. Hollis et al., "Gemcitabine plus bevacizumab compared with gemcitabine plus placebo in patients with advanced pancreatic cancer: phase III trial of the Cancer and Leukemia Group B (CALGB 80303)," *Journal of Clinical Oncology*, vol. 28, no. 22, pp. 3617–3622, 2010.

[122] F. Kullmann, S. Hollerbach, M. M. Dollinger et al., "Cetuximab plus gemcitabine/oxaliplatin (GEMOXCET) in first-line metastatic pancreatic cancer: a multicentre phase II study," *British Journal of Cancer*, vol. 100, no. 7, pp. 1032–1036, 2009.

[123] M. J. Moore, D. Goldstein, J. Hamm et al., "Erlotinib plus gemcitabine compared with gemcitabine alone in patients with advanced pancreatic cancer: a phase III trial of the National Cancer Institute of Canada Clinical Trials Group," *Journal of Clinical Oncology*, vol. 25, no. 15, pp. 1960–1966, 2007.

[124] J. Feliu, P. Borrega, A. León et al., "Phase II study of a fixed dose-rate infusion of gemcitabine associated with erlotinib in advanced pancreatic cancer," *Cancer Chemotherapy and Pharmacology*, vol. 67, no. 1, pp. 215–221, 2011.

[125] C. C. Hsu, J. M. Herman, M. M. Corsini et al., "Adjuvant chemoradiation for pancreatic adenocarcinoma: the Johns Hopkins Hospital-Mayo Clinic collaborative study," *Annals of Surgical Oncology*, vol. 17, no. 4, pp. 981–990, 2010.

[126] M. H. Kalser and S. S. Ellenberg, "Pancreatic cancer. Adjuvant combined radiation and chemotherapy following curative resection," *Archives of Surgery*, vol. 120, no. 8, pp. 899–903, 1985.

[127] J. H. Klinkenbijl, J. Jeekel, T. Sahmoud et al., "Adjuvant radiotherapy and 5-fluorouracil after curative resection of cancer of the pancreas and periampullary region: phase III trial of the EORTC gastrointestinal tract cancer cooperative group," *Annals of Surgery*, vol. 230, no. 6, pp. 776–784, 1999.

[128] K. M. Brown, V. Siripurapu, M. Davidson et al., "Chemoradiation followed by chemotherapy before resection for borderline pancreatic adenocarcinoma," *American Journal of Surgery*, vol. 195, no. 3, pp. 318–321, 2008.

[129] T. B. Brunner, G. G. Grabenbauer, S. Kastl et al., "Preoperative chemoradiation in locally advanced pancreatic carcinoma: a phase II study," *Onkologie*, vol. 23, no. 5, pp. 436–442, 2000.

[130] J. M. Jessup, G. Steele, R. J. Mayer et al., "Neoadjuvant therapy for unresectable pancreatic adenocarcinoma," *Archives of Surgery*, vol. 128, no. 5, pp. 559–564, 1993.

[131] M. V. Pilepich and H. H. Miller, "Preoperative irradiation in carcinoma of the pancreas," *Cancer*, vol. 46, no. 9, pp. 1945–1949, 1980.

[132] S. L. Showalter, S. Charles, J. Belin et al., "Identifying pancreatic cancer patients for targeted treatment: the challenges and limitations of the current selection process and vision for the future," *Expert Opinion on Drug Delivery*, vol. 7, no. 3, pp. 273–284, 2010.

[133] M. S. Van Der Heijden, J. R. Brody, D. A. Dezentje et al., "In vivo therapeutic responses contingent on fanconi anemia/BRCA2 status of the tumor," *Clinical Cancer Research*, vol. 11, no. 20, pp. 7508–7515, 2005.

[134] H. J. Klümpen, K. C. Queiroz, C. A. Spek et al., "mTOR inhibitor treatment of pancreatic cancer in a patient with Peutz-Jeghers syndrome," *Journal of Clinical Oncology*, vol. 29, no. 6, pp. 150–153, 2011.

[135] E. D. Saad and P. M. Hoff, "Molecular-targeted agents in pancreatic cancer," *Cancer Control*, vol. 11, no. 1, pp. 32–38, 2004.

[136] M. L. Scotti, W. R. Bamlet, T. C. Smyrk, A. P. Fields, and N. R. Murray, "Protein kinase Ciota is required for pancreatic cancer cell transformed growth and tumorigenesis," *Cancer Research*, vol. 70, no. 5, pp. 2064–2074, 2010.

[137] H. Harasawa, Y. Yamada, M. Kudoh et al., "Chemotherapy targeting methylthioadenosine phosphorylase (MTAP) deficiency in adult T cell leukemia (ATL)," *Leukemia*, vol. 16, no. 9, pp. 1799–1807, 2002.

[138] S. R. Hustinx, R. H. Hruban, L. M. Leoni et al., "Homozygous deletion of the MTAP gene in invasive adenocarcinoma of the pancreas and in periampullary cancer: a potential new target for therapy," *Cancer Biology and Therapy*, vol. 4, no. 1, pp. 83–86, 2005.

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Review Article

Impact of KRAS Mutations on Management of Colorectal Carcinoma

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The epidermal growth factor receptor (EGFR) pathway is a therapeutic target in the management of colorectal cancer (CRC). EGFR antagonists are active in this disease; however, only a subset of patients respond to such therapy. A Kirsten ras sarcoma viral oncogene (KRAS) wild-type (WT) status of the tumor is necessary, but possibly not sufficient, for a response to anti-EGFR monoclonal antibody therapy. Mechanisms of primary resistance to such therapy in patients harboring KRAS WT tumors are discussed. Strategies to overcome resistance to anti-EGFR monoclonal antibody therapy, including novel agents and combinations of novel therapies, are explored. Also, the use of anti-EGFR monoclonal antibodies in the adjuvant and neoadjuvant setting is reviewed.

1. Introduction

Tumor growth and progression depends in part on the activity of cell surface membrane receptors which control signal transduction pathways. These growth factor receptors can have aberrations in their expression and regulation and activation of growth factor pathways is common in many malignancies [1]. The EGFR, a transmembrane glycoprotein also called ERBB-1 or HER1, is a member of a family of receptor tyrosine kinases (TKs). The EGFR is involved in signaling pathways controlling cell growth, differentiation, and proliferation and is expressed in many different types of normal tissues as well as several tumor types, including CRC [2, 3]. Figure 1 illustrates the main EGFR signaling pathways described [4]. When a ligand binds to the EGFR, the receptor forms a dimer resulting in a signaling cascade within the cell via tyrosine kinase activity [5]. This signaling cascade occurs by the activation of receptor autophosphorylation which triggers a number of intracellular pathways regulating cell proliferation, prevention of apoptosis, and promotion of invasion, metastasis, and neovascularization [6]. The proto-oncogene c-erb-B encodes the EGFR, and activation of the proto-oncogene results in EGFR expression in many tumors [7, 8]. There was therefore interest in investigating this pathway as a potential anticancer therapy target.

Pharmacologically, there are two classes of EGFR antagonists currently in clinical use: antiEGFR monoclonal antibodies directed against the extracellular domain of the receptor and oral small-molecule EGFR TK inhibitors which block the receptor TK activity competitively [10]. The antiEGFR monoclonal antibodies, cetuximab and panitumumab, act by binding to the extracellular region of the EGFR and therefore block the ligand-binding region which prevents ligand-induced TK activation [11]. These monoclonal antibodies solely recognize the EGFR, making them very selective for their target [5]. The small-molecule EGFR TK inhibitors, erlotinib and gefitinib, inhibit the catalytic activity of the TK by competing with adenosine triphosphate (ATP) to bind to the intracellular domain [10]. These small-molecule inhibitors are not exclusive to the EGFR pathway and can block different receptor tyrosine kinases, such as the vascular endothelial growth factor (VEGF) receptor and other members of the EGFR family.

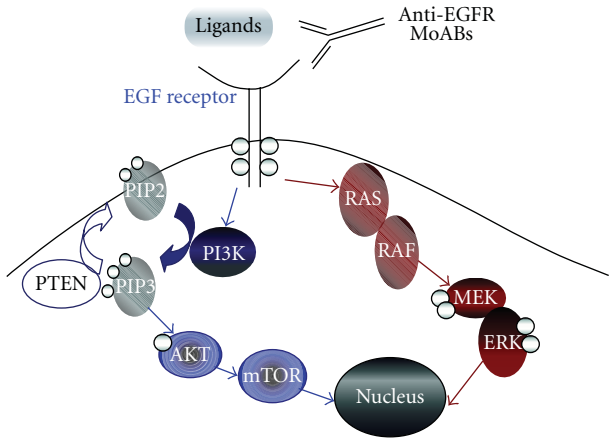

Figure 1: EGFR signaling pathway [4]. (Reprinted with permission from American Society of Clinical Oncology 2008. All rights reserved.)

Anti-EGFR monoclonal antibodies have been evaluated in both untreated metastatic CRC and chemotherapy refractory disease. Figure 2 summarizes the current treatment paradigm for metastatic colorectal cancer including the appropriate incorporation of antiEGFR monoclonal antibody therapy which improves survival for appropriately selected patients [9]. Table 1 summarizes selected clinical trials of antiEGFR monoclonal antibodies in metastatic CRC. Response rates with single-agent antiEGFR monoclonal antibodies range from 9–12%, with much higher response rates observed when cetuximab is used in combination with chemotherapy [12–22]. When administered to unselected metastatic CRC patients, only a minority responded to EGFR inhibitor therapy. Therefore, a method to identify and predict sensitivity to these drugs was needed.

2. Prediction of Response to Anti-EGFR Monoclonal Antibodies

The RAS family of proto-oncogenes include HRAS, KRAS, and NRAS [23]. KRAS (Kirsten ras sarcoma viral oncogene) is a guanosine triphosphate-(GTP-) binding protein downstream of the EGFR and is a central component of the mitogen-activated protein kinase (MAPK) pathway, a component of the EGFR signaling cascade [23]. Roughly 40% of colorectal cancers are characterized by a mutation in the KRAS gene [24]. About 90% of these mutations occur in codons 12 and 13 in exon 2 of the KRAS gene, with the remaining mutations occurring in codons 61 and 146 (roughly 5% each) [25, 26]. Such KRAS mutations lead to EGFR-independent constitutive activation of the signaling pathway and predict for a lack of response and benefit from antiEGFR monoclonal antibodies cetuximab and panitumumab [27–35]. De Roock et al. showed that codon 61 mutations predicted for lack of response to cetuximab similar to codon 12 and 13 mutations; however, codon 146 mutations did not affect cetuximab efficacy [26]. Failure to test for codon 61 mutations may miss a significant mutation which would confer resistance to antiEGFR monoclonal antibody therapy. There is a very high concordance of KRAS mutational status between the primary tumor and metastasis, ranging from 92–100% [36–38]. However, KRAS mutation status heterogeneity between primary tumors, lymph nodes and distant metastases in 5–10% of patients has been reported, with mixed responses to antiEGFR monoclonal antibody therapy in those with metastatic CRC [37, 39, 40]. Because of this, some clinicians have called for a reassessment of KRAS mutation status on metastatic foci in situations where only the primary tumor was assessed for KRAS status [41].

Table 2 summarizes clinical trials of antiEGFR monoclonal antibodies which included analysis of treatment effect and KRAS mutation status. Amado et al. assessed the predictive role of KRAS mutational status in a randomized phase III trial comparing panitumumab monotherapy with best supportive care (BSC) in patients with chemotherapy refractory metastatic CRC [24]. This trial showed that the clinical benefit associated with panitumumab was restricted to the KRAS WT population. KRAS mutations predicted for lack of clinical benefit to panitumumab [24]. Similarly, Karapetis et al. showed that treatment with cetuximab significantly improved OS and PFS in patients with KRAS WT tumors; however, in this chemotherapy-resistant patient population, those with mutated KRAS tumors did not benefit [38]. Use of cetuximab as first-line treatment for metastatic disease was investigated by Van Cutsem et al.; patients were randomly assigned to receive FOLFIRI with or without cetuximab [36]. A statistically significant benefit in PFS for patients with KRAS WT tumors receiving cetuximab and chemotherapy was confirmed in a final presentation of this trial [42]. Bokemeyer et al. investigated the use of cetuximab in combination with FOLFOX chemotherapy as initial treatment for metastatic disease [34]. A retrospective analysis of this data revealed that cetuximab and chemotherapy had a statistically significant increased response rate and lower risk of disease progression compared with chemotherapy alone in patients with KRAS WT tumors [43]. Prospectively, panitumumab has been investigated with either FOLFOX or FOLFIRI chemotherapy in the first-line metastatic setting [44, 45]. The addition of panitumumab to FOLFOX chemotherapy was associated with a statistically significant improvement in PFS [44]. Taken together, the data in Table 2 supports that in metastatic CRC, KRAS WT and mutation status predict for potential sensitivity to, and definite resistance to, respectively, both antiEGFR monoclonal antibodies, regardless of prior treatment and irrespective of use as monotherapy or in combination. Notably, while KRAS status is an established predictor of response to antiEGFR monoclonal antibody therapy, it has been disproven as a prognostic marker. In contrast to KRAS mutational status, evaluation of EGFR expression of CRC cells has failed to demonstrate predictive value for antiEGFR monoclonal antibody therapy. Cunningham et al. reported that the intensity of EGFR staining by immunohistochemical analysis did not correlate with response rate to cetuximab [13]. Similar data has also been reported with panitumumab [46]. KRAS mutated CRC absent of antiEGFR monoclonal

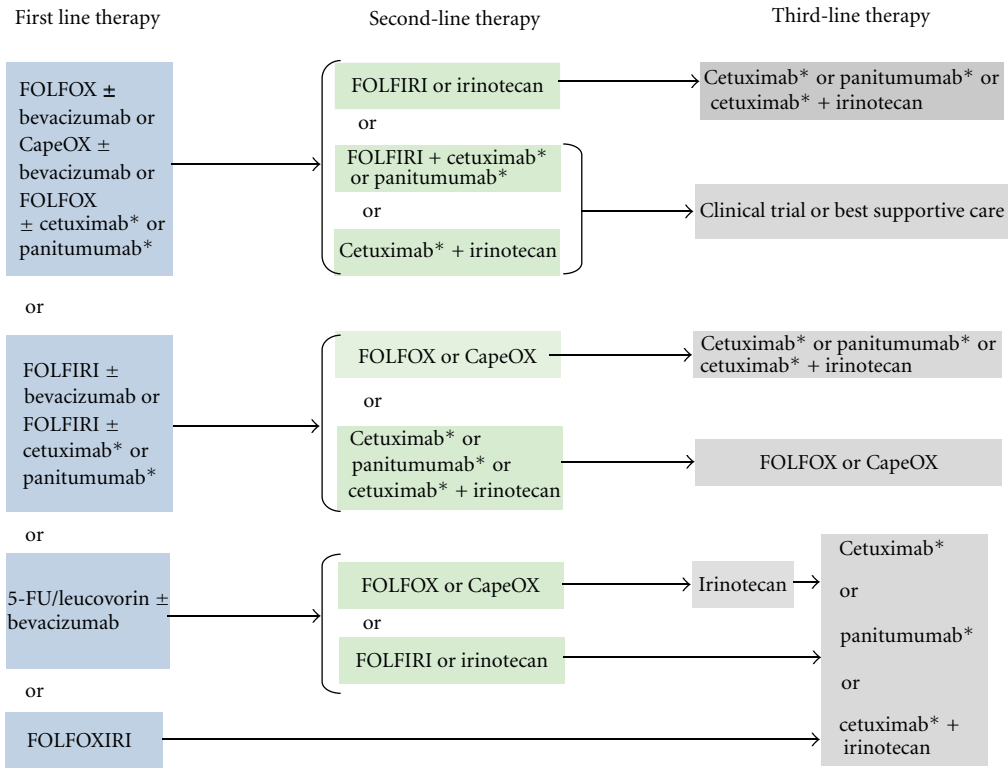

Figure 2: The current treatment paradigm for patients with metastatic colorectal cancer who are appropriate for intensive therapy [9]. *For patients with KRAS WT gene only. CapeOX: capecitabine + oxaliplatin.

antibody therapy is not inferior to patients with KRAS WT disease. The evaluation of KRAS mutational status is a mandatory aspect of management of patients at the time of diagnosis of metastatic CRC.

3. Mechanisms of Resistance

While KRAS mutations are a major mechanism of primary resistance to antiEGFR monoclonal antibody therapies, resistance mechanisms in KRAS WT patients are also being defined. Forty-sixty percent of patients with KRAS WT tumors fail to respond to treatment with antiEGFR monoclonal antibodies [51]. Therefore, other possible molecular determinants of response are being identified in those patients with EGFR monoclonal antibody-resistant WT KRAS disease.

The importance and frequency of NRAS (a ras oncogene variant) mutations in CRC remains under-investigated [52, 53]. Lambrechts et al. found that NRAS, KRAS, and BRAF mutations were all mutually exclusive events, with combined WT status of these genes associated with higher response rates and longer progression-free survival times [54]. Lambrechts et al. also reported that an NRAS mutation was associated with a lack of response to cetuximab. Irahara et al. investigated the relationship between NRAS mutations and clinical outcome in a collection of 225 colorectal cancers from two prospective cohort studies [55]. NRAS mutations were detected in 2.2% of the CRCs. There was no apparent association between the NRAS mutations and any clinical or pathologic features, including patient survival. However, the low frequency of NRAS mutations may obscure a significant relation. De Roock et al. conducted a retrospective analysis of over 700 tumor samples from patients treated with cetuximab plus chemotherapy and found a NRAS mutation frequency of 2.6%. Additionally, in KRAS wild types, carriers of NRAS mutations had a significantly lower response rate to cetuximab than NRAS wild types (7.7% versus 38.1%, $P = .013$) [26]. There was, however, no significant difference in median PFS (14 versus 26 weeks, $P = .055$) and median OS (38 versus 50 weeks, $P = .051$) between NRAS wild types and mutants [26].

B-type Raf kinase (BRAF) is a component of the RAS-RAF-MEK signaling cascade of the EGFR (see Figure 1) [56]. A specific mutation in the BRAF gene (V600E) is present in approximately 5–8% of CRCs and is thought to be limited to those tumors without mutations in exon 2 of KRAS [42, 57]. BRAF, which is located directly downstream from RAS, can have activating mutations leading to stimulation of the MEK pathway [56, 58]. BRAF mutations appear to confer a poor prognosis, and it appears that BRAF mutations also predict for a lack of response to antiEGFR monoclonal antibodies [42, 57, 59, 60]. Loupakis et al. analyzed 87 patients with KRAS WT tumors for the BRAF V600E mutation who were receiving irinotecan and cetuximab for refractory metastatic

Table 1: Clinical trials of antiEGFR monoclonal antibodies in metastatic CRC.

Study	Setting	Treatment	No. of patients	ORR (%)	mTTP (mos)	mPFS (mos)	mOS (mos)
Single Arm phase II [12]	Irinotecan-refractory	Cetuximab monotherapy	57	9	1.4	N.R.	6.4
Randomized phase II [13]	Refractory disease to 5-FU and Irinotecan	Cetuximab monotherapy vs. Cetuximab + Irinotecan	111 vs. 218	10.8* vs. 22.9	1.5* vs. 4.1	N.R.	6.9 vs. 8.6
Single Arm phase II [14]	Refractory disease to 5-FU, Irinotecan, and Oxaliplatin	Cetuximab monotherapy	346	12.4	1.4	N.R.	6.6
Single Arm phase II [15]	First-line treatment	Cetuximab + Irinotecan + 5-FU/FA	21	67	9.9	N.R.	33.0
Randomized phase III [16]	Refractory disease to 5-FU, Irinotecan and Oxaliplatin	Cetuximab monotherapy vs. BSC	287 vs. 285	8* vs. 0	N.R.	1.9* vs. 1.8	6.1* vs. 4.6
Single Arm phase II [17]	First-line treatment	Cetuximab + FOLFOX-4	43	72	N.R.	12.3	30
Randomized phase III [18]	Refractory to Oxaliplatin	Cetuximab + Irinotecan vs. Irinotecan	648 vs. 650	16.4* vs. 4.2	N.R.	4.0* vs. 2.6	10.7 vs. 10.0
Randomized phase III [19]	First-line treatment	Cetuximab + FOLFIRI vs. FOLFIRI	602 vs. 600	46.9* vs. 38.7	N.R.	8.9* vs. 8.0	N.R.
Randomized phase III [20]	Refractory disease to 5-FU, Irinotecan and Oxaliplatin	Panitumumab monotherapy versus BSC	231 vs. 232	10.0* vs. 0	N.R.	8 weeks* vs. 7.3 weeks	6.5 vs. 6.5
Randomized phase II [21]	Refractory to Irinotecan	Irinotecan + Cetuximab+ Bevacizumab vs. Cetuximab + Bevacizumab	43 vs. 40	37 vs. 20	7.3 vs. 4.9	N.R.	14.5 vs. 11.4
Single Arm phase II [22]	Refractory to Irinotecan + Bevacizumab	Cetuximab + Bevacizumab + Irinotecan	33	9	3.9	N.R.	10.6

* Statistically significant improvement.
ORR: overall response rate; mTTP: median time to progression; mPFS: median progression-free survival; mOS: median overall survival; N.R.: not reported; 5-FU: 5-fluorouracil; BSC: best supportive care; FA: folinic acid; NS: not significant.

CRC. This mutation was found in 15% of the patients and was associated with a lack of response to therapy (0% versus 32%, $P = .016$) and a shorter overall survival (4.1 months versus 13.9 months, $P = .037$) [61]. An additional retrospective analysis of 113 patients treated with antiEGFR monoclonal antibodies found the V600E BRAF mutation in 14% of the KRAS WT patients and was associated with no response to therapy and a statistically significant shorter progression-free survival and overall survival compared with BRAF WT patients [59]. In De Roock's retrospective analysis of tumor samples from patients treated with cetuximab plus chemotherapy, a BRAF mutation was discovered in 4.7% of tumors [26]. In KRAS wild types, carriers of BRAF mutations had a significantly lower response rate to cetuximab than in BRAF wild types (8.3% versus 38.0%, $P = .0012$), a significantly shorter PFS (8 versus 26 weeks, $P < .0001$), and a significantly shorter OS (26 versus 54 weeks, $P < .0001$) [26]. KRAS and BRAF mutation status do not, however, appear to affect the clinical benefit of oxaliplatin or irinotecan on PFS or OS [62]. Several compounds (PLX4032, PLX4720, and GDC-0879) which selectively inhibit the kinase enzyme BRAF containing the V600E mutation are in clinical development [63]. In BRAF mutant cancer cell lines, these selective BRAF inhibitors potently block RAF-MEK-ERK signaling. However, in those tumors that are BRAF WT, but possess a KRAS mutation, these BRAF inhibitors activate this same pathway and therefore should be avoided in those cancers with RAS mutations [64–66].

Table 2: Clinical trials with retrospective subset analyses of antiEGFR efficacy in relation to KRAS mutation status.

Study	Setting	Treatment	KRAS genotype	No. of patients	ORR (%)	mPFS (mos)	mOS (mos)
Single arm studies							
Lièvre et al. [34]	Second-line treatment	Cetuximab	WT Mut	65 24	40* 0	31.4 wk* 10.1	14.3* 10.1
De Roock et al. [29]	Irinotecan refractory	Cetuximab or cetuximab + irinotecan	WT Mut	57 46	41† 0	34 wk† (combo) 12 (cetux) 12	44.7 wk† (combo) 27 wk (cetux) 25.3–27.3
Khambata-Ford et al. [28]	Second or third-line treatment	Cetuximab	WT Mut	50 30	10 0	N.R.	N.R.
Di Fiore et al. [47]	Refractory disease	Cetuximab + chemotherapy	WT Mut	43 16	20.3 0	N.R.	N.R.
Benvenuti et al. [48]	Various lines of treatment	Cetuximab or panitumumab or cetuximab + chemotherapy	WT Mut	32 16	31 6	N.R.	N.R.
Randomized studies							
Amado et al. [24]	Refractory disease	Panitumumab + BSC vs. BSC	WT Mut vs. WT Mut	124 84 119 100	17 0 0 0	12.3 wk* 7.4 wk 7.3 wk 7.3 wk	8.1 4.9 7.6 4.4
Van Cutsem et al. [33]	First-line treatment	FOLFIRI + cetuximab vs. FOLFIRI	WT Mut vs. WT Mut	172 105 176 87	59.3 36.2 43.2 40.2	9.9* 7.6 8.7 8.1	24.9 17.5 21.0 17.7
Van Cutsem et al. [42]	First-line treatment	FOLFIRI + cetuximab vs. FOLFIRI	WT Mut vs. WT Mut	316 214 350 183	57.3* 31.3 39.7 36.1	9.9* 7.4 8.4 7.7	23.5* 16.2 20.0 16.7
Bokemeyer et al. [31]	First-line treatment	FOLFOX + cetuximab vs. FOLFOX	WT Mut vs. WT Mut	61 52 73 47	61* 33 37 49	7.7* 5.5 7.2 8.6	N.R.
Bokemeyer et al. [43]	First-line treatment	FOLFOX + cetuximab vs. FOLFOX	WT Mut vs. WT Mut	82 77 97 59	57* 34 34 53	8.3* 5.5 7.2 8.6	22.8 18.5 13.4 17.5
Karapetis et al. [35]	Refractory disease	Cetuximab + BSC vs. BSC	WT Mut vs. WT Mut	115 81 113 83	12.8 1.2 0 0	3.7* 1.8 1.9 1.8	9.5* 4.5 4.8 4.6
Siena et al. [44]	First-line treatment	FOLFOX + panitumumab vs. FOLFOX	WT = 656 Mut = 440		55 48	9.6 (wt)* 7.3 (mut) 8.0 (wt) 8.8 (mut)	N.R.
Kohne et al. [45]	First-line treatment	FOLFIRI + panitumumab	WT Mut	85 57	48 29	N.R.	N.R.

Table 2: Continued.

Study	Setting	Treatment	KRAS genotype	No. of patients	ORR (%)	mPFS (mos)	mOS (mos)
Tol et al. [49]	First-line treatment	CAPOX + bevacizumab + cetuximab vs. CAPOX + bevacizumab	WT	158	50.0	10.5*	21.8
			Mut	98	59.2	8.1	17.2
			WT	156	61.4*	10.6	22.4
			Mut	108	45.9	12.5	24.9
Hecht et al. [50]	First-line treatment	FOLFOX + bevacizumab + panitumumab vs. FOLFOX + bevacizumab	WT	201	50	9.8	20.7
			Mut	135	47	10.4	19.3
			WT	203	56	11.5	24.5
			Mut	125	44	11.0	19.3

* Statistically significant improvement
† Statistically significant improvement for the combination of cetuximab and irinotecan only.
ORR: overall response rate; mPFS: median progression-free survival; mOS: median overall survival; N.R.: not reported; BSC: best supportive care.

The mitogen-activated protein kinase kinase (MEK, also known as MAP2K) is downstream of BRAF in the RAS-RAF-MEK signaling cascade of the EGFR and uses extracellular signal-regulated kinase (ERK) as a substrate (see Figure 1) [67]. A number of MEK inhibitors such as AS703026, AZD6244 and RO5068760 have been or currently are being investigated in phase 1 and 2 clinical trials [68, 69]. The development of several MEK inhibitors has been halted because of either very low response rates or due to ocular toxicity [70]. These agents have however shown substantial preclinical activity in tumor cell lines harboring the BRAF V600E gene mutation [71]. It has been established that KRAS has a number of downstream effectors that are not blocked by MEK inhibition, and indeed BRAF mutant cell lines were found to be more sensitive to MEK inhibitors than KRAS mutant cells [71]. It is imperative to be able to identify which patients are likely to respond to MEK inhibitors, and it appears that those with BRAF mutations are a good start. Given that KRAS signaling operates through a number of downstream effectors, those with KRAS mutations may require a combination of targeted agents. Preclinical evidence suggests that BRAF gene amplification is a mechanism of resistance to both MEK and BRAF inhibitors and a combination of these inhibitors may be a strategy to overcome this [72].

An additional EGFR pathway is the PTEN/PI3K/AKT pathway [phosphatase and tensin homologue gene (PTEN)]. PTEN encodes a phosphatase which uses phosphatidylinositol-3,4,5-triphosphate (PIP-3) as a major substrate [73]. Loss of PTEN function leads to increased PIP-3 concentration, with resultant AKT hyperphosphorylation protecting tumor cells from apoptosis [73]. Roughly 60% of primary CRCs have a hyperphosphorylated AKT [74]. PTEN loss, activating mutations of phosphatidylinositol 3-kinase catalytic alpha polypeptide (PIK3CA) and activating mutations in KRAS/BRAF/MAPK confer resistance to apoptosis induced by cetuximab [75]. In patients with KRAS WT tumors treated with a cetuximab-based regimen, PTEN loss was associated with a significantly shorter OS [60]. Approximately one third of CRCs harbor activating somatic mutations in PIK3CA, and it has been reported that these mutations are predictive of lack of benefit from antiEGFR therapy [76]. Additional genetic alterations which could confer resistance to antiEGFR monoclonal antibodies include an inhibitor of PI3K signaling; coamplification of PAK4 (p-21-activated protein kinase) and AKT, which are downstream mediators of PI3K signaling; and amplification of IRS2 (insulin receptor substrate 2), which is an upstream activator of PI3K signaling [77, 78].

4. Strategies to Overcome Resistance

A number of approaches to the problem of resistance to antiEGFR monoclonal antibody therapy have been studied and are ongoing. Combining antiEGFR monoclonal antibodies with cytotoxic chemotherapy has already been discussed. Erlotinib and gefitinib, two oral small molecule EGFR inhibitors, are inactive by themselves [79, 80]. The combination of erlotinib with capecitabine and oxaliplatin in previously treated patients and the combination of gefitinib with FOLFOX were investigated in small phase II studies with favorable results, however randomized trials with chemotherapy alone as a control are needed [81–83]. Dual antiEGFR therapy with antiEGFR monoclonal antibodies plus antiEGFR TK inhibitors may overcome resistance to either drug alone. A 41% response rate was reported for the combination of cetuximab and erlotinib in patients with refractory disease, however this was limited to patients with KRAS and BRAF WT tumors [84].

EGFR and vascular endothelial growth factor (VEGF) have several signal transduction pathways in common, with preclinical data revealing that antiEGFR and antiVEGF drug combinations have synergistic activity [85]. The BOND-2 study randomized patients with irinotecan- and oxaliplatin-refractory but bevacizumab naïve disease to cetuximab and bevacizumab with or without irinotecan [21]. Response rates, TTP and OS favored the triple drug regimen, however, these results did not hold up in a subsequent study of this combination [21, 22]. Two subsequent randomized phase III trials have shown that combinations of antiEGFR

monoclonal antibodies plus bevacizumab do not improve outcomes and can actually cause increased toxicity irrespective of KRAS mutational status. The PACCE trial evaluated panitumumab combined with oxaliplatin- or irinotecan-based chemotherapy plus bevacizumab. The dual monoclonal antibody arm was associated with increased toxicity and significantly shorter PFS in patients with both KRAS WT and mutant tumors [50]. Similar results were observed with the combination of cetuximab to a regimen containing capecitabine, oxaliplatin, and bevacizumab in the CAIRO2 trial [49].

Novel agents and combinations are being employed in an attempt to overcome antiEGFR monoclonal antibody resistance. Motesanib, an oral inhibitor of VEGF, platelet derived growth factor (PDFG) and Kit receptors is being investigated with or without panitumumab in patients with refractory disease [86]. A number of inhibitors of the mutant BRAF kinase are in clinical development, as discussed above [87]. AMG 102 is an investigational monoclonal antibody against human hepatocyte growth factor (also known as cMET, of which overexpression correlates with cetuximab resistance) is being studied in combination with panitumumab in patients with metastatic CRC [88, 89].

5. Neoadjuvant and Adjuvant Therapy

Given the clinical benefit of antiEGFR monoclonal antibodies in patients with metastatic disease, evaluation of these therapies as postoperative (adjuvant) treatment was warranted. In the adjuvant setting, eradication of micrometastatic disease is associated with increased cure rates. N0147 randomized 1760 patients with resected stage III KRAS WT colon cancer to FOLFOX with or without cetuximab [90]. Interim analysis led to premature closure of this trial after it was determined that no group of patients benefited from cetuximab [90]. Initially this trial enrolled patients regardless of KRAS mutational status, and among 658 patients with mutant KRAS, the addition of cetuximab to FOLFOX resulted in impaired disease-free survival (DFS) and a trend toward impaired OS [91].

In patients with rectal cancer, EGFR is a logical target in combination with neoadjuvant radiotherapy (RT). Retrospective analyses have demonstrated lower pathologic complete response (pCR) rates and shorter DFS in patients with rectal cancer expressing EGFR who were treated with neoadjuvant RT, suggesting that radiosensitivity might be increased by targeting the EGFR [92, 93]. Several phase I/II studies have investigated the combinations of cetuximab and chemoradiotherapy in the neoadjuvant setting for patients with rectal cancer. These studies have demonstrated that cetuximab could be safely combined with preoperative chemoradiotherapy but the pCR rates have been low (5–12%) [94–100]. In two of these studies [96, 99], subsequent analyses were done to correlate KRAS mutation status with response rate. Among patients with KRAS WT tumors, Bengala et al. reported a trend toward a greater rate of tumor regression (36.7% for KRAS WT versus 11% for KRAS mutant), however it did not reach statistical significance ($P = .12$) [101]. Debucquoy et al. also did not find a correlation between KRAS WT tumors and pathologic response to therapy [102]. To our knowledge, panitumumab has not been studied in combination with RT in patients with rectal cancer. Given the failure of antiEGFR monoclonal antibodies to demonstrate a benefit in the adjuvant setting for stage III WT KRAS colon cancer, the value of further study of these agents for rectal cancer is doubtful.

Preclinically gefitinib has demonstrated improved radiosensitization [103]. Valentini et al. investigated the combination of gefitinib, continuous infusion 5-fluorouracil (5-FU) and pelvic RT in 41 patients with locally advanced rectal cancer and reported a pCR rate of 30%, however toxicity was an issue and further studies are necessary to establish the safety of this combination [104].

The effect of combined antiEGFR and antiVEGF therapy in combination with preoperative chemoradiotherapy for rectal cancer remains unknown, however given the negative results reported for combined EGFR and VEGF blockade in patients with metastatic CRC in combination with chemotherapy, studies investigating this avenue are unlikely [22, 49, 50]. Blaszkowsky et al. performed a small study investigating the combination of bevacizumab, erlotinib and 5-FU with RT in patients with locally advanced rectal cancer [105]. The regimen was found to be well-tolerated and highly active with a pCR rate of 47% and may deserve further investigation. However, the value of pCR as a surrogate for DFS and OS is uncertain.

6. Conclusion

Anti-EGFR monoclonal antibodies are among the standard treatment options for patients with metastatic CRC given their established efficacy. It is now clear that the benefit of antiEGFR monoclonal antibodies is isolated to patients with KRAS WT tumors. It appears that KRAS mutational status is just the beginning of our understanding of the EGFR as an integral component of the biology of CRC. Given that only a subset of patients respond to antiEGFR therapy, there is a need for better predictors to guide patient selection for such therapy. Several important components of the EGFR signaling pathway have been discovered, including BRAF, PTEN, AKT and PI3K, which deserve further study as predictors of response to existing treatments, or as targets of new interventions. The unexpected detrimental outcome associated with combined EGFR and VEGF blockade is a reminder of how much there is still to learn. New combinations and novel agents will continue to shed light on how to overcome resistance to inhibitors of the EGFR pathway, and hopefully new targets will be identified. Further study of how to employ our knowledge of EGFR pathway inhibitors to improve outcomes in the adjuvant and neoadjuvant setting is also warranted.

References

[1] C. H. Heldin, "Dimerization of cell surface receptors in signal transduction," *Cell*, vol. 80, no. 2, pp. 213–223, 1995.

[2] G. Carpenter, "Receptors for epidermal growth factor and other polypeptide mitogens," *Annual Review of Biochemistry*, vol. 56, pp. 881–914, 1987.

[3] G. Carpenter and S. Cohen, "Epidermal growth factor," *The Journal of Biological Chemistry*, vol. 265, no. 14, pp. 7709–7712, 1990.

[4] F. Loupakis, L. Pollina, I. Stasi et al., "PTEN expression and KRAS mutations on primary tumors and metastases in the prediction of benefit from cetuximab plus irinotecan for patients with metastatic colorectal cancer," *Journal of Clinical Oncology*, vol. 27, no. 16, pp. 2622–2629, 2009.

[5] A. Citri and Y. Yarden, "EGF-ERBB signalling: towards the systems level," *Nature Reviews Molecular Cell Biology*, vol. 7, no. 7, pp. 505–516, 2006.

[6] N. E. Hynes and H. A. Lane, "ERBB receptors and cancer: the complexity of targeted inhibitors," *Nature Reviews Cancer*, vol. 5, no. 5, pp. 341–354, 2005.

[7] J. Downward, Y. Yarden, E. Mayes et al., "Close similarity of epidermal growth factor receptor and v-erb-B oncogene protein sequences," *Nature*, vol. 307, no. 5951, pp. 521–527, 1984.

[8] F. X. Real, W. J. Rettig, and P. G. Chesa, "Expression of epidermal growth factor receptor in human cultured cells and tissues: relationship to cell lineage and stage of differentiation," *Cancer Research*, vol. 46, no. 9, pp. 4726–4731, 1986.

[9] *NCCN Clinical Practice Guidelines in Oncology. Colon Cancer*, vol. 3, NCCN, Gaithersburg, Md, USA, 2010.

[10] C. M. Croce, "Oncogenes and cancer," *The New England Journal of Medicine*, vol. 358, no. 5, pp. 502–511, 2008.

[11] N. Normanno, C. Bianco, A. De Luca, M. R. Maiello, and D. S. Salomon, "Target-based agents against ErbB receptors and their ligands: a novel approach to cancer treatment," *Endocrine-Related Cancer*, vol. 10, no. 1, pp. 1–21, 2003.

[12] L. B. Saltz, N. J. Meropol, P. J. Loehrer, M. N. Needle, J. Kopit, and R. J. Mayer, "Phase II trial of cetuximab in patients with refractory colorectal cancer that expresses the epidermal growth factor receptor," *Journal of Clinical Oncology*, vol. 22, no. 7, pp. 1201–1208, 2004.

[13] D. Cunningham, Y. Humblet, S. Siena et al., "Cetuximab monotherapy and cetuximab plus irinotecan in irinotecan-refractory metastatic colorectal cancer," *The New England Journal of Medicine*, vol. 351, no. 4, pp. 337–345, 2004.

[14] H. J. Lenz, E. Van Cutsem, S. Khambata-Ford et al., "Multicenter phase II and translational study of cetuximab in metastatic colorectal carcinoma refractory to irinotecan, oxaliplatin, and fluoropyrimidines," *Journal of Clinical Oncology*, vol. 24, no. 30, pp. 4914–4921, 2006.

[15] G. Folprecht, M. P. Lutz, P. Schöffski et al., "Cetuximab and irinotecan/5-fluorouracil/folinic acid is a safe combination for the first-line treatment of patients with epidermal growth factor receptor expressing metastatic colorectal carcinoma," *Annals of Oncology*, vol. 17, no. 3, pp. 450–456, 2006.

[16] D. J. Jonker, C. J. O'Callaghan, C. S. Karapetis et al., "Cetuximab for the treatment of colorectal cancer," *The New England Journal of Medicine*, vol. 357, no. 20, pp. 2040–2048, 2007.

[17] J. Tabernero, E. Van Cutsem, E. Díaz-Rubio et al., "Phase II trial of cetuximab in combination with fluorouracil, leucovorin, and oxaliplatin in the first-line treatment of metastatic colorectal cancer," *Journal of Clinical Oncology*, vol. 25, no. 33, pp. 5225–5232, 2007.

[18] A. F. Sobrero, J. Maurel, L. Fehrenbacher et al., "EPIC: phase III trial of cetuximab plus irinotecan after fluoropyrimidine and oxaliplatin failure in patients with metastatic colorectal cancer," *Journal of Clinical Oncology*, vol. 26, no. 14, pp. 2311–2319, 2008.

[19] E. Van Cutsem, M. Nowacki, I. Lang et al., "Randomized phase III study of irinotecan and 5-FU/FA with or without cetuximab in the first-line treatment of patients with metastatic colorectal cancer (mCRC): the CRYSTAL trial," *Journal of Clinical Oncology*, vol. 25, no. 18, supplement, p. 164s, 2007.

[20] E. Van Cutsem, M. Peeters, S. Siena et al., "Open-label phase III trial of panitumumab plus best supportive care compared with best supportive care alone in patients with chemotherapy-refractory metastatic colorectal cancer," *Journal of Clinical Oncology*, vol. 25, no. 13, pp. 1658–1664, 2007.

[21] L. B. Saltz, H. J. Lenz, H. L. Kindler et al., "Randomized phase II trial of cetuximab, bevacizumab, and irinotecan compared with cetuximab and bevacizumab alone in irinotecan-refractory colorectal cancer: the BOND-2 study," *Journal of Clinical Oncology*, vol. 25, no. 29, pp. 4557–4561, 2007.

[22] N. H. Segal, D. Reidy-Lagunes, M. Capanu et al., "Phase II study of bevacizumab in combination with cetuximab plus irinotecan in irinotecan-refractory colorectal cancer (CRC) patients who have progressed on a bevacizumab-containing regimen (The BOND 2.5 Study)," *Journal of Clinical Oncology*, vol. 27, no. 15, supplement, 2009, ASCO Annual Meeting, abstract no. 4087.

