

# The genetic basis of sporadic pancreatic cancer

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## 1. Introduction

Adenocarcinomas of the pancreatic gland are the fifth leading cause of cancer death in the USA and western Europe with a 5-year survival rate of less than 5% and a median survival of less than 6 months [76]. They bear histological resemblance to the pancreatic ducts and are referred to as pancreatic ductal adenocarcinoma [5,34,53]. Due to the late occurrence of symptoms and the high metastatic potential, pancreatic adenocarcinomas have a dismal prognosis. Since the pancreaticoduodenectomy, a surgical resection that includes the pancreatic head, the duodenum, common bile duct and the gallbladder was introduced in 1912 by Kausch, surgery still remains the primary curative treatment. However, the 5-year survival for patients undergoing resection, is only about 20% [76] and about 80% of all cancers are unresectable at the time of diagnosis [2]. In order to improve the clinical management of cancer patients, it is mandatory to understand the genetic basis of pancreatic cancers. This review describes the histological progression of pancreatic adenocarcinomas and summarizes the underlying chromosomal and genetic alterations.

## 2. Histological progression model

The cells of pancreatic adenocarcinomas show morphological similarity to pancreatic ductal cells. Therefore, the latter are believed to be the progenitors of pancreatic adenocarcinomas. They account for approximately 5% of the pancreatic cell population and are

cubically shaped with basal nuclei. Usually, pancreatic ductal adenocarcinomas develop from intraductal pre-cancerous lesions, which are characterized by an increase of histoarchitectural disorder and cell or nuclear dysplasia [70]. Intraductal proliferation of epithelial cells was more frequently found in pancreatic tissues with invasive ductal carcinoma than in non-neoplastic tissue, and it was always accompanied by lesions of increased levels of dysplasia [8,43,45,70]. These findings led to the development of a progression-model as well as a standardized nomenclature and classification-system [16,37].

The first histological change, called squamous or transitional metaplasia describes the replacement of the normal ductal epithelium by mature squamous or transitional epithelium without atypia. PanIN-1A (**pan**creatic **in**traepithelial **ne**oplasia) denotes the transition to tall columnar cells with basally located non-atypical nuclei (Fig. 1b). PanIN-1B is cytologically indistinguishable from PanIN-1A, but shows a papillary, micropapillary or basally pseudostratified architecture (Fig. 1c). PanIN-2 lesions are flat or papillary and, by definition, these lesions must exhibit nuclear abnormalities, which can include loss of polarity, nuclear crowding, enlarged nuclei, pseudo-stratification and hyperchromatism (Fig. 1d). Cytologically, the PanIN-3 lesions resemble carcinoma cells without invasion of the basal membrane, hence they are called “carcinoma *in situ*”. In PanIN-3 lesions the epithelium is usually papillary or micro-papillary and rarely flat. True cribriforming, budding-off of small clusters of epithelial cells into the lumen and luminal necroses can be detected. The cells are characterized by profound signs of nuclear atypia, like loss of nuclear polarity, irregularities in shape, prominent nucleoli and mitoses. Additionally cellular disorders like dystrophic goblet cells with nuclei oriented towards the lumen and mucinous cytoplasm orientated towards the basement membrane can be found [37] (Fig. 1e).

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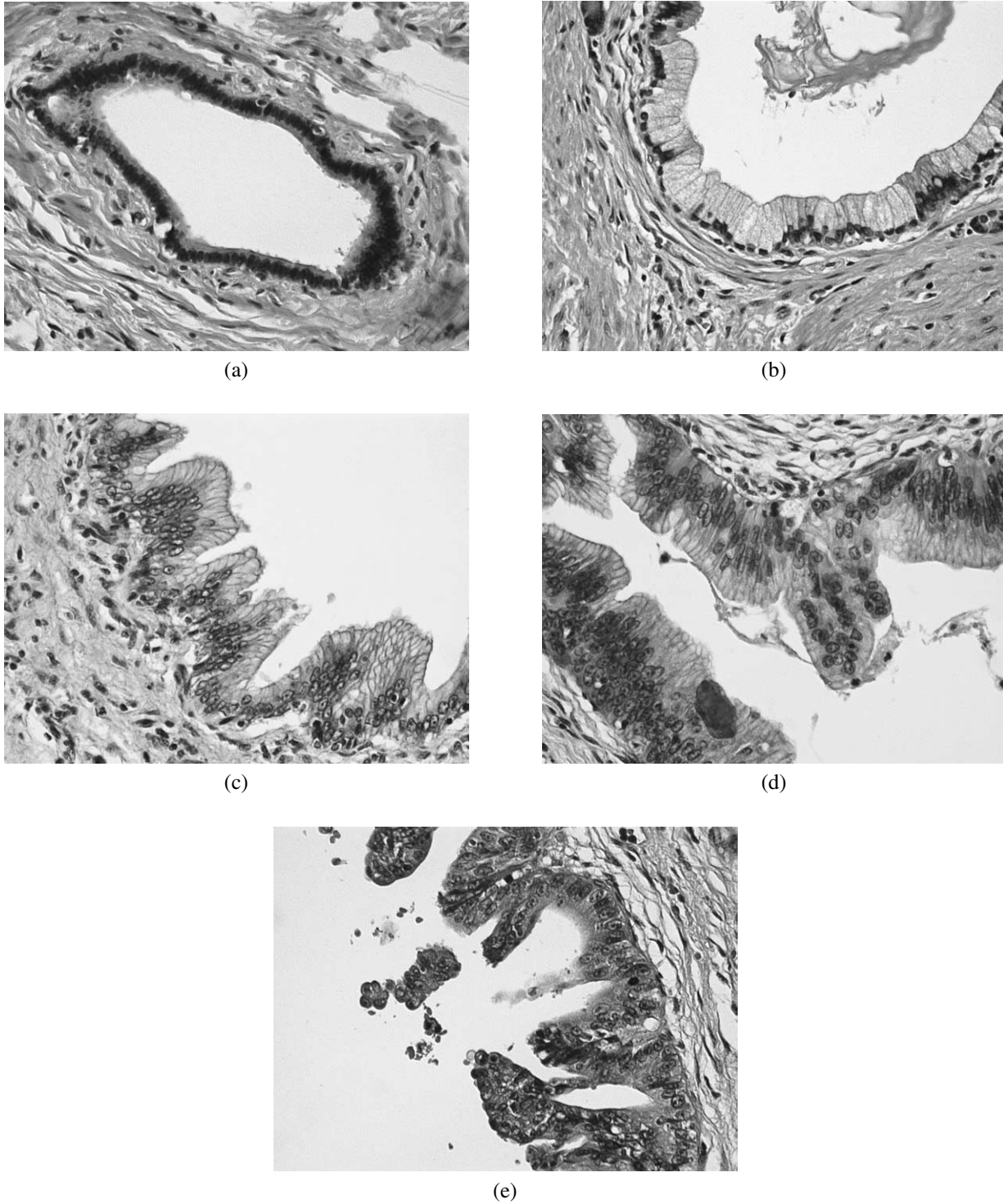


Fig. 1. Representative examples of pancreatic intraepithelial lesions (PanIN), H&E,  $\times 400$ : (a) normal ductal epithelium, (b) PanIN-1A, (c) PanIN-1B, (d) PanIN-2, (e) PanIN-3.