[23] M. Malumbres and M. Barbacid, "RAS oncogenes: the first 30 years," *Nature Reviews Cancer*, vol. 3, no. 6, pp. 459–465, 2003.

[24] R. G. Amado, M. Wolf, M. Peeters et al., "Wild-type KRAS is required for panitumumab efficacy in patients with metastatic colorectal cancer," *Journal of Clinical Oncology*, vol. 26, no. 10, pp. 1626–1634, 2008.

[25] C. J. Allegra, J. M. Jessup, M. R. Somerfield et al., "American society of clinical oncology provisional clinical opinion: testing for KRAS gene mutations in patients with metastatic colorectal carcinoma to predict response to anti-epidermal growth factor receptor monoclonal antibody therapy," *Journal of Clinical Oncology*, vol. 27, no. 12, pp. 2091–2096, 2009.

[26] W. De Roock, B. Claes, D. Bernasconi et al., "Effects of KRAS, BRAF, NRAS, and PIK3CA mutations on the efficacy of cetuximab plus chemotherapy in chemotherapy-refractory metastatic colorectal cancer: a retrospective consortium analysis," *The Lancet Oncology*, vol. 11, no. 8, pp. 753–762, 2010.

[27] J. Baselga and N. Rosen, "Determinants of RASistance to anti-epidermal growth factor receptor agents," *Journal of Clinical Oncology*, vol. 26, no. 10, pp. 1582–1584, 2008.

[28] S. Khambata-Ford, C. R. Garrett, N. J. Meropol et al., "Expression of epiregulin and amphiregulin and K-ras mutation status predict disease control in metastatic colorectal cancer patients treated with cetuximab," *Journal of Clinical Oncology*, vol. 25, no. 22, pp. 3230–3237, 2007.

[29] W. De Roock, H. Piessevaux, J. De Schutter et al., "KRAS wild-type state predicts survival and is associated to early radiological response in metastatic colorectal cancer treated with cetuximab," *Annals of Oncology*, vol. 19, no. 3, pp. 508–515, 2008.

[30] C. J. Punt, J. Tol, C. J. Rodenburg et al., "Randomized phase III study of capecitabine, oxaliplatin, and bevacizumab with or without cetuximab in advanced colorectal cancer, the CAIRO2 study of the Dutch Colorectal Cancer Group," *Journal of Clinical Oncology*, vol. 26, supplement, 2008, abstract LBA 4011.

[31] C. Bokemeyer, I. Bondarenko, A. Makhson et al., "Fluorouracil, leucovorin, and oxaliplatin with and without cetuximab in the first-line treatment of metastatic colorectal

cancer," *Journal of Clinical Oncology*, vol. 27, no. 5, pp. 663–671, 2009.

[32] S. Tejpar, M. Peeters, Y. Humblet et al., "Relationship of efficacy with KRAS status (wild type versus mutant) in patients with irinotecan-refractory metastatic colorectal cancer, treated with irinotecan and escalating doses of cetuximab: the EVEREST experience (preliminary data)," *Journal of Clinical Oncology*, vol. 26, supplement, 2008, abstract no. 4001.

[33] E. Van Cutsem, C. H. Köhne, E. Hitre et al., "Cetuximab and chemotherapy as initial treatment for metastatic colorectal cancer," *The New England Journal of Medicine*, vol. 360, no. 14, pp. 1408–1417, 2009.

[34] A. Lièvre, J. B. Bachet, V. Boige et al., "KRAS mutations as an independent prognostic factor in patients with advanced colorectal cancer treated with cetuximab," *Journal of Clinical Oncology*, vol. 26, no. 3, pp. 374–379, 2008.

[35] C. S. Karapetis, S. Khambata-Ford, D. J. Jonker et al., "K-ras mutations and benefit from cetuximab in advanced colorectal cancer," *The New England Journal of Medicine*, vol. 359, no. 17, pp. 1757–1765, 2008.

[36] D. Santini, F. Loupakis, B. Vincenzi et al., "High concordance of KRAS status between primary colorectal tumors and related metastatic sites: implications for clinical practice," *Oncologist*, vol. 13, no. 12, pp. 1270–1275, 2008.

[37] S. Artale, A. Sartore-Bianchi, S. M. Veronese et al., "Mutations of KRAS and BRAF in primary and matched metastatic sites of colorectal cancer," *Journal of Clinical Oncology*, vol. 26, no. 25, pp. 4217–4219, 2008.

[38] M. C. Etienne-Grimaldi, J. L. Formento, M. Francoual et al., "K-Ras mutations and treatment outcome in colorectal cancer patients receiving exclusive fluoropyrimidine therapy," *Clinical Cancer Research*, vol. 14, no. 15, pp. 4830–4835, 2008.

[39] G. Folprecht, T. Gruenberger, W. O. Bechstein et al., "Tumour response and secondary resectability of colorectal liver metastases following neoadjuvant chemotherapy with cetuximab: the CELIM randomised phase 2 trial," *The Lancet Oncology*, vol. 11, no. 1, pp. 38–47, 2010.

[40] S. E. Baldus, K. L. Schaefer, R. Engers, D. Hartleb, N. H. Stoecklein, and H. E. Gabbert, "Prevalence and heterogeneity of KRAS, BRAF, and PIK3CA mutations in primary colorectal adenocarcinomas and their corresponding metastases," *Clinical Cancer Research*, vol. 16, no. 3, pp. 790–799, 2010.

[41] A. Mancuso, R. Sollami, F. Recine et al., "Patient with colorectal cancer with heterogeneous KRAS molecular status responding to cetuximab-based chemotherapy," *Journal of Clinical Oncology*, vol. 28, no. 36, pp. e756–e758, 2010.

[42] E. Van Cutsem, I. Lang, G. Folprecht et al., "Cetuximab plus FOLFIRI in the treatment of metastatic colorectal cancer: the influence of KRAS and BRAF biomarkers on outcome: updated data from the CRYSTAL trial," in *Proceedings of the Gastrointestinal Cancers Symposium*, Orlando, Fla, USA, January 2010, abstract no. 281.

[43] C. Bokemeyer, I. Bondarenko, J. T. Hartmann et al., "Biomarkers predictive for outcome in patients with metastatic colorectal cancer treated with first-line FOLFOX4 plus or minus cetuximab: updated data from the OPUS study," in *Proceedings of the ASCO Gastrointestinal Cancers Symposium*, Orlando, Fla, USA, January 2010, abstract no. 428.

[44] S. Siena, J. Cassidy, J. Tabernero et al., "Randomized phase III study of panitumumab with FOLFOX4 compared to FOLFOX4 alone as first-line treatment for metastatic colorectal cancer: PRIME trial," in *Proceedings of the Gastrointestinal Cancers Symposium*, Orlando, Fla, USA, January 2010, abstract no. 283.

[45] C. Kohne, K. Mineur, R. Greil et al., "Primary analysis of a phase II study (20060314) combining first-line panitumumab with FOLFIRI in the treatment of patients with metastatic colorectal cancer," in *Proceedings of the Gastrointestinal Cancers Symposium*, Orlando, Fla, USA, January 2010, abstract no. 414.

[46] J. Hecht, E. Mitchell, J. Baranda et al., "Panitumumab antitumor activity in patients (pts) with metastatic colorectal cancer (mCRC) expressing low (1–9%) or negative (<1%) levels of epidermal growth receptor (EGFr)," *Journal of Clinical Oncology*, vol. 24, supplement, 2006, abstract no. 3506.

[47] F. Di Fiore, F. Blanchard, F. Charbonnier et al., "Clinical relevance of KRAS mutation detection in metastatic colorectal cancer treated by Cetuximab plus chemotherapy," *British Journal of Cancer*, vol. 96, no. 8, pp. 1166–1169, 2007.

[48] S. Benvenuti, A. Sartore-Bianchi, F. Di Nicolantonio et al., "Oncogenic activation of the RAS/RAF signaling pathway impairs the response of metastatic colorectal cancers to anti-epidermal growth factor receptor antibody therapies," *Cancer Research*, vol. 67, no. 6, pp. 2643–2648, 2007.

[49] J. Tol, M. Koopman, A. Cats et al., "Chemotherapy, bevacizumab, and cetuximab in metastatic colorectal cancer," *The New England Journal of Medicine*, vol. 360, pp. 563–572, 2009.

[50] J. R. Hecht, E. Mitchell, T. Chidiac et al., "A randomized phase IIIB trial of chemotherapy, bevacizumab, and panitumumab compared with chemotherapy and bevacizumab alone for metastatic colorectal cancer," *Journal of Clinical Oncology*, vol. 27, no. 5, pp. 672–680, 2009.

[51] H. Linardou, I. J. Dahabreh, D. Kanaloupiti et al., "Assessment of somatic k-RAS mutations as a mechanism associated with resistance to EGFR-targeted agents: a systematic review and meta-analysis of studies in advanced non-small-cell lung cancer and metastatic colorectal cancer," *The Lancet Oncology*, vol. 9, no. 10, pp. 962–972, 2008.

[52] J. L. Bos, E. R. Fearon, S. R. Hamilton et al., "Prevalence of ras gene mutations in human colorectal cancers," *Nature*, vol. 327, no. 6120, pp. 293–297, 1987.

[53] B. Vogelstein, E. R. Fearon, S. R. Hamilton et al., "Genetic alterations during colorectal-tumor development," *The New England Journal of Medicine*, vol. 319, no. 9, pp. 525–532, 1988.

[54] D. Lambrechts, W. De Roock, H. Prenen et al., "The role of KRAS, BRAF, NRAS, and PIK3CA mutations as markers of resistance to cetuximab in chemorefractory metastatic colorectal cancer," *Journal of Clinical Oncology*, vol. 27, no. 15, supplement, 2009, abstract no. 4020.

[55] N. Irahara, Y. Baba, K. Nosho et al., "NRAS mutations are rare in colorectal cancer," *Diagnostic Molecular Pathology*, vol. 19, no. 3, pp. 157–163, 2010.

[56] H. Davies, G. R. Bignell, C. Cox et al., "Mutations of the BRAF gene in human cancer," *Nature*, vol. 417, no. 6892, pp. 949–954, 2002.

[57] J. Tol, I. D. Nagtegaal, and C. J. A. Punt, "BRAF mutation in metastatic colorectal cancer," *The New England Journal of Medicine*, vol. 361, no. 1, pp. 98–99, 2009.

[58] S. Siena, A. Sartore-Bianchi, F. Di Nicolantonio, J. Balfour, and A. Bardelli, "Biomarkers predicting clinical outcome of epidermal growth factor receptor-targeted therapy in metastatic colorectal cancer," *Journal of the National Cancer Institute*, vol. 101, no. 19, pp. 1308–1324, 2009.

[59] F. Di Nicolantonio, M. Martini, F. Molinari et al., "Wild-type BRAF is required for response to panitumumab or cetuximab in metastatic colorectal cancer," *Journal of Clinical Oncology*, vol. 26, no. 35, pp. 5705–5712, 2008.

[60] P. Laurent-Puig, A. Cayre, G. Manceau et al., "Analysis of PTEN, BRAF, and EGFR status in determining benefit from cetuximab therapy in wild-type KRAS metastatic colon cancer," *Journal of Clinical Oncology*, vol. 27, no. 35, pp. 5924–5930, 2009.

[61] F. Loupakis, A. Ruzzo, C. Cremolini et al., "KRAS codon 61, 146 and BRAF mutations predict resistance to cetuximab plus irinotecan in KRAS codon 12 and 13 wild-type metastatic colorectal cancer," *British Journal of Cancer*, vol. 101, no. 4, pp. 715–721, 2009.

[62] S. D. Richman, M. T. Seymour, P. Chambers et al., "KRAS and BRAF mutations in advanced colorectal cancer are associated with poor prognosis but do not preclude benefit from oxaliplatin or irinotecan: results from the MRC FOCUS trial," *Journal of Clinical Oncology*, vol. 27, no. 35, pp. 5931–5937, 2009.

[63] K. Cichowski and P. A. Jänne, "Drug discovery: inhibitors that activate," *Nature*, vol. 464, no. 7287, pp. 358–359, 2010.

[64] P. I. Poulikakos, C. Zhang, G. Bollag, K. M. Shokat, and N. Rosen, "RAF inhibitors transactivate RAF dimers and ERK signalling in cells with wild-type BRAF," *Nature*, vol. 464, no. 7287, pp. 427–430, 2010.

[65] G. Hatzivassiliou, K. Song, I. Yen et al., "RAF inhibitors prime wild-type RAF to activate the MAPK pathway and enhance growth," *Nature*, vol. 464, no. 7287, pp. 431–435, 2010.

[66] S. J. Heidorn, C. Milagre, S. Whittaker et al., "Kinase-dead BRAF and oncogenic RAS cooperate to drive tumor progression through CRAF," *Cell*, vol. 140, no. 2, pp. 209–221, 2010.

[67] A. Duffy and S. Kummar, "Targeting mitogen-activated protein kinase kinase (MEK) in solid tumors," *Targeted Oncology*, vol. 4, no. 4, pp. 267–273, 2009.

[68] S. Daouti, B. Higgins, K. Kolinsky et al., "Preclinical in vivo evaluation of efficacy, pharmacokinetics, and pharmacodynamics of a novel MEK1/2 kinase inhibitor RO5068760 in multiple tumor models," *Molecular Cancer Therapeutics*, vol. 9, no. 1, pp. 134–144, 2010.

[69] J. Y. Yoon, K. H. Koo, and K. Y. Choi, "MEK1/2 inhibitors AS703026 and AZD6244 may be potential therapies for KRAS mutated colorectal cancer that is resistant to EGFR monoclonal antibody therapy," *Cancer Research*, vol. 71, no. 2, pp. 445–453, 2011.

[70] C. Montagut and J. Settleman, "Targeting the RAF-MEK-ERK pathway in cancer therapy," *Cancer Letters*, vol. 283, no. 2, pp. 125–134, 2009.

[71] D. B. Solit, L. A. Garraway, C. A. Pratilas et al., "BRAF mutation predicts sensitivity to MEK inhibition," *Nature*, vol. 439, no. 7074, pp. 358–362, 2006.

[72] R. B. Corcoran, D. Dias-Santagata, K. Bergethon, A. J. Iafrate, J. Settleman, and J. A. Engelman, "BRAF gene amplification can promote acquired resistance to MEK inhibitors in cancer cells harboring the BRAF V600E mutation," *Science Signaling*, vol. 3, no. 149, article ra84, 2010.

[73] A. Di Cristofano and P. P. Pandolfi, "The multiple roles of PTEN in tumor suppression," *Cell*, vol. 100, no. 4, pp. 387–390, 2000.

[74] H. K. Roy, B. F. Olusola, D. L. Clemens et al., "AKT proto-oncogene overexpression is an early event during sporadic colon carcinogenesis," *Carcinogenesis*, vol. 23, no. 1, pp. 201–205, 2002.

[75] M. Jhawer, S. Goel, A. J. Wilson et al., "PIK3CA mutation/PTEN expression status predicts response of colon cancer cells to the epidermal growth factor receptor inhibitor cetuximab," *Cancer Research*, vol. 68, no. 6, pp. 1953–1961, 2008.

[76] Y. Samuels, Z. Wang, A. Bardelli et al., "High frequency of mutations of the PIK3CA gene in human cancers," *Science*, vol. 304, no. 5670, p. 554, 2004.

[77] A. Sood, D. McClain, R. Seetharam et al., "Beyond KRAS: the quest for novel genetic markers predictive for response to anti-epidermal growth factor receptor (EGFR) therapy in patients with metastatic colorectal cancer (mCRC)," *Journal of Clinical Oncology*, vol. 28, no. 15, supplement, 2010, ASCO Annual Meeting, abstract no. 3567.

[78] D. W. Parsons, T. L. Wang, Y. Samuels et al., "Colorectal cancer: mutations in a signalling pathway," *Nature*, vol. 436, no. 7052, p. 792, 2005.

[79] C. A. Townsley, P. Major, L. L. Siu et al., "Phase II study of erlotinib (OSI-774) in patients with metastatic colorectal cancer," *British Journal of Cancer*, vol. 94, no. 8, pp. 1136–1143, 2006.

[80] M. L. Rothenberg, B. LaFleur, D. E. Levy et al., "Randomized phase II trial of the clinical and biological effects of two dose levels of gefitinib in patients with recurrent colorectal adenocarcinoma," *Journal of Clinical Oncology*, vol. 23, no. 36, pp. 9265–9274, 2005.

[81] J. A. Meyerhardt, A. X. Zhu, P. C. Enzinger et al., "Phase II study of capecitabine, oxaliplatin, and erlotinib in previously treated patients with metastastic colorectal cancer," *Journal of Clinical Oncology*, vol. 24, no. 12, pp. 1892–1897, 2006.

[82] T. Kuo, C. D. Cho, J. Halsey et al., "Phase II study of gefitinib, fluorouracil, leucovorin, and oxaliplatin therapy in previously treated patients with metastatic colorectal cancer," *Journal of Clinical Oncology*, vol. 23, no. 24, pp. 5613–5619, 2005.

[83] M. G. Zampino, E. Magni, C. Massacesi et al., "First clinical experience of orally active epidermal growth factor receptor inhibitor combined with simplified FOLFOX6 as first-line treatment for metastatic colorectal cancer," *Cancer*, vol. 110, no. 4, pp. 752–758, 2007.

[84] A. J. Weickhardt, T. J. Price, N. Pavlakis et al., "DUX study: a phase II study of evaluating dual targeting of the EGFR using the combination of cetuximab and erlotinib in patients with chemotherapy refractory metastatic colorectal cancer," *Journal of Clinical Oncology*, vol. 28, no. 15, supplement, 2010, ASCO Annual Meeting Proceedings, abstract no. 3533.

[85] J. R. Tonra, D. S. Deevi, E. Corcoran et al., "Synergistic antitumor effects of combined epidermal growth factor receptor and vascular endothelial growth factor receptor-2 targeted therapy," *Clinical Cancer Research*, vol. 12, no. 7, pp. 2197–2207, 2006.

[86] N. C. Tebbutt, D. Kotasek, H. A. Burris et al., "Motesanib with or without panitumumab (pmab) plus FOLFIRI or FOLFOX for the treatment of metastatic colorectal cancer (mCRC)," in *Proceedings of the ASCO Annual Meeting*, 2010, abstract no. 3538.

[87] S. Kopetz, J. Desai, E. Chan et al., "PLX4032 in metastatic colorectal cancer patients with mutant BRAF tumors," *Journal of Clinical Oncology*, vol. 28, no. 15, supplement, 2010, abstract no. 3534.

[88] C. Eng, J. Tabernero, E. Nowara et al., "Panitumumab (pmab) plus AMG 102 in patients (pts) with wild-type KRAS metastatic colorectal cancer (mCRC): updated safety

results," *Journal of Clinical Oncology*, vol. 28, supplement, 2010, ASCO Annual Meeting, abstract no. e14083.

[89] M. Di Salvatore, A. Inno, A. Orlandi et al., "KRAS and BRAF mutational status and PTEN, cMET, and IGF1R expression as predictive markers of response to cetuximab plus chemotherapy in metastatic colorectal cancer (mCRC)," *Journal of Clinical Oncology*, vol. 28, supplement, 2010, ASCO Annual Meeting, abstract no. e14065.

[90] S. R. Alberts, D. J. Sargent, T. C. Smyrk et al., "Adjuvant mFOLFOX6 with or without cetuximab (Cmab) in KRAS wild-type (WT) patients (pts) with resected stage III colon cancer (CC): results from NCCTG Intergroup Phase III Trial N0147," *Journal of Clinical Oncology*, vol. 28, no. 15, supplement, 2010, ASCO Annual Meeting, abstract no. CRA3507.

[91] R. M. Goldberg, D. J. Sargent, and S. N. Thibodeau, "Adjuvant mFOLFOX6 plus or minus cetuximab (Cmab) in patients (pts) with KRAS mutant (m) resected stage III colon cancer (CC): NCCTG Intergroup Phase III Trial N0147," *Journal of Clinical Oncology*, vol. 28, no. 15, supplement, 2010, ASCO Annual Meeting, abstract no. 3508.

[92] J. Giralt, M. de las Heras, L. Cerezo et al., "The expression of epidermal growth factor receptor results in a worse prognosis for patients with rectal cancer treated with preoperative radiotherapy: a multicenter, retrospective analysis," *Radiotherapy and Oncology*, vol. 74, no. 2, pp. 101–108, 2005.

[93] J. S. Kim, J. M. Kim, S. Li et al., "Epidermal growth factor receptor as a predictor of tumor downstaging in locally advanced rectal cancer patients treated with preoperative chemoradiotherapy," *International Journal of Radiation Oncology Biology Physics*, vol. 66, no. 1, pp. 195–200, 2006.

[94] K. Y. Chung, B. Minsky, D. Schrag et al., "Phase I trial of preoperative cetuximab with concurrent continuous infusion 5-fluorouracil and pelvic radiation in patients with locally-regionally advanced rectal cancer," *Journal of Clinical Oncology*, vol. 24, no. 18, supplement, 2006, abstract no. 3560.

[95] R. D. Hofheinz, K. Horisberger, C. Woernle et al., "Phase I trial of cetuximab in combination with capecitabine, weekly irinotecan, and radiotherapy as neoadjuvant therapy for rectal cancer," *International Journal of Radiation Oncology Biology Physics*, vol. 66, no. 5, pp. 1384–1390, 2006.

[96] J. P. Machiels, C. Sempoux, P. Scalliet et al., "Phase I/II study of preoperative cetuximab, capecitabine, and external beam radiotherapy in patients with rectal cancer," *Annals of Oncology*, vol. 18, no. 4, pp. 738–744, 2007.

[97] C. Rödel, D. Arnold, M. Hipp et al., "Phase I-II Trial of cetuximab, capecitabine, oxaliplatin, and radiotherapy as preoperative treatment in rectal cancer," *International Journal of Radiation Oncology Biology Physics*, vol. 70, no. 4, pp. 1081–1086, 2008.

[98] K. Horisberger, A. Treschl, S. Mai et al., "Cetuximab in combination with capecitabine, irinotecan, and radiotherapy for patients with locally advanced rectal cancer: results of a phase II MARGIT trial," *International Journal of Radiation Oncology Biology Physics*, vol. 74, no. 5, pp. 1487–1493, 2009.

[99] F. Bertolini, S. Chiara, C. Bengala et al., "Neoadjuvant treatment with single-agent cetuximab followed by 5-FU, cetuximab, and pelvic radiotherapy: a phase II study in locally advanced rectal cancer," *International Journal of Radiation Oncology Biology Physics*, vol. 73, no. 2, pp. 466–472, 2009.

[100] V. Velenik, J. Ocvirk, I. Oblak, and F. Anderluh, "A phase II study of cetuximab, capecitabine and radiotherapy in neoadjuvant treatment of patients with locally advanced resectable rectal cancer," *European Journal of Surgical Oncology*, vol. 36, no. 3, pp. 244–250, 2010.

[101] C. Bengala, S. Bettelli, F. Bertolini et al., "Epidermal growth factor receptor gene copy number, K-ras mutation and pathological response to preoperative cetuximab, 5-FU and radiation therapy in locally advanced rectal cancer," *Annals of Oncology*, vol. 20, no. 3, pp. 469–474, 2009.

[102] A. Debucquoy, K. Haustermans, A. Daemen et al., "Molecular response to cetuximab and efficacy of preoperative cetuximab-based chemoradiation in rectal cancer," *Journal of Clinical Oncology*, vol. 27, no. 17, pp. 2751–2757, 2009.

[103] K. J. Williams, B. A. Telfer, I. J. Stratford, and S. R. Wedge, "ZD1839 ('Iressa'), a specific oral epidermal growth factor receptor-tyrosine kinase inhibitor, potentiates radiotherapy in a human colorectal cancer xenograft model," *British Journal of Cancer*, vol. 86, no. 7, pp. 1157–1161, 2002.

[104] V. Valentini, A. De Paoli, M. A. Gambacorta et al., "Infusional 5-fluorouracil and ZD1839 (Gefitinib-Iressa) in combination with preoperative radiotherapy in patients with locally advanced rectal cancer: a phase I and II trial (1839IL/0092)," *International Journal of Radiation Oncology Biology Physics*, vol. 72, no. 3, pp. 644–649, 2008.

[105] L. S. Blaszkowsky, T. S. Hong, A. X. Zhu et al., "A phase I/II study of bevacizumab, erlotinib, and 5-fluorouracil with concurrent external beam radiation therapy in locally advanced rectal cancer," *Journal of Clinical Oncology*, vol. 27, no. 15, supplement, p. 194S, 2009, abstract no. 4106.

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Review Article

Gastrointestinal Mesenchymal Neoplasms other than Gastrointestinal Stromal Tumors: Focusing on Their Molecular Aspects

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Gastrointestinal (GI) mesenchymal tumors other than gastrointestinal stromal tumor (GIST) are rare neoplasms, but they often enter the differential diagnosis of more common GI lesions. Some of these mesenchymal tumors in the GI tract have well understood molecular pathologic aspects, including desmoid tumors, inflammatory myofibroblastic tumor (IMT), clear cell sarcoma (CCS), inflammatory fibroid polyp (IFP), and synovial sarcoma (SS). Molecular pathology is fast becoming a mainstream focus in laboratories because it aids in the precise classification of tumors, may be prognostic, and may help predict response to therapy. The following review is not intended as an exhaustive summary of all mesenchymal tumors that have been reported to involve the GI tract, but instead will highlight the current knowledge of the most important non-GIST GI mesenchymal neoplasms, focusing on those tumors with well-characterized molecular pathology and how the molecular pathologic features impact current diagnostic, therapeutic, and prognostic standards.

1. Introduction

Gastrointestinal (GI) mesenchymal tumors are rare, and the molecular pathology of many of these tumors is unknown or poorly characterized. However, some mesenchymal tumors in the GI tract have well-understood molecular pathologic aspects. Molecular pathology is fast becoming a mainstream focus in laboratories because it aids in the precise classification of tumors, may be prognostic, and may help predict response to therapy. A search of the catalogue of somatic mutations in cancer (COSMIC) database for all mesenchymal tumors in the tubular GI tract and adjacent soft tissues, including esophagus, stomach, small intestine, large intestine, peritoneum, and retroperitoneum reveals meaningful data on three tumor types: gastrointestinal stromal tumor (GIST), inflammatory fibroid polyp (IFP), and desmoid tumors. Other mesenchymal tumors that occur in or around the tubular GI tract with well-characterized molecular pathologic features include synovial sarcoma (SS), inflammatory myofibroblastic tumor (IMT), and clear cell sarcoma (CCS); these tumors are characterized by translocations rather than mutations. The following paper is not intended as an exhaustive summary of all mesenchymal tumors that have been reported to involve the GI tract, but instead will highlight the current knowledge of the most important non-GIST GI mesenchymal neoplasms, focusing on those tumors with well-characterized molecular pathology.

2. Intraabdominal Desmoid Tumors

Intraabdominal desmoid tumors arise in the mesentery or retroperitoneum, predominantly in young patients. Approximately 10% of desmoids occur in patients with familial adenomatous polyposis [1] as one of the extracolonic manifestations of Gardner syndrome. Desmoids do not metastasize, but they often recur locally [1, 2]. The histologic features of desmoids are quite characteristic. In particular, these tumors show low to moderate cellularity and are composed of uniform spindle cells with a small, distinct

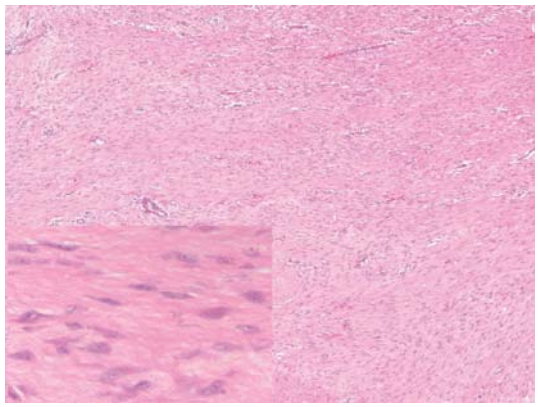

Figure 1: Photomicrograph of H&E-stained section from a desmoid tumor. Note the moderately cellular sweeping fascicles of bland spindle cells. The lower left inset contains a high-power photomicrograph of the same tumor to demonstrate the characteristic pinpoint nucleoli and collagenous stroma of desmoids.

nucleolus arranged in long, sweeping fascicles (Figure 1) [1]. The vasculature shows small arteries with accompanying veins and a mild perivascular lymphoid infiltrate. The associated stroma is quite collagenous. Mitotic figures may be 10/50 high power field (HPF) or more [3, 4], but these tumors lack other histologic features of malignancy such as dense cellularity, cytologic atypia, or atypical mitotic figures. Important differential diagnostic considerations include sclerosing mesenteritis, which does not invade bowel wall [5] and IgG4-related sclerosing disorders, which are rich in IgG4 plasma cells [6].

Most desmoid tumors arise via perturbations within the wnt signaling pathway (Figure 2). In FAP, desmoid tumors arise from mutations in the adenomatous polyposis coli (*APC*) gene, located on chromosome 5q21-22, which encodes a tumor suppressor protein, although its function may be more complex than simply a tumor suppressor [7]. Inactivation of *APC* leads to nuclear accumulation of β-catenin, causing increased transcription and cell proliferation [1]. Most *APC* mutations associated with desmoid tumors are found 3′ to codon 1400 [8, 9]. Some sporadic desmoid tumors also arise from *APC* inactivation [10] but most (>80%) are *APC* wild type with activating mutations of the *CTNNB1* gene, which is located on chromosome 3p22-p21.3. *CTNNB1* encodes β-catenin [11]. Mutations almost exclusively occur at codons 41 and 45 in exon 3 of *CTNNB1* [12]; mutational analysis of *CTNNB1* is usually not necessary in typical cases of desmoids tumors but can be helpful in unusual cases as well as helpful in distinguishing recurrent desmoid from scar. It remains to be determined whether particular *CTNNB1* mutations help predict local recurrence after surgical resection. One report [13] found that tumors harboring S45F mutations in exon 3 of *CTNNB1* had significantly poorer disease-free survival compared to wild-type tumors or codon 41 mutants, whereas another report showed no significant differences in recurrence-free survival among *CTNNB1* mutants but did show worse outcome among all mutants compared to wild-type tumors [14].

Regardless of inciting molecular event, the final common pathway is accumulation of β-catenin protein within the nuclei of the tumor cells, and although most desmoids do not require immunohistochemistry for diagnosis, nuclear staining of with β-catenin characterizes >90% of desmoid tumors [1]. In our experience, β-catenin immunohistochemistry is less reliable than the literature reports, particularly in needle biopsies, and molecular testing for mutations in the β-catenin gene may be more reliable. Immunoreactivity with CD117 has been reported in the literature [4], but this is generally considered an anomaly; when appropriate titers and antigen retrieval methods are used, desmoids show no CD117 positivity [15–18]. Although some investigators have reported response to imatinib [19], others have found that imatinib showed the lowest response rates in comparison to other forms of systemic therapy [20]. Furthermore, members of the PDGFR family are expressed in the majority of desmoids, but this expression does not correlate with response to imatinib. Since no clear target is present on desmoid tumors, imatinib therapy is controversial at best. Surgery remains the mainstay of treatment, but surgical resection is often incomplete because of the infiltrative growth of desmoid tumors, with recurrences up to 38% [13]. In these patients, systemic therapies such as anti-inflammatory, hormonal, cytotoxic chemotherapy, and radiation are considered. In patients with FAP, surgery such as ileal pouch procedures may trigger desmoid tumor formation [1].

3. Inflammatory Myofibroblastic Tumor (IMT)

IMTs are a heterogenous group of spindle cell proliferations with admixed lymphocytes and plasma cells that tend to occur in children and young adults. The omentum and mesentery are the most common extra-pulmonary sites for IMT [21, 22], and these tumors may have a more aggressive biologic behavior, with more frequent recurrences [23]. Most IMTs harbor a heterogenous microscopic appearance and may contain any combination of nodular fasciitis-like areas, compact spindle cell proliferations, or paucicellular scar-like areas, making them a challenging diagnostic entity. In all tumors, inflammatory cells are a distinctive feature, and the infiltrate is often rich in plasma cells (Figure 3) [22].

Some authorities have differentiated IMTs from reactive pseudosarcomatous processes and other neoplasms based on ALK immunoreactivity or evidence of the *ALK* translocation, but many consider ALK-negative IMTs a valid diagnostic category. Tumors with *ALK* rearrangements are associated with younger age and strongly correlate with ALK protein expression detected by immunohistochemistry in some labs, but in other labs ALK immunohistochemistry is nearly always negative. Cook et al. found that 12/20 (60%) of cases expressed ALK by immunohistochemistry [24]. In fact, the literature reports that only about half of IMT harbor an *ALK* translocation, and these may behave more indolently than their ALK-negative counterparts [23], although this may not be the case for all *ALK*-rearranged tumors. For example, a very recent series described 11 intra-abdominal tumors characterized by epithelioid morphology, abundant

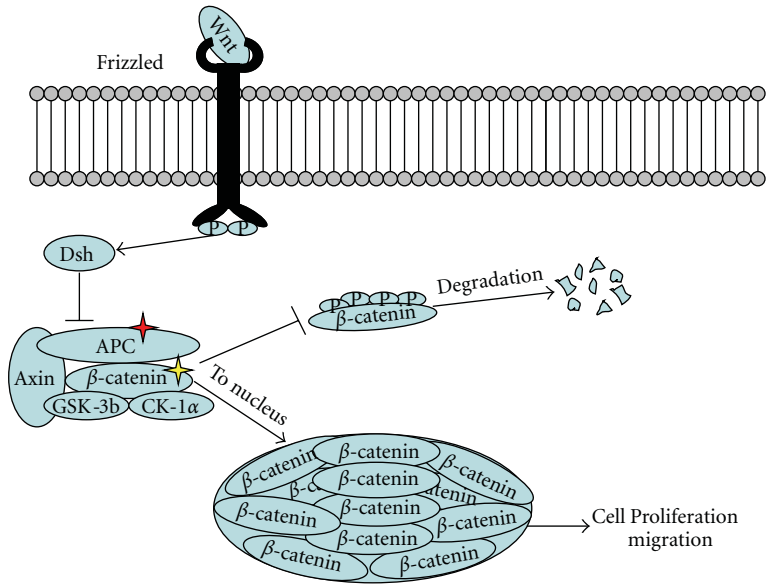

Figure 2: Schematic of Wnt signaling pathway. In demoid tumors, mutations are usually found in the *APC* gene or *CTNNB1* gene, which encodes β-catenin. Regardless of the primary defect, the end result is nuclear accumulation of β-catenin, which fails to undergo cytoplasmic degradation.

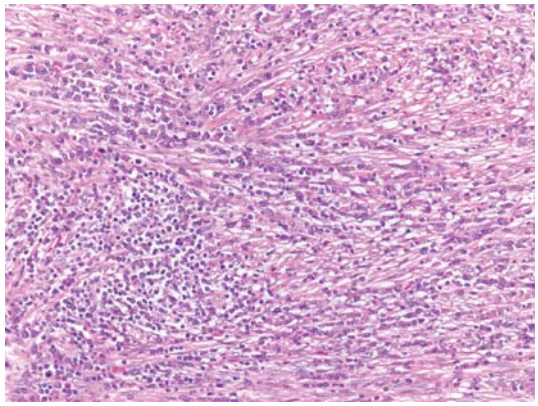

Figure 3: Photomicrograph of H&E-stained section from an inflammatory myofibroblastic tumor. The tumor is relatively cellular and composed of a mixture of plump spindle cells and inflammatory cells, particularly lymphocytes and plasma cells.

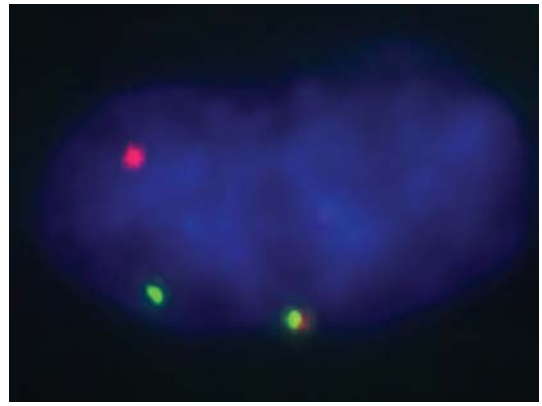

Figure 4: Photomicrograph of FISH break apart probe that targets the *ALK* gene. The normal chromosome (lower middle) shows a green signal and red signal in close proximity, whereas the green and red signals are far apart in the left aspect of the photomicrograph, confirming 1 copy of the *ALK* translocation.

myxoid stroma, and frequent neutrophils [25]. These tumors demonstrated aggressive behavior with rapid local recurrence, death in 5, and metastasis in 2. All 11 showed nuclear membrane or peri-nuclear ALK immunoreactivity. Nine tumors showed *ALK* translocation, and 3 tested showed *ALK/RANBP2* rearrangement.

Tumors other than IMTs have been recognized to demonstrate immunohistochemical expression of ALK, so genetic confirmation of the ALK translocation may be needed in problematic cases. At our institution, we find break-apart FISH testing to be quite useful in this setting (Figure 4). It should be noted, however, that a very recent report of response to the ALK inhibitor crizotinib in one patient with confirmed *ALK* translocation and no response in an IMT without *ALK* rearrangement [26] provides preliminary justification for FISH testing on all suspected IMTs. Secondary resistance to crizotinib in an IMT with ALK translocation (*ALK/RANBP2*) has been recently documented in another patient. This resistance was suspected to occur via the neuroblastoma-associated F1174L *ALK* mutation that has been well studied in neuroblastomas as a mechanism of resistance to some ALK inhibitors [27].

ALK perturbations in IMTs occur via fusion of the C-terminal kinase domain of anaplastic lymphoma kinase (*ALK*) gene located on 2p23. The *ALK* gene encodes a tyrosine kinase receptor that is normally only expressed in

Table 1: Summary of various ALK fusions in IMT.

Fusion partner	Chromosomal location	ALK staining location (Gleason 2008)
TPM3 [30]	1q22-23	Cytoplasmic
TPM4 [30]	19p13.1	Cytoplasmic
CARS [31]	11p15	Cytoplasmic
ATIC [32]	2q35	Cytoplasmic
SEC31L1 [33]	4q21	Cytoplasmic
RANBP2 [34]	2q13	Nuclear membrane
CLTC [35]	17q23	Granular cytoplasmic

the developing nervous system [28]. In IMTs, the *ALK* gene fusion partner is most commonly tropomyosin 3 (*TPM3/ALK*) or tropomyosin 4 (*TPM4/ALK*), leading to constitutive activation. Other reported fusion partners include *CLTC*, *ATIC, RANBP2, CARS*, and *SEC31L1* (Table 1). ALK along with its fusion partner tends to localize to the cytoplasm, but ALK/RANBP2 localizes to the nuclear membrane [29]. ALK function is poorly characterized at this time, so it is difficult to postulate the exact mechanism of oncogenesis; nevertheless, these fusions clearly lead to a survival and growth advantage to the cells harboring the translocation.

4. Inflammatory Fibroid Polyp (IFP)

IFPs are most often encountered in the stomach, usually presenting in the antrum, but are found throughout the GI tract. IFPs tend to arise within the submucosa and frequently extend into the overlying mucosa. These tumors are composed of bland spindle cells that often form perivascular cuffs (Figure 5). The tumor cells are embedded in a distinctive, granulation-type or fibromyxoid stroma with abundant capillary-type vessels. Characteristically, IFPs show a prominent eosinophilic infiltrate, but other inflammatory cells such as lymphocytes, mast cells, plasma cells, and histiocytes are encountered [1]. Given their gastrointestinal location, overlapping molecular features, and characteristic CD34 immunoreactivity, IFPs may be confused with GISTs [17]. Importantly, IFPs do not express other GIST-specific markers such as KIT or DOG-1 [36]. A few patients with germline PDGFRA mutations have been reported in the literature. The reports tend to consider these patients within the spectrum of familial GIST, but they present with a variety of tumors including GIST, GI neurofibromas, lipomas, and IFP-like polyps, some of which demonstrate a lipomatous stroma [12, 37, 38].

IFPs are rare benign tumors of uncertain histogenesis and were considered reactive processes, but recently, mutations in platelet-derived growth factor receptor alpha (*PDGFRA*, chromosome 4q12) were described in IFPs located in the stomach and small bowel [39]. In the stomach, 16/23 (70%) IFPs harbored activating mutations of *PDGFRA* [40]. Six of the mutations were located in exon 12, and 10 were located in exon 18. In the small bowel series, 33/60 (55%) harbored mutations in *PDGFRA*, 31 of which were located in exon 12, and 2 were in exon 18 [41]. Most of these mutations had been previously described in GIST. Extrapolating from the GIST literature, 41 of the 49 mutations detected in these series have been shown to cause at least in vitro ligand-independent activation. In addition, 100% of the stomach IFPs and 95% of small bowel IFPs showed expression of PDGFRA detected by immunohistochemistry, leading to the hypothesis that the cell of origin is a PDGFRA-positive mesenchymal cell [41].

PDGFRA is a receptor tyrosine kinase that is highly homologous to KIT [41]. Activation is normally ligand-dependent, but activating mutations cause constitutive activation (Figure 6). Ligand binding can cause homodimerization with another PDGFRA or hetero-dimerization with a PDGFR-beta. PDGFRA's interaction with several signaling pathways such as RAS/MAPK, PI3K, and JAK/STAT

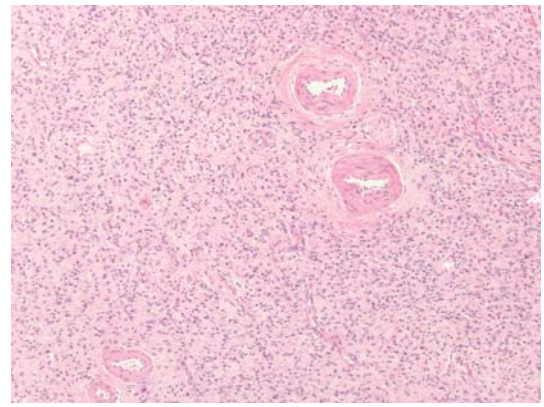

Figure 5: Photomicrograph of H&E-stained section from an inflammatory fibroid polyp. The lesion is moderately cellular with concentric whorls of spindle cells around blood vessels and numerous interspersed eosinophils.

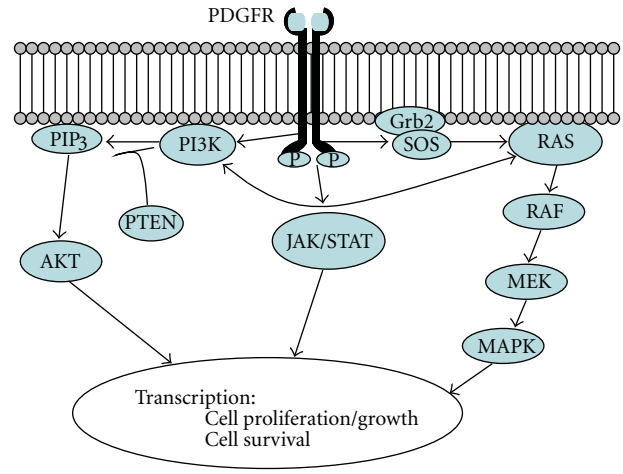

Figure 6: Schematic of platelet-derived growth factor receptor-(PDGFR-)related signaling pathways. Mutations can lead to activation of PDGFR independent of ligand binding. Numerous downstream pathways may lead to neoplastic advantages such as cell proliferation, growth, and survival.

allows for the acquisition of numerous tumorigenic cell functions such as cell growth, migration, inhibition of apoptosis, and angiogenesis [42] when the receptor is constantly activated. The utility of *PDGFRA* mutation testing to confirm the diagnosis of IFP is minimal, but it is important to realize that not all *PDGFRA*-mutated mesenchymal neoplasms in the GI tract are GISTs.

5. Clear Cell Sarcoma (CCS)

Primary GI CCSs are extremely rare, with only about 20 total cases reported in the literature. Age range is quite variable, with the youngest diagnosed at age 13 years and the oldest at 85 [43]. The small bowel is most frequently involved, but reports of gastric, colonic, and mesenteric tumors also exist. These tumors are often metastatic to peritoneum, lymph nodes, or liver at presentation and are at significant risk for local recurrence after surgery [43]. Morphologically and immunophenotypically, there appear to be two CCS-like malignancies that occur in the GI tract.

The first type of GI CCS constitutes those tumors that are morphologically, ultrastructurally, and immunohistochemically indistinguishable from CCSs of soft tissue. CCSs are typically composed of nests and fascicles of pale spindled to epithelioid cells, separated by delicate fibrous septa, forming a lobulated or organoid growth pattern (Figure 7). Cellular pleomorphism is typically uncommon, and multinucleated giant cells may be present. Nucleoli are typically prominent. Immunohistochemically, these tumors are indistinguishable from melanoma, being S-100 protein as well as melanocytic markers such as HMB-45 and Melan-A positive in the majority of cases [43]. In the soft tissue, these tumors are often referred to as "melanoma of soft parts," because of their morphologic and immunohistochemical resemblance to malignant melanoma. Unfortunately, immunohistochemistry is of no help distinguishing the two malignancies, but the characteristic growth pattern and bland cytologic features with pale cytoplasm are useful clues to the diagnosis. As discussed below, rearrangements in the *EWRS1* gene occur in CCS, not melanoma, which can be invaluable in separating CCS from melanoma [44].

The second tumor type encountered in the GI tract is characterized by mostly epithelioid cells with pale cytoplasm without the characteristic tumor cell nesting or compartmentalization of soft tissue CCS. Osteoclast-like multinucleate giant cells are by definition admixed with the tumor cells such that the tumor has been described as "Osteoclast-rich tumor of the GI tract resembling CCS of soft parts." The tumor cells reveal S-100 protein expression but lack any ultrastructural or immunohistochemical evidence of melanocytic differentiation. Other important morphologic differences with conventional CCS of soft tissue include indistinct nucleoli, more mitotic activity, and prominent cellular pleomorphism [43].

More than 90% of CCS of soft tissue are associated with the reciprocal translocation t(12;22)(q13;q12), which results in fusion of the *EWSR1* gene and the *ATF1* gene. This translocation links the N-terminal domain of *EWSR1* to the basic leucine zipper of *ATF1*. [43] Four fusion transcripts

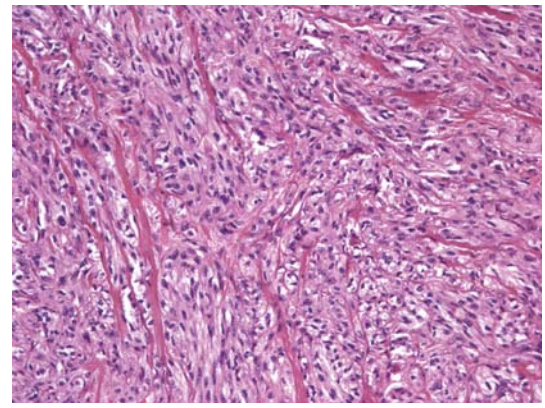

Figure 7: Photomicrograph of H&E-stained section from a conventional clear cell sarcoma. The cells are relatively bland with variably prominent nucleoli, low mitotic activity, and pale to clear cytoplasm. Fibrous bands illicit a compartmentalized appearance.

Table 2: Summary of various EWSR1 fusions in CCS [45, 46].

EWSR1/ATF1 transcript	Relative frequency	GI tract
EWSR1 exon 8/ *ATF1* exon 4	common	Yes
EWSR1 exon 7/ *ATF1* exon 5	common	No
EWSR1 exon 10/ *ATF1* exon 5	uncommon	No
EWSR1 exon 7 *ATF1* exon 7	uncommon	No
EWSR1 exon 7/ *CREB1* exon 7	uncommon	Yes

(Table 2) have been described in soft tissue tumors, the most common, or type 1, is a fusion of exon 8 of *EWSR1* and exon 4 of *ATF1* [45]. This translocation thus far has been the only described *EWSR1/ATF1* translocation in CCS of the GI tract. Another *EWSR1* translocation, *EWSR1/CREB1*, representing the t(2;22)(q34;q12) translocation, recently has been identified in a subset of GI and soft tissue CCS [46–48].

CREB1 and ATF1 belong to the basic leucine zipper superfamily of basic leucine zipper transcription factors. In normal melanocytes, CREB and ATF1 are involved in driving melanocytic differentiation. Both EWSR1/ATF1 and EWSR1/CREB1 fusion transcripts retain the basic leucine zipper domain, which mediates DNA binding and dimerization. In *EWSR1/ATF1* translocations, the activating domain of EWSR1 replaces the kinase inducible domain of ATF1, and the protein product has been shown to bind to microphthalmia-associated transcription factor, which in turn activates melanocyte stimulating hormone [29]. Overexpression of CREB contributes to metastatic potential in melanoma cells and is oncogenic in myeloid lines [46]. Genotype-phenotype correlation is imperfect in these GI tract CCS-like tumors such that either the *EWSR1/ATF1* or *EWSR1/CREB1* may occur in either tumor morphology described above [46].

Due to the rarity of CCS cases occurring in the GI tract, the first hurdle is considering the diagnosis. RT-PCR or FISH analyses are critical to the diagnosis of CCS, given its morphologic and immunophenotypic overlap with melanoma (Figure 8). It remains to be determined whether these

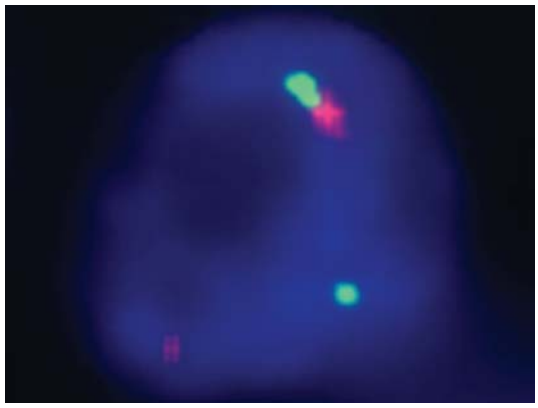

Figure 8: Photomicrograph of FISH break apart probe that targets the *EWSR1* gene. The normal chromosome (upper middle) shows a green signal and red signal in close proximity, whereas the green and red signals are far apart in the lower aspect of the photomicrograph, confirming *EWSR1* translocation.

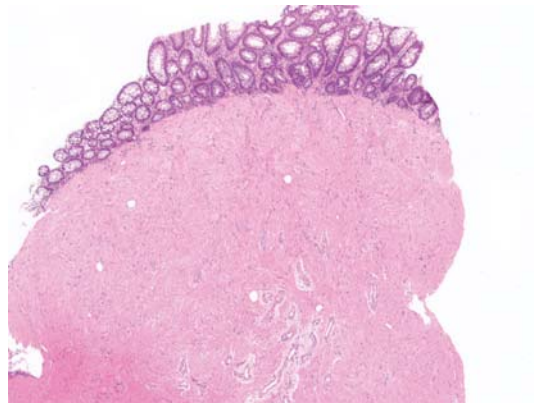

Figure 9: Photomicrograph of H&E-stained section from a leiomyomatous polyp. The leiomyoma is composed of bland smooth muscle cells with eosinophilic cytoplasm. The tumor is directly apposed to the colonic mucosa, suggesting derivation from the muscularis mucosae.

S-100 protein positive tumors harboring *EWSR1* translocations represent a clinical, morphologic, immunophenotype, and genetic spectrum of one tumor [46] or are two distinct tumors [43].

6. Synovial Sarcoma (SS)

Primary GI SSs are quite uncommon. The Armed Forces Institute of Pathology (AFIP) reported a series of 10 SSs after undertaking a 30-year review of stomach mesenchymal neoplasms from 3 large centers. Prior to this series, there were 7 total reports of SS, 6 of which involved the esophagus, 1 involved the stomach, and all were biphasic. The AFIP series found 4 males and 6 females with an age range of 29–68 years. Two of the 8 patients with adequate followup died of their disease and two more showed local recurrence. Most tumors form a plaque-like mucosal mass and show uniform spindle cells in a haphazard arrangement with a collagenous background. Calcification or osseous metaplasia may be present. Nine of the 10 cases were of the monophasic type with one showing a poorly differentiated round cell component with high mitotic activity. The final case was biphasic, demonstrating a mixture of epithelial and spindled components. Mitotic activity is variable and can be greater than 50 per 10 HPF. All 7 tumors tested in the AFIP series showed the character *SYT/SSX* fusion transcripts (3 with *SYT/SSX1* and 4 with *SYT/SSX2*) [49].

The characteristic molecular alteration in SS is the t(X;18)(p11;q11) translocation, which usually fuses *SYT* on chromosome 18 with *SSX1* or *SSX2* on the X chromosome. Other less common fusion partners include *SSX4*, also located on the X chromosome. An *SYT* homolog, *SS18L1*, located on chromosome 20 also has been described as a fusion partner of *SSX1* in SS [50]. The functions of these gene products are unknown, and the oncogenic effects of the *SYT/SSX* fusion protein are unclear. Other genetic events may be necessary for sarcomagenesis and other molecular alterations have been described, including ERBB2 expression, IGF2 upregulation, CD44 repression, PTEN inactivation, and mutations associated with the wnt pathway [28]. Break-apart FISH probes are invaluable at our institution in confirming the diagnosis of SS, particularly in monophasic fibrous types, but PCR-based assays are used effectively at other institutions [50].

7. Smooth Muscle Tumors

Leiomyomas of the GI tract show a male predominance and are most common in the colon and rectum, where they actually outnumber GIST [1]. The vast majority of GI leiomyomas are benign smooth muscle proliferations arising from the muscularis mucosae, often giving a polypoid endoscopic appearance (Figure 9) [51]. Rarely, cytologic atypia ("symplastic" leiomyoma) or even more rarely, mitotic activity may occur [18]. The second most common location for GI leiomyoma is in the distal esophagus, where they also outnumber GIST [51]. Esophageal leiomyomas usually are intramural in location, arising in the muscularis propria. Esophageal leiomyomas often show undulating borders and can range in size from <1 mm to >10 cm [1]. Various X chromosomal abnormalities, including collagen type IV alpha 5 and alpha 6, have been described in esophageal leiomyomas, which potentially accounts for the male predominance in these neoplasms [18]. GI leiomyomas may be confused with GISTs, and immunohistochemistry in this differential diagnosis can be quite helpful. Leiomyomas are invariably strongly positive for desmin, but are negative for CD117. One must not confuse CD117-positive mast cells that may be seen in between smooth muscles cells from true CD117 immunoreactivity in the smooth muscle cells [52].

GI leiomyosarcomas are quite rare [18, 53]. They are generally large masses and are characterized by cytologic atypia, high mitotic rate, and necrosis. Extra-intestinal leiomyosarcoma may show complex cytogenetic abnormalities [29] and have been recently shown to have loss of chromosomes 10q

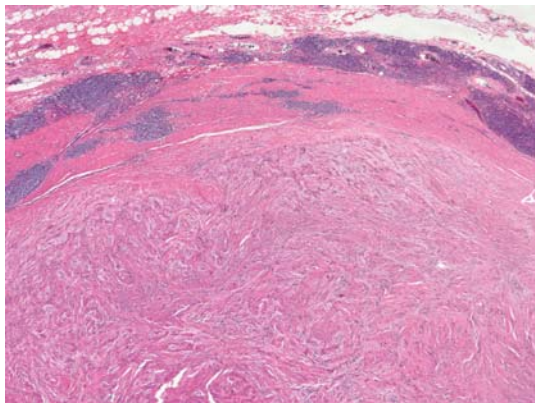

Figure 10: Photomicrograph of H&E-stained section from a gastric schwannoma. These tumors are often more easily recognized by their prominent lymphoid reaction at the periphery and lack of encapsulation rather than classic soft tissue schwannoma features such as Verocay bodies or thick, hyalinized vessels.

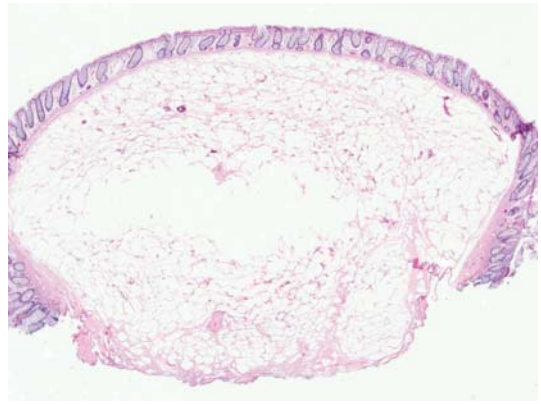

Figure 11: Photomicrograph of H&E-stained section from a submucosal lipoma in the colon. The tumor is composed of mature fibroadipose tissue with sharp demarcation from the overlying mucosa.

and 13q along with amplification of 17p13.1 to 11.2 [54], but the molecular pathology of GI leiomyosarcoma is unknown.

8. Schwannoma

Schwannomas are most commonly encountered in the stomach [55] but are found throughout the GI tract [56, 57]. They may present as polyps or intramural masses. Their histologic appearance is often somewhat different than their extra-GI counterparts (Figure 10). GI schwannomas usually lack well-developed nuclear palisading, hyalinized vessel walls, or a capsule. Only rarely are the characteristic Antoni A and B patterns well developed, and Verocay bodies usually are not encountered. Distinguishing most schwannomas requires observation of the typical "wavy" nuclei with tapered ends; however, a minority may demonstrate an epithelioid morphology [57]. Regardless of morphology, all tumors demonstrate diffuse S-100 protein immunoreactivity while lacking CD117 positivity. Another characteristic feature of GI schwannomas is a prominent lymphoid infiltrate at the tumor periphery. GI schwannomas are benign and do not seem to occur in the setting of NF2 [1]. In addition, GI schwannomas show no *NF2* mutations and only rare LOH of ch. 22q [56], which is different than their soft tissue counterparts. Rare examples tested for *KIT* mutations have been wild type [58].

9. Mesenchymal Polyps

A variety of mesenchymal lesions present as incidentally identified polyps during routine endoscopic procedures (usually colonoscopy), including perineuriomas/fibroblastic polyps [59], muscularis mucosae leiomyomas [51], Schwann cell "hamartomas," [60], granular cell tumors [61], elastofibromatous polyps [62], and ganglioneuromas [63]. Colonic perineuriomas/fibroblastic polyps are often associated with a hyperplastic polyp-like component with serrated epithelial crypts. A recent study reported BRAF mutations in 63% of the epithelial component of these polyps. The authors concluded that the polyps are true mixed epithelial-mesenchymal neoplasms, but there remains no conclusive evidence that the spindle cell proliferation is truly neoplastic [64]. Ganglioneuromas are benign lesions composed of ganglion cells, nerve fibers, and Schwann cells [60] that often arise in the setting of inherited tumor syndromes, including Cowden syndrome (PTEN mutations), MEN 2B, and NF1 [60], among others. Solitary ganglioneuromas are not considered to be a marker of an inherited syndrome. Aside from syndrome-associated ganglioneuromas, the molecular pathologic aspects of these benign polypoid mesenchymal lesions are uncharacterized.

10. Adipocytic Tumors

Lipomas are most common in the right colon and usually are identified as small intramural polypoid lesions. They are usually centered within the submucosa and composed of mature-appearing adipocytes that are relatively uniform in size and lack cytologic atypia (Figure 11). These lesions are often endoscopically suspected after eliciting the "pillow sign" with closed biopsy forceps [65]. No molecular characterization of GI lipomas exists; conventional soft tissue lipomas exhibit abnormal karyotypes in about 60% of cases, most commonly involving rearrangement of chromosome 12q13~15 encompassing the chromatin remodeling gene *HGMA2* [66].

Primary GI liposarcomas are exceptionally rare tumors. Most liposarcomas that involve the gut arise within the retroperitoneum, and this is one of the more common causes of a sarcoma presenting as a GI mass. Typical well-differentiated liposarcomas are fatty tumors that demonstrate large, atypical cells embedded within fibrous septa or between the fat cells, but other morphologies such as inflammatory or sclerosing exist. Conventional liposarcoma may de-differentiate and at least partially loose their typical well-differentiated component. About 80% of well-differentiated

liposarcomas are characterized by ring or giant marker chromosomes derived from chromosome 12q13~q15, including *MDM2, HGMA2*, and other genes [67]. When more sensitive methods such as FISH, PCR, or immunohistochemistry are employed, >95% of tumors show MDM2 amplification [68, 69]. Therefore, assessing for amplification of *MDM2* by FISH or immunohistochemistry is a powerful tool in supporting the diagnosis liposarcoma secondarily involving the GI tract.

11. Glomus Tumors

These tumors are morphologically similar to their counterparts that are most commonly encountered in the distal extremities. The vast majority of GI glomus tumors have been documented to occur in the stomach with fewer occurring in the intestines and <150 cases have been reported in the English literature [70]. Glomus tumors are composed of a proliferation of sharply demarcated modified smooth muscle cells, which are often arranged around dilated staghorn vessels. The cells contain a round nucleus and pale cytoplasm and generally low mitotic activity. Focal atypia and vascular invasion reportedly are common. In the AFIP series of 32 gastric glomus tumors, none of the 5 tumors tested for mutations in exons 9 or 11 of the KIT gene showed a mutation [70]. Otherwise, the molecular pathology of glomus tumors is unknown.

12. Conclusion

Primary mesenchymal tumors of the GI tract are rare, but like their soft tissue counterparts, molecular pathology often plays a critical role in the work-up of these tumors. Molecular pathology plays a major role the diagnosis of these tumors, particularly in clear cell sarcoma, inflammatory myofibroblastic tumor, synovial sarcoma, and liposarcoma, among others. Powerful prognostic data also is emerging such as in inflammatory myofibroblastic tumor with *ALK* rearrangements and possibly *CTNNB1* mutations in desmoids. The future of molecular pathology is in predictive molecular testing—molecular pathology tests aimed at aiding our clinical colleagues in selecting the best treatments for our patients. Although personalized therapy is not standard of care yet in these rare tumors, the recent ALK antagonist case reports in IMTs suggest that it is only a matter of time.

References

[1] S. C. Abraham, "Distinguishing gastrointestinal stromal tumors from their mimics: an update," *Advances in Anatomic Pathology*, vol. 14, no. 3, pp. 178–188, 2007.

[2] A. R. Latchford, N. J. H. Sturt, K. Neale, P. A. Rogers, and R. K. S. Phillips, "A 10-year review of surgery for desmoid disease associated with familial adenomatous polyposis," *British Journal of Surgery*, vol. 93, no. 10, pp. 1258–1264, 2006.

[3] J. A. Rodriguez, L. A. Guarda, and J. Rosai, "Mesenteric fibromatosis with involvement of the gastrointestinal tract. A GIST simulator: a study of 25 cases," *American Journal of Clinical Pathology*, vol. 121, no. 1, pp. 93–98, 2004.

[4] R. K. Yantiss, I. J. Spiro, C. C. Compton, and A. E. Rosenberg, "Gastrointestinal stromal tumor versus intra-abdominal fibromatosis of the bowel wall: a clinically important differential diagnosis," *American Journal of Surgical Pathology*, vol. 24, no. 7, pp. 947–957, 2000.

[5] T. S. Emory, J. M. Monihan, N. J. Carr, and L. H. Sobin, "Sclerosing mesenteritis, mesenteric panniculitis and mesenteric lipodystrophy: a single entity?" *American Journal of Surgical Pathology*, vol. 21, no. 4, pp. 392–398, 1997.

[6] Y. Zen, M. Onodera, D. Inoue et al., "Retroperitoneal fibrosis: a clinicopathologic study with respect to immunoglobulin G4," *American Journal of Surgical Pathology*, vol. 33, no. 12, pp. 1833–1839, 2009.

[7] H. Lamlum, M. Ilyas, A. Rowan et al., "The type of somatic mutation at APC in familial adenomatous polyposis is determined by the site of the germline mutation: a new facet to Knudson's 'two-hit' hypothesis," *Nature Medicine*, vol. 5, no. 9, pp. 1071–1075, 1999.

[8] R. Caspari, S. Olschwang, W. Friedl et al., "Familial adenomatous polyposis: desmoid tumours and lack of ophthalmic lesions (CHRPE) associated with APC mutations beyond codon 1444," *Human Molecular Genetics*, vol. 4, no. 3, pp. 337–340, 1995.

[9] Y. L. Wallis, D. G. Morton, C. M. McKeown, and F. Macdonald, "Molecular analysis of the APC gene in 205 families: extended genotype-phenotype correlations in FAP and evidence for the role of APC amino acid changes in colorectal cancer predisposition," *Journal of Medical Genetics*, vol. 36, no. 1, pp. 14–20, 1999.

[10] B. A. Alman, C. Li, M. E. Pajerski, S. Diaz-Cano, and H. J. Wolfe, "Increased β-catenin protein and somatic APC mutations in sporadic aggressive fibromatoses (desmoid tumors)," *American Journal of Pathology*, vol. 151, no. 2, pp. 329–334, 1997.

[11] S. Tejpar, F. Nollet, C. Li et al., "Predominance of beta-catenin mutations and beta-catenin dysregulation in sporadic aggressive fibromatosis (desmoid tumor)," *Oncogene*, vol. 18, no. 47, pp. 6615–6620, 1999.

[12] S. Salas, F. Chibon, T. Noguchi et al., "Molecular characterization by array comparative genomic hybridization and DNA sequencing of 194 desmoid tumors," *Genes Chromosomes and Cancer*, vol. 49, no. 6, pp. 560–568, 2010.

[13] A. J. F. Lazar, D. Tuvin, S. Hajibashi et al., "Specific mutations in the β-Catenin gene (CTNNB1) correlate with local recurrence in sporadic desmoid tumors," *American Journal of Pathology*, vol. 173, no. 5, pp. 1518–1527, 2008.

[14] J. Dômont, S. Salas, L. Lacroix et al., "High frequency of β-catenin heterozygous mutations in extra-abdominal fibromatosis: a potential molecular tool for disease management," *British Journal of Cancer*, vol. 102, no. 6, pp. 1032–1036, 2010.

[15] M. C. Heinrich, H. Joensuu, G. D. Demetri et al., "Phase II, open-label study evaluating the activity of imatinib in treating life-threatening malignancies known to be associated with imatinib- sensitivetyrosine kinases," *Clinical Cancer Research*, vol. 14, no. 9, pp. 2717–2725, 2008.

[16] M. C. Heinrich, G. A. McArthur, G. D. Demetri et al., "Clinical and molecular studies of the effect of imatinib on advanced aggressive fibromatosis (desmoid tumor)," *Journal of Clinical Oncology*, vol. 24, no. 7, pp. 1195–1203, 2006.

[17] J. K. Greenson, "Gastrointestinal stromal tumors and other mesenchymal lesions of the gut," *Modern Pathology*, vol. 16, no. 4, pp. 366–375, 2003.

[18] M. Miettinen, M. Furlong, M. Sarlomo-Rikala, A. Burke, L. H. Sobin, and J. Lasota, "Gastrointestinal stromal tumors, intramural leiomyomas, and leiomyosarcomas in the rectum and anus: a clinicopathologic, immunohistochemical, and

molecular genetic study of 144 cases," *American Journal of Surgical Pathology*, vol. 25, no. 9, pp. 1121–1133, 2001.

[19] R. Chugh, J. K. Wathen, S. R. Patel et al., "Efficacy of imatinib in aggressive fibromatosis: results of a phase II multicenter Sarcoma Alliance for Research through Collaboration (SARC) trial," *Clinical Cancer Research*, vol. 16, no. 19, pp. 4884–4891, 2010.

[20] V. P. De Camargo, M. L. Keohan, D. R. D'Adamo et al., "Clinical outcomes of systemic therapy for patients with deep fibromatosis (desmoid tumor)," *Cancer*, vol. 116, no. 9, pp. 2258–2265, 2010.

[21] C. M. Coffin, P. A. Humphrey, and L. P. Dehner, "Extrapulmonary inflammatory myofibroblastic tumor: a clinical and pathological survey," *Seminars in Diagnostic Pathology*, vol. 15, no. 2, pp. 85–101, 1998.

[22] C. M. Coffin, J. Watterson, J. R. Priest, and L. P. Dehner, "Extrapulmonary inflammatory myofibroblastic tumor (inflammatory pseudotumor): a clinicopathologic and immunohistochemical study of 84 cases," *American Journal of Surgical Pathology*, vol. 19, no. 8, pp. 859–872, 1995.

[23] C. M. Coffin, J. L. Hornick, and C. D. M. Fletcher, "Inflammatory myofibroblastic tumor: comparison of clinicopathologic, histologic, and immunohistochemical features including ALK expression in atypical and aggressive cases," *American Journal of Surgical Pathology*, vol. 31, no. 4, pp. 509–520, 2007.

[24] J. R. Cook, L. P. Dehner, M. H. Collins et al., "Anaplastic lymphoma kinase (ALK) expression in the inflammatory myofibroblastic tumor: a comparative immunohistochemical study," *American Journal of Surgical Pathology*, vol. 25, no. 11, pp. 1364–1371, 2001.

[25] A. Marino-Enriquez, W. L. Wang, A. Roy et al., "Epithelioid inflammatory myofibroblastic sarcoma: an aggressive intra-abdominal variant of inflammatory myofibroblastic tumor with nuclear membrane or perinuclear ALK," *American Journal of Surgical Pathology*, vol. 35, no. 1, pp. 135–144, 2011.

[26] J. E. Butrynski, D. R. D'Adamo, J. L. Hornick et al., "Crizotinib in ALK-rearranged inflammatory myofibroblastic tumor," *New England Journal of Medicine*, vol. 363, no. 18, pp. 1727–1733, 2010.

[27] T. Sasaki, K. Okuda, W. Zheng et al., "The neuroblastoma-associated F1174L ALK mutation causes resistance to an ALK kinase inhibitor in ALK-translocated cancers," *Cancer Research*, vol. 70, no. 24, pp. 10038–10043, 2010.

[28] C. Fisher, "Soft tissue sarcomas with non-EWS translocations: molecular genetic features and pathologic and clinical correlations," *Virchows Archiv*, vol. 456, no. 2, pp. 153–166, 2010.

[29] B. P. Rubin, A. J. Lazar, and A. M. Oliviera, "Molecular pathology of bone and soft tissue tumors," in *Cell and Tissue Based Molecular Pathology*, R. T. Tubbs and M. H. Stoler, Eds., pp. 325–359, Churchill Livingstone, Philadelphia, Pa, USA, 2009.

[30] B. Lawrence, A. Perez-Atayde, M. K. Hibbard et al., "TPM3-ALK and TPM4-ALK oncogenes in inflammatory myofibroblastic tumors," *American Journal of Pathology*, vol. 157, no. 2, pp. 377–384, 2000.

[31] J. Cools, I. Wlodarska, R. Somers et al., "Identification of novel fusion partners of ALK, the anaplastic lymphoma kinase, in anaplastic large-cell lymphoma and inflammatory myofibroblastic tumor," *Genes Chromosomes and Cancer*, vol. 34, no. 4, pp. 354–362, 2002.

[32] M. Debiec-Rychter, P. Marynen, A. Hagemeijer, and P. Pauwels, "ALK-ATIC fusion in urinary bladder inflammatory myofibroblastic tumor," *Genes Chromosomes and Cancer*, vol. 38, no. 2, pp. 187–190, 2003.

[33] I. Panagopoulos, T. Nilsson, H. A. Domanski et al., "Fusion of the SEC31L1 and ALK genes in an inflammatory myofibroblastic tumor," *International Journal of Cancer*, vol. 118, no. 5, pp. 1181–1186, 2006.

[34] Z. Ma, D. A. Hill, M. H. Collins et al., "Fusion of ALK to the Ran-binding protein 2 (RANBP2) gene in inflammatory myofibroblastic tumor," *Genes Chromosomes and Cancer*, vol. 37, no. 1, pp. 98–105, 2003.

[35] J. A. Bridge, M. Kanamori, Z. Ma et al., "Fusion of the ALK gene to the clathrin heavy chain gene, CLTC, in inflammatory myofibroblastic tumor," *American Journal of Pathology*, vol. 159, no. 2, pp. 411–415, 2001.

[36] M. Miettinen, Z. F. Wang, and J. Lasota, "DOG1 antibody in the differential diagnosis of gastrointestinal stromal tumors: a study of 1840 cases," *American Journal of Surgical Pathology*, vol. 33, no. 9, pp. 1401–1408, 2009.

[37] J. A. Carney and C. A. Stratakis, "Stromal, fibrous, and fatty gastrointestinal tumors in a patient with a PDGFRA gene mutation," *American Journal of Surgical Pathology*, vol. 32, no. 9, pp. 1412–1420, 2008.

[38] T. de Raedt, J. Cools, M. Debiec-Rychter et al., "Intestinal neurofibromatosis is a subtype of familial GIST and results from a dominant activating mutation in PDGFRA," *Gastroenterology*, vol. 131, no. 6, pp. 1907–1912, 2006.

[39] O. Daum, J. Hatlova, V. Mandys et al., "Comparison of morphological, immunohistochemical, and molecular genetic features of inflammatory fibroid polyps (Vanek's tumors)," *Virchows Archiv*, vol. 456, no. 5, pp. 491–497, 2010.

[40] H. U. Schildhaus, T. Caviar, E. Binot, R. Büttner, E. Wardelmann, and S. Merkelbach-Bruse, "Inflammatory fibroid polyps harbour mutations in the platelet-derived growth factor receptor alpha (PDGFRA) gene," *Journal of Pathology*, vol. 216, no. 2, pp. 176–182, 2008.

[41] J. Lasota, Z. F. Wang, L. H. Sobin, and M. Miettinen, "Gain-of-function PDGFRA mutations, earlier reported in gastrointestinal stromal tumors, are common in small intestinal inflammatory fibroid polyps. A study of 60 cases," *Modern Pathology*, vol. 22, no. 8, pp. 1049–1056, 2009.

[42] J. Andrae, R. Gallini, and C. Betsholtz, "Role of platelet-derived growth factors in physiology and medicine," *Genes and Development*, vol. 22, no. 10, pp. 1276–1312, 2008.

[43] K. Kosemehmetoglu and A. L. Folpe, "Clear cell sarcoma of tendons and aponeuroses, and osteoclast-rich tumour of the gastrointestinal tract with features resembling clear cell sarcoma of soft parts: a review and update," *Journal of Clinical Pathology*, vol. 63, no. 5, pp. 416–423, 2010.

[44] M. Hantschke, T. Mentzel, A. Rütten et al., "Cutaneous clear cell sarcoma: a clinicopathologic, immunohistochemical, and molecular analysis of 12 cases emphasizing its distinction from dermal melanoma," *American Journal of Surgical Pathology*, vol. 34, no. 2, pp. 216–222, 2010.

[45] I. Panagopoulos, F. Mertens, M. Dêbiec-Rychter et al., "Molecular genetic characterization of the EWS/ATF1 fusion gene in clear cell sarcoma of tendons and aponeuroses," *International Journal of Cancer*, vol. 99, no. 4, pp. 560–567, 2002.

[46] C. R. Antonescu, K. Nafa, N. H. Segal, P. Dal Cin, and M. Ladanyi, "EWS-CREB1: a recurrent variant fusion in clear cell sarcoma—association with gastrointestinal location and absence of melanocytic differentiation," *Clinical Cancer Research*, vol. 12, no. 18, pp. 5356–5362, 2006.

[47] M. Hisaoka, T. Ishida, T. T. Kuo et al., "Clear cell sarcoma of soft tissue: a clinicopathologic, immunohistochemical, and molecular analysis of 33 cases," *American Journal of Surgical Pathology*, vol. 32, no. 3, pp. 452–460, 2008.

[48] W. L. Wang, E. Mayordomo, W. Zhang et al., "Detection and characterization of EWSR1/ATF1 and EWSR1/CREB1 chimeric transcripts in clear cell sarcoma (melanoma of soft parts)," *Modern Pathology*, vol. 22, no. 9, pp. 1201–1209, 2009.

[49] H. R. Makhlouf, W. Ahrens, B. Agarwal et al., "Synovial sarcoma of the stomach: a clinicopathologic, immunohistochemical, and molecular genetic study of 10 cases," *American Journal of Surgical Pathology*, vol. 32, no. 2, pp. 275–281, 2008.

[50] M. R. Tanas and J. R. Goldblum, "Fluorescence in situ hybridization in the diagnosis of soft tissue neoplasms: a review," *Advances in Anatomic Pathology*, vol. 16, no. 6, pp. 383–391, 2009.

[51] A. Agaimy and P. H. Wünsch, "True smooth muscle neoplasms of the gastrointestinal tract: morphological spectrum and classification in a series of 85 cases from a single institute," *Langenbeck's Archives of Surgery*, vol. 392, no. 1, pp. 75–81, 2007.

[52] N. A. C. S. Wong and J. Pawade, "Mast cell-rich leiomyomas should not be mistaken for gastrointestinal stromal tumours," *Histopathology*, vol. 51, no. 2, pp. 273–275, 2007.

[53] M. Miettinen, J. Kopczynski, H. R. Makhlouf et al., "Gastrointestinal stromal tumors, intramural leiomyomas, and leiomyosarcomas in the duodenum: a clinicopathologic, immunohistochemical, and molecular genetic study of 167 cases," *American Journal of Surgical Pathology*, vol. 27, no. 5, pp. 625–641, 2003.

[54] L. A. Meza-Zepeda, S. H. Kresse, A. H. Barragan-Polania et al., "Array comparative genomic hybridization reveals distinct DNA copy number differences between gastrointestinal stromal tumors and leiomyosarcomas," *Cancer Research*, vol. 66, no. 18, pp. 8984–8993, 2006.

[55] A. Agaimy, B. Märkl, J. Kitz et al., "Peripheral nerve sheath tumors of the gastrointestinal tract: a multicenter study of 58 patients including NF1-associated gastric schwannoma and unusual morphologic variants," *Virchows Archiv*, vol. 456, no. 4, pp. 411–422, 2010.

[56] J. Lasota, B. Wasag, A. Dansonka-Mieszkowska et al., "Evaluation of NF2 and NF1 tumor suppressor genes in distinctive gastrointestinal nerve sheath tumors traditionally diagnosed as benign schwannomas: a study of 20 cases," *Laboratory Investigation*, vol. 83, no. 9, pp. 1361–1371, 2003.

[57] M. Miettinen, K. M. Shekitka, and L. H. Sobin, "Schwannomas in the colon and rectum: a clinicopathologic and immunohistochemical study of 20 cases," *American Journal of Surgical Pathology*, vol. 25, no. 7, pp. 846–855, 2001.

[58] Y. Y. Hou, Y. S. Tan, J. F. Xu et al., "Schwannoma of the gastrointestinal tract: a clinicopathological, immunohistochemical and ultrastructural study of 33 cases," *Histopathology*, vol. 48, no. 5, pp. 536–545, 2006.

[59] G. M. Groisman and S. Polak-Charcon, "Fibroblastic polyp of the colon and colonic perineurioma: names for a single entity?" *American Journal of Surgical Pathology*, vol. 32, no. 7, pp. 1088–1094, 2008.

[60] J. A. Gibson and J. L. Hornick, "Mucosal schwann cell "hamartoma": clinicopathologic study of 26 neural colorectal polyps distinct from neurofibromas and mucosal neuromas," *American Journal of Surgical Pathology*, vol. 33, no. 5, pp. 781–787, 2009.

[61] A. D. Singhi and E. A. Montgomery, "Colorectal granular cell tumor: a clinicopathologic study of 26 cases," *American Journal of Surgical Pathology*, vol. 34, no. 8, pp. 1186–1192, 2010.

[62] C. M. Hobbs, D. M. Burch, and L. H. Sobin, "Elastosis and elastofibromatous change in the gastrointestinal tract: a clinicopathologic study of 13 cases and a review of the literature," *American Journal of Clinical Pathology*, vol. 122, no. 2, pp. 232–237, 2004.

[63] K. M. Shekitka and L. H. Sobin, "Ganglioneuromas of the gastrointestinal tract: relation to von Recklinghausen disease and other multiple tumor syndromes," *American Journal of Surgical Pathology*, vol. 18, no. 3, pp. 250–257, 1994.

[64] A. Agaimy, R. Stoehr, M. Vieth, and A. Hartmann, "Benign serrated colorectal fibroblastic polyps/intramucosal perineuriomas are true mixed epithelial-stromal polyps (hybrid hyperplastic polyp/mucosal perineurioma) with frequent BRAF mutations," *American Journal of Surgical Pathology*, vol. 34, no. 11, pp. 1663–1671, 2010.

[65] M. A. Murray, V. Kwan, S. J. Williams, and M. J. Bourke, "Detachable nylon loop assisted removal of large clinically significant colonic lipomas," *Gastrointestinal Endoscopy*, vol. 61, no. 6, pp. 756–759, 2005.

[66] A. A. Sandberg, "Updates on the cytogenetics and molecular genetics of bone and soft tissue tumors: lipoma," *Cancer Genetics and Cytogenetics*, vol. 150, no. 2, pp. 93–115, 2004.

[67] A. A. Sandberg, "Updates on the cytogenetics and molecular genetics of bone and soft tissue tumors: liposarcoma," *Cancer Genetics and Cytogenetics*, vol. 155, no. 1, pp. 1–24, 2004.

[68] M. B. N. Binh, X. Sastre-Garau, L. Guillou et al., "MDM2 and CDK4 immunostainings are useful adjuncts in diagnosing well-differentiated and dedifferentiated liposarcoma subtypes: a comparative analysis of 559 soft tissue neoplasms with genetic data," *American Journal of Surgical Pathology*, vol. 29, no. 10, pp. 1340–1347, 2005.

[69] J. Weaver, E. Downs-Kelly, J. R. Goldblum et al., "Fluorescence in situ hybridization for MDM2 gene amplification as a diagnostic tool in lipomatous neoplasms," *Modern Pathology*, vol. 21, no. 8, pp. 943–949, 2008.

[70] M. Miettinen, E. Paal, J. Lasota, and L. H. Sobin, "Gastrointestinal glomus tumors: a clinicopathologic, immunohistochemical, and molecular genetic study of 32 cases," *American Journal of Surgical Pathology*, vol. 26, no. 3, pp. 301–311, 2002.

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Review Article

EGFR Signaling in Colorectal Carcinoma

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The epidermal growth factor receptor (EGFR) and its downstream signaling pathways are involved in the development and progression of several human tumors, including colorectal cancer. Much attention has been given to the EGFR pathway as of lately because both EGFR and some downstream components serve as targets for anticancer therapy. In addition to playing a critical role in targeted therapy, alterations in this pathway can have prognostic implications. The EGFR pathway and its impact on colorectal carcinogenesis and prognosis are the emphasis of this paper. Since prognosis is tightly related to response to various therapies, the predictive value of the components of this pathway will be briefly discussed, but this is not the focus of this paper.