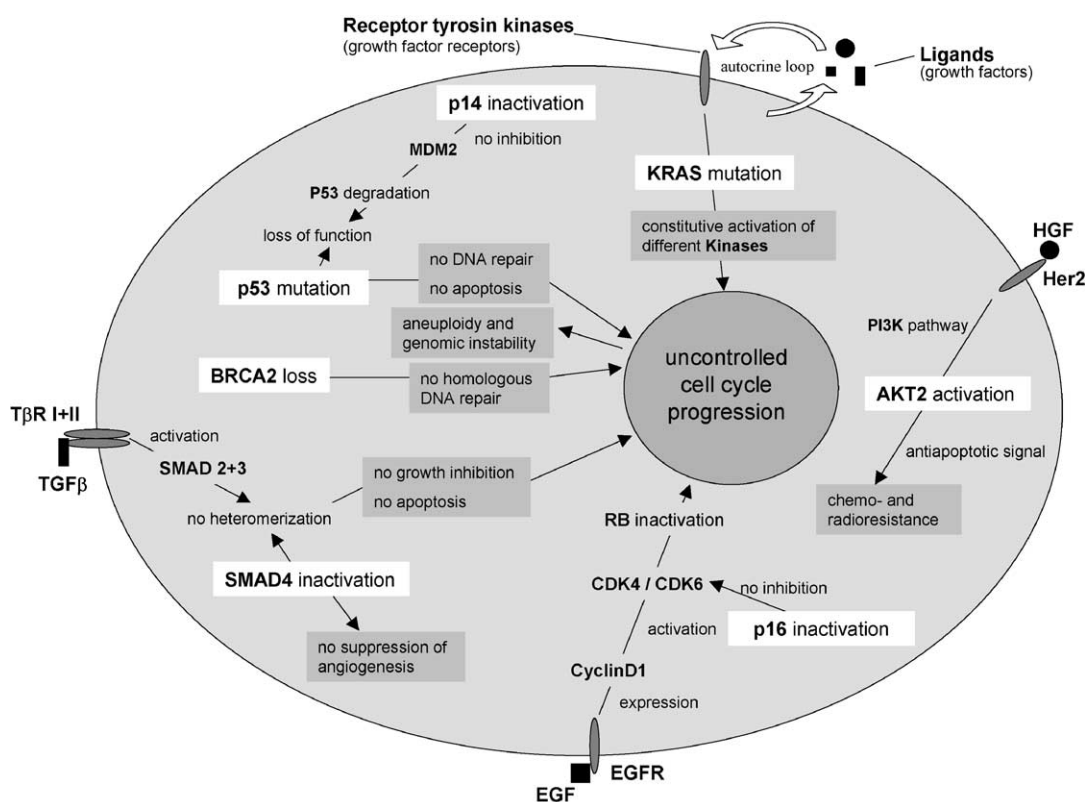


Fig. 2. Schematic diagram of major pathways in the development of pancreatic adenocarcinomas as discussed in the text. Key events (white boxes) and their consequences (grey boxes), which finally lead to the loss of cell cycle control.

### 3. Genetic alterations

The majority of pancreatic carcinomas show alterations of oncogenes such as *KRAS*, *EGFR* and *HER-2* as well as tumor suppressor genes such as *CDKN2A*, *P53*, *DPC4* and *BRCA2* (often referred to as signature mutations) [5]. According to the suggested progression model, mutations of *KRAS* and activation of *EGFR* and *HER-2* are supposed to be early changes that arise in low-grade PanINs with minimal atypia. In high-level PanINs, losses of *CDKN2A* can be detected, followed by inactivation of *P53*, *DPC4* and *BRCA2* [5,36].

#### 3.1. *KRAS*

The oncogene *KRAS* (kirsten rat sarcoma) is located on chromosome 12p12. It encodes a small GTP-binding protein, which is involved in signaling pathways between membrane located receptor tyrosine kinases like TGFR, VGFR, EGFR and HER2 and cytoplasmic kinases. As a consequence of genetic mutations the protein loses its GTPase function and therefore the capability of self-deactivation. This results

in continuous stimulation of different pathways with a number of cellular effects, like induction of DNA synthesis and cell proliferation (Fig. 2). Cells with *KRAS* mutations gain growth advantage through inflammatory stimuli and expression of transforming growth factor alpha ( $TGF\alpha$ ) and epithelial growth factor (EGF). If additional activation of EGFR (erbB) occurs, it will cause uncontrolled proliferation as a result of autocrine stimulation [5].

Almost all *KRAS* mutations of the pancreatic duct affect codon 12, whereas mutations in codon 13 and 61 are rare [78]. They can be identified in early neoplastic lesions of the pancreatic ducts but also at high frequencies in normal epithelium (19% to 38% [6,48]) and in patients with chronic pancreatitis (18% to 62.5% [49, 56]). The mutation rate increases with disease progression and almost all pancreatic cancers exhibit *KRAS* mutations [5].

#### 3.2. *EGFR* and *HER-2/neu*

EGFR (epidermal growth factor receptor) and Her-2/neu (heregulin-, neuregulin- or glial growth factor-

receptor) belong to the *ERBB*-family. All receptors of this gene family are transmembrane proteins and contain two cysteine-rich regions in the extra cellular domain and a tyrosine-kinase domain in the cytoplasmic part.

The *HER-2* oncogene is located on chromosome 17q21.2 and is amplified and over-expressed in many solid tumors [10,67,68]. In normal pancreatic tissue and chronic pancreatitis *HER-2* expression remains unaffected, whereas in patients with pancreatic cancer over-expression of *HER-2* can be found in early morphologic duct lesions [4,10]. Using immunohistochemistry, over-expression of *HER-2* ranges from 21% to 80%, and only 27% of the immunohistochemical positive cases showed amplification of *HER-2* as detected by FISH [4,60].

The *EGFR* gene is located on chromosome 7p12. Expression of *EGFR* can be detected in about 50% of pancreatic carcinomas [54,72,80]. *EGFR* expression seems to play an important role in metastasis (26% in patients without and in 64% of patients with metastasis), especially liver metastasis and was found more often in male (48%) than in female patients (17%) [72]. Analyses of the pancreatic cancer cell lines BxPC-3 and AsPC-1 exhibited evidence that Cyclin-D1 (*CCND1*) dependent cell cycle progression occurs through the induction of Cyclin-D1 by *EGFR*. *EGF* induces *CCND1* mRNA expression, followed by cell cycle progression to G1/S. Expression of *EGF*, *EGFR* or Cyclin-D1 could not be detected in normal pancreatic tissue, but at high frequencies in pancreatic carcinomas [54]. These findings indicate that the mitogenic effect of *EGF*-signaling through *EGFR* might be jointly responsible for Cyclin-D1 over-expression (Fig. 2). Amplified and unregulated expression of D-cyclins causes phosphorylation and inactivation of the RB protein by the cyclin-dependent-kinases 4 and 6 (*CDK4*, *CDK6*). This leads to the unimpeded progression from the G1 to the S phase.

### 3.3. *CDKN2A*

*CDKN2A* (cyclin-dependent kinase inhibitor 2A) is located on chromosome 9p21 and encodes for two tumor suppressor genes via distinct first exons and alternative reading frames in shared downstream exons [66]. The first product is p14<sup>ARF</sup> and the second is p16<sup>INK4a</sup>, often referred to as p16. P16 is an inhibitor of *CDK4* and *CDK6* and counteracts inactivation of RB. P14<sup>ARF</sup> inhibits MDM2, a protein that is responsible for the proteolytic deactivation of p53. Losses of

p14<sup>ARF</sup> and p16<sup>INK4a</sup> lead to the inactivation of the RB and p53 pathways [66] (Fig. 2). Even up to 30% of PanIN-1A and B lesions show inactivating mutations of p16<sup>INK4a</sup> [32,50,77]. Loss of both gene products of *CDKN2A* by mutation, deletion or promoter hypermethylation can be found in almost all sporadic pancreatic cancers [57,64]. Germline mutations in the first exon of *p16* are associated with the Familial Atypical Mole-Malignant Melanoma syndrome (FAMMM), a disease associated with a very high penetrance and early onset [5]. This mutation also predisposes to pancreatic cancer but the penetrance is very low and displays latency similar to the sporadic disease [24].

### 3.4. *p53*

The tumor suppressor gene *p53* is localized on chromosome 17p13. The “guardian of the genome” maintains genetic stability through its function in cell cycle control [46]. As a response to DNA damage p53 can arrest the cell cycle in order to either initiate DNA repair or, in case of serious damage, through the induction of apoptosis [41]. As a transcription factor, p53 controls the expression of growth inhibiting genes like *p21<sup>CIP</sup>*, *GADD45* or *IGFBP3* [44]. *P53* mutations can be found in 50% of all human tumors and in up to 81% of pancreatic adenocarcinomas [32, 57]. Loss of p53 function usually results from missense mutations of one and loss of heterozygosity (LOH) of the other allele [50]. As a consequence the adjacent cells accumulate further DNA damages leading to genomic instability and the development of cancer [30] (Fig. 2). The ability of DNA binding is the crucial requirement for the tumor suppressor function of p53, which is found to be lost in all human tumors [42]. Even mutation of one allele can completely disable p53 function since wild-type p53 is deactivated when complexed with mutated p53 [42]. Furthermore, the proteolytic degradation of mutated p53 is delayed, resulting in cellular accumulation. Mutated p53 was found in PanIN-3 with a frequency up to 41% [50], whereas LOH was already found in PanIN-1 lesions with frequencies between 13% and 18% [32,50].

### 3.5. *DPC4/SMAD4*

The loss of the long arm of chromosome 18 (18q) is one of the most frequent chromosomal alteration in the development of pancreatic cancer [29]. The gene *DPC4* (deleted in pancreatic cancer, locus 4) is located at chromosome 18q21 and also referred to

as *SMAD4* [29]. As a member of the *SMAD*-family of genes it is involved in transforming growth factor beta (TGF $\beta$ )-mediated growth and differentiation signal transduction. TGF $\beta$  is an inhibitor of cellular proliferation of most human cells. It induces the transcription of plasminogen-activator-inhibitor-1 (PAI-1) and inhibitors of cyclin dependent kinases such as p15<sup>INK4b</sup> and p21<sup>CIP</sup>. Therefore, disturbance of this signaling pathway results in uncontrolled growth regulation and carcinogenesis [9,33]. If TGF $\beta$  binds to the TGF $\beta$  type I and II receptors, the SMAD proteins 2 and 3 get phosphorylated, form heteromeric complexes with SMAD4 and translocate to the nucleus, where they function as transcription factors [33]. Additionally, SMAD4 has been shown to suppress angiogenesis [65] (Fig. 2). A loss of SMAD4/DPC4 can be found in up to 60% of pancreatic adenocarcinomas, in nearly 30% through homozygous deletion of both alleles [32, 50]. Nevertheless, complete loss of gene function determined by immunohistochemical expression analyses could not be detected in early PanIN lesions [79]. The *DPC4* locus is more commonly deleted in PanIN-3 lesions than in PanIN-2 lesions, whereas a significant difference could not be established when PanIN-3 lesions and invasive carcinoma were compared [32,50].

### 3.6. *AKT2*

Another gene with possible oncogenic function is *AKT2* (v-akt murine thymoma viral oncogene homologue 2). It maps to chromosome 19q13 and is a downstream effector of the phosphatidylinositol 3-kinase (PI3K), which is activated by EGF, PDGF and FGF. The latter are all known to be over-expressed in pancreatic cancer [13]. *AKT2* is amplified and over-expressed in 20% of pancreatic adenocarcinomas [58]. Activation due to phosphorylation was found in 59% of pancreatic adenocarcinomas and seems to be associated with *HER2* over-expression [62]. Constitutive activation of *AKT2* provides a potent antiapoptotic signal in cancer cells conferring chemo- and radioresistance [62] (Fig. 2). Additionally it can increase the expression of the insulin-like growth factor I receptor and can promote the invasiveness of human pancreatic cancer cells [71].

### 3.7. *BRCA2*

*BRCA2* (breast cancer 2) is located on chromosome 13q and encodes for a protein that regulates homologous-recombination-based DNA-repair processes. It

therefore maintains genomic stability, whereas *BRCA2*-deficient cells accumulate chromosomal aberrations [75] (Fig. 2). Patients with germline mutations have a familial predisposition for developing breast and ovarian cancer at younger ages [22]. Additionally, germline *BRCA2*-mutations increase the risk for the development of pancreatic cancers, although the age of onset and the clinical outcome are similar to sporadic pancreatic cancer [22]. *BRCA2* is inactivated in about 7% of sporadic pancreatic carcinomas, and loss of both alleles was already found in PanIN-3 lesions [23].

### 3.8. *LKB1/STK11*

*LKB1/STK11* (serine–threonine kinase 11) is known to play a role in the autosomal dominant inherited Peutz–Jeghers syndrome, which is associated with increased risk of cancer of many organ systems [19]. It is located on chromosome 19p13 and encodes for a cytoplasmic serine–threonine kinase, which may be a member of a yet unidentified signaling pathway. Germline mutations increase risk of developing pancreatic cancers [19]. Additionally *STK11* is deactivated in 7% of the sporadic pancreatic cancers and may play a role in tumor growth inhibition [59].

## 4. Epigenetic changes

Another cause of gene expression changes in the development of pancreatic cancer is DNA-methylation. About 50% of the human genes have 5' CpG islands, typically in the 5' regulatory region of the genes. Methylation of these regions usually causes gene silencing of tumor suppressor genes and demethylation can cause transcription of oncogenes. The methylation of the promoter regions of different genes (e.g., *p16*, *RB*, *VHL*, *hMLH1*, *hMSH2*) was also found in pancreatic carcinomas [73]. Beside *p16*, the gene preproenkephalin (*ppENK*), which has growth inhibiting function, was found methylated in 90% of the pancreatic cancers [74]. Methylation of genes with a significant role in carcinogenesis occur early in cancer development. The methylation of *p16* can be observed from 12% in PanIN-1A to 21.4% in PanIN-3 lesions, whereas an increase from 7.7% to 46.2% of *ppENK* methylation can be found in these lesions. The number of methylated loci increases with the size of the tumour and the age of the patients [15,74]. Non-neoplastic epithelium is not methylated [15].