1. Introduction

The epidermal growth factor receptor (EGFR) and its downstream signaling pathways regulate key cellular events that drive the progression of many neoplasms. EGFR is expressed in a variety of human tumors, including gliomas and carcinomas of the lung, colon, head and neck, pancreas, breast, ovary, bladder, and kidney. Mutations, gene amplification, and protein overexpression of various elements of this pathway not only contribute to carcinogenesis but also impact prognosis and provide specific targets for therapeutic intervention. The importance of EGFR and its signaling pathway in colorectal carcinogenesis is the topic of this paper. Since prognosis is tightly related to response to various therapies, the predictive value of the components of this pathway will be discussed, but only briefly. There is another paper in this series, "Impact of *KRas* mutations on management of colorectal cancer" by Sullivan and Kozuch, which provides an in-depth review of the predictive value of *KRas* and other members of the EGFR signaling pathway.

2. EGFR and the EGFR Signaling Pathway

EGFR is a 170-kDa transmembrane tyrosine kinase receptor that belongs to the ErbB family of cell membrane receptors. In addition to EGFR (also known as HER1 and ErbB-1), other receptors in this family include HER2/c-neu (ErbB-2), Her 3 (ErbB-3), and Her 4 (ErbB-4). All of these receptors contain an extracellular ligand-binding region, a single membrane-spanning region, and a cytoplasmic tyrosine-kinase-containing domain.

In normal cells, the EGFR signaling cascade begins with ligand activation of EGFR (Figure 1). Up to eleven ligands can bind the ErbB family of receptors, including EGF and transforming growth factoralpha [1]. Ligand binding induces dimerization of the receptor with formation of homodimers and heterodimers, which leads to the activation of tyrosine kinase. The intracellular tyrosine kinase residues then become autophosphorylated, inducing activation of multiple signal transduction pathways. Two main intracellular pathways activated by EGFR are the mitogen-activated protein kinase (MAPK) pathway and the phosphatidylinositol 3-kinase- (PI3K-) protein kinase B (AKT) pathway. These pathways lead to the activation of various transcription factors that then impact cellular responses such as proliferation, migration, differentiation, and apoptosis [2].

Signaling through the EGFR pathway is a complex process that requires tight regulation [2]. The first level of complexity is encountered at the receptor level, where

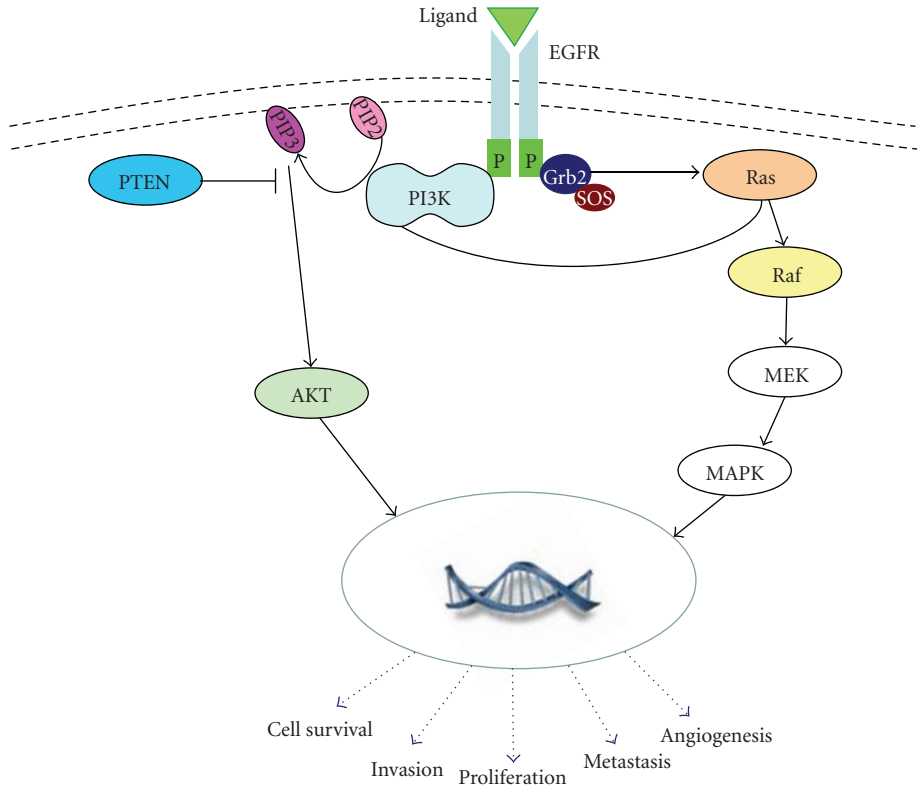

Figure 1: EGFR signaling pathway. Ligand binding induces dimerization and activates the EGFR. Subsequent autophosphorylation of tyrosine residues activates downstream signaling. In the Ras-Raf-MEK-MAPK, one axis of the EGFR signaling cascade, an adaptor protein complex composed of growth factor receptor-bound protein 2 adapter protein (Grb2), which harbors a tyrosine phosphate-docking site, and son of sevenless (SOS), a Ras GDP/GTP exchange factor, then activates the Ras GTPase. After activation, Ras (i.e., KRas) recruits and activates the serine protein Raf (i.e., B-Raf), and subsequent phosphorylation and activation of MEK and then MAPK occurs, resulting in activation of transcription factors in the cell nucleus. The Ras-Raf-MAPK signaling pathway is thought to control cell growth, differentiation, and survival (?apoptosis). The other axis of the EGFR signaling cascade that is important in colorectal carcinogenesis is the PI3K-AKT pathway. Once the EFGR tyrosine residues are phosphorylated, PI3K is translocated to the cell membrane and binds to tyrosine phosphate (through its adaptor subunit p85) which triggers the PI3K catalytic subunit p110 to produce phosphatidylinositol-3,4,5-triphosphate (PIP3). PI3K then promotes AKT activation. Activated AKT (p-AKT), present within the cytoplasm, then activates various targets that result in cell growth, proliferation, and survival (paralleling the Ras-Raf-MEK-MAPK signaling pathway). Importantly, these two axes are closely related and have some overlap. For example, the p110 subunit of PI3K can also be activated via interaction with Ras. Of note, phosphatase with tensin homology (PTEN) is a phosphatase that converts PIP3 back to phosphatidylinositol (4, 5) bisphosphate (PIP2), thereby negatively regulating the PI3K-AKT pathway.

multiple ligands are shared and lateral signaling occurs between members of the ErbB family. Then there are positive and negative feedback loops built into the pathways and differential activation of transcription factors, depending upon the cell type. When this tightly regulated system goes awry, it can contribute to malignant transformation and tumor progression through increased cell proliferation, prolonged survival, angiogenesis, antiapoptosis, invasion, and metastasis [3, 4].

3. The EGFR Pathway and Colorectal Carcinogenesis (Table 1)

3.1. EGFR Protein Expression. EGFR expression (or overexpression), typically determined by immunohistochemistry, has been found to be associated with tumor progression and poor survival in various malignancies, such as carcinomas of the head and neck [5]. However, the significance of EGFR protein expression is controversial in other tumors, such as lung carcinomas [6]. Although EGFR has been reported to be overexpressed in anywhere from 25% to 82% of colorectal cancers [4], some recent studies report protein overexpression (defined as 2+ and/or 3+ staining or in >50% of cells) in 35 to 49% of cases [7–9]. However, the clinical significance of EGFR overexpression in colorectal cancer is uncertain. While one study of 249 colorectal cancers demonstrated an association of EGFR overexpression with tumor grade (poor differentiation) ($P = .014$) [8], another group found no association with grade in 134 tumors [9]. Similarly, some studies have found an association between EGFR overexpression (defined as 2+ or 3+ intensity) and reduced survival [7, 9], while others have not [4].

Due to the known expression of EGFR in colorectal cancer, a phase II trial of cetuximab, an anti-EGFR monoclonal antibody, in patients with refractory EGFR-positive

Table 1: Components of the EGFR signaling pathway important in colorectal cancer.

Component (gene/protein)	Protein function	Defect in CRC	Frequency	Impact	
				Prognostic	Predictive (to anti-EGFR therapy)
EGFR/EGFR	Transmembrane tyrosine kinase receptor	Protein expression	25–90%	Controversial	No correlation
		Mutation	Rare	Unknown	Unknown
		Increased copy number	0–50%*	Uncertain	Uncertain
KRas/KRas	GDP-/GTP-binding protein; facilitates ligand-dependent signaling	Activating mutation (codons 12, 13, 61, 146); leads to activation of MAPK pathway	30–40%	Controversial	No response (if *KRas* is mutated)
BRAF/B-Raf	Serine-threonine protein kinase downstream of KRas	Activating mutation (V600E)	5–12%	Poor prognosis in MSS tumors	No response (if *BRAF* is mutated)
PIK3CA/PI3K	A key signal transducer in the PI3K-AKT pathway	Activating mutation (exons 9 and 20)	14–18%	Poor rognosis in KRas wt tumors	No response (if exon 20 is mutated)
PTEN/PTEN	A protein tyrosine phosphatase enzyme; inactivates PI3K pathway	Loss of protein expression; mutation; LOH	13–19%	Poor prognosis in KRas wt tumors	No response (possibly)

CRC: colorectal cancer; LOH: loss of heterozygosity; wt: wild-type.
*Low % for high (>10 copies) amplification; higher % for low number of copies (3–5 copies).

(assessed by immunohistochemistry) colorectal cancer was undertaken [10]. The results of this trial, reported in 2004, were promising. It was soon discovered, however, that there was no correlation between EGFR expression in the tumor and response to therapy [11, 12]. In the study by Chung et al., four of 16 (25%) patients with EGFR-negative tumors who received cetuximab-plus-irinotecan therapy achieved a partial response with a greater than 50% reduction in the size of measurable lesions [11]. This response rate is nearly identical to the 23% response rate seen in a separate cetuximab-plus-irinotecan clinical trial in EGFR-positive patients [12]. As a result, cetuximab is now administered as indicated without the need for EGFR testing.

The wide range of EGFR expression in colorectal cancer reported in the literature, as well as the uncertain significance of EGFR expression as a prognostic indicator, may be related to the methodology used to detect EGFR. Most studies use immunohistochemistry to detect EGFR expression in colorectal cancers. As demonstrated by the experience of HER2 expression in breast cancer, immunohistochemistry is highly dependent on the antibody clone that is used, staining protocols, selection of scoring methods, and selection of cutoff values. Until a standard method of EGFR staining and reporting is adopted, the significance of EGFR protein expression in colorectal cancer remains controversial.

3.2. EGFR Mutations, Gene Amplification, and Copy Number. Mutations affecting the extracellular domain of EGFR, often accompanied by gene amplification, are frequent in glioblastomas [13], while mutations in the tyrosine kinase domain of EGFR, also frequently associated with increased *EGFR* gene copy numbers, are clinically relevant in lung adenocarcinoma [6, 14–16]. Unlike lung cancer and other tumors, *EGFR* gene mutations are uncommon in colorectal cancers [17, 18].

The significance of *EGFR* gene amplification/increased *EGFR* copy number is more difficult to summarize. Some studies report that *EGFR* gene amplification (assessed by in situ hybridization methods) is uncommon in colorectal cancer [19, 20]. In contrast, in recent studies on chemorefractory colon cancers, it appears that modest increases in copy number (three- to fivefold) are present in up to 50% of cases [21]. It appears, however, that increased *EGFR* protein expression does not always translate into increased *EGFR* gene dosage [19, 21, 22]. For example, a study by Shia et al. found that only a small fraction (17 of 124 or 14%) of EGFR-positive (defined as 1+, 2+, or 3+) colorectal carcinomas detected by immunohistochemistry were associated with *EGFR* gene amplification (defined as >5 gene copies/nucleus) [19].

Similarly, the predictive significance of *EGFR* gene amplification is also confusing and uncertain. One study of 47 patients with metastatic colorectal cancer treated with a cetuximab-based regimen showed that EGFR gene copy gain, as assessed by fluorescence in situ hybridization, had no correlation with objective response rate, disease control rate, progression-free survival, or overall survival [23]. Conversely, another study of 173 patients with *KRas* wild-type metastatic colorectal cancer treated with a cetuximab-based regimen found that *EGFR* amplification/increased *EGFR* copy number, present in 17.7% of patients, was associated with response to anti-EGFR therapy [24]. These conflicting results may be related to the fact that there are no established guidelines for EGFR gene amplification. But since there are no guidelines, testing for EGFR gene amplification in colorectal cancer is not routinely performed.

In addition to molecular alterations of the *EGFR* gene, activation of EGFR downstream effectors can lead to tumor formation/progression. Specific alterations can impact prognosis and predict response to anti-EGFR therapy.

3.3. KRas Mutations. The *KRas* proto-oncogene encodes a 21-kDa guanosine 5′-triphosphate- (GTP-) binding protein at the beginning of the MAPK signaling pathway. Somatic *KRas* mutations are found in many cancers, including 30%–40% of colorectal cancers, and are an early event in carcinogenesis [25–29]. *KRas* mutations, most commonly codon 12/13 missense mutations, lead to constitutive activation of the KRas protein by abrogating GTPase activity. These mutations result in unregulated downstream signaling that will not be blocked by antibodies that target the EGFR receptor.

The prognostic significance of *KRas* mutations is controversial. *KRas* mutation status is associated with shorter survival in some studies [28, 30–32], but not others [29, 32, 33]. The results of one study, which showed increased mortality with codon 13 G-A mutations but not with *KRas* mutations in general, suggest that prognosis may be related to specific mutations in the *KRas* gene [28]. Although not predictive of outcome with standard chemotherapy, *KRas* mutation status is a strong predictive marker of resistance to EGFR-targeted therapy in patients with metastatic colorectal cancer (i.e., *KRas* mutations predict a lack of response to anti-EGFR monoclonal antibodies cetuximab and panitumumab) [34–39]; this topic is discussed in detail in another paper in this series, "Impact of *KRas* mutations on management of colorectal cancer" by Sullivan and Kozuch.

3.4. BRAF Mutations. The *BRAF* gene encodes a serine-threonine protein kinase that is downstream of KRas in the MAPK signaling pathway. *BRAF* mutations occur in 5–22% of all colorectal cancers [40, 41]. When separated by microsatellite instability status, *BRAF* mutations are present in 40–52% of colorectal cancers that arise through the microsatellite instability pathway (MSI) pathway (microsatellite unstable tumors) [41–44], but only 5% of cancers are microsatellite stable [42]. The most frequently reported *BRAF* mutation is a valine-to-glutamic acid amino acid (V600E) substitution [45]. *BRAF* mutations are mutually exclusive with *KRas* mutations [41].

Unlike *KRas* mutations, *BRAF* mutations do have an impact on prognosis and survival. In some studies, the effect is dependent upon the microsatellite status of the colorectal cancer. Patients with a *BRAF* mutation in a microsatellite-stable colon cancer have significantly poorer survival than those without the mutation, but the *BRAF* status does not affect survival of patients with microsatellite-unstable tumors [29, 42]. In patients with metastatic *KRas* wild type tumors, *BRAF* mutations have been associated with shorter progression-free and shorter overall survival [24]. *BRAF* status also predicts response to anti-EGFR therapy. Of metastatic colorectal cancers that are found to be *KRas* wild type at codons 12/13, 5% to 15% can harbor *BRAF* mutations and show resistance to anti-EGFR therapy [46, 47]. The predictive role of *BRAF* mutations is further covered in another article in this series, "Impact of *KRas* mutations on management of colorectal cancer."

3.5. The PI3K Pathway-PIK3CA Mutations and Expression of PTEN and p-AKT. The PI3K-AKT pathway can be deregulated by activating mutations in the *PIK3CA* gene (p110 subunit), by inactivation (often by epigenetic mechanisms) of the phosphatase and tensin homolog (*PTEN*) gene, or by activation of AKT [1, 48]. The *PIK3CA* gene encodes phosphatidylinositol 3-kinase (PI3K), a key signal transducer in the PI3K-AKT pathway. Mutations in *PIK3CA* occur in 14% to 18% of colon cancers, and most mutations involve hotspots on exons 9 and 20 [47, 49]. Interestingly, there is a strong association between *PIK3CA* exon 9 mutations and *KRas* mutations [47]. As a prognostic marker, *PIK3CA* mutations are associated with shorter cancer-specific survival, but this effect may be limited to patients with *KRas* wild-type tumors [49]. Briefly, as a predictive marker, only *PIK3CA* exon 20 mutations appear to be associated with worse outcome after cetuximab [47].

The *PTEN* gene encodes a protein tyrosine phosphatase enzyme (PTEN) that dephosphorylates phosphatidylinositol-3,4,5 triphosphate (PIP3) and thereby inhibits PI3K function [1]. Loss of PTEN results in constitutive activation of the PI3K-AKT pathway. *PTEN* mutations and loss of heterozygosity (LOH) of the *PTEN* locus have been reported in 13%–18% and 17%–19% of colon cancers, respectively [50, 51]. It appears that loss of PTEN has prognostic value. Loss of PTEN protein expression (assessed by IHC) is associated with shorter overall survival in patients with *KRas* wild-type tumors [24]. It appears that there is an association with *PTEN* mutations/LOH with MSI status, but the current published results are conflicting [50, 51]. PTEN protein inactivation may also be a negative predictor of response to anti-EGFR therapy [22, 52].

AKT is a major downstream effector of PI3K. A recent study by Baba et al. examined the role of activated (phosphorylated) AKT expression in a large cohort of colorectal cancers [48]. They demonstrated that p-AKT expression is associated with early stage disease and good prognosis. They also showed that p-AKT expression is associated with *PIK3CA* mutation, as expected from their relationship in the EGFR pathway, but that the prognostic effect of p-AKT expression was independent of *PIK3CA* mutation. It is possible that p-AKT expression could serve as positive prognostic marker in patients with colorectal cancer.

In summary, the EGFR signaling pathway is a complex and tightly regulated process that is involved in growth, proliferation, and survival of normal cells. When this system goes awry and unchecked, it can lead to growth, proliferation, survival, and metastasis of neoplastic cells. Alterations within the EGFR signaling cascade, such as gene mutations, gene amplification, and protein overexpression, have been shown to contribute to colorectal carcinogenesis. Some alterations also portend a poor prognosis in patients with colorectal cancer. Due to the complex interaction of EGFR and its downstream regulators, the study of individual

components of this pathway often yields conflicting results, as noted in this paper. Hence, there are still many questions that need to be answered before we can fully understand the impact of the EGFR signaling pathway on colorectal carcinogenesis and the prognosis of patients with colorectal cancer.

References

[1] N. E. Hynes and H. A. Lane, "ERBB receptors and cancer: the complexity of targeted inhibitors," *Nature Reviews Cancer*, vol. 5, no. 5, pp. 341–354, 2005.

[2] A. Citri and Y. Yarden, "EGF-ERBB signalling: towards the systems level," *Nature Reviews Molecular Cell Biology*, vol. 7, no. 7, pp. 505–516, 2006.

[3] T. Mitsudomi and Y. Yatabe, "Epidermal growth factor receptor in relation to tumor development: EGFR gene and cancer," *FEBS Journal*, vol. 277, no. 2, pp. 301–308, 2010.

[4] J. P. Spano, R. Fagard, J. C. Soria, O. Rixe, D. Khayat, and G. Milano, "Epidermal growth factor receptor signaling in colorectal cancer: preclinical data and therapeutic perspectives," *Annals of Oncology*, vol. 16, no. 2, pp. 189–194, 2005.

[5] S. S. Chang and J. Califano, "Current status of biomarkers in head and neck cancer," *Journal of Surgical Oncology*, vol. 97, no. 8, pp. 640–643, 2008.

[6] S. Dacic, "EGFR assays in lung cancer," *Advances in Anatomic Pathology*, vol. 15, no. 4, pp. 241–247, 2008.

[7] N. S. Goldstein and M. Armin, "Epidermal growth factor receptor immunohistochemical reactivity in patients with American Joint Committee on Cancer Stage IV colon adenocarcinoma: implications for a standardized scoring system," *Cancer*, vol. 92, pp. 1331–1346, 2001.

[8] J. A. McKay, L. J. Murray, S. Curran et al., "Evaluation of the epidermal growth factor receptor (EGFR) in colorectal tumours and lymph node metastases," *European Journal of Cancer*, vol. 38, no. 17, pp. 2258–2264, 2002.

[9] M. B. Resnick, J. Routhier, T. Konkin, E. Sabo, and V. E. Pricolo, "Epidermal growth factor receptor, c-MET, β-catenin, and p53 expression as prognostic indicators in stage II colon cancer: a tissue microarray study," *Clinical Cancer Research*, vol. 10, no. 9, pp. 3069–3075, 2004.

[10] L. B. Saltz, N. J. Meropol, P. J. Loehrer Sr., M. N. Needle, J. Kopit, and R. J. Mayer, "Phase II trial of cetuximab in patients with refractory colorectal cancer that expresses the epidermal growth factor receptor," *Journal of Clinical Oncology*, vol. 22, no. 7, pp. 1201–1208, 2004.

[11] K. Y. Chung, J. Shia, N. E. Kemeny et al., "Cetuximab shows activity in colorectal cancer patients with tumors that do not express the epidermal growth factor receptor by immunohistochemistry," *Journal of Clinical Oncology*, vol. 23, no. 9, pp. 1803–1810, 2005.

[12] D. Cunningham, Y. Humblet, S. Siena et al., "Cetuximab monotherapy and cetuximab plus irinotecan in irinotecan-refractory metastatic colorectal cancer," *New England Journal of Medicine*, vol. 351, no. 4, pp. 337–345, 2004.

[13] L. Frederick, X. Y. Wang, G. Eley, and C. D. James, "Diversity and frequency of epidermal growth factor receptor mutations in human glioblastomas," *Cancer Research*, vol. 60, no. 5, pp. 1383–1387, 2000.

[14] T. J. Lynch, D. W. Bell, R. Sordella et al., "Activating mutations in the epidermal growth factor receptor underlying responsiveness of non-small-cell lung cancer to gefitinib," *New England Journal of Medicine*, vol. 350, no. 21, pp. 2129–2139, 2004.

[15] J. G. Paez, P. A. Jänne, J. C. Lee et al., "EGFR mutations in lung, cancer: correlation with clinical response to gefitinib therapy," *Science*, vol. 304, no. 5676, pp. 1497–1500, 2004.

[16] W. Pao, V. Miller, M. Zakowski et al., "EGF receptor gene mutations are common in lung cancers from "never smokers" and are associated with sensitivity of tumors to gefitinib and erlotinib," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 101, no. 36, pp. 13306–13311, 2004.

[17] J. W. Lee, Y. H. Soung, S. Y. Kim et al., "Absence of EGFR mutation in the kinase domain in common human cancers besides non-small cell lung cancer," *International Journal of Cancer*, vol. 113, no. 3, pp. 510–511, 2005.

[18] T. D. Barber, B. Vogelstein, K. W. Kinzler, and V. E. Velculescu, "Somatic mutations of EGFR in colorectal cancers and glioblastomas," *New England Journal of Medicine*, vol. 351, no. 27, p. 2883, 2004.

[19] J. Shia, D. S. Klimstra, A. R. Li et al., "Epidermal growth factor receptor expression and gene amplification in colorectal carcinoma: an immunohistochemical and chromogenic in situ hybridization study," *Modern Pathology*, vol. 18, no. 10, pp. 1350–1356, 2005.

[20] K. L. Spindler, J. Lindebjerg, J. N. Nielsen et al., "Epidermal growth factor receptor analyses in colorectal cancer: a comparison of methods," *International Journal of Oncology*, vol. 29, no. 5, pp. 1159–1165, 2006.

[21] F. Cappuzzo, G. Finocchiaro, E. Rossi et al., "EGFR FISH assay predicts for response to cetuximab in chemotherapy refractory colorectal cancer patients," *Annals of Oncology*, vol. 19, no. 4, pp. 717–723, 2008.

[22] A. Bardelli and S. Siena, "Molecular mechanisms of resistance to cetuximab and panitumumab in colorectal cancer," *Journal of Clinical Oncology*, vol. 28, no. 7, pp. 1254–1261, 2010.

[23] A. Italiano, P. Follana, F. X. Caroli et al., "Cetuximab shows activity in colorectal cancer patients with tumors for which FISH analysis does not detect an increase in EGFR gene copy number," *Annals of Surgical Oncology*, vol. 15, no. 2, pp. 649–654, 2008.

[24] P. Laurent-Puig, A. Cayre, G. Manceau et al., "Analysis of PTEN, BRAF, and EGFR status in determining benefit from cetuximab therapy in wild-type KRAS metastatic colon cancer," *Journal of Clinical Oncology*, vol. 27, no. 35, pp. 5924–5930, 2009.

[25] G. C. Burmer and L. A. Loeb, "Mutations in the KRAS2 oncogene during progressive stages of human colon carcinoma," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 86, no. 7, pp. 2403–2407, 1989.

[26] A. Leslie, F. A. Carey, N. R. Pratt, and R. J. C. Steele, "The colorectal adenoma-carcinoma sequence," *British Journal of Surgery*, vol. 89, no. 7, pp. 845–860, 2002.

[27] M. Brink, A. F. P. M. de Goeij, M. P. Weijenberg et al., "K-ras oncogene mutations in sporadic colorectal cancer in The Netherlands Cohort Study," *Carcinogenesis*, vol. 24, no. 4, pp. 703–710, 2003.

[28] W. S. Samowitz, K. Curtin, D. Schaffer, M. Robertson, M. Leppert, and M. L. Slattery, "Relationship of Ki-ras mutations in colon cancers to tumor location, stage, and survival: a population-based study," *Cancer Epidemiology Biomarkers and Prevention*, vol. 9, no. 11, pp. 1193–1197, 2000.

[29] A. D. Roth, S. Tejpar, M. Delorenzi et al., "Prognostic role of KRAS and BRAF in stage II and III resected colon cancer: results of the translational study on the PETACC-3, EORTC

40993, SAKK 60-00 trial," *Journal of Clinical Oncology*, vol. 28, no. 3, pp. 466–474, 2010.

[30] H. J. N. Andreyev, A. R. Norman, D. Cunningham et al., "Kirsten ras mutations in patients with colorectal cancer: the 'RASCAL II' study," *British Journal of Cancer*, vol. 85, no. 5, pp. 692–696, 2001.

[31] R. T. Belly, J. D. Rosenblatt, M. Steinmann et al., "Detection of mutated K12-ras in histologically negative lymph nodes as an indicator of poor prognosis in stage II colorectal cancer," *Clinical Colorectal Cancer*, vol. 1, no. 2, pp. 110–116, 2001.

[32] S. Tejpar, M. Bertagnolli, F. Bosman et al., "Prognostic and predictive biomarkers in resected colon cancer: current status and future perspectives for integrating genomics into biomarker discovery," *Oncologist*, vol. 15, no. 4, pp. 390–404, 2010.

[33] S. Ogino, J. A. Meyerhardt, N. Irahara et al., "KRAS mutation in stage III colon cancer and clinical outcome following intergroup trial CALGB 89803," *Clinical Cancer Research*, vol. 15, no. 23, pp. 7322–7329, 2009.

[34] R. G. Amado, M. Wolf, M. Peeters et al., "Wild-type KRAS is required for panitumumab efficacy in patients with metastatic colorectal cancer," *Journal of Clinical Oncology*, vol. 26, no. 10, pp. 1626–1634, 2008.

[35] W. de Roock, H. Piessevaux, J. de Schutter et al., "KRAS wild-type state predicts survival and is associated to early radiological response in metastatic colorectal cancer treated with cetuximab," *Annals of Oncology*, vol. 19, no. 3, pp. 508–515, 2008.

[36] F. Di Fiore, F. Blanchard, F. Charbonnier et al., "Clinical relevance of KRAS mutation detection in metastatic colorectal cancer treated by Cetuximab plus chemotherapy," *British Journal of Cancer*, vol. 96, no. 8, pp. 1166–1169, 2007.

[37] C. S. Karapetis, S. Khambata-Ford, D. J. Jonker et al., "K-ras mutations and benefit from cetuximab in advanced colorectal cancer," *New England Journal of Medicine*, vol. 359, no. 17, pp. 1757–1765, 2008.

[38] A. Lièvre, J. B. Bachet, V. Boige et al., "KRAS mutations as an independent prognostic factor in patients with advanced colorectal cancer treated with cetuximab," *Journal of Clinical Oncology*, vol. 26, no. 3, pp. 374–379, 2008.

[39] A. Lièvre, J. B. Bachet, D. Le Corre et al., "KRAS mutation status is predictive of response to cetuximab therapy in colorectal cancer," *Cancer Research*, vol. 66, no. 8, pp. 3992–3995, 2006.

[40] M. J. Garnett and R. Marais, "Guilty as charged: B-RAF is a human oncogene," *Cancer Cell*, vol. 6, no. 4, pp. 313–319, 2004.

[41] H. Rajagopalan, A. Bardelli, C. Lengauer, K. W. Kinzler, B. Vogelstein, and V. E. Velculescu, "RAF/RAS oncogenes and mismatch-repair status," *Nature*, vol. 418, no. 6901, article 934, 2002.

[42] W. S. Samowitz, C. Sweeney, J. Herrick et al., "Poor survival associated with the BRAF V600E mutation in microsatellite-stable colon cancers," *Cancer Research*, vol. 65, no. 14, pp. 6063–6070, 2005.

[43] E. Domingo, P. Laiho, M. Ollikainen et al., "BRAF screening as a low-cost effective strategy for simplifying HNPCC genetic testing," *Journal of Medical Genetics*, vol. 41, no. 9, pp. 664–668, 2004.

[44] M. B. Loughrey, P. M. Waring, A. Tan et al., "Incorporation of somatic BRAF mutation testing into an algorithm for the investigation of hereditary non-polyposis colorectal cancer," *Familial Cancer*, vol. 6, no. 3, pp. 301–310, 2007.

[45] H. Davies, G. R. Bignell, C. Cox et al., "Mutations of the BRAF gene in human cancer," *Nature*, vol. 417, no. 6892, pp. 949–954, 2002.

[46] F. Loupakis, A. Ruzzo, C. Cremolini et al., "KRAS codon 61, 146 and BRAF mutations predict resistance to cetuximab plus irinotecan in KRAS codon 12 and 13 wild-type metastatic colorectal cancer," *British Journal of Cancer*, vol. 101, no. 4, pp. 715–721, 2009.

[47] W. de Roock, B. Claes, D. Bernasconi et al., "Effects of KRAS, BRAF, NRAS, and PIK3CA mutations on the efficacy of cetuximab plus chemotherapy in chemotherapy-refractory metastatic colorectal cancer: a retrospective consortium analysis," *Lancet Oncology*, vol. 11, pp. 753–762, 2010.

[48] Y. Baba, K. Nosho, K. Shima et al., "Phosphorylated AKT expression is associated with PIK3CA mutation, low stage, and favorable outcome in 717 colorectal cancers," *Cancer*. In press.

[49] S. Ogino, K. Nosho, G. J. Kirkner et al., "PIK3CA mutation is associated with poor prognosis among patients with curatively resected colon cancer," *Journal of Clinical Oncology*, vol. 27, no. 9, pp. 1477–1484, 2009.

[50] X. P. Zhou, A. Loukola, R. Salovaara et al., "PTEN mutational spectra, expression levels, and subcellular localization in microsatellite stable and unstable colorectal cancers," *American Journal of Pathology*, vol. 161, no. 2, pp. 439–447, 2002.

[51] N. T. Nassif, G. P. Lobo, X. Wu et al., "PTEN mutations are common in sporadic microsatellite stable colorectal cancer," *Oncogene*, vol. 23, no. 2, pp. 617–628, 2004.

[52] P. M. Wilson, M. J. LaBonte, and H. J. Lenz, "Molecular markers in the treatment of metastatic colorectal cancer," *Cancer Journal*, vol. 16, no. 3, pp. 262–272, 2010.

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Review Article

MicroRNA Expression in Selected Carcinomas of the Gastrointestinal Tract

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MicroRNAs (miRNAs) comprise a recently discovered class of small, 18–25 nucleotide, noncoding RNA sequences that regulate gene expression at the posttranscriptional level by binding to and inhibiting the translation of target messenger RNAs (mRNAs). Characteristic patterns of miRNA expression have been described in several malignancies of the gastrointestinal tract, and numerous investigators have demonstrated interactions between specific miRNA species and target oncogenes or tumor-suppressor genes. It is clear that miRNAs play an important role in regulating expression of a number of genes involved in gastrointestinal carcinogenesis, and, thus, these molecules may represent either diagnostic markers of, or therapeutic targets for, some types of malignancy. This paper summarizes the literature regarding miRNA expression in carcinomas of the colon, pancreas, and liver and discusses some of the mechanisms by which these molecules participate in gastrointestinal oncogenesis.

1. Introduction

MicroRNAs are small, 18–25 nucleotide, noncoding RNA sequences that regulate gene expression at the posttranscriptional level by binding to and inhibiting translation of target messenger RNAs (mRNAs). Over 1,000 human miRNAs have been identified to date, and many have tissue-specific expression profiles. Several studies have shown that miRNAs demonstrate characteristic patterns of expression in cancers. Some species show overexpression in cancers relative to nonneoplastic tissues, whereas others display decreased expression, similar to oncogenes and tumor suppressor genes, respectively [1–9]. Emerging data indicate that virtually every type of human malignancy displays dysregulated miRNA expression, and, in fact, potential applications of miRNA expression profiling to the diagnosis, prognosis, and treatment of gastrointestinal cancers have been the subject of extensive recent investigation. Some investigators have suggested that panels of miRNAs may be used for diagnostic purposes among patients with suspected gastrointestinal malignancies, and a subset of these represent prognostically important markers or even potential therapeutic targets. The purpose of this paper is to provide the readership with a comprehensive overview of published data regarding miRNA expression in gastrointestinal malignances with emphasis on colorectal, pancreatic, and hepatocellular carcinomas.

2. Overview

MicroRNAs were first recognized as regulatory agents of gene expression in 1993 when they were discovered in *Caenorhabditis elegans* [10]. Each miRNA molecule can potentially bind to either the 3′- or 5′-untranslated region (UTR) of hundreds of mRNAs by sequence complementarity. Binding of miRNA to mRNA suppresses expression by either inducing mRNA degradation or inhibiting translational machinery. Primary miRNA transcripts within the nucleus are processed by the nuclear RNAse III complex, Drosha, to become miRNA precursors termed "pre-miRNAs". Pre-miRNAs are exported to the cytoplasm where the endoribonuclease, Dicer, processes them into mature miRNAs. These mature molecules are subsequently integrated into the RNA-induced silencing complex (RISC), the functional unit of which inhibits mRNA translation [11, 12].

A growing body of evidence indicates that a subset of miRNAs are functionally important to the development of human cancers. Many investigators have identified tumor-specific miRNA signatures that accurately distinguish malignancies from benign tissues in multiple different sites, suggesting that some miRNAs are oncogenic, and their potency depends on other gene mutations that are present in the tumor. Manipulation of miRNAs in cancer cell lines directly affects cell proliferation and apoptosis, and many researchers have demonstrated links between miRNA dysregulation and cell signaling pathway abnormalities [9, 13–15]. Thus, miRNAs comprise a recently described class of molecules that contributes to cancer formation through interactions with mRNAs derived from oncogenes and tumor suppressor genes.

3. MicroRNAs in Colorectal Cancer

MicroRNA expression in colon cancer and nonneoplastic colonic tissues has been extensively studied (Table 1). Cummins et al. performed serial analysis of gene expression (miRAGE) in colorectal cancer cell lines and identified 133 miRAGE tags that corresponded to previously unrecognized miRNAs. They also detected differential expression of 52 miRAGE tags in colon cancer cells relative to normal colonic epithelium. These results provided evidence that the number of miRNAs in the human genome is likely much larger than had been previously predicted and that their expression is frequently dysregulated in colorectal cancer [25]. Subsequent studies provided data indicating that miRNA dysregulation is important to colon cancer development. Bandres et al. studied expression of 156 miRNAs in colon cancer cell lines as well as paired tumoral and nontumoral tissues. They identified a subset of 13 differentially expressed species [16]. Wang et al. used miRNA microarrays to identify 12 miRNAs that were upregulated in colon cancer and 2 that were downregulated compared to nonneoplastic colonic tissues [17].

The expression levels of several miRNA species have been associated with clinicopathologic features and prognosis in colon cancer. MicroRNA-31 was first identified by Bandres et al. as one of the most substantially dysregulated miRNAs in colon cancer cell lines and resected colon cancers. The authors of that study found that miR-31 expression was significantly higher in stage IV tumors compared to stage II carcinomas [16]. Wang et al. later demonstrated an association between miR-31 upregulation and advanced TNM stage as well as deeper invasion of the primary tumor [26]. Slaby et al. failed to identify any correlation between miR-31 expression and tumor stage in their analysis of 29 colon carcinomas, but they did note that miR-31 levels were significantly higher in high-grade carcinomas, compared to low-grade tumors [19]. MicroRNA-21, a species with antiapoptotic properties, is dysregulated in many human cancers including tumors of the head and neck, lung, breast, prostate, brain, thyroid, pancreas, stomach, colon, and esophagus [9, 27–32]. Its high expression has been associated with regional lymph node and distant metastases in colorectal cancer patients [19]. Schetter et al. analyzed 197 colonic adenocarcinomas using microarray assays and qRT-PCR and found that high miR-21 levels predicted poor survival prognosis and higher TNM stage [21]. This same group later demonstrated a relationship between high miR-21 expression and increased levels of IL-6, a proinflammatory cytokine and lower levels of IL-12a in colonic adenocarcinomas. They postulated that IL-6 drives miR-21 expression whereas IL-12a is a negatively regulated target of miR-21 [21]. Presumably, IL-12a activity is important for host resistance to malignancy. Thus, its downregulation by miR-21 may account for some of the negative impact of miR-21 on prognosis among patients with colorectal cancer. Finally, miR-145 is normally expressed in colonic epithelium, but it shows decreased expression in colon cancer [23]. Decreased miR-145 is more commonly observed in tumors of the proximal colon and those of large size (>50 mm) [22]. Akao et al. found decreased expression of miR-143 and miR-145 in adenomas and carcinomas, but they did not find any correlation between their expression and any other clinical prognostic factors, indicating that these miRNAs may primarily contribute to initiation, but not progression, of colonic tumorigenesis. Notably, synthetic miR-143 has a suppressive effect on growth of xenografted tumors comprised of human colon cancer cells [33].

Expression of a number of other miRNA species has been reported to correlate with clinicopathologic features and prognosis among patients with colon cancer. Schepeler et al. found that stage II colon cancers with high miR-320 or miR-498 expression showed significant differences with respect to progression-free survival compared to tumors with low expression of these species [18]. High expression of miR-200c has also been reported to predict shorter survival and is associated with frequent p53 mutations [20]. Diaz et al. studied 110 patients with colon cancer and reported that downregulation of miR-106a predicted shorter disease-free survival [34]. Sarver et al. identified 6 miRNAs (HS-29, miR-135b, miR-32, miR-33, miR-542-5p, and miR-96) that were more highly expressed in stage IV microsatellite stable cancers relative to stage II tumors [22]. Huang et al. studied nonneoplastic colonic mucosa adjacent to colon cancers in three patients with lymph node metastases and three patients with node negative disease. They found 6-fold higher expression of miR-137 in lymph node positive tumors compared to node-negative cases [35]. Finally, Yantiss et al. studied miRNA expression in colorectal cancers obtained from 24 patients <40 years of age and 45 patients >40 years old. They found significantly increased expression of miR-21, miR-20a, miR-145, miR-181b, and miR-203 in tumors from young patients compared to older adults [36].

Data from several studies have documented interactions between specific miRNA species, oncogenes, and tumor suppressor genes relevant to colonic carcinogenesis. Chen et al. observed an inverse correlation between miR-143 and *KRAS* expression in 13 colonic adenocarcinomas. They also used semiquantitative RT-PCR to show that *KRAS* transcript levels were decreased in cell lines transfected with pre-miR-143 whereas addition of anti-miR-143 oligonucleotides increased *KRAS* transcript levels. These findings suggest that miR-143 downregulation in colon carcinoma promotes cancer cell growth by disinhibiting *KRAS* translation [37].

Table 1: Summary of microRNA expression in colorectal cancer.