## 5. Chromosomal alterations

Pancreatic adenocarcinomas are highly aneuploid and genetically heterogeneous. They exhibit a high degree of CIN (chromosomal instability) reflected by ANCA (average number of copy alterations [55]) values of up to 22, the highest known for solid tumors [17, 25, 27, 39, 55]. Patients with complex chromosomal abnormalities have a worse prognosis than those with only a few aberrations [39].

FISH (fluorescence *in situ* hybridization) based techniques like CGH (comparative genomic hybridization) or SKY (spectral karyotyping) established recurrent patterns of chromosomal alterations [17, 40, 63] (Fig. 3). Genetic losses usually involve chromosome arms and chromosomes 8p, 9p, 17p, 18q, 19p and 21, whereas gains can be mapped to 3q, 5p, 7p, 8q, 12p and 20q [17, 51, 69]. These chromosomal regions are affected in up to 90% of pancreatic adenocarcinomas and correlate very well with those regions harboring oncogenes and tumor suppressor genes such as *DPC4* at 18q, *p16* at 9p, *p53* at 17p and *Kras* at 12p.

Chromosomal instability occurs early in the development of pancreatic cancers [52]. Caused by inflammation and regenerative processes, the epithelial cells in PanIN lesions show a high degree of proliferation, leading to shortened telomeres [31]. This can lead to chromosomal breakage-fusion-bridge events which lead to chromosomal instability and intratumor heterogeneity [20, 21]. Another important point is that chromosomal instability is often accompanied by centrosome abnormalities and amplification [18, 61]. A dividing cell with anaphase chromosome bridges and centrosome anomalies could acquire a growth advantage due to partly disrupted chromosomes, if these rearrangements delete one or both alleles of a suppressor gene or would cause an extra copy of an oncogene. This could probably explain the high frequency and recurrence of chromosomal alterations despite intratumor heterogeneity.

## 6. Summary and future directions

The development of pancreatic adenocarcinomas is associated with inflammation and regeneration of the ductal epithelium, leading to a stepwise accumulation of genetic and histological alterations. But even though many of these mutations can already be found in precursor lesions, it has still not been possible to use these markers to detect pancreatic cancer at early stages or to

develop new therapeutical strategies [26]. As discussed above, the onset of chromosomal instability may be the critical point for cancer development. The design of FISH probes to uncover chromosomal aberrations often found in pancreatic adenocarcinomas may be useful for early detection. The association between telomere shortening and cancer development has also been shown. But even if this may improve diagnostics, for the therapeutical use one has to look more closer. Since the identification of recurrent patterns of chromosomal gains and losses indicates that numerous loci are involved in pancreatic carcinogenesis, high-throughput technologies like SAGE (serial analysis of gene expression) and gene expression microarrays [7, 14, 28, 38] will probably improve the classification of tumor types, unveil new prognostic markers and identify new genetic targets [7]. For example the expression profiling of cancer cell lines with different metastatic potential identifies those genes associated with the metastatic or invasive phenotype of pancreatic cancer cells and when done with cells treated or untreated with drugs or, e.g., growth factors, the affected pathways become visible [7]. Additionally, the investigation of the genetic interaction between neoplastic epithelial cells and stromal tissue and the associated desmoplastic reaction could be of relevance for a rational therapeutic approach. In the future, it may be possible to choose the appropriate regimens for the palliative and adjuvant treatment of pancreatic cancer patients based on the individual expression signatures of tissue samples [7]. The main problem of array technology is the generation of a large amount of expression data, which is difficult to handle and to analyze [7]. Many genes have been found to be over expressed in resected cancer specimens like cystatin S, MAT-8, S100A, mesothelin, hsp47, ADAM9 and many more [14, 28, 38]. However, the issue of selecting the appropriate genes as targets for pharmacological or diagnostic approaches, is still not solved [7]. Further investigations hopefully will contribute to a better understanding of pancreatic tumorigenesis. Especially new animal models that allow the development of premalignant lesions are believed to improve early detection methods [1, 35, 47]. Mouse models of pancreatic cancer now have reached the level of their counterparts in other organs [47]. The generation of progressive PanIN lesions and low-frequency progression to invasive and metastatic adenocarcinoma following activation of oncogenic KRAS in the mouse pancreas have been made [35, 47]. When tissue-specific ink4a/arf deficiency is combined with activation of oncogenic KRAS, accelerated PanIN for-