Study	Differentially expressed miRNAs (no./total)	Most significantly overexpressed miRNAs in colorectal cancer	Most significantly underexpressed miRNAs in colorectal cancer
Bandres et al. [16]	13/156	miR-31, miR-96, miR-133b, miR-135b, miR-145, miR-183	
Wang et al. [17]	14/723	miR-106b, miR-135b, miR-18a, miR-18b, miR-196b, miR-19a, miR-224, miR-335, miR-424, miR-20a*, miR-301b, miR-734a	miR-378, miR-378*
Schepeler et al. [18]	60/315	miR-20a, miR-510, miR-92, miR-513	miR-145, miR-455, miR-484, miR-101
Slaby et al. [19]	4/4	miR-31, miR-21	miR-145, miR-143
Xi et al. [20]	4/10	miR-15b, miR-181b, miR-191, miR-200c	
Schetter et al. [21]	37/389	miR-20a, miR-21, miR-106a, miR-181b, miR-203	
Sarver et al. [22]	39/735	miR-135b, miR-96, miR-182, miR-182*, miR-183	miR-1, miR-133a, miR-30a-3p, miR-30a-5p, miR-20b, miR-363
Michael et al. [23]	2/28		miR-143, miR-145
Guo et al. [24]	45/262	miR-93, miR-92, miR-520h, miR-508, miR-505, miR-449, miR-429, miR-384, miR-373, miR-34c, miR-326, miR-25, miR-224, miR-210, miR-200a, miR-19b, miR-19a, miR-18a, miR-183, miR-182, miR-181b, miR-181a, miR-181c, miR-17-5p, miR-148a, miR-141, miR-130b, miR-128a, miR-106b, miR-106a, let-7d	miR-96, miR-485-5p, miR-422b, miR-342, miR-214, miR-199a, miR-195, miR-150, miR-145, miR-143, miR-133a, miR-126, miR-125b, miR-100

MicroRNAs also impact the Wnt signaling pathway. The adenomatous polyposis coli (*APC*) gene normally functions as a tumor suppressor by regulating Wnt signaling. In the absence of functional *APC*, β-catenin accumulates in the cytoplasm and is transported to the nucleus, where it facilitates transcription of genes involved in proliferation, such as *cyclin D1*. Germline *APC* mutations are responsible for familial adenomatous polyposis syndrome, and biallelic inactivation of *APC* occurs in the majority of sporadic colonic adenocarcinomas [38–41]. Transduction of colon cancer cell lines with miR-135a and miR-135b results in diminished *APC* expression and accumulation of β-catenin. Colon cancers with high levels of miR-135a and miR-135b show lower *APC* expression. In this situation, qRT-PCR data demonstrate reduced *APC* mRNA, suggesting that miR-135a and miR-135b regulate the Wnt signaling pathway by promoting mRNA decay [42].

MicroRNA-34, a species that is lost in multiple types of cancer including those of the colon and pancreas, is inducible by p53, and its overexpression is associated with p53 effects including cell cycle arrest and apoptosis [43]. Guo et al. found that restoration of miR-126, a species that shows low-to-absent expression in colon cancer, impedes cancer cell growth by targeting the p85B subunit of phosphatidylinositol-3-kinase (PI3K). Phosphatidylinositol-3-kinase activates AKT, a protein kinase involved in the PI3K/AKT/mTOR pathway, which triggers a variety of downstream responses related to cell growth, proliferation, and motility. Presumably, loss of miR-126 removes a critical checkpoint in the PI3K/AKT/mTOR pathway and facilitates tumor growth [24]. Continued research will likely uncover additional regulatory roles for miRNAs in colorectal carcinogenesis and other neoplasms throughout the gastrointestinal tract.

Most colorectal cancers are characterized by aneuploidy, allelic imbalance, and mutations in *KRAS*, *TP53*, and *APC* although approximately 15% of sporadic colonic adenocarcinomas develop *via* microsatellite instability (MSI) and have defective DNA mismatch repair mechanisms. Such tumors are often located in the proximal colon, show high-grade histology, and contain infiltrating lymphocytes. They are generally diploid and have a better prognosis than non-MSI tumors, but they are probably less responsive to conventional 5-flourouracil-based chemotherapy [44, 45]. Patients with germline mutations in one of the mismatch repair genes (*MLH1*, *MSH2*, *MSH6*, and *PMS2*) are at risk for acquired mutations of the second allele and development of heritable microsatellite unstable carcinomas of the gastrointestinal, genitourinary, and gynecologic tracts, termed Lynch syndrome. Sporadic colonic carcinomas with MSI may also occur, but in this situation, tumorigenesis results from biallelic epigenetic methylation and silencing of *MLH1*. Several authors have studied differences in miRNA expression patterns between microsatellite stable (MSS) colon cancers and those with MSI-H (Table 2). Lanza et al. used microarray data to profile 23 MSS and 16 sporadic MSI-H colon cancers. They identified 72 mRNAs and 14 miRNAs that were differentially expressed between these two tumor types and used their combined expression to distinguish between MSS and MSI-H cancers [14]. Sarver et al. studied expression of 735 miRNAs in 12 MSI-H and 68 MSS colon tumors and found that miR-552, miR-592,

Table 2: MicroRNA expression in microsatellite stable *versus* microsatellite unstable colorectal cancer.

Study	MSS colorectal cancers		MSI-H colorectal cancers	
	Overexpressed	Underexpressed	Overexpressed	Underexpressed
Schepeler et al. [18]	miR-20a, miR-510, miR-92, miR-513	miR-142-3p, miR-212, miR-146b, miR-217	miR-492, miR-20a, miR-432*, miR-21	miR-145, miR-455, miR-484, miR-101
Sarver et al. [22]		miR-552, miR-592, miR-181c, miR-196b	miR-625, miR-31	
Lanza et al. [14]	miR-215, miR-192, miR-191, miR-203, miR-32, miR-17, miR-25, miR-106a, miR-92-1, miR-92-2, miR-93-1, miR-20		miR-223, miR-155	

miR-181c, and miR-196b were significantly increased in MSS tumors relative to MSI-H tumors, whereas miR-625 and miR-31 levels were higher in MSI-H tumors [22]. Schepeler et al. compared miRNA expression in 37 MSS relative to 12 MSI-H cancers and identified a 4-miRNA signature (miR-142-3p, miR-212, miR-151, and miR-144) that predicted MSI with 81% specificity and 92% sensitivity [18]. These data indicate that the type of genetic instability in colorectal cancer is reflected at the miRNA level.

5-Flourouracil (5-FU) has been a mainstay of colorectal cancer therapy for the past several decades [46]. Some patients have a suboptimal response to therapy for unclear reasons, so identification of molecular markers that predict the likelihood of a therapeutic response is important. *In vitro* studies evaluating miRNA expression in colon cancer cell lines treated with 5-FU have generated promising data regarding the potential use of miRNAs as markers of chemosensitivity. Borralho et al. showed that stable expression of miR-143, a species known to be downregulated in colon cancer, was associated with increased death in cell lines after exposure to 5-FU [23, 47]. Others have applied this concept to colon cancer resection specimens. Nakajima et al. used qRT-PCR to study miRNA expression in residual or recurrent colon cancers from 46 patients who were treated with oral 5-FU alone or in combination with cisplatin. Twenty-seven patients who experienced complete disease remission showed a partial response or maintained stable disease after treatment had significantly lower levels of let-7g and miR-181b, compared to 19 patients who suffered disease progression [48]. Schetter et al. analyzed associations between miR-21 expression and therapeutic outcomes in 56 stage II or stage III colorectal cancer patients treated with 5-FU. High miR-21 expression was associated with worse overall survival, lending preliminary support to the notion that miR-21 overexpression predicts a poor response to therapy [21]. Boni et al. investigated associations between polymorphisms in miRNA-containing genomic regions and genes related to miRNA biogenesis and clinical outcome in patients with metastatic colon cancer who were treated with 5-FU and irinotecan. Single-nucleotide polymorphisms in the miR-26-a-1 gene and 5′UTR of pre-miR-100 correlated with better overall survival and disease control, respectively, and both were associated with a prolonged interval to progression [49]. The mechanisms by which miRNAs modulate efficacy of therapy are not understood, but these early data support the hypothesis that changes in miRNA expression levels and in the miRNA genome impact tumor response to therapy.

4. MicroRNAs in Pancreatic Neoplasia

Aberrant miRNA expression has been described in pancreatic ductal adenocarcinoma and benign pancreatobiliary disease (Table 3). Early studies exploited differences in miRNA expression patterns to distinguish between benign and malignant pancreatic diseases. Bloomston et al. examined 65 pancreatic ductal adenocarcinomas and benign adjacent pancreatic tissue, as well as 42 cases of chronic pancreatitis for miRNA expression. They found 21 miRNAs to be dysregulated in cancer compared to benign tissues and noted that a panel of 11 miRNAs (miR-148a, miR-148b, miR-155, miR-181a, miR-181b, miR-181b-1, miR-181c, miR-181d, miR-21, miR-221, and miR-375) distinguished pancreatic ductal adenocarcinoma from chronic pancreatitis and normal pancreatic tissue [49]. Lee et al. analyzed 28 pancreatic ductal adenocarcinomas and 21 nonneoplastic pancreatic tissues and found miR-155, miR-181b, miR-181c, miR-21, and miR-221 to be among the top 20 of 100 miRNAs overexpressed in pancreatic cancer [31]. Szafranska et al. identified 26 dysregulated miRNAs in pancreatic adenocarcinoma using resection specimens and pancreatic cancer cell lines. They reported that miR-196a upregulation combined with miR-217 downregulation reliably distinguished pancreatic cancer from benign pancreas and chronic pancreatitis [50]. In a later study, this signature correctly identified malignancy in 9/10 fine needle aspiration biopsies of pancreatic cancer [51].

The roles of dysregulated miRNAs in neoplastic pancreatic lesions have also been examined. du Rieu et al. used qRT-PCR to study miRNA expression in pancreatic intraepithelial neoplasia (PanIN) samples from human and mouse pancreas and found that levels of miR-21, miR-221, miR-22, and let-7a increased in the progression of PanIN to carcinoma [52]. Dilhoff et al. found that strong miR-21 expression by *in situ* hybridization was associated with decreased survival among patients with lymph node-negative pancreatic carcinoma [53]. Ikenaga et al. used qRT-PCR to evaluate miR-203 levels in resection specimens from patients with pancreatic ductal adenocarcinoma ($n = 113$), chronic pancreatitis ($n = 20$), and samples of nondiseased pancreas ($n = 8$) and found higher expression of miR-203 in pancreatic cancer.

Table 3: Summary of microRNA expression in pancreatic ductal adenocarcinoma.

Study	Differentially expressed miRNAs (no./total)	Most significantly overexpressed miRNAs compared with normal controls and/or chronic pancreatitis	Most significantly underexpressed miRNAs compared with normal controls and/or chronic pancreatitis
Bloomston et al. [30]	46/326	miR-221, miR-181a, miR-155, miR-210, miR-213, miR-181b, miR-222, miR-181b-2, miR-21, miR-181b-1, miR-181c, miR-220, miR-181d, miR-223, miR-100-1/2, miR-125a, miR-143, miR-10a, miR-146, miR-99, miR-100, miR-199a-1, miR-10b, miR-199a-2, miR-107, miR-103-2, miR-125b-1, miR-205, miR-23b, miR-23a, miR-96, miR-34, miR-497, miR-203, miR-453, miR-92, miR-93, miR-21	miR-148a, miR-148b, miR-375, miR-494, miR-483, miR-339, miR-218-2, miR-409-3p
Lee et al. [31]	100/222	miR-221, miR-424, miR-301, miR-100, miR-376a, miR-125b-1, miR-21, miR-16-1, miR-181a, miR-181c, miR-92-1, miR-15b, miR-155, let-7f-1, miR-212, miR-107, miR-24-1, miR-24-2, let-7d	miR-345, miR-142-p, miR-139
Szafranska et al. [50]	26/377	miR-205, miR-143, miR-145, miR-146a, miR-148a, miR-196b, miR-93, miR-31, miR-210, miR-196a, miR-18a, miR-203, miR-150, miR-155, miR-221, miR-222, miR-223, miR-224	miR-29c, miR-216, miR-217, miR-375, miR-148a, miR-96, miR-148b, miR-141, miR-130b
du Rieu et al. [52]	7/7	miR-21, miR-221, miR-222, miR-200, miR-205, miR-29c	let-7a

Results of a multivariate analysis showed miR-203 expression to be an independent predictor of poor prognosis in patients who had undergone complete tumor resection [54]. Wang et al. investigated expression of miR-21, -210, -155, and 196a by qRT-PCR in the sera of 49 patients with pancreatic adenocarcinoma and 36 healthy controls and found higher expression of all four markers in sera of pancreatic cancer patients [55]. In a later study, Kong et al. analyzed serum levels of miR-196a, miR-21, and miR-155 in 35 patients with pancreatic adenocarcinoma, 15 patients with chronic pancreatitis, and 15 healthy controls. They found that higher miR-21 expression distinguished cancer patients from those with chronic pancreatitis and healthy subjects, whereas miR-155 and miR-196a discriminated between patients with chronic pancreatitis and healthy controls. They also noted that serum miR-196a levels were significantly higher in patients with unresectable cancer than in those amenable to surgery and that higher miR-196a levels predicted shorter survival in pancreatic cancer patients [56]. Others have shown increased miR-196a expression to be associated with a 2-year survival of 17%, compared to 64% among tumors with low expression of this marker [30].

The relative tissue-specificity of miRNAs makes them attractive targets for molecular therapy among patients with pancreatic cancer. Park et al. analyzed the effects of miR-21 and miR-221 antisense oligonucleotides on pancreatic cancer cell lines and found that treated cells showed increased apoptosis and cell cycle arrest compared to cells treated with control oligonucleotides. MicroRNA-21 targets two tumor suppressor molecules, PTEN and RECK, both of which were found to be increased in extracts from cell lines treated with antisense oligonucleotides to miR-21. Similarly, p27, the target of miR-221, increased when this molecule was inhibited. Park et al. also found that cells pretreated with antisense sequences against miR-21 and miR-221 showed decreased viability by colorimetric analysis following gemcitabine treatment compared to those treated with control oligonucleotides [57].

Few studies have investigated miRNA expression in pancreatic endocrine and acinar tumors. Roldo et al. performed microarray and northern blot analyses of 40 endocrine tumors, 4 acinar cell carcinomas, and 12 samples of nonneoplastic pancreas. They found that stable expression of miR-103 and miR-107, in combination with a lack of miR-155 expression, discriminated all tumor samples from nonneoplastic tissues. They also identified 10 miRNAs that distinguished endocrine from acinar tumors and 28 miRNA species that were aberrantly increased in both tumor types [29]. These preliminary data suggest that altered miRNA expression occurs in endocrine and acinar neoplasms of the pancreas.

5. MicroRNAs in Liver Disease

Early studies evaluating miRNA expression in hepatocellular carcinoma identified approximately 69 dysregulated species

Table 4: Summary of microRNA expression in hepatocellular carcinoma.

Study	Differentially expressed miRNAs (no./total)	Most significantly overexpressed miRNAs compared with nonneoplastic liver (no disease or chronic viral hepatitis)	Most significantly underexpressed miRNAs compared with nonneoplastic liver (no disease or chronic viral hepatitis)
Murakami et al. [58]	7/180	miR-18, miR-224	miR-199a*, miR-195, miR-199, miR-200a, miR-125a
Li et al. [59]	84/509	miR-106b, miR-15b, miR-18a, miR-221, miR-222, miR-224	miR-125b, miR-101
Ladeiro et al. [67]	130/250	miR-224, miR-200c, miR-203, miR-21, miR-222, miR-10b	miR-422b, miR-122a
Varnholt et al. [60]	29/80	miR-122, miR-100, miR-10a	miR-198, miR-145
Li et al. [75]	8/8	miR-17-5p, miR-18a, miR-19a, miR-20a, miR-92-1, miR-106b, miR-93, miR-25	
Pineau et al. [61]	12/215	miR-106b, miR-21, miR-210, miR-210, miR-221, miR-222, miR-224, miR-34a, miR-425, miR-519a, miR-93, miR-96	let-7c

(Table 4) [58–63]. Many of those miRNAs, including miR-122, miR-221, and miR-222, were later recognized as carcinogenic catalysts and prognostic markers in hepatocellular carcinoma. MicroRNA-122 is the most abundant miRNA in hepatic parenchyma and is relatively specific for hepatocyte differentiation, showing rare expression outside of liver [64–66]. Downregulation of miR-122 is frequently observed in hepatocellular carcinoma, and hepatocellular carcinoma cell lines treated with miR-122 oligonucleotides show increased apoptosis and decreased viability [58, 59, 67–72]. Coulouarn et al. correlated miR-122 tissue levels with the clinicopathologic features of 64 hepatocellular carcinomas and found that low expression of this marker predicted shorter survival, high-grade histology, and large tumor size. They also noted that loss of miR-122 was associated with higher expression of genes involved in cell motility, angiogenesis, hypoxia, and epithelial-mesenchymal transition [73]. Tsai et al. reported lower levels of miR-122 in hepatocellular carcinomas with intrahepatic metastases compared to solitary tumors [74].

The roles of microRNA species in hepatocellular carcinogenesis and their molecular targets are under current investigation. Wong et al. found that high miR-222 levels correlated with advanced tumor stage and shorter overall survival in patients with hepatocellular carcinoma independent of stage. They also noted PI3K/AKT/mTOR pathway inhibition occurred in hepatocellular carcinoma cell lines transfected with anti-miR-222 [62]. MicroRNA-221, another frequently dysregulated species in hepatocellular carcinoma, participates in the modulation of key molecules related to hepatocarcinogenesis. Pineau et al. found that expression levels of the cyclin-dependent kinase inhibitor, p27, and the PI3K/AKT/mTOR pathway regulator, DDIT4, were decreased in liver cancer cell lines that overexpressed miR-221 [61]. Gramantieri et al. showed an inverse correlation between miR-221 upregulation and levels of the proapoptotic protein, Bmf, in hepatocellular carcinoma samples [76]. Fornari et al. showed increased CDKN1C/p57 and CDKN1B/p27 protein levels by western blot analysis in hepatocellular carcinoma cell lines transfected with anti-miR-221 compared to controls. Conversely, cell lines treated with miR-221 showed decreased CDKN1C/p57 and CDKN1B/p27 protein levels [77]. Finally, Mneg et al. reported that inhibition of miR-21 in hepatocellular carcinoma cell lines increased expression of PTEN and decreased tumor cell proliferation, suggesting that increased miR-21 levels promote carcinogenesis [78]. Other identified, but less-well-studied, modulators of apoptosis in hepatocellular carcinoma include miR-29, miR-15b, miR-152, miR-101, and the miR-106b-25 cluster [75, 79–82].

Ji et al. evaluated a cohort of 214 patients with hepatocellular carcinoma and found that tumors with reduced miR-26 expression had a favorable response to adjuvant therapy with interferon alpha, whereas those with high miR-26 did not respond to therapy, suggesting that miR-26 may be used to select patients who may benefit from interferon alpha treatment [83]. Connelly et al. reported that miR-21 and the miR-17-92 polycistron are consistently upregulated in human and animal hepatocellular carcinoma cell lines and that their inhibition by antisense oligonucleotides causes reduced tumor cell proliferation [84].

Recent studies have established a role for miRNAs in regulation of hepatitis C virus (HCV) infection and offer promise for new treatment modalitites. The most frequently implicated miRNA in HCV modulation is the liver-specific species, miR-122. Jopling et al. first described a physical interaction between miR-122 and the HCV genome by showing that miR-122 binds to the 5′ UTR of viral RNA and stimulates viral replication [85]. Henke et al. showed that miR-122 drives HCV translation by enhancing the association between a small ribosomal subunit and HCV RNA [86]. Both mechanisms of HCV potentiation were later validated by Jangra et al. who demonstrated that viruses with mutations in miR-122 binding sites failed to replicate [87]. Young et al. reported decreased viral replication in liver cells

treated with inhibitors of miR-122 and suggested that these small molecules may represent a new target for HCV therapy, which has already been successfully tested in HCV-infected chimpanzees [72, 88].

The potential impact of miRNA analysis on patient selection for specific therapies was underscored by Sarasin-Filipowicz et al. These authors assessed miR-122 levels by qRT-PCR in pre- and posttreatment liver biopsies from 42 patients with HCV. They found that patients with decreased miR-122 levels in pretreatment liver biopsies showed a poor response to interferon therapy [89]. Other miRNA species such as miR-24, miR-149, miR-638, and miR-1181 have also been implicated in HCV-related liver disease and may facilitate viral entry, replication, and propagation [90].

MicroRNA dysregulation also occurs in association with hepatitis B virus (HBV) infection and may provide clues to the pathogenesis of HBV-related disease in infected patients. Yang et al. found that miR-602 expression increased with progression of HBV-related hepatitis to cirrhosis and hepatocellular carcinoma and noted that the tumor suppressor gene *RASSF1A* was inhibited in cell lines that highly expressed miR-602 [91]. Ura et al. studied 12 patients with HBV-related hepatocellular carcinoma and 14 with HCV-related hepatocellular carcinoma. They identified 19 differentially expressed miRNAs between patients with HBV and HCV infection. Microarray analysis also identified separate target genes for HBV- and HCV-related cancers. MicroRNAs important to HBV-related carcinoma regulate genes involved in cell death, DNA damage, recombination, and signal transduction whereas those important to HCV-related carcinoma were related to immune response, antigen presentation, cell cycle, and proteasome and lipid metabolism [92]. These findings provide insight into the differences between HBV- and HCV-infection and disease progression and may help identify potential therapeutic target molecules in the future.

6. MicroRNAs and *In Vitro* Cancer Models

Several strategies utilizing miRNAs as *in vivo* therapeutic targets are currently under development. Use of antisense oligonucleotides has been most extensively studied *in vitro*, and was recently shown to be an effective suppressor of miRNA expression *in vivo*. Krutzfeldt et al. engineered synthetic RNA analogues, termed "antagomiRs," to miR-16, miR-122, miR-192, and miR-194. These compounds were administered to mice intravenously and corresponding miRNA levels were measured by northern blot assay 24 hours after injection. These authors reported a marked reduction in target miRNA levels in various tissues including liver, lung, kidney, heart, intestine, fat, skin, bone marrow, muscle, ovaries, and adrenal glands [93]. Locked nucleic acid (LNA) constructs represent another promising approach to suppressing miRNA expression. These molecules are nucleic acid analogues that are "locked" by a methylene bridge connecting the 2'O and 4'C atoms. This structural modification enables LNA oligonucleotides to bind complementary nucleotide sequences with high affinity and excellent mismatch discrimination. Elmen et al. administered an LNA-antimiR to African green monkeys in order to study its effect on plasma cholesterol levels and miR-122 levels in liver tissue by northern blot analysis. They observed a dose-dependent decrease in total plasma cholesterol and depletion of mature miR-122 in liver biopsies from these monkeys [94]. Finally, some investigators have employed adenovirus vectors to increase expression of tumor-suppressor miRNAs. Kota et al. showed that the adenovirus vector-mediated introduction of miR-26, a species downregulated in hepatocellular carcinoma, into mice with liver cancer caused cancer cell apotosis and tumor regression. This therapy had no adverse effect on benign hepatocytes, underscoring the potential applications of this approach [95]. Future *in vivo* progress in this field will depend upon increased understanding of miRNA function in mammals, improved chemical design of antimiRs and synthetic miRNAs, and development of more efficient methods for delivery of these molecules to target tissues.

7. Conclusion

MicroRNAs represent an important class of molecules with profound diagnostic and therapeutic implications. Emerging evidence suggests that they may be useful diagnostic adjuncts that aid identification of tumors of unknown origin or even ascertain the presence of malignancy in scant biopsy specimens or sera of patients with suspected cancer. Specific miRNA expression profiles clearly correlate with prognosis, so it is highly likely that miRNA analysis will play an important role in determining the management of patients in the future. Preliminary studies utilizing antisense oligonucleotides against cancer-specific miRNAs have shown that some tumors respond to therapy while minimally damaging healthy tissues. These findings suggest that targeted therapies against selected miRNAs represent a new treatment modality for patients with gastrointestinal malignancies. Advances in this field have improved our understanding of the heterogeneity of human malignancies and will contribute to the growing trend toward individualized management strategies for cancer patients.

References

[1] W. Tam, "The emergent role of microRNAs in molecular diagnostics of cancer," *Journal of Molecular Diagnostics*, vol. 10, no. 5, pp. 415–423, 2008.

[2] W. Zhang, J. E. Dahlberg, and W. Tam, "MicroRNAs in tumorigenesis: a primer," *American Journal of Pathology*, vol. 171, no. 3, pp. 728–738, 2007.

[3] A. E. Szafranska, T. S. Davison, J. Shingara et al., "Accurate molecular characterization of formalin-fixed, paraffin-embedded tissues by microRNA expression profiling," *Journal of Molecular Diagnostics*, vol. 10, no. 5, pp. 415–423, 2008.

[4] S. S. Jeffrey, "Cancer biomarker profiling with microRNAs," *Nature Biotechnology*, vol. 26, no. 4, pp. 400–401, 2008.

[5] J. Lu, G. Getz, E. A. Miska et al., "MicroRNA expression profiles classify human cancers," *Nature*, vol. 435, no. 7043, pp. 834–838, 2005.

[6] N. Rosenfeld, R. Aharonov, E. Meiri et al., "MicroRNAs accurately identify cancer tissue origin," *Nature Biotechnology*, vol. 26, no. 4, pp. 462–469, 2008.

[7] G. Bloom, I. V. Yang, D. Boulware et al., "Multi-platform, multi-site, microarray-based human tumor classification," *American Journal of Pathology*, vol. 164, no. 1, pp. 9–16, 2004.

[8] P. Sood, A. Krek, M. Zavolan, G. Macino, and N. Rajewsky, "Cell-type-specific signatures of microRNAs on target mRNA expression," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 103, no. 8, pp. 2746–2751, 2006.

[9] S. Volinia, G. A. Calin, C. G. Liu et al., "A microRNA expression signature of human solid tumors defines cancer gene targets," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 103, no. 7, pp. 2257–2261, 2006.

[10] R. C. Lee, R. L. Feinbaum, and V. Ambros, "The *C. elegans* heterochronic gene lin-4 encodes small RNAs with antisense complementarity to lin-14," *Cell*, vol. 75, no. 5, pp. 843–854, 1993.

[11] A. Esquela-Kerscher, S. M. Johnson, L. Bai et al., "Post-embryonic expression of *C. elegans* microRNAs belonging to the lin-4 let-7 families in the hypodermis and the reproductive system," *Developmental Dynamics*, vol. 234, no. 4, pp. 868–877, 2005.

[12] S. Singh, S. C. Bevan, K. Patil, D. C. Newton, and P. A. Marsden, "Extensive variation in the 5′-UTR of Dicer mRNAs influences translational efficiency," *Biochemical and Biophysical Research Communications*, vol. 335, no. 3, pp. 643–650, 2005.

[13] W. M. Grady, R. K. Parkin, P. S. Mitchell et al., "Epigenetic silencing of the intronic microRNA hsa-miR-342 and its host gene EVL in colorectal cancer," *Oncogene*, vol. 27, no. 27, pp. 3880–3888, 2008.

[14] G. Lanza, M. Ferracin, R. Gafà et al., "mRNA/microRNA gene expression profile in microsatellite unstable colorectal cancer," *Molecular Cancer*, vol. 6, article 54, 2007.

[15] A. Gaur, D. A. Jewell, Y. Liang et al., "Characterization of microRNA expression levels and their biological correlates in human cancer cell lines," *Cancer Research*, vol. 67, no. 6, pp. 2456–2468, 2007.

[16] E. Bandres, E. Cubedo, X. Agirre et al., "Identification by Real-time PCR of 13 mature microRNAs differentially expressed in colorectal cancer and non-tumoral tissues," *Molecular Cancer*, vol. 5, article 29, 2006.

[17] Y. X. Wang, X. Y. Zhang, B. F. Zhang, C. Q. Yang, X. M. Chen, and H. J. Gao, "Initial study of microRNA expression profiles of colonic cancer without lymph node metastasis," *Journal of Digestive Diseases*, vol. 11, no. 1, pp. 50–54, 2010.

[18] T. Schepeler, J. T. Reinert, M. S. Ostenfeld et al., "Diagnostic and prognostic microRNAs in stage II colon cancer," *Cancer Research*, vol. 68, no. 15, pp. 6416–6424, 2008.

[19] O. Slaby, M. Svoboda, P. Fabian et al., "Altered expression of miR-21, miR-31, miR-143 and miR-145 is related to clinicopathologic features of colorectal cancer," *Oncology*, vol. 72, no. 5-6, pp. 397–402, 2008.

[20] Y. Xi, A. Formentini, M. Chien et al., "Prognostic values of microRNAs in colorectal cancer," *Biomarker Insights*, vol. 1, pp. 113–121, 2006.

[21] A. J. Schetter, H. N. Giang, E. D. Bowman et al., "Association of inflammation-related and microRNA gene expression with cancer-specific mortality of colon adenocarcinoma," *Clinical Cancer Research*, vol. 15, no. 18, pp. 5878–5887, 2009.

[22] A. L. Sarver, A. J. French, P. M. Borralho et al., "Human colon cancer profiles show differential microRNA expression depending on mismatch repair status and are characteristic of undifferentiated proliferative states," *BMC Cancer*, vol. 9, article 401, 2009.

[23] M. Z. Michael, S. M. O'Connor, N. G. Van Holst Pellekaan, G. P. Young, and R. J. James, "Reduced accumulation of specific microRNAs in colorectal neoplasia," *Molecular Cancer Research*, vol. 1, no. 12, pp. 882–891, 2003.

[24] C. Guo, J. F. Sah, L. Beard, J. K. V. Willson, S. D. Markowitz, and K. Guda, "The noncoding RNA, miR-126, suppresses the growth of neoplastic cells by targeting phosphatidylinositol 3-kinase signaling and is frequently lost in colon cancers," *Genes Chromosomes and Cancer*, vol. 47, no. 11, pp. 939–946, 2008.

[25] J. M. Cummins, Y. He, R. J. Leary et al., "The colorectal microRNAome," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 103, no. 10, pp. 3687–3692, 2006.

[26] C. J. Wang, Z. G. Zhou, L. Wang et al., "Clinicopathological significance of microRNA-31, -143 and -145 expression in colorectal cancer," *Disease Markers*, vol. 26, no. 1, pp. 27–34, 2009.

[27] J. A. Chan, A. M. Krichevsky, and K. S. Kosik, "MicroRNA-21 is an antiapoptotic factor in human glioblastoma cells," *Cancer Research*, vol. 65, no. 14, pp. 6029–6033, 2005.

[28] M. V. Iorio, M. Ferracin, C. G. Liu et al., "MicroRNA gene expression deregulation in human breast cancer," *Cancer Research*, vol. 65, no. 16, pp. 7065–7070, 2005.

[29] C. Roldo, E. Missiaglia, J. P. Hagan et al., "MicroRNA expression abnormalities in pancreatic endocrine and acinar tumors are associated with distinctive pathologic features and clinical behavior," *Journal of Clinical Oncology*, vol. 24, no. 29, pp. 4677–4684, 2006.

[30] M. Bloomston, W. L. Frankel, F. Petrocca et al., "MicroRNA expression patterns to differentiate pancreatic adenocarcinoma from normal pancreas and chronic pancreatitis," *Journal of the American Medical Association*, vol. 297, no. 17, pp. 1901–1908, 2007.

[31] E. J. Lee, Y. Gusev, J. Jiang et al., "Expression profiling identifies microRNA signature in pancreatic cancer," *International Journal of Cancer*, vol. 120, no. 5, pp. 1046–1054, 2007.

[32] W. A. Mardin and S. T. Mees, "MicroRNAs: novel diagnostic and therapeutic tools for pancreatic ductal adenocarcinoma?" *Annals of Surgical Oncology*, vol. 16, no. 11, pp. 3183–3189, 2009.

[33] Y. Akao, Y. Nakagawa, and T. Naoe, "let-7 microRNA functions as a potential growth suppressor in human colon cancer cells," *Biological and Pharmaceutical Bulletin*, vol. 29, no. 5, pp. 903–906, 2006.

[34] R. Diaz, J. Silva, J. M. García et al., "Deregulated expression of miR-106a predicts survival in human colon cancer patients," *Genes Chromosomes and Cancer*, vol. 47, no. 9, pp. 794–802, 2008.

[35] Z. M. Huang, J. Yang, X. Y. Shen et al., "MicroRNA expression profile in non-cancerous colonic tissue associated with lymph node metastasis of colon cancer," *Journal of Digestive Diseases*, vol. 10, no. 3, pp. 188–194, 2009.

[36] R. K. Yantiss, M. Goodarzi, X. K. Zhou et al., "Clinical, pathologic, and molecular features of early-onset colorectal carcinoma," *The American Journal of Surgical Pathology*, vol. 33, no. 4, pp. 572–582, 2009.

[37] X. Chen, X. Guo, H. Zhang et al., "Role of miR-143 targeting KRAS in colorectal tumorigenesis," *Oncogene*, vol. 28, no. 10, pp. 1385–1392, 2009.

[38] S. M. Powell, "Direct analysis for familial adenomatous polyposis mutations," *Applied Biochemistry and Biotechnology. Part B*, vol. 20, no. 2, pp. 197–207, 2002.

[39] M. Hermsen, C. Postma, J. Baak et al., "Colorectal adenoma to carcinoma progression follows multiple pathways of chromosomal instability," *Gastroenterology*, vol. 123, no. 4, pp. 1109–1119, 2002.

[40] E. R. Fearon, "Genetic alterations underlying colorectal tumorigenesis," *Cancer Surveys*, vol. 12, pp. 119–136, 1992.

[41] K. W. Kinzler, M. C. Nilbert, L. K. Su et al., "Identification of FAP locus genes from chromosome 5q21," *Science*, vol. 253, no. 5020, pp. 661–665, 1991.

[42] R. Nagel, C. Le Sage, B. Diosdado et al., "Regulation of the adenomatous polyposis coli gene by the miR-135 family in colorectal cancer," *Cancer Research*, vol. 68, no. 14, pp. 5795–5802, 2008.

[43] T. C. Chang, E. A. Wentzel, O. A. Kent et al., "Transactivation of miR-34a by p53 broadly influences gene expression and promotes apoptosis," *Molecular Cell*, vol. 26, no. 5, pp. 745–752, 2007.

[44] S. Popat, R. Hubner, and R. S. Houlston, "Systematic review of microsatellite instability and colorectal cancer prognosis," *Journal of Clinical Oncology*, vol. 23, no. 3, pp. 609–618, 2005.

[45] C. M. Ribic, D. J. Sargent, M. J. Moore et al., "Tumor microsatellite-instability status as a predictor of benefit from fluorouracil-based adjuvant chemotherapy for colon cancer," *New England Journal of Medicine*, vol. 349, no. 3, pp. 247–257, 2003.

[46] C. Heidelberger, "Fluorinated pyrimidines," *Progress in Nucleic Acid Research and Molecular Biology*, vol. 4, pp. 1–50, 1965.

[47] P. M. Borralho, B. T. Kren, R. E. Castro, I. B. Moreira Da Silva, C. J. Steer, and C. M. P. Rodrigues, "MicroRNA-143 reduces viability and increases sensitivity to 5-fluorouracil in HCT116 human colorectal cancer cells," *FEBS Journal*, vol. 276, no. 22, pp. 6689–6700, 2009.

[48] G. Nakajima, K. Hayashi, Y. Xi et al., "Non-coding microRNAs hsa-let-7g and hsa-miR-181b are associated with chemoresponse to S-1 in colon cancer," *Cancer Genomics and Proteomics*, vol. 3, no. 5, pp. 317–324, 2006.

[49] V. Boni, R. Zarate, J. C. Villa et al., "Role of primary miRNA polymorphic variants in metastatic colon cancer patients treated with 5-fluorouracil and irinotecan," *Pharmacogenomics Journal*. In press.

[50] A. E. Szafranska, T. S. Davison, J. John et al., "MicroRNA expression alterations are linked to tumorigenesis and non-neoplastic processes in pancreatic ductal adenocarcinoma," *Oncogene*, vol. 26, no. 30, pp. 4442–4452, 2007.

[51] A. E. Szafranska, M. Doleshal, H. S. Edmunds et al., "Analysis of microRNAs in pancreatic fine-needle aspirates can classify benign and malignant tissues," *Clinical Chemistry*, vol. 54, no. 10, pp. 1716–1724, 2008.

[52] M. C. Du Rieu, J. Torrisani, J. Selves et al., "MicroRNA-21 is induced early in pancreatic ductal adenocarcinoma precursor lesions," *Clinical Chemistry*, vol. 56, no. 4, pp. 603–612, 2010.

[53] M. Dillhoff, J. Liu, W. Frankel, C. Croce, and M. Bloomston, "MicroRNA-21 is overexpressed in pancreatic cancer and a potential predictor of survival," *Journal of Gastrointestinal Surgery*, vol. 12, no. 12, pp. 2171–2176, 2008.

[54] N. Ikenaga, K. Ohuchida, K. Mizumoto et al., "MicroRNA-203 expression as a new prognostic marker of pancreatic adenocarcinoma," *Annals of Surgical Oncology*, vol. 17, no. 12, pp. 3120–3128, 2010.

[55] J. Wang, J. Chen, P. Chang et al., "MicroRNAs in plasma of pancreatic ductal adenocarcinoma patients as novel blood-based biomarkers of disease," *Cancer Prevention Research*, vol. 2, no. 9, pp. 807–813, 2009.

[56] X. Kong, Y. Du, G. Wang et al., "Detection of differentially expressed microRNAs in serum of pancreaticductal adenocarcinoma patients: miR-196a could be a potential marker for poor prognosis," *Digestive Diseases and Sciences*. In press.

[57] J. K. Park, E. J. Lee, C. Esau, and T. D. Schmittgen, "Antisense inhibition of microRNA-21 or -221 arrests cell cycle, induces apoptosis, and sensitizes the effects of gemcitabine in pancreatic adenocarcinoma," *Pancreas*, vol. 38, no. 7, pp. e190–e199, 2009.

[58] Y. Murakami, T. Yasuda, K. Saigo et al., "Comprehensive analysis of microRNA expression patterns in hepatocellular carcinoma and non-tumorous tissues," *Oncogene*, vol. 25, no. 17, pp. 2537–2545, 2006.

[59] W. Li, L. Xie, X. He et al., "Diagnostic and prognostic implications of microRNAs in human hepatocellular carcinoma," *International Journal of Cancer*, vol. 123, no. 7, pp. 1616–1622, 2008.

[60] H. Varnholt, U. Drebber, F. Schulze et al., "MicroRNA gene expression profile of hepatitis C virus-associated hepatocellular carcinoma," *Hepatology*, vol. 47, no. 4, pp. 1223–1232, 2008.

[61] P. Pineau, S. Volinia, K. McJunkin et al., "miR-221 overexpression contributes to liver tumorigenesis," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 107, no. 1, pp. 264–269, 2010.

[62] Q. W. L. Wong, A. K. K. Ching, A. W. H. Chan et al., "MiR-222 overexpression confers cell migratory advantages in hepatocellular carcinoma through enhancing AKT signaling," *Clinical Cancer Research*, vol. 16, no. 3, pp. 867–875, 2010.

[63] Y. Yamamoto, N. Kosaka, M. Tanaka et al., "MicroRNA-500 as a potential diagnostic marker for hepatocellular carcinoma," *Biomarkers*, vol. 14, no. 7, pp. 529–538, 2009.

[64] M. Lagos-Quintana, R. Rauhut, A. Yalcin, J. Meyer, W. Lendeckel, and T. Tuschl, "Identification of tissue-specific microRNAs from mouse," *Current Biology*, vol. 12, no. 9, pp. 735–739, 2002.

[65] J. Chang, J. T. Guo, D. Jiang, H. Guo, J. M. Taylor, and T. M. Block, "Liver-specific microRNA miR-122 enhances the replication of hepatitis C virus in nonhepatic cells," *Journal of Virology*, vol. 82, no. 16, pp. 8215–8223, 2008.

[66] X. Tang, J. Gal, X. Zhuang, W. Wang, H. Zhu, and G. Tang, "A simple array platform for microRNA analysis and its application in mouse tissues," *RNA*, vol. 13, no. 10, pp. 1803–1822, 2007.

[67] Y. Ladeiro, G. Couchy, C. Balabaud et al., "MicroRNA profiling in hepatocellular tumors is associated with clinical features and oncogene/tumor suppressor gene mutations," *Hepatology*, vol. 47, no. 6, pp. 1955–1963, 2008.

[68] H. Kutay, S. Bai, J. Datta et al., "Downregulation of miR-122 in the rodent and human hepatocellular carcinomas," *Journal of Cellular Biochemistry*, vol. 99, no. 3, pp. 671–678, 2006.

[69] L. Gramantieri, F. Fornari, E. Callegari et al., "MicroRNA involvement in hepatocellular carcinoma: microRNA review series," *Journal of Cellular and Molecular Medicine*, vol. 12, no. 6, pp. 2189–2204, 2008.

[70] A. Budhu, H. L. Jia, M. Forgues et al., "Identification of metastasis-related microRNAs in hepatocellular carcinoma," *Hepatology*, vol. 47, no. 3, pp. 897–907, 2008.

[71] M. Girard, E. Jacquemin, A. Munnich, S. Lyonnet, and A. Henrion-Caude, "miR-122, a paradigm for the role of microRNAs in the liver," *Journal of Hepatology*, vol. 48, no. 4, pp. 648–656, 2008.

[72] D. D. Young, C. M. Connelly, C. Grohmann, and A. Deiters, "Small molecule modifiers of microRNA miR-122 function for the treatment of hepatitis C virus infection and hepatocellular carcinoma," *Journal of the American Chemical Society*, vol. 132, no. 23, pp. 7976–7981, 2010.

[73] C. Coulouarn, V. M. Factor, J. B. Andersen, M. E. Durkin, and S. S. Thorgeirsson, "Loss of miR-122 expression in liver cancer correlates with suppression of the hepatic phenotype and gain of metastatic properties," *Oncogene*, vol. 28, no. 40, pp. 3526–3536, 2009.

[74] W. C. Tsai, P. W. C. Hsu, T. C. Lai et al., "MicroRNA-122, a tumor suppressor MicroRNA that regulates intrahepatic metastasis of hepatocellular carcinoma," *Hepatology*, vol. 49, no. 5, pp. 1571–1582, 2009.

[75] Y. Li, W. Tan, T. W. L. Neo et al., "Role of the miR-106b-25 microRNA cluster in hepatocellular carcinoma," *Cancer Science*, vol. 100, no. 7, pp. 1234–1242, 2009.

[76] L. Gramantieri, F. Fornari, M. Ferracin et al., "MicroRNA-221 targets Bmf in hepatocellular carcinoma and correlates with tumor multifocality," *Clinical Cancer Research*, vol. 15, no. 16, pp. 5073–5081, 2009.

[77] F. Fornari, L. Gramantieri, M. Ferracin et al., "MiR-221 controls CDKN1C/p57 and CDKN1B/p27 expression in human hepatocellular carcinoma," *Oncogene*, vol. 27, no. 43, pp. 5651–5661, 2008.

[78] F. Meng, R. Henson, H. Wehbe-Janek, K. Ghoshal, S. T. Jacob, and T. Patel, "MicroRNA-21 regulates expression of the PTEN tumor suppressor gene in human hepatocellular cancer," *Gastroenterology*, vol. 133, no. 2, pp. 647–658, 2007.

[79] Y. Xiong, J. H. Fang, J. P. Yun et al., "Effects of microrna-29 on apoptosis, tumorigenicity, and prognosis of hepatocellular carcinoma," *Hepatology*, vol. 51, no. 3, pp. 836–845, 2010.

[80] G. E. Chung, J. H. Yoon, S. J. Myung et al., "High expression of microRNA-15b predicts a low risk of tumor recurrence following curative resection of hepatocellular carcinoma," *Oncology Reports*, vol. 23, no. 1, pp. 113–119, 2010.

[81] H. Su, J. R. Yang, T. Xu et al., "MicroRNA-101, down-regulated in hepatocellular carcinoma, promotes apoptosis and suppresses tumorigenicity," *Cancer Research*, vol. 69, no. 3, pp. 1135–1142, 2009.

[82] J. Huang, Y. Wang, Y. Guo, and S. Sun, "Down-regulated microRNA-152 induces aberrant DNA methylation in hepatitis B virus-related hepatocellular carcinoma by targeting DNA methyltransferase 1," *Hepatology*, vol. 52, no. 1, pp. 60–70, 2010.

[83] J. Ji, J. Shi, A. Budhu et al., "MicroRNA expression, survival, and response to interferon in liver cancer," *New England Journal of Medicine*, vol. 361, no. 15, pp. 1437–1447, 2009.

[84] E. Connolly, M. Melegari, P. Landgraf et al., "Elevated expression of the miR-17-92 polycistron and miR-21 in hepadnavirus-associated hepatocellular carcinoma contributes to the malignant phenotype," *American Journal of Pathology*, vol. 173, no. 3, pp. 856–864, 2008.

[85] C. L. Jopling, "Regulation of hepatitis C virus by microRNA-122," *Biochemical Society Transactions*, vol. 36, no. 6, pp. 1220–1223, 2008.

[86] J. I. Henke, D. Goergen, J. Zheng et al., "MicroRNA-122 stimulates translation of hepatitis C virus RNA," *EMBO Journal*, vol. 27, no. 24, pp. 3300–3310, 2008.

[87] R. K. Jangra, M. Yi, and S. M. Lemon, "Regulation of hepatitis C virus translation and infectious virus production by the microRNA miR-122," *Journal of Virology*, vol. 84, no. 13, pp. 6615–6625, 2010.

[88] R. E. Lanford, E. S. Hildebrandt-Eriksen, A. Petri et al., "Therapeutic silencing of microRNA-122 in primates with chronic hepatitis C virus infection," *Science*, vol. 327, no. 5962, pp. 198–201, 2010.

[89] M. Sarasin-Filipowicz, J. Krol, I. Markiewicz, M. H. Heim, and W. Filipowicz, "Decreased levels of microRNA miR-122 in individuals with hepatitis C responding poorly to interferon therapy," *Journal of Hepatology*, vol. 51, pp. 606–609, 2009.

[90] X. Liu, T. Wang, T. Wakita, and W. Yang, "Systematic identification of microRNA and messenger RNA profiles in hepatitis C virus-infected human hepatoma cells," *Virology*, vol. 398, no. 1, pp. 57–67, 2010.

[91] L. Yang, Z. Ma, D. Wang, W. Zhao, L. Chen, and G. Wang, "MicroRNA-602 regulating tumor suppressive gene RASSF1A is overexpressed in hepatitis B virus-infected liver and hepatocellular carcinoma," *Cancer Biology and Therapy*, vol. 9, no. 10, pp. 803–808, 2010.

[92] S. Ura, M. Honda, T. Yamashita et al., "Differential microRNA expression between hepatitis B and hepatitis C leading disease progression to hepatocellular carcinoma," *Hepatology*, vol. 49, no. 4, pp. 1098–1112, 2009.

[93] J. Kota, R. R. Chivukula, K. A. O'Donnell et al., "Therapeutic delivery of miR-26a inhibits cancer cell proliferation and induces tumor-specific apoptosis," *Cell*, vol. 137, pp. 1005–1017, 2009.

[94] J. Krutzfeldt, N. Rajewsky, R. Braich et al., "Silencing of microRNAs in vivo with 'antagomirs'," *Nature*, vol. 438, no. 7068, pp. 685–689, 2005.

[95] J. Elmen, M. Lindow, S. Schütz et al., "LNA-mediated microRNA silencing in non-human primates," *Nature*, vol. 452, no. 7189, pp. 896–899, 2008.

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Review Article

Hereditary Diffuse Gastric Cancer: Multidisciplinary Case Report with Review of the Literature

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Hereditary diffuse gastric cancer (HDGC) is a rare, inherited cancer syndrome with at least one fourth of HDGC patients having an autosomal dominantly inherited mutation of *CDH1* (*E-Cadherin*). Penetrance is relatively high (70–80% lifetime risk for gastric cancer). It is important for pathologists to recognize the syndrome's phenotype in early gastric lesions: patchy intramucosal signet ring cells often associated with pagetoid spread. Due to the insidious nature of this lesion, surveillance is limited and currently prophylactic gastrectomy is an option chosen by many HDGC patients. We present a case report from a multidisciplinary team of authors with a review of the literature that includes the updated guidelines for *CDH1* genetic testing.

1. The Case

An asymptomatic 36-year-old male presented for an elective, increased risk, upper endoscopy (EGD). The patient's remarkable history included at least five family members diagnosed with gastric carcinoma, four known to have occurred at an early age (Figure 1). He had a documented mutation in the *CDH1* (*E-Cadherin*) gene (1212delC) identified approximately one month prior to this surveillance EGD. The EGD was essentially normal. The stomach insufflated and decompressed without difficulty and there was no visual evidence of mucosal changes. EGD biopsies were read as gastric body mucosa without diagnostic abnormality and were negative for *Helicobacter pylori*, intestinal metaplasia, dysplasia, and malignancy.

Three months later, following genetic counseling, the patient was admitted for an exploratory laparotomy with a prophylactic total gastrectomy with D2 lymphadenectomy and end-to-side Roux-en-Y esophagojejunostomy and needle catheter feeding jejunostomy. The patient's sister and two cousins had recently undergone similar prophylactic procedures.

Surgical exploration showed no evidence of metastatic disease. Gross pathological examination of the stomach revealed intact, pink mucosa with normal rugae and no masses. The entire surgical margins, multiple (>40) gastric sections from all anatomic zones, and the entire perigastric adipose tissue were submitted for histologic review. Histological examination revealed multifocal, intramucosal signet-ring cell adenocarcinoma with focal pagetoid spread of the signet ring cells in preserved fundic glands (Figure 2(a)). The sections were negative for gastric dysplasia and *Helicobacter pylori*. All margins and lymph nodes were negative for tumor (AJCC pathologic stage pT1a pN0).

The patient had an uneventful postoperative recovery with an upper gastrointestinal study done on postoperative day #5 that showed no anastomotic leaks or strictures. He was discharged on postoperative day #7. Followup examinations revealed extensive weight loss (approximately 50 lbs) but the patient was otherwise doing well.

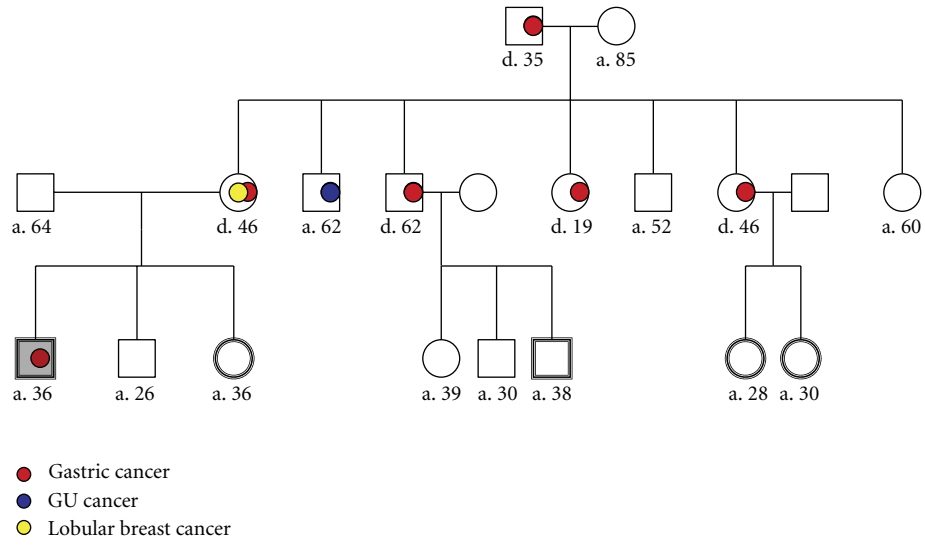
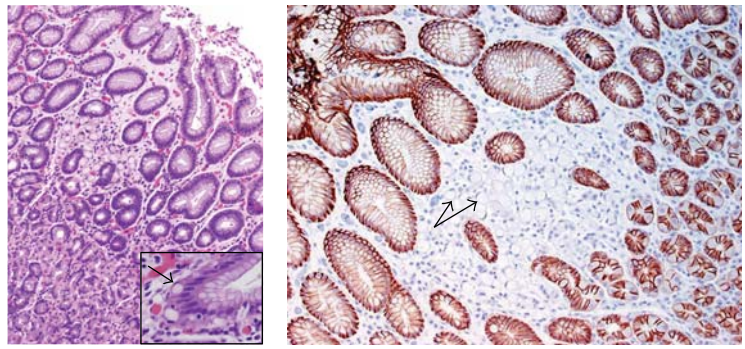


Figure 1: Pedigree of case study (index) patient (shaded grey). Note that five additional family members show a history of gastric cancer with the youngest presentation being 19 years of age. Double outline indicates positive genetic testing for E-Cadherin mutation (1212delC).



(a) (b)

Figure 2: (a) Focal intramucosal signet-ring cell adenocarcinoma in case study patient with known CDH1 mutation. Insert highlights area of pagetoid spread. (b) Focal absence of E-Cadherin staining highlights the tumor cluster in case study patient. In those HDGC cases in which tumor cells show decreased E-cadherin expression, the normal background epithelium serves as an excellent internal control.

2. Molecular Carcinogenesis

Only a small number, 2–8%, of all gastric carcinomas arise from inherited gastric cancer syndromes [1, 2]. The majority of families with autosomal dominant familial gastric carcinoma will have the diffuse, poorly differentiated (linitis plastica) morphologic subtype and are referred to as *hereditary diffuse gastric cancer* (HDGC). A germline mutation in the tumor suppressor gene *CDH1* (*E-Cadherin*) is identified in approximately 25–48% of individuals with HDGC [3, 4]. E-Cadherin is an epithelial cell-cell adhesion molecule essential to cell differentiation and normal epithelial cell architecture. It is therefore not surprising that germline mutations in *E-Cadherin* are highly specific for families who meet HDGC criteria and has not been described in families with inherited intestinal type/morphology gastric carcinoma [3, 5, 6].

In 1999, the International Gastric Cancer Linkage Consortium (IGCLC) met to first define criteria required to select those patients appropriate to receive mutational screening for HDGC [5]. Strict application of these 1999 IGCLC criteria found approximately one third of screened patients to be heterozygous for a germline, point or frameshift, mutation of the *CDH1* gene on chromosome 16q22.1 [6, 7]. This implies that there are either currently unidentifiable *CDH1* mutations or other genes causing HDGC in some families. Medical management recommendations are particularly difficult in these families with undetectable *CDH1* mutations. There are at least twenty-seven documented inactivating (truncating) *CDH1* mutations scattered at various exons along the *E-Cadherin* gene described in a diverse ethnic population [3, 8–10]. In a recent study addressing patients that met HDGC criteria but lacked *CDH1* germline point mutations, Oliveira et al. found that 6.5% of their study patients had large deletions affecting the *CDH1* locus by using multiplex ligation-dependent probe amplification (MLPA) [7]. Therefore, analysis for large

Table 1: (Updated) Criteria for CDH1 molecular genetic testing.

Two or more cases of gastric cancer in a family, with at least one histologically confirmed diffuse gastric cancer (DGC) diagnosed before the age of 50.
Three or more confirmed cases of DGC in 1st or 2nd degree relatives, independent of age of onset.
An individual diagnosed with DGC before the age of 40.
An individual or family members diagnosed with DGC and lobular breast cancer, one being diagnosed before the age of 50.

genomic deletions using alternative techniques such as MLPA or array comparative genomic hybridization (CGH) should be explored in highly suspicious cases in which standard DNA sequencing is negative for point mutations.

The current hypothesis for how HDGC susceptibility cancers lose their CDH1 heterozygosity and thus their E-Cadherin expression follows the "two-hit" mutation theory [11]. Hypermethylation of the *CDH1* promoter is the most common cause of inactivation of the second allele; however, mutation and loss of heterozygosity (LOH) are also well-documented culprits [11–13]. Interestingly, the mechanisms of the "2nd hit" may differ in primary tumors versus metastases, an important consideration for therapeutics. The morphologic expression of the second hit is multifocal clusters of tumor cells that have lost or show abnormal (reduced) E-cadherin expression (Figure 2(b)). The subsequent step, progression to invasion of the submucosa, is less well defined. The current line of thought involves an integrated role between additional genetic events and changes in microenvironment. Unfortunately, there are no current means of predicting the time course between tumor expansion and submucosal invasion.

The *CDH1* mutation carries a high penetrance with carriers of germline mutations having an estimated lifetime risk of 67% in men and 83% in women to develop gastric carcinoma [4, 14]. In HDGC families, the most commonly described malignancy other than diffuse gastric carcinoma is lobular breast adenocarcinoma. Female carriers of a germline *CDH1* mutation have a 40% lifetime risk of developing this subset of breast cancer [14]. In general, susceptibility cancers in HDGC occur at a relatively early age, with the average age of presentation of diffuse gastric carcinoma being 38 years [15].

3. Clinicopathologic Management

The IGCLC guidelines were first developed in 1999 to support clinical management of families felt to be predisposed to gastric carcinoma [5]. This multidisciplinary group has recently provided us with an updated, somewhat broadened version of their original guidelines as to which patients should be offered molecular genetic testing (Table 1) [4]. Any patient who meets the minimum requirements for HDGC listed in Table 1 should be offered genetic counseling and testing for the *CDH1* mutation. Health care professionals experienced in cancer genetics must provide those patients that choose to undergo this testing pre- and posttesting genetic counseling.

In general, annual endoscopy using a white light high-definition endoscope is recommended for surveillance of HDGC patients. Using histopathology mapping in six gastrectomy specimens from New Zealand Maori HDGC families, Charlton et al. showed a preferential pattern of intramucosal diffuse gastric carcinoma in the body-antral transitional zone [16]. Based on their study, they proposed a targeted approach to endoscopy with the goal of minimizing sampling error. However, results from prophylactic gastrectomy specimens from other areas of the world did not show similar findings [17–21]. Due to the discrepancy in study results regarding localization of early gastric lesions, multiple gastric biopsies representing all of the major gastric anatomic zones is recommended. The HDGC endoscopic protocol provided by the most recent consensus guidelines suggests the following biopsies: a single antral biopsy taken first for surveillance of *H. pylori* status, any focal lesions, and in addition at least three biopsies from each anatomical area (prepyloric area, gastric antrum, transitional zone, gastric body, gastric fundus, and gastric cardia) [4]. This extensive sampling is driven by the insidious phenotype of this disease (patchy, diffuse growth pattern of gastric carcinoma under endoscopically normal gastric mucosa), which leads to low sensitivity in currently available surveillance procedures.

Our HDGC patient was found to have occult intramucosal signet ring cell adenocarcinoma on resection despite having a normal endoscopic appearance to the stomach and no diagnostic findings by random endoscopic biopsies. Upon review of the literature representing different, independent HDGC families, the identification of indolent gastric cancer is consistent with the pathology found in the majority of cases following prophylactic gastrectomy [19, 20, 22]. In light of the inherent limitations to the current screening procedures/tests available for HDGC patients, a prophylactic gastrectomy is not only a reasonable option but some may argue a life saving one. The estimated 30-day postgastrectomy mortality rate is cited as 3–6% [23] and most likely reaches even smaller numbers when a gastrointestinal surgeon who routinely performs gastrectomies and other major surgeries performs the procedure. To put this in perspective, the 5-year mortality rate in patients with symptomatic, invasive gastric carcinoma is 90% [24].

However, this procedure is not without long-term complications. All patients who undergo gastrectomy will have postoperative weight loss. Many will have metabolic complications including malabsorption, diarrhea, and/or "dumping syndrome." Dieticians are often required to assist postsurgical patients in nutritional management. There are

also secondary surgical complications such as esophageal strictures. A more thorough understanding of the long-term physical and psychological effects of this surgery will only emerge through followup of these relatively young HDGC patients. The current consensus guidelines have called for a central registry of HDGC patients who have undergone prophylactic gastrectomies [4]. This would provide essential prospective data regarding effects of the surgery as well as long-term followup regarding disease-free status.

Prophylactic gastrectomies for HDGC will most likely occur at major academic institutions in which specialized gastrointestinal pathologist are readily available. The gastric specimen is inked and fixed with lymph node retrieval as in any gastrectomy specimen. However, following adequate (overnight) fixation, these specimens should be photographed with mapped sampling occurring from all anatomical zones as well as submission of the entire surgical margins. If carcinoma is not identified, additional mapped sampling will be required. The focal intramucosal signet ring cells are generally identified on H & E morphology. A combination of cytokeratin (positive immunoreactivity) and E-Cadherin (negative or reduced immunoreactivity; see Figure 2(b)) can highlight these foci, especially in areas of pagetoid spread. Interestingly, in their study of eight total gastrectomy specimens done for germline *E-cadherin* mutations, Rogers et al. found two cases which showed reversion of E-cadherin expression in foci of deeply invasive adenocarcinoma while the superficial signet ring cells cancer showed the expected loss or reduced E-cadherin expression [21]. Additional, nonspecific histologic features described in some cases of HDGC include foveolar hyperplasia with or without tufting, cytoplasmic vacuolization, and clustered histiocytes or vaguely granulomatous reactions occurring around ruptured glands [4, 18, 21].

The IGCLC also suggests that female carriers of the *CDH1* mutation receive high-risk screening for lobular breast adenocarcinoma from the age of 35. Other cancers, such, signet ring cell carcinoma of the colon, have been proposed to be associated with HDGC. Colon cancer screening may therefore be recommended in HDGC patients who have a pedigree showing colon carcinoma presenting before the age of 40. This type of personalized heightened cancer screening emphasizes the need for thorough documentation of family histories as well as the importance of enrolling HDGC patient into cooperative registries for ongoing clinical research.

4. Conclusion

At least one third of all patients with HDGC have an autosomal dominantly inherited mutation of the *CDH1* (*E-Cadherin*) gene. Penetrance is high with a specific phenotype of diffuse, signet ring cell morphology. Pathologists should be aware of this phenotype of patchy clusters of intramucosal signet ring tumor cells associated with a pagetoid spread of individual signet ring cells (Figure 2), although not known to be entirely specific, this finding, especially in a young patient, may warrant a discussion with the treating clinician regarding referral to a genetic counselor. In patients known to have HDGC, a multidisciplinary approach, including genetic counselors, subspecialized pathologists, clinical researchers, dietitians, and experienced gastrointestinal surgeons, is essential in the management of these patients. Due to the insidious nature of early lesions in this disorder, endoscopy is not an adequate screening method. However, annual white light high-definition endoscopy with extensive biopsy sampling may provide a method of surveillance for those patients who are not good surgical candidates, who refuse more aggressive methods, or are carriers of mutations (e.g., missense) where clinical significance is less defined.

The prophylactic gastrectomy specimen of our patient known to carry a *CDH1* mutation was found to have multifocal intramucosal signet ring cell adenocarcinoma despite normal endoscopic exam and biopsies. Previous publications, of at least three major groups, document the same finding in multiple HDGC families, making these risk-reducing gastrectomies simultaneously therapeutic. Currently there is no way of determining the latent period between the intramucosal CDH1 −/− adenocarcinoma and invasion into submucosa. However, it is important to note that our patient had at least two family members that died of HDGC at ages younger (19 and 35 years of age) than his age at the time of gastrectomy (36 years of age). Until better screening tests emerge, gastrectomy may be the only adequate means of lengthening survival in carriers of the *CDH1* mutation.

References

[1] H. F. A. Vasen, J. T. Wijnen, F. H. Menko et al., "Cancer risk in families with hereditary nonpolyposis colorectal cancer diagnosed by mutation analysis," *Gastroenterology*, vol. 110, no. 4, pp. 1020–1027, 1996.

[2] C. La Vecchia, E. Negri, S. Franceschi, and A. Gentile, "Family history and the risk of stomach and colorectal cancer," *Cancer*, vol. 70, no. 1, pp. 50–55, 1992.

[3] A. R. Brooks-Wilson, P. Kaurah, G. Suriano et al., "Germline E-cadherin mutations in hereditary diffuse gastric cancer: assessment of 42 new families and review of genetic screening criteria," *Journal of Medical Genetics*, vol. 41, no. 7, pp. 508–517, 2004.

[4] R. C. Fitzgerald, R. Hardwick, D. Huntsman et al., "Hereditary diffuse gastric cancer: updated consensus guidelines for clinical management and directions for future research," *Journal of Medical Genetics*, vol. 47, no. 7, pp. 436–444, 2010.

[5] C. Caldas, F. Carneiro, H. T. Lynch et al., "Familial gastric cancer: overview and guidelines for management," *Journal of Medical Genetics*, vol. 36, no. 12, pp. 873–880, 1999.

[6] C. Oliveira, M. C. Bordin, N. Grehan et al., "Screening E-cadherin in gastric cancer families reveals germline mutations only in hereditary diffuse gastric cancer kindred," *Human Mutation*, vol. 19, no. 5, pp. 510–517, 2002.

[7] C. Oliveira, J. Senz, P. Kaurah et al., "Germline CDH1 deletions in hereditary diffuse gastric cancer families," *Human Molecular Genetics*, vol. 18, no. 9, pp. 1545–1555, 2009.

[8] S. A. Gayther, K. L. Gorringe, S. J. Ramus et al., "Identification of germ-line E-cadherin mutations in gastric cancer families of

European origin," *Cancer Research*, vol. 58, no. 18, pp. 4086–4089, 1998.

[9] J. C. Rodriguez-Sanjuan, A. Fontalba, M. Mayorga et al., "A novel mutation in the E-cadherin gene in the first family with hereditary diffuse gastric cancer reported in Spain," *European Journal of Surgical Oncology*, vol. 32, no. 10, pp. 1110–1113, 2006.

[10] F. Roviello, G. Corso, C. Pedrazzani et al., "Hereditary diffuse gastric cancer and E-cadherin: description of the first germline mutation in an Italian family," *European Journal of Surgical Oncology*, vol. 33, no. 4, pp. 448–451, 2007.

[11] F. Graziano, B. Humar, and P. Guilford, "The role of the E-cadherin gene (CDH1) in diffuse gastric cancer susceptibility: from the laboratory to clinical practice," *Annals of Oncology*, vol. 14, no. 12, pp. 1705–1713, 2003.

[12] M. Barber, A. Murrell, Y. Ito et al., "Mechanisms and sequelae of E-cadherin silencing in hereditary diffuse gastric cancer," *Journal of Pathology*, vol. 216, no. 3, pp. 295–306, 2008.

[13] C. Oliveira, S. Sousa, H. Pinheiro et al., "Quantification of epigenetic and genetic 2nd hits in CDH1 during hereditary diffuse gastric cancer syndrome progression," *Gastroenterology*, vol. 136, no. 7, pp. 2137–2148, 2009.

[14] P. D. P. Pharoah, P. Guilford, and C. Caldas, "Incidence of gastric cancer and breast cancer in CDH1 (E-cadherin) mutation carriers from hereditary diffuse gastric cancer families," *Gastroenterology*, vol. 121, no. 6, pp. 1348–1353, 2001.

[15] P. Kaurah, A. MacMillan, N. Boyd et al., "Founder and recurrent CDH1 mutations in families with hereditary diffuse gastric cancer," *Journal of the American Medical Association*, vol. 297, no. 21, pp. 2360–2372, 2007.

[16] A. Charlton, V. Blair, D. Shaw, S. Parry, P. Guilford, and I. G. Martin, "Hereditary diffuse gastric cancer: predominance of multiple foci of signet ring cell carcinoma in distal stomach and transitional zone," *Gut*, vol. 53, no. 6, pp. 814–820, 2004.

[17] M. E. Barber, V. Save, F. Carneiro et al., "Histopathological and molecular analysis of gastrectomy specimens from hereditary diffuse gastric cancer patients has implications for endoscopic surveillance of individuals at risk," *Journal of Pathology*, vol. 216, no. 3, pp. 286–294, 2008.

[18] F. Carneiro, D. G. Huntsman, T. C. Smyrk et al., "Model of the early development of diffuse gastric cancer in E-cadherin mutation carriers and its implications for patient screening," *Journal of Pathology*, vol. 203, no. 2, pp. 681–687, 2004.

[19] D. G. Huntsman, F. Carneiro, F. R. Lewis et al., "Early gastric cancer in young, asymptomatic carriers of germ-line E-cadherin mutations," *New England Journal of Medicine*, vol. 344, no. 25, pp. 1904–1909, 2001.

[20] Y. S. Chun, N. M. Lindor, T. C. Smyrk et al., "Germline E-cadherin gene mutations: is prophylactic total gastrectomy indicated?" *Cancer*, vol. 92, no. 1, pp. 181–187, 2001.

[21] W. M. Rogers, E. Dobo, J. A. Norton et al., "Risk-reducing total Gastrectomy for germline mutations in E-cadherin (CDH1): pathologic findings with clinical implications," *American Journal of Surgical Pathology*, vol. 32, no. 6, pp. 799–809, 2008.

[22] H. T. Lynch and J. F. Lynch, "Hereditary diffuse gastric cancer: lifesaving total gastrectomy for CDH1 mutation carriers," *Journal of Medical Genetics*, vol. 47, no. 7, pp. 433–435, 2010.

[23] R. C. Fitzgerald and C. Caldas, "E-cadherin mutations and hereditary gastric cancer: prevention by resection?" *Digestive Diseases*, vol. 20, no. 1, pp. 23–31, 2002.

[24] S. Msika, M. A. Tazi, A. M. Benhamiche, C. Couillault, M. Hard, and J. Faivre, "Population-based study of diagnosis, treatment and prognosis of gastric cancer," *British Journal of Surgery*, vol. 84, no. 10, pp. 1474–1478, 1997.

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Review Article

Molecular Aspects of *H. pylori*-Related MALT Lymphoma

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Helicobacter pylori-related extranodal marginal zone lymphoma of mucosa-associated lymphoid tissue is a paradigm for malignancy arising in an inflammatory background. While the diagnosis of *H. pylori* gastritis is often straightforward, distinction between severe gastritis and early lymphoma can be difficult and requires careful assessment of clinical findings in addition to histological features and immunohistochemical results. A number of cytogenetic abnormalities have been discovered in *H. pylori*-related lymphomas and several have clinical importance, related to the responsiveness of lymphoma to *H. pylori* eradication therapy, but routine molecular studies are not widely utilized. While molecular methods may be used in equivocal cases, a trial of conservative therapy is warranted given the propensity for these lymphomas to regress with eradication of the organism. Once therapy is initiated, care must be taken to avoid a premature assignment of disease refractoriness because complete response can take several months to more than a year. Cases truly refractory to *H. pylori* eradication therapy may be treated with adjuvant chemoradiation with a high response rate.

1. Introduction

Helicobacter pylori is a common pathogen and the most frequent cause of gastric and duodenal ulcers (Figure 1) [1, 2]. This Gram-negative, curved bacillus was first recognized as the cause of human disease by Marshall and Warren in the 1980s and has since been classified as a class I carcinogen, potentially leading to gastric adenocarcinoma and, more commonly, extranodal marginal zone lymphoma of mucosa-associated lymphoid tissue (MALT lymphoma) [2–6]. Most *H. pylori*-related MALT lymphomas arise in the stomach, but extragastric lymphomas may also be related to the organism, particularly in the duodenum [7]. The gastritis caused by *H. pylori* is morphologically distinctive, with a band-like infiltrate of plasma cells and lymphocytes in the superficial gastric mucosa, typically in the antrum, accompanied by active (neutrophilic) inflammation in the mucus neck region of the mucosa, at the interface between the foveolae and glands. While *H. pylori* gastritis is most often easily recognized, the dividing line between severe gastritis and early MALT lymphoma is frequently indistinct [6, 7].

Several cytogenetic abnormalities have been described in gastrointestinal (GI) MALT lymphoma, and inappropriate activation of the NF-κB pathway is a common thread [8–11]. While molecular assays for these abnormalities are available, guidelines for their routine use are not widely accepted, and the vast majority of cases of gastric as well as many extragastric, MALT lymphomas respond to conservative therapy aimed at eradication of infection [8, 12–17]. Some cytogenetic translocations seen in a subset of MALT lymphomas, however, are associated with resistance to antibiotic therapy. The routine use of molecular assays in diagnosis and prognosis of MALT lymphomas is controversial, and many cases are negative for any known cytogenetic abnormality [18–20].

This paper summarizes the current knowledge of cytogenetic abnormalities in GI MALT lymphoma, with particular attention to *H. pylori*-related gastric lymphomas. Currently available molecular testing methods are discussed, followed by a practical approach to their use in diagnosis. Finally, recommendations for disease followup are offered, with an emphasis on the utility of conservative therapy and

avoidance of a premature determination of refractoriness to *H. pylori* eradication therapy.

2. MALT Lymphoma: A Review of Concepts

MALT lymphoma (Figure 2) is a low-grade B-cell lymphoma composed predominantly of small lymphocytes, first described around the same time as *H. pylori* by Isaacson and Wright [21]. Morphologically, the lymphoma cells may be centrocyte-like (with small nuclei and scant cytoplasm, resembling follicle center cells) or monocytoid (with ample pale cytoplasm and indented nuclei), often with admixed centroblast-like cells (large cells that may have prominent nucleoli) [6]. Monocytoid cells are fairly characteristic of MALT lymphoma, but individual cases are commonly a mixture of the three cell types, with centroblast-like large cells typically being individually scattered. Plasmacytic differentiation is common, and some cases are almost completely plasmacytic in appearance [6, 22]. While immunohistochemistry (IHC) is useful in diagnosis, there remains no specific IHC marker for MALT lymphoma [7, 18].

MALT lymphoma accounts for about 8% of all B-cell lymphomas and tends to occur in older individuals with a nearly equal sex distribution [22]. About 85% of all GI MALT lymphomas occur in the stomach, and isolated involvement of the small intestine is rare [7]. Conversely, about 25% of gastric MALT lymphomas are accompanied by involvement of other GI sites, and there is evidence that it is a truly systemic disease [6, 18, 23]. The vast majority of GI MALT lymphomas are thought to be related to *H. pylori* infection, which is believed to lead to malignant transformation via chronic antigenic stimulation resulting in the clonal expansion of subpopulations of B cells [18, 24–27]. The noninfected stomach is largely devoid of lymphoid tissue; *H. pylori* gastritis owes its appearance to the acquisition of a lymphoplasmacytic infiltrate in the gastric lamina propria, which may or may not be accompanied by B-cell nodules and even germinal centers [6, 7, 18]. This type of tissue is termed "acquired MALT" to distinguish it from "native MALT" of the type seen, for example, in the distal ileum, where germinal centers make up the Peyer's patches.

Interestingly, while MALT lymphoma is a clonal B-cell neoplasm, the process of lymphomagenesis is believed to be driven by activated T cells [7, 27–29]. *H. pylori* strains expressing the *CagA* gene have been associated with significant morbidity, and this gene may play a role in lymphomagenesis [30]. The transformation of *H. pylori*-related MALT lymphoma to diffuse large B-cell lymphoma (DLBCL) has been postulated, although the exact mechanism of such a transformation remains obscure [6, 11, 18]. DLBCL is the most common GI lymphoma overall and the presence of a large cell component should be mentioned in the diagnosis when it occurs in association with MALT lymphomas [7, 22]. Sheets of large (centroblast-like) cells should prompt an unequivocal diagnosis of DLBCL, even when a recognizable low-grade MALT component is also present. Furthermore, such a low-grade component should be mentioned with the diagnosis of DLBCL when present. The former diagnosis of "high-grade MALT lymphoma" is no longer recognized and should not be used.

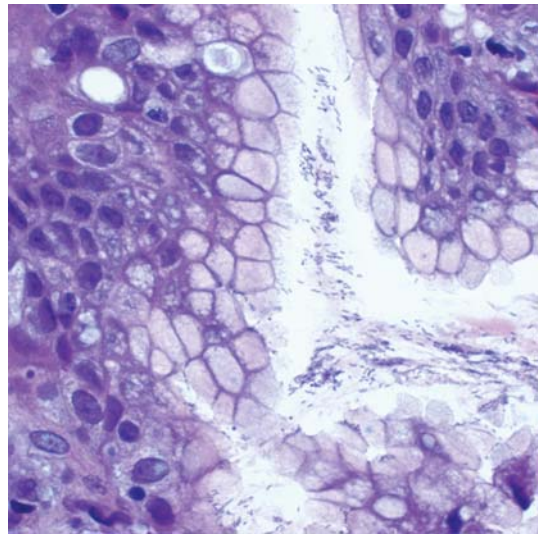

Figure 1: *Helicobacter pylori*, the underlying cause of most cases of gastric MALT lymphoma. The curved bacteria are seen in the surface mucus layer of the gastric pits and have a characteristic, curvilinear, "gull-wing" appearance (600x original magnification).

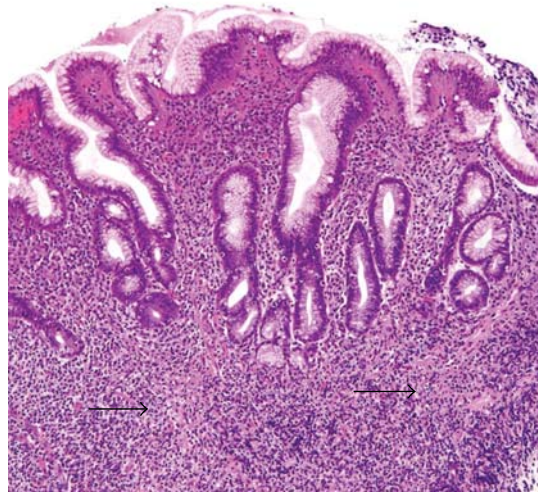

Figure 2: MALT lymphoma. The histomorphology in this case closely resembles severe *H. pylori* gastritis. Note the infiltration of the muscularis mucosae at the base of the mucosa (arrows). The clinical information was crucial in this case, as diffusely thickened gastric folds were seen endoscopically (400x original magnification).

3. Cytogenetic Abnormalities in Gastrointestinal MALT Lymphoma

3.1. t(11;18)(q21;q21) API2/MALT1. The t(11;18) translocation involves the fusion of the N-terminus of *API2* (apoptosis

inhibitor-2) on chromosome 11 and the C-terminus of *MALT1* (MALT lymphoma associated translocation) on chromosome 18. It is the most common translocation found in MALT lymphomas of the GI tract, involving up to 25% of gastric MALT lymphoma and 40%–60% of MALT lymphoma occurring in the small intestine [31, 32].

The presence of this translocation correlates with resistance to antibiotic therapy and MALT occurring without concomitant chronic active *H. pylori* gastritis, although it has been seen in specific strains of *H. pylori* infection [32, 33]. Cases with this translocation are more likely to have disseminated rather than stage I disease, but it is infrequently associated with diffuse large B-cell lymphoma [34, 35]. Some evidence suggests that plasmacytic morphology may not be seen in such cases [36].

Assays for the presence of t(11;18) are widely available. Methods include fluorescence in situ hybridization (FISH) or reverse transcription polymerase chain reaction (RT-PCR), both of which can be performed on fresh tissue or archival formalin-fixed paraffin-embedded tissue. FISH is more sensitive in the rare cases that do not involve the most common breakpoint. Dual color dual fusion FISH has been in use for the past ten years utilizing metaphase chromosomes or interphase nuclei [37]. In addition, break-apart MALT1 FISH probes are available, relatively easily interpreted in comparison to fusion probes, and may have the advantage of being useful in detecting MALT1 rearrangements in either t(11;18) or t(14;18) (see below) using one assay. IHC for BCL10 protein will show nuclear expression in many cases with the translocation; however, this is not specific and can be seen in t(1;14)(p22;q32) in association with the *BCL10/IGH* fusion [32]. The vast majority of cases with t(11;18) will show weak cytoplasmic MALT1 protein expression using IHC [38].

3.2. t(14;18)(q32;q21) IgH/MALT1. The t(14;18) translocation involves the immunoglobulin heavy chain gene (*IgH*) on chromosome 14 and *MALT1* on chromosome 18 which activates the NF-κB pathway [39]. This translocation may coexist with trisomies (3, 12, or 18). While the breakpoint on chromosome 18 is in the same region as that seen in follicular lymphoma, the gene involved is *MALT1* rather than *BCL2*; rare cases of MALT lymphoma have reportedly been associated with the *IgH-BCL2* fusion [40]. While IgH/MALT1 is present in a large minority of MALT lymphomas, it occurs more commonly in non-GI sites. It has been reported, however, in unusual GI sites such as the liver [41].

Assays to detect t(14;18) include dual fusion FISH and PCR. IHC for MALT1 and BCL10 is highly sensitive, typically exhibiting strong, uniform cytoplasmic expression [38]; however, neither of these immunostains is specific for this translocation, as weak cytoplasmic staining for MALT1 and strong nuclear staining for BCL10 are characteristic of t(11;18). As these immunostains can be expressed in other translocations, they are not specific markers.

3.3. t(1;14)(p22;q32) BCL10/IGH. The t(1;14) translocation juxtaposes the *BCL10* gene on chromosome 1 with the *IgH* gene on chromosome 14. A variant translocation involving lambda light chain on chromosome 2 has also been reported [42]. These translocations are present in a minority of MALT lymphoma (1%–3%); however, it is important as gastric MALT lymphoma with this translocation can also demonstrate antibiotic resistance [43]. It has not been reported in other non-Hodgkin lymphomas. As mentioned previously, IHC for BCL10 shows strong nuclear expression. MALT1 is weakly expressed in the cytoplasm [38].

3.4. t(3;14)(q27;q32) BCL6/IGH and Other BCL6 Rearrangements. BCL6 translocations are involved in a small number of MALT lymphomas, approximately 1%-2% in one large study [44]. The translocation can involve the immunoglobulin heavy chain, light chains, or other partners. Of relevance in GI cases, it has been reported in cases of diffuse large B-cell lymphoma with concurrent MALT in the stomach [45].

BCL6 rearrangements can be identified by FISH using dual color break-apart probes. In a subset of *BCL6* rearranged cases, IHC for BCL6 protein will show nuclear staining of the lymphocytes which can cause diagnostic confusion with follicular lymphoma [44]. Only 25%–30% of the cases with the translocation in this study showed BCL6 expression by IHC. The number of cases is small, and additional large studies are needed.

3.5. t(3;14)(p14.1;q32) FOXP1/IGH. This translocation can be seen in diffuse large B-cell lymphoma and MALT lymphomas (ocular, thyroid, and cutaneous) without the t(11;18) [46]; it has not been commonly associated with MALT lymphomas of the GI tract.

3.6. Trisomy 3 and 18. Chromosomal trisomies are commonly detected in gastrointestinal marginal zone lymphomas but are nonspecific. Trisomy 3q27 is the most common chromosomal abnormality in gastrointestinal lymphomas. In one large series, it was present in 50%–65% of low-grade MALT lymphomas from stomach and small intestine [47]; however, other series showed a lower prevalence [31, 48]. It occurs in both low-grade marginal zone lymphomas and DLBCL and is more common in patients with higher stage disease [49]. Trisomy 18 can be seen independently or in association with trisomy 3 and has also been correlated with more aggressive disease, especially in gastrointestinal lymphomas classified as diffuse large B-cell lymphoma [50]. Both trisomies can be detected using FISH with chromosomal enumeration probes, and break-apart probes for BCL6 and MALT1 may also potentially identify these abnormalities.