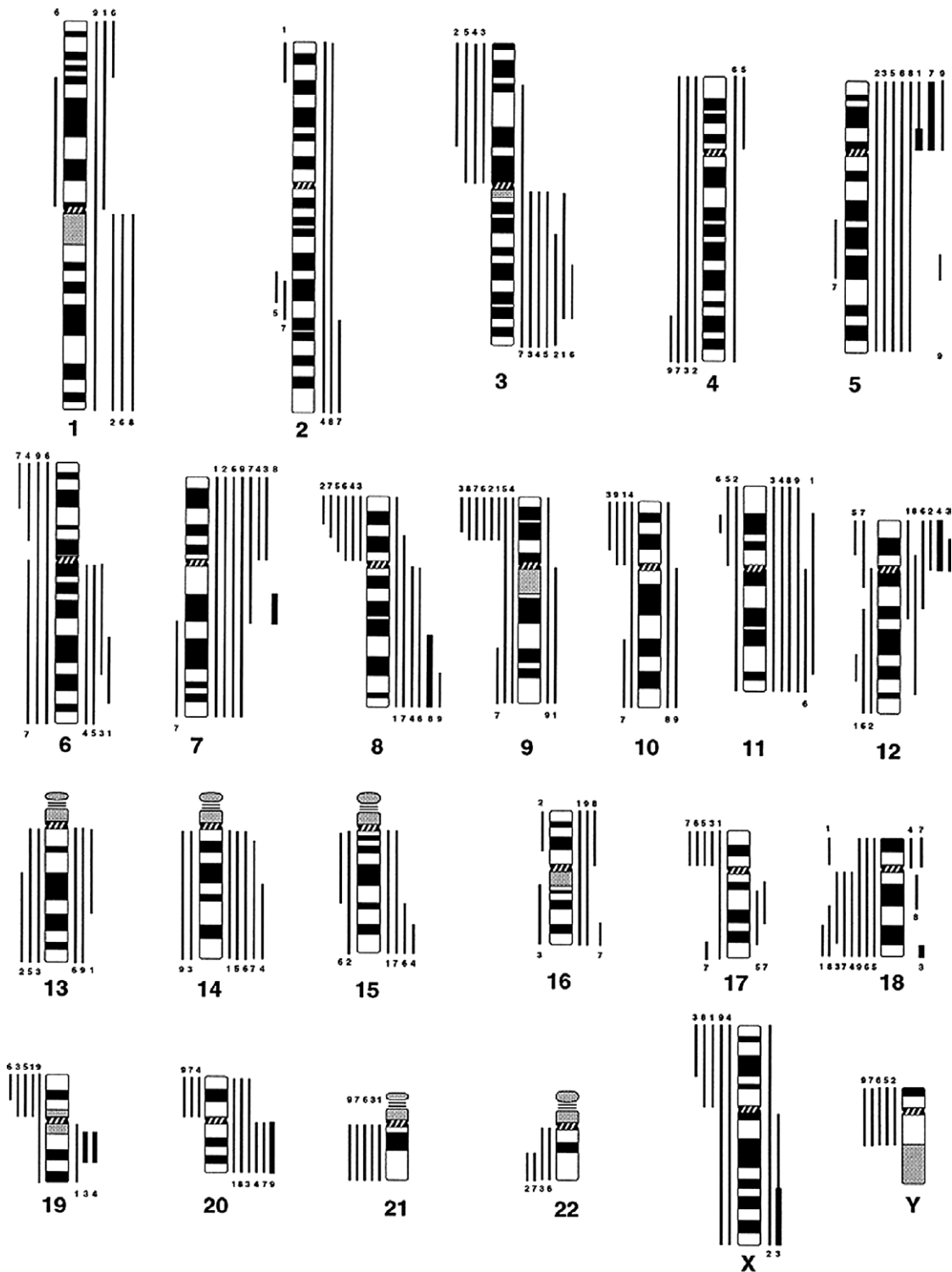


Fig. 3. Karyogram of chromosomal gains and losses in nine pancreatic carcinoma cell lines obtained by CGH. Vertical lines on the left side of each chromosome ideogram represent loss of genetic material, whereas those on the right side correspond to a gain. Amplification sites are represented as solid squares or bars. Each cell line can be identified by the case number on the top of each line: BxPC-3 (1), MIA-PaCa-2 (2), PANC-1 (3), SU.86.86 (4), CFPAC-1 (5), Capan-2 (6), Capan-1 (7), AsPC-1 (8), Hs766T (9) [17].

mation, rapid tumor progression and fatal metastasis occurs [1,47]. Additionally an amplification of the mutated KRAS allele and evolving expression of EGFR and Her2 were observed [1]. These findings in the mouse provide experimental support for the widely accepted model of human pancreatic cancer and may serve as a platform for the identification of early disease markers and for the efficient testing of novel therapeutic strategies [35,47]. For instance, the use of RNA interference (RNAi), mediated by small interfering RNA (siRNA), to silence genes overexpressed in pancreatic cancer has been reported as a new, non-toxic therapy. Carcinoembryonic antigen-related cell adhesion molecule 6 (CEACAM6) [11], KRAS [3] and ribonucleotide reductase M2 subunit (RRM2) [12] siRNAs were tested using mouse models and showed complete inhibition of metastasis and suppression of tumor growth [11], inhibition of tumor dissemination in the murine peritoneal cavity [3], attenuated cellular invasiveness and gemcitabine chemo-resistance [12]. These results give hope for the development of rational therapies in the human disease. Even if many discoveries were made in the last few years, still further investigations have to be made to improve our understanding of pancreatic cancer.

## References

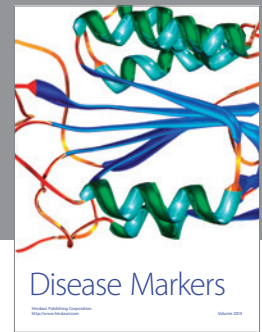
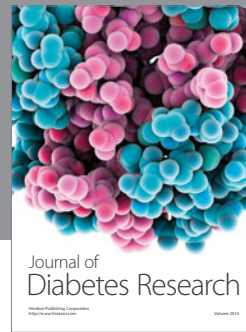
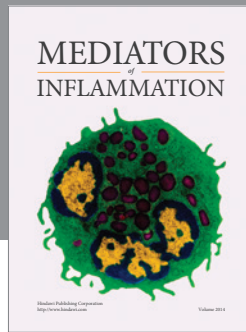
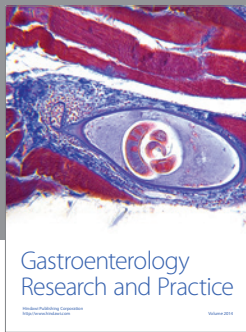
- [1] A.J. Aguirre, N. Bardeesy, M. Sinha, L. Lopez, D.A. Tuveson, J. Horner, M.S. Redston and R.A. DePinho, Activated Kras and Ink4a/Arf deficiency cooperate to produce metastatic pancreatic ductal adenocarcinoma, *Genes Dev.* **17**(24) (2003), 3112–3126.
- [2] S.A. Ahrendt and H.A. Pitt, Surgical management of pancreatic cancer, *Oncology* **16** (2002), 725–734.
- [3] K. Aoki, S. Ohnami and T. Yoshida, Suppression of pancreatic and colon cancer by antisense K-ras RNA expression vectors, *Methods Mol. Med.* **106** (2004), 192–204.
- [4] S.K. Apple, J.R. Hecht, D.N. Lewin, S.A. Jahromi, W.W. Grody and R.K. Nieberg, Immunohistochemical evaluation of K-ras, p53, and HER-2/neu expression in hyperplastic, dysplastic, and carcinomatous lesions of the pancreas: evidence for multistep carcinogenesis. *Hum. Pathol.* **30**(2) (1999), 123–129.
- [5] N. Bardeesy and R.A. DePinho, Pancreatic cancer biology and genetics, *Nat. Rev. Cancer* **2**(12) (2002), 897–909.
- [6] D.H. Berger, H. Chang, M. Wood, L. Huang, C.W. Heath, T. Lehman and B.A. Ruggeri, Mutational activation of K-ras in nonneoplastic exocrine pancreatic lesions in relation to cigarette smoking status, *Cancer* **85**(2) (1999), 326–332.
- [7] M. Buchholz, W. Boeck, H. Fensterer, F. Muller, C. Wenger, P. Michl, G. Adler and T.M. Gress, Use of DNA arrays/microarrays in pancreatic research, *Pancreatology* **1**(6) (2001), 581–586.
- [8] A.L. Cubilla and P.J. Fitzgerald, Morphological lesions associated with human primary invasive nonendocrine pancreas cancer, *Cancer Res.* **36**(7.2) (1976), 2690–2698.
- [9] J.L. Dai, K.K. Turnacioglu, M. Schutte, A.Y. Sugar and S.E. Kern, Dpc4 transcriptional activation and dysfunction in cancer cells, *Cancer Res.* **58**(20) (1998), 4592–4597.
- [10] J.D. Day, J.A. Digiuseppe, C. Yeo, M. Lai-Goldman, S.M. Anderson, S.N. Goodman, S.E. Kern and R.H. Hruban, Immunohistochemical evaluation of HER-2/neu expression in pancreatic adenocarcinoma and pancreatic intraepithelial neoplasms, *Hum. Pathol.* **27**(2) (1996), 119–124.
- [11] M.S. Duxbury, E. Matros, H. Ito, M.J. Zinner, S.W. Ashley and E.E. Whang, Systematic siRNA-mediated gene silencing: A new approach to targeted therapy of cancer, *Ann. Surg.* **240**(4) (2004), 667–676.
- [12] M.S. Duxbury, H. Ito, E. Benoit, M.J. Zinner, S.W. Ashley and E.E. Whang, Retrovirally mediated RNA interference targeting the M2 subunit of ribonucleotide reductase: A novel therapeutic strategy in pancreatic cancer, *Surgery* **136**(2) (2004), 261–269.
- [13] H. Friess, P. Berberat, M. Schilling, J. Kunz, M. Korc and M.W. Buchler, Pancreatic cancer: the potential clinical relevance of alterations in growth factors and their receptors, *J. Mol. Med.* **74**(1) (1996), 35–42.
- [14] H. Friess, J. Ding, J. Kleeff, L. Fenkell, J.A. Rosinski, A. Guweidhi, J.F. Reidhaar-Olson, M. Korc, J. Hammer and M.W. Buchler, Microarray-based identification of differentially expressed growth- and metastasis-associated genes in pancreatic cancer, *Cell. Mol. Life Sci.* **60**(6) (2003), 1180–1199.
- [15] N. Fukushima, N. Sato, T. Ueki, C. Rosty, K.M. Walter, R.E. Wilentz, C.J. Yeo, R.H. Hruban and M. Goggins, Aberrant methylation of preproenkephalin and p16 genes in pancreatic intraepithelial neoplasia and pancreatic ductal adenocarcinoma, *Am. J. Pathol.* **160**(5) (2002), 1573–1581.
- [16] T. Furukawa, R. Chiba, M. Kobari, S. Matsuno, H. Nagur and T. Takahashi, Varying grades of epithelial atypia in the pancreatic ducts of humans. Classification based on morphometry and multivariate analysis and correlated with positive reactions of carcinoembryonic antigen, *Arch. Pathol. Lab. Med.* **118**(3) (1994), 227–234.
- [17] B.M. Ghadimi, E. Schrock, R.L. Walker, D. Wangsa, A. Jauho, P.S. Meltzer and T. Ried, Specific chromosomal aberrations and amplification of the AIB1 nuclear receptor coactivator gene in pancreatic carcinomas, *Am. J. Pathol.* **154**(2) (1999), 525–536.
- [18] B.M. Ghadimi, D.L. Sackett, M.J. Difilippantonio, E. Schrock, T. Neumann, A. Jauho, G. Auer and T. Ried, Centrosome amplification and instability occurs exclusively in aneuploid, but not in diploid colorectal cancer cell lines, and correlates with numerical chromosomal aberrations, *Gen. Chrom. Canc.* **27**(2) (2000), 183–190.
- [19] F.M. Giardiello, J.D. Brensinger, A.C. Tersmette, S.N. Goodman, G.M. Petersen, S.V. Booker, M. Cruz-Correa and J.A. Offerhaus, Very high risk of cancer in familial Peutz–Jeghers syndrome, *Gastroenterology* **119**(6) (2000), 1447–1453.
- [20] D. Gisselsson, L. Pettersson, M. Hoglund, M. Heidenblad, L. Gorunova, J. Wiegant, F. Mertens, P. Dal Cin, F. Mitelman and N. Mandahl, Chromosomal breakage-fusion-bridge events cause genetic intratumor heterogeneity, *PNAS USA* **97**(10) (2000), 5357–5362.



- [21] D. Gisselsson, T. Jonson, A. Petersen, B. Strombeck, P. Dal Cin, M. Hoglund, F. Mitelman, F. Mertens and N. Mandahl, Telomere dysfunction triggers extensive DNA fragmentation and evolution of complex chromosome abnormalities in human malignant tumors, *PNAS USA* **98**(22) (2001), 12683–12688.
- [22] M. Goggins, M. Schutte, J. Lu, C.A. Moskaluk, C.L. Weinstein, G.M. Petersen, C.J. Yeo, C.E. Jackson, H.T. Lynch, R.H. Hruban and S.E. Kern, Germline BRCA2 gene mutations in patients with apparently sporadic pancreatic carcinomas, *Cancer Res.* **56**(23) (1996), 5360–5364.
- [23] M. Goggins, R.H. Hruban and S.E. Kern, BRCA2 is inactivated late in the development of pancreatic intraepithelial neoplasia: evidence and implications, *Am. J. Pathol.* **156**(5) (2000), 1767–1771.
- [24] A.M. Goldstein, J.P. Struewing, M.C. Fraser, M.W. Smith and M.A. Tucker, Prospective risk of cancer in CDKN2A germline mutation carriers, *J. Med. Genet.* **41** (2004), 421–424.
- [25] L. Gorunova, M. Hoglund, A. Andren-Sandberg, S. Dawiskiba, Y. Jin, F. Mitelman and B. Johansson, Cytogenetic analysis of pancreatic carcinomas: intratumor heterogeneity and nonrandom pattern of chromosome aberrations, *Gen. Chrom. Canc.* **23**(2) (1998), 81–99.
- [26] M. Grade, H. Becker and B.M. Ghadimi, The future impact of molecular oncology: A clinicians perspective, *Cellular Oncology*, in press.
- [27] C.A. Griffin, R.H. Hruban, L.A. Morsberger, T. Ellingham, P.P. Long, E.M. Jaffee, K.M. Hauda, S.K. Bohlander and C.J. Yeo, Consistent chromosome abnormalities in adenocarcinoma of the pancreas, *Cancer Res.* **55**(11) (1995), 2394–2399.
- [28] R. Grutzmann, M. Foerder, I. Alldinger, E. Staub, T. Brummendorf, S. Ropcke, X. Li, G. Kristiansen, R. Jesnowski, B. Sipos, M. Lohr, J. Luttges, D. Ockert, G. Kloppel, H.D. Saeger and C. Pilarsky, Gene expression profiles of microdissected pancreatic ductal adenocarcinoma, *Virchows Arch.* **443**(4) (2003), 508–517.
- [29] S.A. Hahn, M. Schutte, A.T. Hoque, C.A. Moskaluk, L.T. da Costa, E. Rozenblum, C.L. Weinstein, A. Fischer, C.J. Yeo, R.H. Hruban and S.E. Kern, DPC4, a candidate tumor suppressor gene at human chromosome 18q21.1, *Science* **271**(5247) (1996), 350–353.
- [30] P. Hainaut and M. Hollstein, p53 and human cancer: the first ten thousand mutations, *Adv. Cancer Res.* **77** (2000), 81–137.
- [31] N.T. van Heek, A.K. Meeker, S.E. Kern, C.J. Yeo, K.D. Lillemoe, J.L. Cameron, G.J. Offerhaus, J.L. Hicks, R.E. Wilentz, M.G. Goggins, A.M. De Marzo, R.H. Hruban and A. Maitra, Telomere shortening is nearly universal in pancreatic intraepithelial neoplasia, *Am. J. Pathol.* **161**(5) (2002), 1541–1547.
- [32] E. Heinmoller, W. Dietmaier, H. Zirngibl, P. Heinmoller, W. Scaringe, K.W. Jauch, F. Hofstadter and J. Ruschoff, Molecular analysis of microdissected tumors and preneoplastic intraductal lesions in pancreatic carcinoma, *Am. J. Pathol.* **157**(1) (2000), 83–92.
- [33] C.H. Heldin, K. Miyazono and P. ten Dijke, TGF-beta signalling from cell membrane to nucleus through SMAD proteins, *Nature* **390**(6659) (1997), 465–471.
- [34] W. Hilgers and S.E. Kern, Molecular genetic basis of pancreatic adenocarcinoma, *Gen. Chrom. Canc.* **26**(1) (1999), 1–12.
- [35] S.R. Hingorani, E.F. Petricoin, A. Maitra, V. Rajapakse, C. King, M.A. Jacobetz, S. Ross, T.P. Conrads, T.D. Veenstra, B.A. Hitt, Y. Kawaguchi, D. Johann, L.A. Liotta, H.C. Crawford, M.E. Putt, T. Jacks, C.V. Wright, R.H. Hruban, A.M. Lowy and D.A. Tuveson, Preinvasive and invasive ductal pancreatic cancer and its early detection in the mouse, *Cancer Cell* **4**(6) (2003), 437–450.
- [36] R.H. Hruban, M. Goggins, J. Parsons and S.E. Kern, Progression model for pancreatic cancer, *Clin. Canc. Res.* **6** (2000), 2969–2972.
- [37] R.H. Hruban, N.V. Adsay, J. Albores-Saavedra, C. Compton, E.S. Garrett, S.N. Goodman, S.E. Kern, D.S. Klimstra, G. Kloppel, D.S. Longnecker, J. Luttges and G.J. Offerhaus, Pancreatic intraepithelial neoplasia: a new nomenclature and classification system for pancreatic duct lesions, *Am. J. Surg. Pathol.* **25**(5) (2001), 579–86.
- [38] C.A. Iacobuzio-Donahue, A. Maitra, M. Olsen, A.W. Lowe, N.T. van Heek, C. Rosty, K. Walter, N. Sato, A. Parker, R. Ashfaq, E. Jaffee, B. Ryu, J. Jones, J.R. Eshleman, C.J. Yeo, J.L. Cameron, S.E. Kern, R.H. Hruban, P.O. Brown and M. Goggins, Exploration of global gene expression patterns in pancreatic adenocarcinoma using cDNA microarrays, *Am. J. Pathol.* **162**(4) (2003), 1151–1162.
- [39] B. Johansson, G. Bardi, N. Pandis, L. Gorunova, P.L. Backman, N. Mandahl, S. Dawiskiba, A. Andren-Sandberg, S. Heim and F. Mitelman, Karyotypic pattern of pancreatic adenocarcinomas correlates with survival and tumour grade, *Int. J. Cancer* **58**(1) (1994), 8–13.
- [40] A. Kallioniemi, O.P. Kallioniemi, D. Sudar, D. Rutovitz, J.W. Gray, F. Waldman and D. Pinkel, Comparative genomic hybridization for molecular cytogenetic analysis of solid tumors, *Science* **258** (1992), 818–821.
- [41] M.B. Kastan, C.E. Canman and C.J. Leonard, P53, cell cycle control and apoptosis: implications for cancer, *Cancer Metastasis Rev.* **14**(1) (1995), 3–15.
- [42] S.E. Kern, J.A. Pietenpol, S. Thiagalingam, A. Seymour, K.W. Kinzler and B. Vogelstein, Oncogenic forms of p53 inhibit p53-regulated gene expression, *Science* **256**(5058) (1992), 827–830.
- [43] G. Kloppel, G. Bommer, K. Ruckert and G. Seifert, Intraductal proliferation in the pancreas and its relationship to human and experimental carcinogenesis, *Virchows Arch. A Pathol. Anat. Histol.* **387**(2) (1980), 221–233.
- [44] L.J. Ko and C. Prives, p53: puzzle and paradigm, *Genes Dev.* **10**(9) (1996), 1054–1072.
- [45] S. Kozuka, R. Sassa, T. Taki, K. Masamoto, S. Nagasawa, S. Saga, K. Hasegawa and M. Takeuchi, Relation of pancreatic duct hyperplasia to carcinoma, *Cancer* **43**(4) (1979), 1418–1428.
- [46] D.P. Lane, p53, guardian of the genome, *Nature* **358** (1992), 15–16.
- [47] S.D. Leach, Mouse models of pancreatic cancer: The fur is finally flying!, *Cancer Cell* **5**(1) (2004), 7–11.
- [48] J. Luttges, A. Reinecke-Luthge, B. Mollmann, M.A. Menke, A. Clemens, M. Klimpfing, B. Sipos and G. Kloppel, Duct changes and K-ras mutations in the disease-free pancreas: analysis of type, age relation and spatial distribution, *Virchows Arch.* **435**(5) (1999), 461–468.