4. Diagnosis of MALT Lymphoma and Use of Molecular Assays: A Pragmatic Approach

The distinction between severe *H. pylori* gastritis and early MALT lymphoma is often difficult and requires careful assessment of clinical findings as well as histomorphology and IHC. A carefully reasoned and evidence-based diagnosis

of an incipient MALT lymphoma may not be clinically helpful when the lack of endoscopic findings makes it impossible for the gastroenterologist to find an appropriate site for rebiopsy to assess the effectiveness of therapy. Thus, discussion with clinical colleagues and a conscientious search of the clinical and endoscopic record for lesions including masses, malignant-appearing ulcers, and diffusely thickened gastric folds are helpful in evaluating the patient for malignancy. In reality, the vast majority of gastric MALT lymphomas—possibly as many as 80% or more—will regress in time with conservative *H. pylori* eradication therapy, including cases that are organism negative by IHC [51]. Some evidence suggests that MALT lymphomas in locations outside the stomach may also regress with conservative therapy. As a result, erring on the side of diagnostic caution in the setting of dubious clinical findings is prudent.

Morphological features helpful in the diagnosis of MALT lymphoma include *bona fide* epithelial and mucosal injury, typified by the so-called "lymphoepithelial lesion", a characteristic but nonspecific infiltration of epithelial structures by lymphoma cells (Figure 3) [6, 7, 52]. Care must be taken to avoid overinterpretation of such lesions, however, as they may also appear in benign settings including reactive lymphoid infiltrates and in crypts adjacent to normal Peyer's patches. Reactive germinal centers, common in the deeper mucosa in *H. pylori* gastritis, may be colonized by lymphoma cells, with destruction of the mantle zone and the appearance of so-called "naked" follicles [6, 7]. In more extensive cases, the lymphoma can create mucosal ulcers and can infiltrate the muscularis mucosae, the submucosa, and even the muscularis propria. Muscularis mucosae infiltration and disruption can be a useful clue to the diagnosis in small biopsy specimens. While no specific IHC marker is available, an overabundance of B cells is present on CD20 stain, and aberrant coexpression of CD43 by neoplastic B-cells is found in up to 50% of cases in large series [6, 53]. In cases with extensive (or nearly complete) plasmacytic differentiation, IHC for kappa and lambda light chains can be extremely useful in highlighting a restricted plasma cell population, as such cases can closely mimic the intense plasma cell infiltrate of severe *H. pylori* gastritis. The standard application of light chain IHC, however, is not particularly helpful for determining the clonality of small lymphocytes, in our experience.

Studies of clonality can be useful in lymphoma diagnosis, but routine use of molecular studies such as heavy-chain gene rearrangement assays to aid in a determination of lymphocyte clonality in MALT lymphoma is not our practice. Clonal populations have been demonstrated in nonneoplastic *H. pylori* gastritis and, as noted earlier, equivocal cases are probably best treated conservatively as most will respond to *H. pylori* eradication [54, 55]. In addition, a significant number of MALT lymphomas may not exhibit detectable clonal *IgH* rearrangements [54, 56]. Furthermore, biopsy specimens are usually small and easily exhausted, putting the slides cut for adequate morphological and IHC analysis at a premium. Thus, the differential diagnosis of severe gastritis and incipient lymphoma is best made based on a combination of clinical information, histomorphology, and IHC.

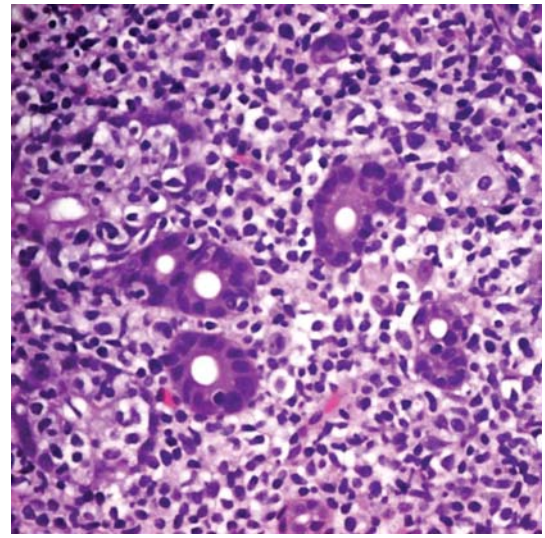

Figure 3: Lymphoepithelial lesions. This infiltration and destruction of the gastric gland epithelium by lymphocytes is a characteristic, but not specific, feature of MALT lymphoma (400x original magnification).

Whether pretreatment analysis of molecular cytogenetics to determine the likelihood of treatment response is useful remains a topic of debate, although some reports indicate that it can be used in equivocal cases to help determine malignancy [36]. Reported cytogenetic abnormalities in gastric MALT lymphomas are summarized in Table 1.

Once the diagnosis of MALT lymphoma has been made and *H. pylori* eradication therapy initiated, the definition of treatment failure must be considered. While the majority of cases will respond to conservative therapy, the timing of rebiopsy for assessment of response is crucial in avoiding an inappropriate judgment of failure. Complete resolution of the lymphoid infiltrate typically takes several months, and periods as long as two years to complete resolution have been reported [14]. While it is typical practice in the setting of colonic adenomas to rebiopsy for assessment of complete polyp removal after a few weeks' time to allow for mucosal healing, at least eight to twelve weeks between initiation of therapy and followup biopsy for MALT lymphoma is more prudent. Certainly, an assessment of failure is inappropriate before at least eight to twelve weeks have elapsed, as the lymphomatous infiltrate may not look substantively different in such a short time. Even at two to three months' treatment duration (some observers suggest as long as a year), only a morphologically obvious worsening of the infiltrate or endoscopically visible increase in mass or lesion size should be considered a likely failure. In addition, molecular evidence of clonality by *IgH* gene rearrangement studies may persist for years after morphological remission is achieved, apparently without affecting patient outcome [14, 57]. Molecular analysis for t(11;18)(q21;q21) may be used in unresponsive cases, although the known association of this translocation with refractoriness to *H. pylori* eradication therapy may make such an assay unnecessary at this point in the disease course. Furthermore, there have been reports of antibiotic response

Table 1: Cytogenetic abnormalities in gastric MALT lymphomas, along with their reported frequencies, known clinical implications and available assays for their detection.

Abnormality	% Gastric MALT	Clinical implications	Available assay(s)
t(11;18)(q21;q21)	Up to 25%	Antibiotic resistance	FISH; RT-PCR; BCL10 IHC (nuclear)
t(14;18)(q32;q21)	Up to 5%	Unknown	FISH; PCR; MALT1 and BCL10 IHC (cytoplasmic/perinuclear)
t(1;14)(p22;q32)	Rare	Antibiotic resistance	BCL10 IHC (nuclear)
t(3;14)(q27;q32)	Rare	Reported in DLBCL with concurrent MALT	FISH (break-apart); BCL6 IHC (only 25%–30%)
Trisomies	5%–65%, depending on series	3q27 most common; associated with high-stage disease	FISH

in cases with this translocation [51, 58]. Thus, further study is needed to determine the utility of molecular assays in determining prognosis and directing therapy for patients refractory to conservative treatment [59].

Refractory cases may require more aggressive adjuvant therapy, including systemic chemotherapy and/or radiotherapy [12, 60]. MALT lymphomas have been found to be very sensitive to such systemic treatment [61, 62]. Cases with minimal residual mucosal disease, however, may be best managed by watchful waiting [63, 64]. Rarely, but particularly in cases that have transformed to DLBCL, surgery may be necessary to deal with complications such as intractable bleeding or perforation, and there is some discussion about whether surgical intervention should be reconsidered for primary therapy [65]. In addition to the presence of certain translocations described earlier, other factors associated with refractoriness to conservative therapy are transmural infiltration and transformation to DLBCL. A small number (around 10%) of cases will relapse, but this is typically associated with *H. pylori* reinfection [60].

To summarize, while the diagnosis of gastric MALT lymphoma and its definitive differentiation from severe *H. pylori* gastritis can be difficult and are not amenable to a specific, algorithmic approach, our diagnostic methodology involves several touchstones. First, the simple presence of a lymphoplasmacytic infiltrate, with or without *H. pylori* organisms, is insufficient for a diagnosis of MALT lymphoma and, indeed, is probably best regarded as *H. pylori*-type gastritis and treated conservatively. We generally require microscopic evidence of significant mucosal disruption and injury—ideally accompanied by macroscopic features such as an endoscopically visible ulcer, mass, or thickened gastric folds—to raise any suspicion of lymphoma. In this setting, we perform an IHC panel that includes CD3, CD20, and CD43 to confirm an excess of CD20-postive B cells, possibly with the addition of kappa and lambda light chain stains if a significant plasmacytic component is suspected. Aberrant coexpression of CD43 by the B cells adds further evidential weight to the diagnosis, but is only present in up to 50% of cases. We rarely, if ever, utilize molecular assays for either clonality or specific chromosomal abnormalities, as biopsy tissue is generally sparse and the vast majority of cases will respond to *H. pylori* eradication therapy. For followup, we recommend rebiopsy after at least 12 weeks, with the expectation that the infiltrate at that point may not be markedly better, but should at least be no worse. We routinely compare follow-up biopsies to previous material and, given the known propensity for MALT lymphoma infiltrates to persist for several months to more than a year, we assiduously avoid an assignment of treatment-refractory status to a lymphoma unless it fails to appreciably improve after several months of therapy or recognizably worsens during therapy. For follow-up biopsies, we mention the status of the infiltrate in our reports, either in the diagnostic line or in a comment, such as "Stomach, biopsy: Residual MALT lymphoma, significantly improved from the prior biopsy on [date]".

5. Conclusion

Surgical pathologists commonly face the diagnosis of *H. pylori* gastritis, and severe cases can be difficult to distinguish from *H. pylori*-associated MALT lymphoma, particularly in the absence of suspicious clinical features. Happily, the large majority of such lymphoma cases respond to conservative antibiotic-based therapy for *H. pylori* eradication. Several cytogenetic abnormalities, including chromosomal translocations and trisomies, have been described in MALT lymphoma, and two translocations [t(11;18)(q21;q21) and t(1;14)(p22;q32)] are associated with resistance to conservative treatment. The routine use of molecular studies, including those for clonality such as *IgH* gene rearrangement, in the diagnosis of MALT lymphoma is controversial, however, and a trial of conservative therapy is probably the best initial approach given the propensity for response to such treatment. Careful assessment of response is crucial, since lymphomas may take several months to more than a year to exhibit a complete resolution of the malignant lymphoid infiltrate. Thus, a premature declaration of treatment refractoriness should be avoided in order to prevent the inappropriate use of more aggressive adjuvant therapy.

References

[1] W. L. Peterson, "*Helicobacter pylori* and peptic ulcer disease," *The New England Journal of Medicine*, vol. 324, no. 15, pp. 1043–1048, 1991.

[2] S. J. O. Veldhuyzen van Zanten and P. M. Sherman, "*Helicobacter pylori* infection as a cause of gastritis, duodenal ulcer, gastric cancer and nonulcer dyspepsia: a systematic overview," *Canadian Medical Association Journal*, vol. 150, no. 2, pp. 177–185, 1994.

[3] B. J. Marshall and J. R. Warren, "Unidentified curved bacilli in the stomach of patients with gastritis and peptic ulceration," *The Lancet*, vol. 1, no. 8390, pp. 1311–1314, 1984.

[4] N. Uemura, S. Okamoto, S. Yamamoto et al., "*Helicobacter pylori* infection and the development of gastric cancer," *The New England Journal of Medicine*, vol. 345, no. 11, pp. 784–789, 2001.

[5] A. Morgner, E. Bayerdörffer, A. Neubauer, and M. Stolte, "Malignant tumors of the stomach: gastric mucosa-associated lymphoid tissue lymphoma and *Helicobacter pylori*," *Gastroenterology Clinics of North America*, vol. 29, no. 3, pp. 593–607, 2000.

[6] P. G. Isaacson and M. Q. Du, "Gastrointestinal lymphoma: where morphology meets molecular biology," *Journal of Pathology*, vol. 205, no. 2, pp. 255–274, 2005.

[7] J. K. Greenson et al., "MALT lymphoma," in *Diagnostic Pathology: Gastrointestinal*, pp. 2:70–2:75, Amirsys, Inc., Salt Lake City, Utah, USA, 2010.

[8] M. Q. Du, "MALT lymphoma : recent advances in aetiology and molecular genetics," *Journal of Clinical and Experimental Hematopathology*, vol. 47, no. 2, pp. 31–42, 2007.

[9] M. Q. Du and P. G. Isaccson, "Gastric MALT lymphoma: from aetiology to treatment," *The Lancet Oncology*, vol. 3, no. 2, pp. 97–104, 2002.

[10] R. A. Hamoudi, A. Appert, H. Ye et al., "Differential expression of NF-κB target genes in MALT lymphoma with and without chromosome translocation: insights into molecular mechanism," *Leukemia*, vol. 24, pp. 1487–1497, 2010.

[11] H. Inagaki, "Mucosa-associated lymphoid tissue lymphoma: molecular pathogenesis and clinicopathological significance," *Pathology International*, vol. 57, no. 8, pp. 474–484, 2007.

[12] A. Andriani, A. Miedico, L. Tedeschi et al., "Management and long-term follow-up of early stage H. pylori-associated gastric MALT-lymphoma in clinical practice: an Italian, multicentre study," *Digestive and Liver Disease*, vol. 41, no. 7, pp. 467–473, 2009.

[13] K. Hori, T. Nishigami, T. Chiba, Y. Matsushima, and T. Kojima, "Regression of MALT lymphomas coexisting in the duodenal bulb and the stomach by eradiction of *Helicobacter pylori*," *Journal of Gastroenterology*, vol. 37, no. 4, pp. 288–292, 2002.

[14] C. Montalban, A. Santon, D. Boixeda et al., "Treatment of low-grade gastric mucosa-associated lymphoid tissue lymphoma in stage I with *Helicobacter pylori* eradication. Long-term results after sequential histologic and molecular follow-up," *Haematologica*, vol. 86, no. 6, pp. 609–617, 2001.

[15] S. Nakamura, T. Matsumoto, S. Nakamura et al., "Duodenal mucosa-associated lymphoid tissue lymphoma treated by eradication of *Helicobacter pylori*: report of 2 cases including EUS findings," *Gastrointestinal Endoscopy*, vol. 54, no. 6, pp. 772–775, 2001.

[16] D. Niino, K. Yamamoto, O. Tsuruta et al., "Regression of rectal mucosa-associated lymphoid tissue (MALT) lymphoma after antibiotic treatments," *Pathology International*, vol. 60, no. 6, pp. 438–442, 2010.

[17] A. C. Wotherspoon, C. Doglioni, T. C. Diss et al., "Regression of primary low-grade-B-cell gastric lymphoma of mucosa-associated lymphoid tissue type after eradication of *Helicobacter pylori*," *The Lancet*, vol. 342, no. 8871, pp. 575–577, 1993.

[18] P. M. Banks, "Gastrointestinal lymphoproliferative disorders," *Histopathology*, vol. 50, no. 1, pp. 42–54, 2007.

[19] M. Q. Du and J. C. Atherton, "Molecular subtyping of gastric malt lymphomas: implications for prognosis and management," *Gut*, vol. 55, no. 6, pp. 886–893, 2006.

[20] T. Nakamura, H. Inagaki, M. Seto, and S. Nakamura, "Gastric low-grade B-cell MALT lymphoma: treatment, response, and genetic alteration," *Journal of Gastroenterology*, vol. 38, no. 10, pp. 921–929, 2003.

[21] P. Isaacson and D. H. Wright, "Malignant lymphoma of mucosa-associated lymphoid tissue. A distinctive type of B-cell lymphoma," *Cancer*, vol. 52, no. 8, pp. 1410–1416, 1983.

[22] P. G. Isaacson, A. Chott, S. Nakamura, H. K. Muller-Hermelink, N. L. Harris, and S. H. Swerdlow, "Extranodal marginal zone lymphoma of mucosa-associated lymphoid tissue (MALT lymphoma)," in *WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues*, S. H. Swerdlow et al., Ed., pp. 214–217, IARCPress, Lyon, France, 2008.

[23] M. P. Siakantaris, G. A. Pangalis, E. Dimitriadou et al., "Early-stage gastric MALT lymphoma: is it a truly localized disease?" *The Oncologist*, vol. 14, no. 2, pp. 148–154, 2009.

[24] S. Nakamura, T. Yao, K. Aoyagi, M. Iida, M. Fujishima, and M. Tsuneyoshi, "*Helicobacter pylori* and primary gastric lymphoma: a histopathologic and immunohistochemical analysis of 237 patients," *Cancer*, vol. 79, no. 1, pp. 3–11, 1997.

[25] P. Farinha and R. D. Gascoyne, "Molecular pathogenesis of mucosa-associated lymphoid tissue lymphoma," *Journal of Clinical Oncology*, vol. 23, no. 26, pp. 6370–6378, 2005.

[26] J. L. O'Rourke, "Gene expression profiling in Helicobacter-induced MALT lymphoma with reference to antigen drive and protective immunization," *Journal of Gastroenterology and Hepatology*, vol. 23, no. 2, pp. S151–S156, 2008.

[27] X. Sagaert, C. De Wolf-Peeters, H. Noels, and M. Baens, "The pathogenesis of MALT lymphomas: where do we stand?" *Leukemia*, vol. 21, no. 3, pp. 389–396, 2007.

[28] V. J. Craig, S. B. Cogliatti, I. Arnold et al., "B-cell receptor signaling and CD40 ligand-independent T cell help cooperate in Helicobacter-induced MALT lymphomagenesis," *Leukemia*, vol. 24, pp. 1186–1196, 2010.

[29] M. M. D'Elios, A. Amedei, M. Benagiano, A. Azzurri, and G. Del Prete, "*Helicobacter pylori*, T cells and cytokines: the "dangerous liaisons"," *FEMS Immunology and Medical Microbiology*, vol. 44, no. 2, pp. 113–119, 2005.

[30] S. Umehara, H. Higashi, N. Ohnishi, M. Asaka, and M. Hatakeyama, "Effects of *Helicobacter pylori* CagA protein on the growth and survival of B lymphocytes, the origin of MALT lymphoma," *Oncogene*, vol. 22, no. 51, pp. 8337–8342, 2003.

[31] E. D. Remstein, A. Dogan, R. R. Einerson et al., "The incidence and anatomic site specificity of chromosomal translocations in primary extranodal marginal zone B-cell lymphoma of mucosa-associated lymphoid tissue (MALT lymphoma) in North America," *American Journal of Surgical Pathology*, vol. 30, no. 12, pp. 1546–1553, 2006.

[32] H. Ye, H. Liu, A. Attygalle et al., "Variable frequencies of t(11;18)(q21;q21) in MALT lymphomas of different sites: significant association with CagA strains of H pylori in gastric MALT lymphoma," *Blood*, vol. 102, no. 3, pp. 1012–1018, 2003.

[33] S. Nakamura, H. Ye, C. M. Bacon et al., "Clinical impact of genetic aberrations in gastric MALT lymphoma: a comprehensive analysis using interphase fluorescence in situ hybridisation," *Gut*, vol. 56, no. 10, pp. 1358–1363, 2007.

[34] S. S. Chuang, C. Lee, R. A. Hamoudi et al., "High frequency of t(11;18) in gastric mucosa-associated lymphoid tissue lymphomas in Taiwan, including one patient with high-grade transformation," *British Journal of Haematology*, vol. 120, no. 1, pp. 97–100, 2003.

[35] H. Inagaki, T. Nakamura, C. Li et al., "Gastric MALT lymphomas are divided into three groups based on responsiveness to *Helicobacter pylori* eradication and detection of API2-MALT1 fusion," *American Journal of Surgical Pathology*, vol. 28, no. 12, pp. 1560–1567, 2004.

[36] G. Wang, A. Auerbach, M. Wei et al., "t(11;18)(q21;q21) in extranodal marginal zone B-cell lymphoma of mucosa-associated lymphoid tissue in stomach: a study of 48 cases," *Modern Pathology*, vol. 22, no. 1, pp. 79–86, 2009.

[37] J. Dierlamm, M. Baens, M. Stefanova-Ouzounova et al., "Detection of t(11;18)(q21;q21) by interphase fluorescence in situ hybridization using API2 and MLT specific probes," *Blood*, vol. 96, no. 6, pp. 2215–2218, 2000.

[38] H. Ye, L. Gong, H. Liu et al., "MALT lymphoma with t(14;18)(q32;q21)/IGH-MALT1 is characterized by strong cytoplasmic MALT1 and BCL10 expression," *Journal of Pathology*, vol. 205, no. 3, pp. 293–301, 2005.

[39] L. Ho, R. E. Davis, B. Conne et al., "MALT1 and the API2-MALT1 fusion act between CD40 and IKK and confer NF-κB-dependent proliferative advantage and resistance against FAS-induced cell death in B cells," *Blood*, vol. 105, no. 7, pp. 2891–2899, 2005.

[40] S. Nakamura, H. Ye, C. M. Bacon et al., "Gastric MALT lymphoma with t(14;18)(q32;q21) involving IGH and BCL2 genes that responded to *Helicobacter pylori* eradication," *Journal of Clinical Pathology*, vol. 60, no. 10, pp. 1171–1173, 2007.

[41] B. Streubel, I. Simonitsch-Klupp, L. Müllauer et al., "Variable frequencies of MALT lymphoma-associated genetic aberrations in MALT lymphomas of different sites," *Leukemia*, vol. 18, no. 10, pp. 1722–1726, 2004.

[42] R. Achuthan, S. M. Bell, J. P. Leek et al., "Novel translocation of the BCL10 gene in a case of mucosa associated lymphoid tissue lymphoma," *Genes Chromosomes and Cancer*, vol. 29, no. 4, pp. 347–349, 2000.

[43] H. Ye, A. Dogan, L. Karran et al., "BCL10 expression in normal and neoplastic lymphoid tissue: nuclear localization in MALT lymphoma," *American Journal of Pathology*, vol. 157, no. 4, pp. 1147–1154, 2000.

[44] H. Ye, E. D. Remstein, C. M. Bacon, A. G. Nicholson, A. Dogan, and M. Q. Du, "Chromosomal translocations involving BCL6 in MALT lymphoma," *Haematologica*, vol. 93, no. 1, pp. 145–146, 2008.

[45] Y. W. Chen, X. T. Hu, A. C. Liang et al., "High BCL6 expression predicts better prognosis, independent of BCL6 translocation status, translocation partner, or BCL6-deregulating mutations, in gastric lymphoma," *Blood*, vol. 108, no. 7, pp. 2373–2383, 2006.

[46] B. Streubel, U. Vinatzer, A. Lamprecht, M. Raderer, and A. Chott, "t(3;14)(p14.1;q32) involving IGH and FOXP1 is a novel recurrent chromosomal aberration in MALT lymphoma," *Leukemia*, vol. 19, no. 4, pp. 652–658, 2005.

[47] A. C. Wotherspoon, T. M. Finn, and P. G. Isaacson, "Trisomy 3 in low-grade B-cell lymphomas of mucosa-associated lymphoid tissue," *Blood*, vol. 85, no. 8, pp. 2000–2004, 1995.

[48] G. Ott, J. Kalla, A. Steinhoff et al., "Trisomy 3 is not a common feature in malignant lymphomas of mucosa-associated lymphoid tissue type," *American Journal of Pathology*, vol. 153, no. 3, pp. 689–694, 1998.

[49] J. Krugmann, A. Tzankov, S. Dirnhofer et al., "Complete or partial trisomy 3 in gastro-intestinal MALT lymphomas co-occurs with aberrations at 18q21 and correlates with advanced disease stage: a study on 25 cases," *World Journal of Gastroenterology*, vol. 11, no. 46, p. 7384, 2005.

[50] J. Krugmann, A. Tzankov, S. Dirnhofer et al., "Unfavourable prognosis of patients with trisomy 18q21 detected by fluorescence in situ hybridisation in t(11;18) negative, surgically resected, gastrointestinal B cell lymphomas," *Journal of Clinical Pathology*, vol. 57, no. 4, pp. 360–364, 2004.

[51] S. Nakamura, T. Matsumoto, H. Ye et al., "*Helicobacter pylori*-negative gastric mucosa-associated lymphoid tissue lymphoma: a clinicopathologic and molecular study with reference to antibiotic treatment," *Cancer*, vol. 107, no. 12, pp. 2770–2778, 2006.

[52] A. Rawal, W. G. Finn, B. Schnitzer, and R. Valdez, "Site-specific morphologic differences in extranodal marginal zone B-cell lymphomas," *Archives of Pathology and Laboratory Medicine*, vol. 131, no. 11, pp. 1673–1678, 2007.

[53] R. Lai, L. M. Weiss, K. L. Chang, and D. A. Arber, "Frequency of CD43 expression in non-Hodgkin lymphoma: a survey of 742 cases and further characterization of rare $CD43^+$ follicular lymphomas," *American Journal of Clinical Pathology*, vol. 111, no. 4, pp. 488–494, 1999.

[54] E. D. Hsi, J. K. Greenson, T. P. Singleton, J. Siddiqui, B. Schnitzer, and C. W. Ross, "Detection of immunoglobulin heavy chain gene rearrangement by polymerase chain reaction in chronic active gastritis associated with *Helicobacter pylori*," *Human Pathology*, vol. 27, no. 3, pp. 290–296, 1996.

[55] S. D. Georgopoulos, K. Triantafyllou, M. Fameli et al., "Molecular analysis of B-cell clonality in *Helicobacter pylori* gastritis," *Digestive Diseases and Sciences*, vol. 50, no. 9, pp. 1616–1620, 2005.

[56] J. N. Steiff, A. Neubauer, M. Stolte, and T. Wündisch, "Clonality analyses in gastric MALT (mucosa-associated lymphoid tissue)," *Pathology Research and Practice*, vol. 202, no. 7, pp. 503–507, 2006.

[57] C. Montalban, A. Santón, C. Redondo et al., "Long-term persistence of molecular disease after histological remission in low-grade gastric MALT lymphoma treated with H. pylori eradication. Lack of association with translocation t(11;18): a 10-year updated follow-up of a prospective study," *Annals of Oncology*, vol. 16, no. 9, pp. 1539–1544, 2005.

[58] T. Wündisch, C. Thiede, A. Morgner et al., "Long-term follow-up of gastric MALT lymphoma after *Helicobacter pylori* eradication," *Journal of Clinical Oncology*, vol. 23, no. 31, pp. 8018–8024, 2005.

[59] H. Suzuki, Y. Saito, and T. Hibi, "*Helicobacter pylori* and gastric mucosa-associated lymphoid tissue (MALT) lymphoma: updated review of clinical outcomes and the molecular pathogenesis," *Gut and Liver*, vol. 3, no. 2, pp. 81–87, 2009.

[60] C. Montalban and F. Norman, "Treatment of gastric mucosa-associated lymphoid tissue lymphoma: *Helicobacter pylori* eradication and beyond," *Expert Review of Anticancer Therapy*, vol. 6, no. 3, pp. 361–371, 2006.

[61] M. Lévy, C. Copie-Bergman, V. Molinier-Frenkel et al., "Treatment of t(11;18)-positive gastric mucosa-associated lymphoid tissue lymphoma with rituximab and chlorambucil: clinical, histological, and molecular follow-up," *Leukemia and Lymphoma*, vol. 51, no. 2, pp. 284–290, 2010.

[62] S. Nakamura, T. Matsumoto, H. Suekane et al., "Long-term clinical outcome of *Helicobacter pylori* eradication for gastric mucosa-associated lymphoid tissue lymphoma with a reference to second-line treatment," *Cancer*, vol. 104, no. 3, pp. 532–540, 2005.

[63] W. Fischbach, M. E. Goebeler-Kolve, B. Dragosics, A. Greiner, and M. Stolte, "Long term outcome of patients with gastric marginal zone B cell lymphoma of mucosa associated lymphoid tissue (MALT) following exclusive *Helicobacter pylori* eradication therapy: experience from a large prospective series," *Gut*, vol. 53, no. 1, pp. 34–37, 2004.

[64] W. Fischbach, M. E. Goebeler, A. Ruskone-Fourmestraux et al., "Most patients with minimal histological residuals of gastric MALT lymphoma after successful eradication of *Helicobacter pylori* can be managed safely by a watch and wait strategy: experience from a large international series," *Gut*, vol. 56, no. 12, pp. 1685–1687, 2007.

[65] S.-H. Kuo, L.-T. Chen, M.-S. Wu et al., "Long-term follow-up of gastrectomized patients with mucosa-associated lymphoid tissue lymphoma: need for a revisit of surgical treatment," *Annals of Surgery*, vol. 247, no. 2, pp. 265–269, 2008.

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Review Article

HER-2/*neu* Testing and Therapy in Gastroesophageal Adenocarcinoma

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Despite ongoing advances in the treatment of gastroesophageal cancer, prognosis remains poor. The best promise to improve this poor survival is provided by new targeted agents. Of these, human epidermal growth factor receptor 2 (HER2) is currently in the spotlight. In this review, we provide an overview of recent developments in HER2 testing and results of clinical trials targeting HER2 in gastroesophageal adenocarcinoma. Based on the encouraging ToGA trial findings it is now expected that routine HER2 testing will be included in the diagnostic work-up of patients with advanced gastric cancer. With regard to this testing, overexpression of the HER2 protein seems to possess the best predictive properties. However, HER2 immunohistochemistry (IHC) is subject to assay and interobserver variability, so standardization and internal and external proficiency testing is an absolute prerequisite, especially as the IHC scoring system in gastric cancer is different from that of breast cancer. Further study is needed to investigate the clinical meaning of the significant heterogeneity observed in both gene amplification and protein overexpression in gastroesophageal cancer. Highly effective therapies for gastroesophageal cancer can only be accomplished by a multi-targeted approach, considering crosstalk between pathways and continuing to optimize chemotherapy.

1. Introduction

Despite ongoing advances in the treatment of gastroesophageal cancer, prognosis remains poor. Esophageal adenocarcinoma incidence has been rapidly increasing in Western countries during the past half century, especially in Caucasian males. This is believed to be attributable to the increased prevalence of gastroesophageal reflux disease and its major determinant, obesity [1], resulting in Barrett's esophagus. Esophageal adenocarcinoma arising in Barrett's esophagus has a poor prognosis with a 5-year relative survival of 10–20%. Gastric cancer affects about one million people per year and is the second leading cause of cancer-related mortality worldwide [2]. Gastric cancer is thought to result from a combination of environmental factors and accumulation of specific genetic alterations, and consequently, mainly affects older patients. Gastric cancer exists as two main histological types, diffuse and intestinal, as described by Lauren [3], and can be subdivided into proximal (cardia) and distal (corpus and pylorus) cancers. Interestingly, there seems to be a trend towards more proximally (cardiac) located gastric cancer. This distal to proximal shift is yet incompletely understood and seems to parallel the observed recent rise in incidence of Barrett's esophagus. This fall in incidence in mid- and distal gastric cancer may be explained by the decline in *Helicobacter pylori* infection and associated atrophic gastritis. When a tumor is located at the gastroesophageal junction, it is often unknown whether the tumor is of esophageal or gastric origin. This group of cancers is therefore called "gastroesophageal junction cancers." Surgery is the mainstay of treatment for resectable adenocarcinomas of stomach and esophagus, but recurrence rates are high even after radical resection. In Western countries, most patients are diagnosed at an advanced (unresectable) stage and, despite benefits of palliative radiotherapy and chemotherapy, survival of patients with advanced tumors remains poor (median survival 7–10 months) [4]. The best promise to improve this poor survival is provided by new agents

acting against specific molecular targets. Of these, HER2 is currently in the spotlight. The aim of this paper is to provide an overview of recent developments in HER2 testing and results of clinical trials targeting HER2 in gastric and esophageal adenocarcinoma. Since most studies combine Barrett's esophagus, gastroesophageal junction cancers, and gastric cancer, the term "gastroesophageal" is used to refer to this combined group.

2. Human Epidermal Growth Factor Receptor 2

HER-2/*neu* (HER2) is a proto-oncogene located on chromosome 17q21 and a member of the human epidermal growth factor receptor (EGFR) family. It encodes a 185 kD transmembrane tyrosine kinase receptor protein that, through dimerisation with other family members, regulates signal transduction in cellular processes including proliferation, differentiation, and cellular survival [5, 6]. Many studies have indicated a role of HER2 in the development of various types of human cancer. HER2 is amplified, and the expression of its receptor protein is increased in about 10–20% of breast carcinomas [7–11]. HER2 amplification and/or overexpression have also been observed in colon [12], bladder [13], ovarian [14], Fallopian tube [15], endometrial [16], lung [17], uterine cervix [18], head and neck [19], prostate [20], pancreatic [21], salivary gland [22], and esophageal [23] and gastric [24] carcinomas.

Patients with HER2-positive (amplification and/or overexpression) primary and metastatic breast tumors have increased survival rates when treated with trastuzumab (Herceptin), a recombinant humanized monoclonal anti-HER2 antibody [25, 26]. The efficacy of trastuzumab in breast cancer patients urged investigation into its antitumor activity in patients with other HER2-positive cancers, including gastroesophageal cancer. Furthermore, overexpression [27] and amplification of HER2 [28] have also been shown to correlate with poor prognosis and with resistance to conventional adjuvant chemotherapy and tamoxifen [29–33] in breast cancer. With the recognition of its prognostic, predictive, and therapeutic implications, assessment of HER2 status has now become of major importance in clinical practice for cancer patients.

2.1. Diagnostic Tests to Detect HER2 Amplification and Overexpression. Since the costs for trastuzumab therapy are high and side effects are significant, accurate selection of eligible patients for this therapy is crucial. Since 1998, trastuzumab has been used to treat more than 740,000 patients with HER2-positive breast cancer worldwide, so there is much to learn from the diagnostic methods used in the selection of breast cancer patients for this treatment. HER2 status is mainly assessed by immunohistochemistry (IHC) and chromogenic (CISH) or fluorescence *in situ* hybridization (FISH).

At present, the most common method to assess HER2 status is IHC [11] which is a routine technique available in most pathology laboratories to detect protein expression levels. Among HER2 IHC scoring systems for breast cancer, the HercepTest (Dako, Glostrup, Denmark) is frequently used to evaluate patterns of membranous immunoreactivity on tumor cells. The scoring system is based on intensity of reactivity, whether complete or incomplete and the percentage of reactive cells. Patterns are scored as IHC 0 (no staining or staining in <10% of tumor cells, negative), IHC 1+ (faint/barely perceptible incomplete membrane staining in >10% of tumor cells, negative), IHC 2+ (weak to moderate complete membrane staining in >10% of tumor cells, equivocal), or IHC 3+ (strong complete membrane staining in >10% (until 2007) or >30% (2007–now) of tumor cells, positive) [34]. Although staining and scoring methodology has been better standardized with the introduction of the Hercep Test than for most IHC assays, IHC is affected by poor tissue fixation, and there are still problems with reproducibility and interpretation of IHC assays [35–37], leading to both false negative and false positive IHC results. In addition, in breast cancer, there is some evidence that testing for HER2 gene amplification provides better predictive information than IHC [38–41]. Gene amplification testing was traditionally mostly done by FISH. For FISH assessments, an HER2:CEP17 (centromeric probe 17) ratio of >2.2 is now used (≥2.0 before 2007) to define HER2 positivity (amplification), and ratios of 1.8–2.2 and <1.8 are used to define equivocal and negative categories, respectively [34].

Comparative studies of FISH and IHC have generally shown a high level of concordance in breast cancer [39, 42, 43]. Discordant results were mainly observed for tumors that were scored 2+ by IHC. However, pathologists have been reluctant to embrace routine FISH testing, because it is a difficult, expensive, and cumbersome technique that requires trained personnel which is not available in every pathology laboratory. Moreover, fluorescence fades upon storage, making it difficult to preserve the slides for future reference, and the fluorescent probes in the kits have a limited half life. Furthermore, detailed morphological features of the tumor are usually difficult to observe due to the required protein digestion and the fluorescent mode, and heterogeneity can be missed since spots are evaluated at ×100 magnification using oil immersion.

Chromogenic *in situ* hybridization (CISH) was introduced as an alternative for FISH in 2000 by Tanner et al. [44], using an immunoperoxidase reaction to detect specific DNA probes, which makes visualization possible with a conventional bright field microscope. Furthermore, similar to IHC, a permanent staining record is retained, and better morphologic examination is possible facilitating detection of heterogeneity. This is important in gastroesophageal cancer since higher rates of heterogeneity have been reported in gastric cancer (5%) compared to breast cancer (1.5%) [45]. CISH is also easier to interpret for pathologists who are not trained in fluorescence microscopy, and it is less expensive than FISH. In CISH scoring, the presence of large peroxidase-positive intranuclear clusters or >10 individual small signals in >50% of tumor cells (counted in at least 20 tumor cells) indicates HER2-positivity (amplification). The presence of small peroxidase-positive intranuclear clusters or 6–10 individual small signals indicates a low-level amplification,

and 5 or less individual small signals are scored as HER2-negative [34].

In several breast cancer studies, HER2 CISH correlated well with FISH and IHC [44, 46–51]. In gastric cancer, one study systematically analyzed the concordance between CISH and FISH assays and observed a perfect correlation in 128 samples [52]. However, one drawback of CISH assays is that amplification can only be assessed semiquantitatively. Therefore, detection of amplification by easier quantitative PCR techniques has been proposed as an alternative. One of the newly introduced techniques for detection of HER2 amplification in breast cancer is multiplex ligation-dependent probe amplification (MLPA) [53]. This technique determines relative copy numbers in a quantitative way and requires only minute quantities of small DNA fragments, which makes it very suitable for DNA isolated from paraffin-embedded material. In previous studies in breast cancer, excellent results were obtained with MLPA in comparison with IHC, CISH and FISH [10, 11, 51, 54, 55]. All currently available Food and Drug Administration approved or Clinical Laboratory Improvement Amendment validated HER2 tests have been recently summarized by Allison [56].

To eliminate discrepancies observed between IHC and FISH, Hofmann et al. [45] established an IHC scoring system specific for gastric cancer. In an international consensus meeting, modifications to the breast scoring system were made mainly based on the more frequent basolateral (incomplete) membrane staining and heterogeneity in gastric cancer. This new scoring system, illustrated in Table 1, has also been used to select patients for a clinical trial to evaluate trastuzumab efficacy and safety in HER2-positive advanced esophageal and gastric cancer [57]. A subsequent study validated these guidelines in terms of interlaboratory and interobserver consensus in a large series of gastric cancer and formulated additional specific recommendations [58]. For example, for reproducible intensity scoring, the degree of microscopic magnification (×-fold) at which membranous (linear intercellular) staining is clearly visible should be considered. Strong tumor HER2 IHC staining is usually already directly visible. In these cases, only low magnification (×2.5–5) is needed to confirm strong staining intensity. In any case where high magnification (×40) is required for unequivocal demonstration of membranous staining, the tumor is scored IHC 1+. The interobserver variation results within a ring-study prior and after application of the magnification rule clearly were in favor of such an approach over nonstandardized wording, for example, of "barely visible" for IHC 1+.

Table 1: Consensus panel recommendations on HER2 scoring for gastric cancer [45, 58].

Reactivity characteristics	Score/classification
No reactivity or membranous reactivity in <10% of tumor cells	0/negative
Faint/ barely perceptible membranous reactivity in >10% of tumor cells; cells are reactive only in part of their membrane, in any case where high magnification (×40) is required for unequivocal demonstration of membranous staining	1+/negative
Weak to moderate complete or basolateral membranous reactivity in >10% of tumor cells	2+/equivocal
Moderate to strong complete or basolateral membranous reactivity in >10% of tumor cells; only low magnification (×2.5–5) is needed to confirm strong staining intensity.	3+/positive
Biopsy (not surgery) samples with cohesive either IHC3+ and/or FISH+ clones (at least 5 cells) are considered positive irrespective of size, that is <10% of tumor area	3+/positive

FISH: fluorescence *in situ* hybridization; HER2: human epidermal growth factor receptor 2; IHC: immunohistochemistry.

2.2. Relationship between HER2 Amplification and HER2 Overexpression. Nine studies (totalling 1,232 samples) examining the frequency of *HER2* amplification in gastroesophageal cancer showed a mean *HER2* positivity rate of 19.2% (range 7–43%) [45], which is similar to the reported percentage of protein overexpression.

In breast cancer, it is generally thought that HER2 overexpression is the direct result of gene amplification [59]. In esophageal and gastric cancer, concordance percentages between amplification and overexpression reported in literature range between 86.9 and 96.4% [60], as illustrated in Figure 1. Nevertheless, primary results from a very recent phase III trial (ToGA) containing >3,800 advanced esophageal and gastric cancer samples showed that the frequency of samples with amplification but without corresponding overexpression was high (23%) compared to that in breast cancer suggesting that FISH testing may be the more relevant procedure to conduct on these tumor specimens [61]. However, preliminary data from this same trial reported that patients with amplified tumors without overexpression (IHC 0 or 1+) did not show a substantial overall survival benefit from trastuzumab (HR 1.07, median overall survival 10.0 months versus 8.7 months) in contrast to patients with IHC 2+/FISH positive or IHC 3+ tumors (HR 0.65, median overall survival 16.0 months versus 11.8 months) [57], suggesting that measuring HER2 at the protein level should be the primary screening method for selecting gastroesophageal cancer patients for trastuzumab therapy. Final publication of these trial data needs to be awaited to draw firmer conclusions, and further research may be necessary to clarify these findings. The pattern seen in breast cancer, where amplification of HER2 leads to an overexpression of the protein, does not seem to have been fully confirmed in gastric cancer yet. If the observations in the ToGA trial are correct, this might be similar to what has been reported with another gene/protein relationship in breast cancer: topoisomerase II alpha (TOP2A). Unlike TOP2A, HER2 protein expression is not cell-cycle dependent, so other mechanisms (increased receptor degradation, transcriptional repression) may lay behind this discrepancy. Also, gastric cancer may have more

inherent genomic instability than breast cancer resulting in more genes which are amplified as a bystander effect and not necessarily resulting in a functional increase of protein expression.