- [49] J. Luttges, A. Diederichs, M.A. Menke, I. Vogel, B. Kremer and G. Kloppel, Ductal lesions in patients with chronic pancreatitis show K-ras mutations in a frequency similar to that in the normal pancreas and lack nuclear immunoreactivity for p53, *Cancer* **88**(11) (2000), 2495–2504.
- [50] J. Luttges, H. Galehdari, V. Brocker, I. Schwarte-Waldhoff, D. Henne-Bruns, G. Kloppel, W. Schmiegel and S.A. Hahn, Allelic loss is often the first hit in the biallelic inactivation of the p53 and DPC4 genes during pancreatic carcinogenesis, *Am. J. Pathol.* **158**(5) (2001), 1677–1683.
- [51] E.H. Mahlamaki, M. Hoglund, L. Gorunova, R. Karhu, S. Dawiskiba, A. Andren-Sandberg, O.P. Kallioniemi and B. Johansson, Comparative genomic hybridization reveals frequent gains of 20q, 8q, 11q, 12p, and 17q, and losses of 18q, 9p, and 15q in pancreatic cancer, *Gen. Chrom. Canc.* **20**(4) (1997), 383–391.
- [52] A.H. Moskovitz, N.J. Linford, T.A. Brentnall, M.P. Bronner, B.E. Storer, J.D. Potter, R.H. Bell, Jr. and P.S. Rabinovitch, Chromosomal instability in pancreatic ductal cells from patients with chronic pancreatitis and pancreatic adenocarcinoma, *Gen. Chrom. Canc.* **37**(2) (2003), 201–206.
- [53] M.M. Murr, M.G. Sarr, A.J. Oishi and J.A. van Heerden, Pancreatic cancer, *CA. Cancer J. Clin.* **44**(5) (1994), 304–318.
- [54] B. Poch, F. Gansauge, A. Schwarz, T. Seufferlein, T. Schnelldorfer, M. Ramadani, H.G. Beger and S. Gansauge, Epidermal growth factor induces cyclin D1 in human pancreatic carcinoma: evidence for a cyclin D1-dependent cell cycle progression, *Pancreas* **23**(3) (2001), 280–287.
- [55] T. Ried, K. Heselmeyer-Haddad, H. Blegen, E. Schrock and G. Auer, Genomic changes defining the genesis, progression, and malignancy potential in solid human tumors: a phenotype/genotype correlation, *Gen. Chrom. Canc.* **25**(3) (1999), 195–204.
- [56] J.A. Rivera, C.J. Rall, F. Graeme-Cook, C. Fernandez-del Castillo, P. Shu, N. Lakey, R. Tepper, D.W. Rattner, A.L. Warsaw and A.K. Rustgi, Analysis of K-ras oncogene mutations in chronic pancreatitis with ductal hyperplasia, *Surgery* **121**(1) (1997), 42–49.
- [57] E. Rozenblum, M. Schutte, M Goggins, S.A. Hahn, S. Panzer, M. Zahurak, S.N. Goodman, T.A. Sohn, R.H. Hruban, C.J. Yeo and S.E. Kern, Tumor-suppressive pathways in pancreatic carcinoma, *Cancer Res.* **57**(9) (1997), 1731–1734.
- [58] 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, *Mol. Carcinog.* **21**(2) (1998), 81–86.
- [59] F. Sahin, A. Maitra, P. Argani, N. Sato, N. Maehara, E. Montgomery, M. Goggins, R.H. Hruban and G.H. Su, Loss of Stk11/Lkb1 expression in pancreatic and biliary neoplasms, *Mod. Pathol.* **16**(7) (2003), 686–691.
- [60] H. Safran, M. Steinhoff, S. Mangray, R. Rathore, T.C. King, L. Chai, K. Berzein, T. Moore, D. Iannitti, P. Reiss, T. Pasquariello, P. Akerman, D. Quirk, R. Mass, L. Goldstein and U. Tantravahi, Overexpression of the HER-2/neu oncogene in pancreatic adenocarcinoma, *Am. J. Clin. Oncol.* **24**(5) (2001), 496–499. Erratum in: *Am. J. Clin. Oncol.* **25**(2) (2002), 181.
- [61] N. Sato, K. Mizumoto, M. Nakamura, N. Maehara, Y.A. Minamishima, S. Nishio, E. Nagai and M. Tanaka, Correlation between centrosome abnormalities and chromosomal instability in human pancreatic cancer cells, *Cancer Genet. Cytogenet.* **126**(1) (2001), 13–19.
- [62] 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, *Brit. J. Cancer* **89** (2003), 2110–2115.
- [63] E. Schrock, S. du Manoir, T. Veldman, B. Schoell, J. Wienberg, M.A. Ferguson-Smith, Y. Ning, D.H. Ledbetter, I. Bar-Am, D. Soenksen, Y. Garini and T. Ried, Multicolor spectral karyotyping of human chromosomes, *Science* **273** (1996), 494–497.
- [64] M. Schutte, R.H. Hruban, J. Geradts, R. Maynard, W. Hilgers, S.K. Rabindran, C.A. Moskaluk, S.A. Hahn, I. Schwarte-Waldhoff, W. Schmiegel, S.B. Baylin, S.E. Kern and J.G. Herman, Abrogation of the Rb/p16 tumor-suppressive pathway in virtually all pancreatic carcinomas, *Cancer Res.* **57**(15) (1997), 3126–3130.
- [65] I. Schwarte-Waldhoff, O.V. Volpert, N.P. Bouck, B. Sipos, S.A. Hahn, S. Klein-Scory, J. Luttges, G. Kloppel, U. Graeven, C. Eilert-Micus, A. Hintelmann and W. Schmiegel, Smad4/DPC4-mediated tumor suppression through suppression of angiogenesis, *PNAS USA* **97**(17) (2000), 9624–9629.
- [66] C.J. Sherr, The INK4A/ARF network in tumor suppression, *Nat. Rev. Mol. Cell Biol.* **2** (2001), 731–737.
- [67] M. Sibilila, A. Fleischmann, A. Behrens, L. Stingl, J. Carroll, F.M. Watt, J. Schlessinger and E.F. Wagner, The EGF receptor provides an essential survival signal for SOS-dependent skin tumor development, *Cell* **102**(2) (2000), 211–220.
- [68] D.J. Slamon, G.M. Clark, S.G. Wong, W.J. Levin, A. Ullrich and W.L. McGuire, Human breast cancer: correlation of relapse and survival with amplification of the HER-2/neu oncogene, *Science* **235**(4785) (1987), 177–182.
- [69] S. Solinas-Toldo, C. Wallrapp, F. Muller-Pillasch, M. Bentz, T. Gress and P. Lichter, Mapping of chromosomal imbalances in pancreatic carcinoma by comparative genomic hybridization, *Cancer Res.* **56**(16) (1996), 3803–3807.
- [70] S.C. Sommers, S.A. Murphy and S. Warren, Pancreatic duct hyperplasia and cancer, *Gastroenterology* **27**(5) (1954), 629–640.
- [71] S. Tanno, S. Tanno, Y. Mitsuchi, D.A. Altomare, G.H. Xiao, J.R. Testa, AKT activation up-regulates insulin-like growth factor I receptor expression and promotes invasiveness of human pancreatic cancer cells, *Cancer Res.* **61**(2) (2001), 589–593.
- [72] K. Tobita, H. Kijima, S. Dowaki, H. Kashiwagi, Y. Ohtani, Y. Oida, H. Yamazaki, M. Nakamura, Y. Ueyama, M. Tanaka, S. Inokuchi and H. Makuuchi, Epidermal growth factor receptor expression in human pancreatic cancer: Significance for liver metastasis, *Int. J. Mol. Med.* **11**(3) (2003), 305–309.
- [73] T. Ueki, M. Toyota, T. Sohn, C.J. Yeo, J.P. Issa, R.H. Hruban and M. Goggins, Hypermethylation of multiple genes in pancreatic adenocarcinoma, *Cancer Res.* **60**(7) (2000), 1835–1839.
- [74] T. Ueki, M. Toyota, H. Skinner, K.M. Walter, C.J. Yeo, J.P. Issa, R.H. Hruban and M. Goggins, Identification and characterization of differentially methylated CpG islands in pancreatic carcinoma, *Cancer Res.* **61**(23) (2001), 8540–8546.
- [75] A.R. Venkataraman, Cancer susceptibility and the functions of BRCA1 and BRCA2, *Cell* **108**(2) (2002), 171–182.
- [76] WHO, incidence, mortality and survival databases: <http://www.who.int/cancer/recources/incidences/en>.

- [77] R.E. Wilentz, J. Geradts, R. Maynard, G.J. Offerhaus, M. Kang, M. Goggins, C.J. Yeo, S.E. Kern and R.H. Hruban, Inactivation of the p16 (INK4A) tumor-suppressor gene in pancreatic duct lesions: loss of intranuclear expression, *Cancer Res.* **58**(20) (1998), 4740–4744.
- [78] R.E. Wilentz, M. Goggins, M. Redston, V.A. Marcus, N.V. Adsay, T.A. Sohn, S.S. Kadkol, C.J. Yeo, M. Choti, M. Zahurak, K. Johnson, M. Tascilar, G.J. Offerhaus, R.H. Hruban and S.E. Kern, Genetic, immunohistochemical, and clinical features of medullary carcinoma of the pancreas: A newly described and characterized entity, *Am. J. Pathol.* **156**(5) (2000), 1641–1651.
- [79] R.E. Wilentz, C.A. Iacobuzio-Donahue, P. Argani, D.M. McCarthy, J.L. Parsons, C.J. Yeo, S.E. Kern and R.H. Hruban, Loss of expression of Dpc4 in pancreatic intraepithelial neoplasia: evidence that DPC4 inactivation occurs late in neoplastic progression, *Cancer Res.* **60**(7) (2000), 2002–2006.
- [80] L. Zhang and S.Z. Yuan, Expression of c-erbB-2 oncogene protein, epidermal growth factor receptor, and TGF-beta1 in human pancreatic ductal adenocarcinoma, *Hepatobil. Pancreat. Dis. Int.* **1**(4) (2002), 620–623.



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