2.3. HER2 Expression in Gastroesophageal Cancer. Reported rates of HER2 overexpression in gastroesophageal cancer vary widely (2–45%) due to small sample sizes, differences in patient populations and methodological and scoring differences between studies [45, 62–67]. In addition, differences between HER2 overexpression in European and Asian/South-American populations (22–28% versus 3–15%, resp.) have been reported by some studies [68], but others have found these differences to be less substantial [69]. The largest data set of >3,800 advanced esophageal and gastric cancer samples found HER2 protein positivity rates of 23% [57, 68]. A recent review combining data from 24 studies (6,542 patients) calculated a weighted mean of 19% HER2 positivity [60]. In the few studies that reported separate HER2 positivity rates for gastroesophageal junction cancers and gastric cancer, HER2 positivity was higher in gastroesophageal junction cancer (24–35%) than in gastric cancer (9.5–21%) [65, 68, 70, 71].

2.4. HER2 in Esophageal Cancer. The data on HER2 overexpression in esophageal cancer are variable, with most studies showing HER2 overexpression in 9%–60% of cases, whereas other reports failed to observe HER2 expression [72]. The differences among reported overexpression rates might depend on stage of the disease, tumor histology (adenocarcinoma versus squamous cell carcinoma), methodology, and interpretation of IHC results. The relationship between HER2 expression and the prognosis of patients with esophageal cancer is not clear. It has been demonstrated that HER2 overexpression correlates with tumor invasion and lymph node metastasis, and thus indicates a poor prognosis [71, 73–75].

Studies that specifically analyzed HER2 expression and/or amplification in Barrett's esophagus reported positivity rates of 38–50% and showed an association with progression from Barrett's esophagus to dysplasia and adenocarcinoma [76–79]. A very small pilot study showed that trastuzumab treatment caused HER2 downregulation and increased apoptosis in patients with dysplasia and adenocarcinoma arising in Barrett's esophagus [80].

2.5. HER2 in Gastric Cancer. Previous studies have shown that early-onset gastric cancer (presenting at the age of 45 years or younger) forms a small (<10%) [81] but distinct group of gastric cancers with a different molecular expression profile than conventional gastric cancer [82–84]. We recently showed that these younger patients show very low (<5%) HER2 amplification and overexpression frequencies (unpublished data). Common gastric tumors classified as intestinal type are more likely to be HER2-positive (16–34%) than diffuse (2–7%) or mixed (5–20%) types [65, 68, 70]. The reason for the selective overexpression of HER2 in intestinal-type gastric cancers is complex and needs further investigation. The association of HER2 with a specific type suggests that intestinal- and diffuse-type gastric cancers develop along different molecular pathways and supports earlier studies showing distinct patterns of genetic alterations in gastric cancers of differing histopathologic features [85]. Some similarities can be drawn with breast cancer: diffuse-type gastric cancers and lobular invasive breast carcinomas are both associated with E-cadherin loss, which is inversely correlated with HER2 amplification/overexpression which is more common in ductal invasive breast carcinomas and intestinal-type gastric cancers.

Although some studies have reported that HER2 amplification and overexpression are highly homogeneous within a tumor and between primary and metastatic gastric cancer [62], others have reported significant heterogeneity in both gene amplification and protein overexpression in individual cancers, even among IHC 3+ cancers [67, 86]. Some studies, including our own findings (unpublished data), showed homogeneous *HER2* gene amplification but heterogeneous HER2 protein expression in certain samples, indicating that false negatives might arise when IHC is employed to predict trastuzumab response, especially when insufficient material is examined, such as in gastric biopsy specimens [52]. Generally, higher rates of HER2 heterogeneity have been reported in gastric cancer (5%) compared to breast cancer (1.5%) [45]. Chromosomal instability is probably one of the major causes of this heterogeneity. It needs to be shown whether patients with small cohesive HER2-positive clones show a different response to trastuzumab compared with patients with extended HER2-positive areas.

Although reports are conflicting, some studies have suggested that HER2-positive status in gastric cancer is associated with poor outcomes and aggressive disease [65, 70].

3. Preclinical and Clinical Data: Anti-HER2 Therapy

Several studies have indicated antitumor activity of trastuzumab and lapatinib in human gastric cancer cell lines (NCI-N87, 4-1ST, SMU-216, MKN-45P) or xenograft models which overexpress HER2 [65, 87–90]. In these preclinical studies, these targeted compounds have been shown to be effective both as single agents and in combination with chemotherapeutic agents that are widely used for the treatment of gastric cancer. The three-drug combination of capecitabine (Xeloda, Roche), cisplatin, and trastuzumab showed a remarkable tumor inhibitory effect in the NCI-N87 tumor xenograft model, and it is this drug combination that was also used in the ToGA trial. This trial was the first randomized, prospective, multicenter, phase III trial to study the efficacy and safety of first-line trastuzumab in HER2-positive advanced gastroesophageal cancer [60, 68]. The modest but clinically significant overall survival benefit indicated that trastuzumab is a new, effective, and well tolerated treatment for HER2-positive gastroesophageal cancer. In this trial, patients with gastroesophageal cancer ($n = 3,807$) were centrally tested for HER2 status by IHC and FISH (patients were eligible if their tumor samples were scored

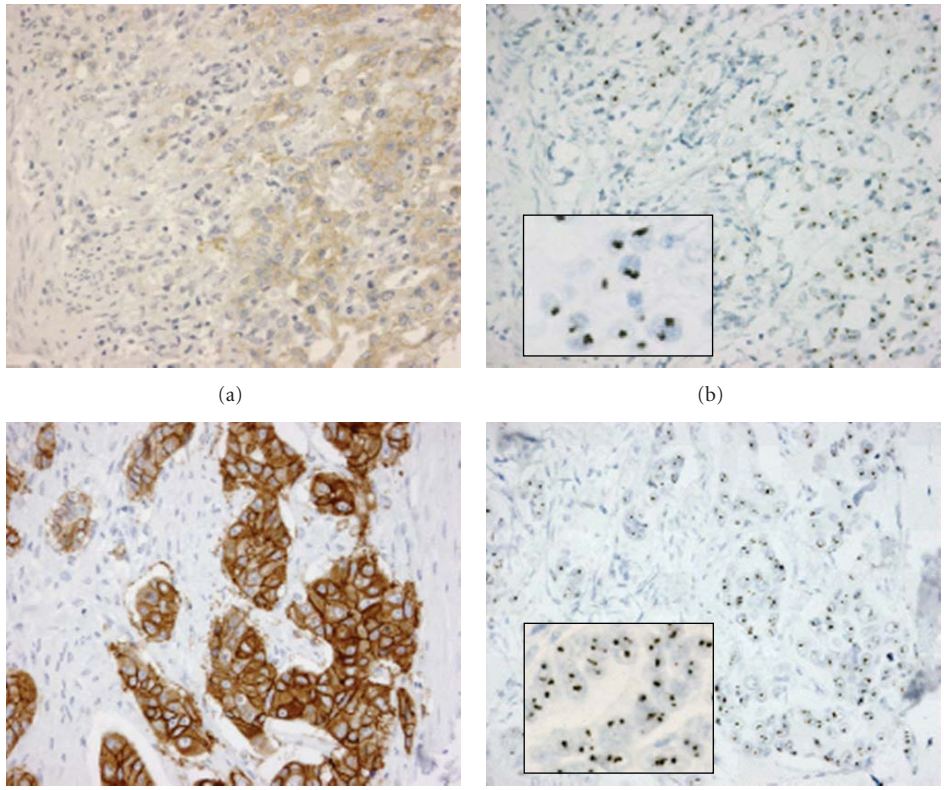

Figure 1: Two gastric tumors analyzed by HER2 immunohistochemistry (IHC, HercepTest) and chromogenic *in situ* hybridization (CISH). (a) Case 1 with IHC 2+ score and corresponding (b) CISH amplification (see inset). (c) Case 2 with IHC 3+ score and corresponding (d) CISH amplification (see inset).

as 3+ on IHC or if they were FISH positive (HER2:CEP17 ratio ≥2.0), and 22% were HER2 positive. HER2 positivity was higher in esophageal junction cancers (33%) than gastric cancer (21%), and tumors classified as intestinal type (32%) were significantly more likely to be HER2-positive than diffuse (only 6%) or mixed (20%) types. Median overall survival, the primary endpoint, was significantly prolonged in the trastuzumab plus chemotherapy arm when compared with chemotherapy alone (13.5 months versus 11.1 months; $P = .0048$), representing a 26% reduction in the risk of death in the trastuzumab group (hazard ratio 0.74, confidence interval 0.60–0.91). The overall response rate was also significantly greater in the trastuzumab arm (47.3% versus 34.5%; $P = .0017$). Safety profiles were similar in the two study groups with no unexpected adverse events being reported with the addition of trastuzumab to chemotherapy.

No clinical data with lapatinib are available so far, but several phase II studies are ongoing and even a phase III trial has been initiated. Compared to trastuzumab, lapatinib is a small molecule tyrosine kinase inhibitor (TKI) that targets both HER2 and epidermal growth factor receptor (EGFR/HER1) and can be administered orally. It would be interesting to see whether the dual action of lapatinib will provide additional benefit over trastuzumab, especially in view of the fact that EGFR also seems to be overexpressed in gastric cancer [91–93].

4. Changing Treatment of Esophageal/Gastric Cancer

Despite advances in clinical diagnostics, surgical techniques, chemotherapy, and radiotherapy regimens, prognosis of gastric cancer remains poor, and novel treatment options as well as predictors of treatment response are urgently needed. Van Cutsem et al. presented preliminary results of the ToGA study at the 2009 ASCO Annual Meeting [94]. Although data from this trial should be considered encouraging and a major step forward in the treatment of advanced gastric cancer, some important considerations should be made. Firstly, the absolute benefit in response to trastuzumab addition to chemotherapy was 12.8%, indicating that—as in breast cancer—there is also resistance to trastuzumab even among HER2-positive selected patients. A better understanding of HER but also of other signalling pathways such as the Wnt and TGFb pathways is therefore crucial. A combination of targeted agents, which ideally target different "crosstalk" pathways, would theoretically lead to highly effective therapies [95]. Secondly, although trastuzumab efficacy is likely in the adjuvant setting, trastuzumab use in early gastric cancer requires the completion of new adjuvant phase III trials. Thirdly, although some studies have reported that HER2 amplification and overexpression are highly homogeneous within tumors and between primary and metastatic gastric

cancer [62], others have reported significant heterogeneity in both gene amplification and protein overexpression in individual cancers, even among IHC 3+ cancers [67, 86]. This could impede predicting treatment response and thus selecting the right patients for treatment.

Future directions of research in HER2-positive gastroesophageal cancer should focus on the evaluation of novel antibodies (such as pertuzumab, a dimerization inhibitor, and T-DM1, a drug that combines trastuzumab with a linked chemotherapy agent called maytansine), irreversible tyrosine kinase inhibitors (such as neratinib and BIBW 2992, both dual EGFR-HER2 inhibitors), and inhibitors of HER2-related downstream signaling (such as mammalian target of rapamycin (mTOR), heat shock protein 90 (Hsp90), and PI3K/Akt) and of receptor crosstalk (such as other HER family members, vascular endothelial growth factor receptor (VEGFR), and insulin-like growth factor receptor (IGFR)).

In the latter category, some promising targeted agents have already been investigated in gastroesophageal cancer. Molecular interactions between HER2 and other members of its family (HER1 or EGFR, HER3 and HER4) have led to the development of new targeted therapies such as the anti-EGFR monoclonal antibody cetuximab, the anti-EGFR oral small molecule tyrosine kinase inhibitors erlotinib and gefitinib, and the dual EGFR-HER2 tyrosine kinase inhibitor lapatinib [96]. Cetuximab has undergone more extensive evaluation in gastroesophageal cancers than any other targeted agent in the locally advanced setting as well as in the first-line metastatic setting, the second-line setting, and beyond. Unfortunately, most of these trial results have only been published in abstract form, and final publication is eagerly awaited. Cetuximab shows promise in the treatment of esophageal and gastric cancers in the locally advanced as well as in the first-line metastatic setting. Both erlotinib and gefitinib have very little single-agent activity in the first- and second-line settings in gastroesophageal cancers. Lapatinib has shown only modest results in the very few studies evaluating its activity in the metastatic setting. However, despite these modest results, the phase III LOGIC trial is currently evaluating the combination of capecitabine/oxaliplatin with or without lapatinib as first-line therapy in HER2-positive locally advanced, unresectable or metastatic gastroesophageal cancer.

HER2 has also been shown to communicate with VEGFR, and studies in breast cancer have shown a synergistic interaction with the combination of trastuzumab and a VEGFR tyrosine kinase inhibitor [97]. Therapies directed against VEGF(R) are the focus of ongoing research in many malignancies including gastroesophageal cancer [98, 99]. Bevacizumab, a monoclonal anti-VEGF antibody, has been investigated in the locally advanced and metastatic first- and second-line setting with encouraging phase II results [96]. A confirmatory international phase III trial of capecitabine/cisplatin with or without bevacizumab in advanced gastric cancer is currently underway. Sunitinib and sorafinib, both multitarget (among which VEGFR) tyrosine kinase inhibitors, have been tested in metastatic gastroesophageal cancers with promising preliminary results [96].

5. Conclusion and Future Directions

Based on the encouraging ToGA trial findings, it is now expected that routine HER2 testing will be included in the diagnostic work-up of patients with advanced gastric cancer. With regard to this testing, overexpression of the HER2 protein seems to possess the best predictive value. However, HER2 IHC is subject to assay and interobserver variability; so standardization and internal and external proficiency testing is an absolute prerequisite, especially because the IHC scoring system in gastric cancer is different from that of breast cancer.

As in breast cancer, trastuzumab research will now most probably move into the adjuvant setting. The combined treatment of chemotherapy and trastuzumab may also be beneficial in decreasing the recurrence of the disease after resection of the tumor.

Given the high degree of primary and acquired resistance to trastuzumab therapy and bearing in mind that most patients with gastroesophageal cancer are HER2-negative, highly effective therapies can only be accomplished by a multitargeted approach, considering crosstalk between pathways and continuing to optimize chemotherapy.

Targeted agents will likely gain an increasingly important role in the treatment of patients with esophageal and gastric cancer in the near future, but how big a part they will play is unclear at this point. Lessons learnt over the past decades in breast cancer can help us maximize therapy benefit.

References

[1] M. Hongo, Y. Nagasaki, and T. Shoji, "Epidemiology of esophageal cancer: orient to Occident. Effects of chronology, geography and ethnicity," *Journal of Gastroenterology and Hepatology*, vol. 24, no. 5, pp. 729–735, 2009.

[2] D. Maxwell Parkin, F. Bray, J. Ferlay, and P. Pisani, "Estimating the world cancer burden: Globocan 2000," *International Journal of Cancer*, vol. 94, no. 2, pp. 153–156, 2001.

[3] P. Lauren, "The two histological main types of gastric carcinoma: diffuse and so-called intestinal-type carcinoma. An attempt at a histo-clinical classification," *Acta Pathologica et Microbiologica Scandinavica*, vol. 64, pp. 31–49, 1965.

[4] P. C. Enzinger and R. J. Mayer, "Esophageal cancer," *New England Journal of Medicine*, vol. 349, no. 23, pp. 2241–2252, 2003.

[5] T. Akiyama, C. Sudo, and H. Ogawara, "The product of the human c-erbB-2 gene: a 185-kilodalton glycoprotein with tyrosine kinase activity," *Science*, vol. 232, no. 4758, pp. 1644–1646, 1986.

[6] N. C. Popescu, C. R. King, and M. H. Kraus, "Localization of the human erbB-2 gene on normal and rearranged chromosomes 17 to bands q12-21.32," *Genomics*, vol. 4, no. 3, pp. 362–366, 1989.

[7] M. A. Owens, B. C. Horten, and M. M. Da Silva, "HER2 amplification ratios by fluorescence in situ hybridization and correlation with immunohistochemistry in a cohort of 6556 breast cancer tissues," *Clinical Breast Cancer*, vol. 5, no. 1, pp. 63–69, 2004.

[8] J. S. Ross, J. A. Fletcher, K. J. Bloom et al., "Targeted therapy in breast cancer: the HER-2/neu gene and protein," *Molecular and Cellular Proteomics*, vol. 3, no. 4, pp. 379–398, 2004.

[9] D. J. Slamon, W. Godolphin, L. A. Jones et al., "Studies of the HER-2/neu proto-oncogene in human breast and ovarian cancer," *Science*, vol. 244, no. 4905, pp. 707–712, 1989.

[10] C. B. Moelans, R. A. de Weger, M. T. van Blokland, E. van der Wall, and P. J. van Diest, "Simultaneous detection of TOP2A and HER2 gene amplification by multiplex ligation-dependent probe amplification in breast cancer," *Modern Pathology*, vol. 23, no. 1, pp. 62–70, 2010.

[11] C. B. Moelans, R. A. De Weger, and P. J. Van Diest, "Absence of chromosome 17 polysomy in breast cancer: analysis by CEP17 chromogenic in situ hybridization and multiplex ligation-dependent probe amplification," *Breast Cancer Research and Treatment*, vol. 120, no. 1, pp. 1–7, 2010.

[12] B. Schuell, T. Gruenberger, W. Scheithauer, Ch. Zielinski, and F. Wrba, "HER 2/neu protein expression in colorectal cancer," *BMC Cancer*, vol. 6, article no. 123, 2006.

[13] E. Eltze, C. Wülfing, D. Von Struensee, H. Piechota, H. Buerger, and L. Hertle, "Cox-2 and Her2/neu co-expression in invasive bladder cancer," *International Journal of Oncology*, vol. 26, no. 6, pp. 1525–1531, 2005.

[14] S. J. McKenzie, K. A. DeSombre, B. S. Bast et al., "Serum levels of HER-2 neu (C-erbB-2) correlate with overexpression of p185(neu) in human ovarian cancer," *Cancer*, vol. 71, no. 12, pp. 3942–3946, 1993.

[15] M. E. Nowee, J. C. Dorsman, J. M. J. Piek et al., "HER-2/neu and p27Kip1 in progression of Fallopian tube carcinoma: an immunohistochemical and array comparative genomic hybridization study," *Histopathology*, vol. 51, no. 5, pp. 666–673, 2007.

[16] D. J. Hetzel, T. O. Wilson, G. L. Keeney, P. C. Roche, S. S. Cha, and K. C. Podratz, "HER-2/neu expression: a major prognostic factor in endometrial cancer," *Gynecologic Oncology*, vol. 47, no. 2, pp. 179–185, 1992.

[17] N. Hirashima, W. Takahashi, S. Yoshii, T. Yamane, and A. Ooi, "Protein overexpression and gene amplification of c-erb B-2 in pulmonary carcinomas: a comparative immunohistochemical and fluorescence in situ hybridization study," *Modern Pathology*, vol. 14, no. 6, pp. 556–562, 2001.

[18] A. B. Mitra, V. V. V. S. Murty, M. Pratap, P. Sodhani, and R. S. K. Chaganti, "ERBB2 (HER2/neu) oncogene is frequently amplified in squamous cell carcinoma of the uterine cervix," *Cancer Research*, vol. 54, no. 3, pp. 637–639, 1994.

[19] R. N. Beckhardt, N. Kiyokawa, L. Xi et al., "HER-2/neu oncogene characterization in head and neck squamous cell carcinoma," *Archives of Otolaryngology*, vol. 121, no. 11, pp. 1265–1270, 1995.

[20] M. Jorda, A. Morales, Z. Ghorab, G. Fernandez, M. Nadji, and N. Block, "HER2 expression in prostatic cancer: a comparison with mammary carcinoma," *Journal of Urology*, vol. 168, no. 4, pp. 1412–1414, 2002.

[21] Y. Yamanaka, H. Friess, M. S. Kobrin et al., "Overexpression of HER2/neu oncogene in human pancreatic carcinoma," *Human Pathology*, vol. 24, no. 10, pp. 1127–1134, 1993.

[22] G. Cornolti, M. Ungari, M. L. Morassi et al., "Amplification and overexpression of Her2/neu gene and HER2/neu protein in salivary duct carcinoma of the parotid gland," *Archives of Otolaryngology*, vol. 133, no. 10, pp. 1031–1036, 2007.

[23] U. Reichelt, P. Duesedau, M. CH. Tsourlakis et al., "Frequent homogeneous HER-2 amplification in primary and metastatic adenocarcinoma of the esophagus," *Modern Pathology*, vol. 20, no. 1, pp. 120–129, 2007.

[24] J. Jaehne, C. Urmacher, H. T. Thaler, H. Friedlander-Klar, C. Cordon-Cardo, and H. J. Meyer, "Expression of Her2/neu oncogene product p185 in correlation to clinicopathological and prognostic factors of gastric carcinoma," *Journal of Cancer Research and Clinical Oncology*, vol. 118, no. 6, pp. 474–479, 1992.

[25] D. J. Slamon, B. Leyland-Jones, S. Shak et al., "Use of chemotherapy plus a monoclonal antibody against her2 for metastatic breast cancer that overexpresses HER2," *New England Journal of Medicine*, vol. 344, no. 11, pp. 783–792, 2001.

[26] C. A. Hudis, "Trastuzumab—mechanism of action and use in clinical practice," *New England Journal of Medicine*, vol. 357, no. 1, pp. 39–51, 2007.

[27] J. P. A. Baak, D. Chin, P. J. Van Diest, R. Ortiz, P. Matze-Cok, and S. S. Bacus, "Comparative long-term prognostic value of quantitative HER-2/neu protein expression, DNA ploidy, and morphometric and clinical features in paraffin-embedded invasive breast cancer," *Laboratory Investigation*, vol. 64, no. 2, pp. 215–223, 1991.

[28] H. Joensuu, P.-L. Kellokumpu-Lehtinen, P. Bono et al., "Adjuvant docetaxel or vinorelbine with or without trastuzumab for breast cancer," *New England Journal of Medicine*, vol. 354, no. 8, pp. 809–820, 2006.

[29] D. J. Slamon, G. M. Clark, and S. G. Wong, "Human breast cancer: correlation of relapse and survival with amplification of the HER-2/neu oncogene," *Science*, vol. 235, no. 4785, pp. 177–182, 1987.

[30] S. Sjögren, M. Inganäs, A. Lindgren, L. Holmberg, and J. Bergh, "Prognostic and predictive value of c-erbB-2 overexpression in primary breast cancer, alone and in combination with other prognostic markers," *Journal of Clinical Oncology*, vol. 16, no. 2, pp. 462–469, 1998.

[31] A. Borg, B. Baldetorp, M. Ferno et al., "ERBB2 amplification is associated with tamoxifen resistance in steroid-receptor positive breast cancer," *Cancer Letters*, vol. 81, no. 2, pp. 137–144, 1994.

[32] C. Carlomagno, F. Perrone, C. Gallo et al., "c-erbB2 overexpression decreases the benefit of adjuvant tamoxifen in early-stage breast cancer without axillary lymph node metastases," *Journal of Clinical Oncology*, vol. 14, no. 10, pp. 2702–2708, 1996.

[33] B. Têtu, J. Brisson, V. Plante, and P. Bernard, "p53 and c-erbB-2 as markers of resistance to adjuvant chemotherapy in breast cancer," *Modern Pathology*, vol. 11, no. 9, pp. 823–830, 1998.

[34] A. C. Wolff, M. E. H. Hammond, J. N. Schwartz et al., "American society of clinical oncology/college of American pathologists guideline recommendations for human epidermal growth factor receptor 2 testing in breast cancer," *Archives of Pathology and Laboratory Medicine*, vol. 131, no. 1, pp. 18–43, 2007.

[35] M. F. Press, G. Hung, W. Godolphin, and D. J. Slamon, "Sensitivity of HER-2/neu antibodies in archival tissue samples: potential source of error in immunohistochemical studies of oncogene expression," *Cancer Research*, vol. 54, no. 10, pp. 2771–2777, 1994.

[36] T. W. Jacobs, A. M. Gown, H. Yaziji, M. J. Barnes, and S. J. Schnitt, "Specificity of HercepTest in determining HER-2/neu status of breast cancers using the United States Food and Drug Administration-approved scoring system," *Journal of Clinical Oncology*, vol. 17, no. 7, pp. 1983–1987, 1999.

[37] R. R. Tubbs, J. D. Pettay, P. C. Roche, M. H. Stoler, R. B. Jenkins, and T. M. Grogan, "Discrepancies in clinical laboratory testing of eligibility for trastuzumab therapy: apparent immunohistochemical false-positives do not get the message," *Journal of Clinical Oncology*, vol. 19, no. 10, pp. 2714–2721, 2001.

[38] R. Mass, "The role of HER-2 expression in predicting response to therapy in breast cancer," *Seminars in Oncology*, vol. 27, no. 6, pp. 46–52, 2000.

[39] G. Pauletti, S. Dandekar, H. M. Rong et al., "Assessment of methods for tissue-based detection of the HER-2/neu alteration in human breast cancer: a direct comparison of fluorescence in situ hybridization and immunohistochemistry," *Journal of Clinical Oncology*, vol. 18, no. 21, pp. 3651–3664, 2000.

[40] J. M. S. Bartlett, J. J. Going, E. A. Mallon et al., "Evaluating HER2 amplification and overexpression in breast cancer," *Journal of Pathology*, vol. 195, no. 4, pp. 422–428, 2001.

[41] C. L. Vogel, M. A. Cobleigh, D. Tripathy et al., "Efficacy and safety of trastuzumab as a single agent in first-line treatment of HER2-overexpressing metastatic breast cancer," *Journal of Clinical Oncology*, vol. 20, no. 3, pp. 719–726, 2002.

[42] R. E. Jimenez, T. Wallis, P. Tabasczka, and D. W. Visscher, "Determination of Her-2/neu status in breast carcinoma: comparative analysis of immunohistochemistry and fluorescent in situ hybridization," *Modern Pathology*, vol. 13, no. 1, pp. 37–45, 2000.

[43] A. Lebeau, D. Deimling, C. Kaltz et al., "HER-2/neu analysis in archival tissue samples of human breast cancer: comparison of immunohistochemistry and fluorescence in situ hybridization," *Journal of Clinical Oncology*, vol. 19, no. 2, pp. 354–363, 2001.

[44] M. Tanner, D. Gancberg, B. A. D. Leo et al., "Chromogenic in situ hybridization: a practical alternative for fluorescence in situ hybridization to detect HER-2/neu oncogene amplification in archival breast cancer samples," *American Journal of Pathology*, vol. 157, no. 5, pp. 1467–1472, 2000.

[45] M. Hofmann, O. Stoss, D. Shi et al., "Assessment of a HER2 scoring system for gastric cancer: results from a validation study," *Histopathology*, vol. 52, no. 7, pp. 797–805, 2008.

[46] N. Dandachi, O. Dietze, and C. Hauser-Kronberger, "Chromogenic in situ hybridization: a novel approach to a practical and sensitive method for the detection of HER2 oncogene in archival human breast carcinoma," *Laboratory Investigation*, vol. 82, no. 8, pp. 1007–1014, 2002.

[47] J. Zhao, R. Wu, A. Au, A. Marquez, Y. Yu, and Z. Shi, "Determination of HER2 gene amplification by chromogenic in situ hybridization (CISH) in archival breast carcinoma," *Modern Pathology*, vol. 15, no. 6, pp. 657–665, 2002.

[48] M. van de Vijver, M. Bilous, and W. Hanna, "Correlation of chromogenic in-situ hybridisation (CISH) with FISH and IHC for assessment of HER2 gene amplification: an international validation ring study," *European Journal of Cancer*, vol. 2, supplement 99, 2004, abstract no. 157.

[49] Y. Gong, M. Gilcrease, and N. Sneige, "Reliability of chromogenic in situ hybridization for detecting HER-2 gene status in breast cancer: comparison with fluorescence in situ hybridization and assessment of interobserver reproducibility," *Modern Pathology*, vol. 18, no. 8, pp. 1015–1021, 2005.

[50] W. M. Hanna and K. Kwok, "Chromogenic in-situ hybridization: a viable alternative to fluorescence in-situ hybridization in the HER2 testing algorithm," *Modern Pathology*, vol. 19, no. 4, pp. 481–487, 2006.

[51] C. B. Moelans, R. A. De Weger, M. T. M. Van Blokland et al., "HER-2/neu amplification testing in breast cancer by multiplex ligation-dependent probe amplification in comparison with immunohistochemistry and in situ hybridization," *Cellular Oncology*, vol. 31, no. 1, pp. 1–10, 2009.

[52] B. Yan, E. X. Yau, S. S.B. Omar et al., "A study of HER2 gene amplification and protein expression in gastric cancer," *Journal of Clinical Pathology*, vol. 63, no. 9, pp. 839–842, 2010.

[53] J. P. Schouten, C. J. McElgunn, R. Waaijer, D. Zwijnenburg, F. Diepvens, and G. Pals, "Relative quantification of 40 nucleic acid sequences by multiplex ligation-dependent probe amplification," *Nucleic Acids Research*, vol. 30, no. 12, p. e57, 2002.

[54] C. B. Moelans, R. A. de Weger, C. Ezendam, and P. J. van Diest, "HER-2/neu amplification testing in breast cancer by Multiplex Ligation-dependent Probe Amplification: influence of manual- and laser microdissection," *BMC Cancer*, vol. 9, article no. 4, 2009.

[55] C. B. Moelans, R. A. De Weger, and P. J. Van Diest, "Multiplex ligation-dependent probe amplification to detect HER2 amplification in breast cancer: new insights in optimal cut-off value," *Cellular Oncology*, vol. 32, no. 4, pp. 311–312, 2010.

[56] M. Allison, "The HER2 testing conundrum," *Nature Biotechnology*, vol. 28, no. 2, pp. 117–119, 2010.

[57] Y.-J. Bang, E. Van Cutsem, A. Feyereislova et al., "Trastuzumab in combination with chemotherapy versus chemotherapy alone for treatment of HER2-positive advanced gastric or gastro-oesophageal junction cancer (ToGA): a phase 3, open-label, randomised controlled trial," *The Lancet*, vol. 376, no. 9742, pp. 687–697, 2010.

[58] J. Rüschoff, M. Dietel, G. Baretton et al., "HER2 diagnostics in gastric cancer-guideline validation and development of standardized immunohistochemical testing," *Virchows Archiv*, vol. 457, no. 3, pp. 299–307, 2010.

[59] J. S. Ross, "Breast cancer biomarkers and HER2 testing after 10 years of anti-HER2 therapy," *Drug News and Perspectives*, vol. 22, no. 2, pp. 93–106, 2009.

[60] J. T. Jørgensen, "Targeted HER2 treatment in advanced gastric cancer," *Oncology*, vol. 78, no. 1, pp. 26–33, 2010.

[61] Y. Bang, H. Chung, J. Xu et al., "Pathological features of advanced gastric cancer (GC): relationship to human epidermal growth factor receptor 2 (HER2) positivity in the global screening programme of the ToGA trial," *Journal of Clinical Oncology*, vol. 27, supplement 15S, 2009, abstract 4556.

[62] A. H. Marx, L. Tharun, J. Muth et al., "HER-2 amplification is highly homogenous in gastric cancer," *Human Pathology*, vol. 40, no. 6, pp. 769–777, 2009.

[63] D. I. Park, J. W. Yun, J. H. Park et al., "HER-2/neu amplification is an independent prognostic factor in gastric cancer," *Digestive Diseases and Sciences*, vol. 51, no. 8, pp. 1371–1379, 2006.

[64] J. D. Barros-Silva, D. Leit~o, L. Afonso et al., "Association of ERBB2 gene status with histopathological parameters and disease-specific survival in gastric carcinoma patients," *British Journal of Cancer*, vol. 100, no. 3, pp. 487–493, 2009.

[65] M. Tanner, M. Hollmén, T. T. Junttila et al., "Amplification of HER-2 in gastric carcinoma: association with Topoisomerase IIα gene amplification, intestinal type, poor prognosis and sensitivity to trastuzumab," *Annals of Oncology*, vol. 16, no. 2, pp. 273–278, 2005.

[66] G. Z. Yu, Y. Chen, and J. J. Wang, "Overexpression of Grb2/HER2 signaling in Chinese gastric cancer: their relationship with clinicopathological parameters and prognostic significance," *Journal of Cancer Research and Clinical Oncology*, vol. 135, no. 10, pp. 1331–1339, 2009.

[67] H. Grabsch, S. Sivakumar, S. Gray, H. E. Gabbert, and W. Müller, "HER2 expression in gastric cancer: rare, heterogeneous and of no prognostic value-conclusions from 924 cases

of two independent series," *Cellular Oncology*, vol. 32, no. 1-2, pp. 57–65, 2010.

[68] K. Hede, "Gastric cancer: trastuzumab trial results spur search for other targets," *Journal of the National Cancer Institute*, vol. 101, no. 19, pp. 1306–1307, 2009.

[69] Y. Bang, H. Chung, A. Sawaki et al., "HER2-positivity rates in advanced gastric cancer (GC): results from a large international phase III trial," *Journal of Clinical Oncology*, vol. 26, supplement 15S, 2008, Abstract no. 4526.

[70] C. Gravalos and A. Jimeno, "HER2 in gastric cancer: a new prognostic factor and a novel therapeutic target," *Annals of Oncology*, vol. 19, no. 9, pp. 1523–1529, 2008.

[71] W. Polkowski, J. W. Van Sandick, G. J. A. Offerhaus et al., "Prognostic value of Lauren classification and c-erbB-2 oncogene overexpression in adenocarcinoma of the esophagus and gastroesophageal junction," *Annals of Surgical Oncology*, vol. 6, no. 3, pp. 290–297, 1999.

[72] S. Ekman, M. Bergqvist, C.-H. Heldin, and J. Lennartsson, "Activation of growth factor receptors in esophageal cancer—implications for therapy," *Oncologist*, vol. 12, no. 10, pp. 1165–1177, 2007.

[73] J.-F. Flejou, F. Paraf, F. Muzeau et al., "Expression of c-erbB-2 oncogene product in Barrett's adenocarcinoma: pathological and prognostic correlations," *Journal of Clinical Pathology*, vol. 47, no. 1, pp. 23–26, 1994.

[74] T. Nakamura, H. Nekarda, A. H. Hoelscher et al., "Prognostic value of DNA ploidy and c-erbB-2 oncoprotein overexpression in adenocarcinoma of Barrett's esophagus," *Cancer*, vol. 73, no. 7, pp. 1785–1794, 1994.

[75] T. P. Brien, R. D. Odze, C. E. Sheehan, B. J. McKenna, and J. S. Ross, "Her-2/neu gene amplification by FISH predicts poor survival in Barrett's esophagus-associated adenocarcinoma," *Human Pathology*, vol. 31, no. 1, pp. 35–39, 2000.

[76] E. Rossi, S. Grisanti, V. Villanacci et al., "HER-2 overexpression/amplification in Barrett's oesophagus predicts early transition from dysplasia to adenocarcinoma: a clinico-pathologic study," *Journal of Cellular and Molecular Medicine*, vol. 13, no. 9 B, pp. 3826–3833, 2009.

[77] E. Rossi, V. Villanacci, G. Bassotti et al., "Her-2/neu in Barrett esophagus: a comparative study between histology, immunohistochemistry, and fluorescence in situ hybridization," *Diagnostic Molecular Pathology*, vol. 15, no. 3, pp. 125–130, 2006.

[78] S. Rauser, R. Weis, H. Braselmann et al., "Significance of HER2 low-level copy gain in Barrett's cancer: implications for fluorescence in situ hybridization testing in tissues," *Clinical Cancer Research*, vol. 13, no. 17, pp. 5115–5123, 2007.

[79] B. Albrecht, M. Hausmann, H. Zitzelsberger et al., "Array-based comparative genomic hybridization for the detection of DNA sequence copy number changes in Barrett's adenocarcinoma," *Journal of Pathology*, vol. 203, no. 3, pp. 780–788, 2004.

[80] V. Villanacci, E. Rossi, S. Grisanti et al., "Targeted therapy with trastuzumab in dysplasia and adenocarcinoma arising in Barrett's esophagus: a translational approach," *Minerva Gastroenterologica e Dietologica*, vol. 54, no. 4, pp. 347–353, 2008.

[81] A. Kokkola and P. Sipponen, "Gastric carcinoma in young adults," *Hepato-Gastroenterology*, vol. 48, no. 42, pp. 1552–1555, 2001.

[82] R. Carvalho, A. N. A. Milne, B. P. van Rees et al., "Early-onset gastric carcinomas display molecular characteristics distinct from gastric carcinomas occuring at a later age," *Journal of Pathology*, vol. 204, no. 1, pp. 75–83, 2004.

[83] A. N. A. Milne, R. Carvalho, F. M. Morsink et al., "Early-onset gastric cancers have a different molecular expression profile than conventional gastric cancers," *Modern Pathology*, vol. 19, no. 4, pp. 564–572, 2006.

[84] A. N. Milne, R. Sitarz, R. Carvalho, F. Carneiro, and G. J. A. Offerhaus, "Early onset gastric cancer: on the road to unraveling gastric carcinogenesis," *Current Molecular Medicine*, vol. 7, no. 1, pp. 15–28, 2007.

[85] C.-W. Wu, G.-D. Chen, C. S.-J. Fann et al., "Clinical implications of chromosomal abnormalities in gastric adenocarcinomas," *Genes Chromosomes and Cancer*, vol. 35, no. 3, pp. 219–231, 2002.

[86] M. Bilous, R. Y. Osamura, J. Ruschoff et al., "HER-2 amplification is highly homogenous in gastric cancer," *Human Pathology*, vol. 41, no. 2, pp. 304–305, 2010.

[87] K. Fujimoto-Ouchi, F. Sekiguchi, H. Yasuno, Y. Moriya, K. Mori, and Y. Tanaka, "Antitumor activity of trastuzumab in combination with chemotherapy in human gastric cancer xenograft models," *Cancer Chemotherapy and Pharmacology*, vol. 59, no. 6, pp. 795–805, 2007.

[88] S. Y. Kim, H. P. Kim, Y. J. Kim et al., "Trastuzumab inhibits the growth of human gastric cancer cell lines with HER2 amplification synergistically with cisplatin," *International Journal of Oncology*, vol. 32, no. 1, pp. 89–95, 2008.

[89] S. J. Gong, C. J. Jin, S. Y. Rha, and H. C. Chung, "Growth inhibitory effects of trastuzumab and chemotherapeutic drugs in gastric cancer cell lines," *Cancer Letters*, vol. 214, no. 2, pp. 215–224, 2004.

[90] Y. Matsui, M. Inomata, M. Tojigamori, K. Sonoda, N. Shiraishi, and S. Kitano, "Suppression of tumor growth in human gastric cancer with HER2 overexpression by an anti-HER2 antibody in a murine model," *International Journal of Oncology*, vol. 27, no. 3, pp. 681–685, 2005.

[91] Y. Itakura, H. Sasano, C. Shiga et al., "Epidermal growth factor receptor overexpression in esophageal carcinoma: an immunohistochemical study correlated with clinicopathologic findings and DNA amplification," *Cancer*, vol. 74, no. 3, pp. 795–804, 1994.

[92] N. W. Wilkinson, J. D. Black, E. Roukhadze et al., "Epidermal growth factor receptor expression correlates with histologic grade in resected esophageal adenocarcinoma," *Journal of Gastrointestinal Surgery*, vol. 8, no. 4, pp. 448–453, 2004.

[93] M. A. Kim, H. S. Lee, H. E. Lee, Y. K. Jeon, H. K. Yang, and W. H. Kim, "EGFR in gastric carcinomas: prognostic significance of protein overexpression and high gene copy number," *Histopathology*, vol. 52, no. 6, pp. 738–746, 2008.

[94] E. van Cutsem, Y. Kang, H. Chung et al., "Efficacy results from the ToGA trial: a phase III study of trastuzumab added to standard chemotherapy (CT) in first-line human epidermal growth factor receptor 2 (HER2)-positive advanced gastric cancer (GC)," *Journal of Clinical Oncology*, vol. 27, supplement 18S, 2009, Abstract LBA4509.

[95] D. H. Roukos, "Targeting gastric cancer with trastuzumab: new clinical practice and innovative developments to overcome resistance," *Annals of Surgical Oncology*, vol. 17, no. 1, pp. 14–17, 2010.

[96] G. Y. Ku and D. H. Ilson, "Esophagogastric cancer: targeted agents," *Cancer Treatment Reviews*, vol. 36, no. 3, pp. 235–248, 2010.

[97] E. M. H. Kim, C. Lobocki, L. Dubay, and V. K. Mittal, "A specific vascular endothelial growth factor receptor tyrosine kinase inhibitor enhances the antiproliferative effect of trastuzumab in human epidermal growth factor receptor 2

overexpressing breast cancer cell lines," *American Journal of Surgery*, vol. 197, no. 3, pp. 331–336, 2009.

[98] W. Liu, X. Zhang, and W. Sun, "Developments in treatment of esophageal/gastric cancer," *Current Treatment Options in Oncology*, vol. 9, no. 4–6, pp. 375–387, 2008.

[99] J. Meza-Junco, H.-J. Au, and M. B. Sawyer, "Trastuzumab for gastric cancer," *Expert Opinion on Biological Therapy*, vol. 9, no. 12, pp. 1543–1551, 2009.