

Review Article

Profiling of GEPNETs

Ola Nilsson

Sahlgrenska Cancer Centre and Department of Biomedicine, The Sahlgrenska Academy, University of Gothenburg, 405 30 Gothenburg, Sweden

Correspondence should be addressed to Ola Nilsson, ola.nilsson@lcr.med.gu.se

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The tumorigenesis of gastrointestinal and pancreatic tumors (GEPNETs) is poorly understood. We need a better understanding of the molecular alterations in GEPNETs to obtain an accurate classification, and it may also provide targets for therapeutic intervention. *Purpose of Paper.* The purpose of this paper was to critically examine recent advances in the molecular understanding of GEPNETs gained from genome-wide and transcriptome-wide profiling studies. Special emphasis was put on diagnostic, predictive, and therapeutic implications of profiling studies. *Results.* Pancreatic neuroendocrine tumours (PNETs) were characterised by a distinct pattern of chromosomal alterations and a higher degree of chromosomal instability (CIN) than ileal carcinoids. Subgroups of PNETs and ileal carcinoids were identified on the basis of specific chromosomal alterations. Exome sequencing identified mutations in *MEN1*, *ATRX/DAXX*, and mTOR pathway genes as being frequent events in sporadic PNETs. Expression profiles of PNETs and ileal carcinoids were found to be different, and allowed identification of subgroups of tumors, as well discrimination between benign and malignant tumors. The molecular data provided a number of candidate genes and pathways suitable for targeted therapy. For PNETs, candidate targets include BRAF, KRAS, TERT, EGFR, RET, MDM2, IGF, MET/HGF, ANG2, LCK, PDGFRB, AKT-mTOR, and SSTR2. Some of these targets have already been evaluated in clinical trials (mTOR and SSTR2). For ileal carcinoids, significantly fewer candidate targets were provided, including ERBB2 (HER2), RET, APLP1, and Notch. *Conclusion.* Profiling of GEPNETs is a powerful tool for discovery of novel targets for therapeutic intervention. Further studies, combining genome, epigenome, transcriptome, and proteome data are needed to enable us to identify clinically relevant targets in GEPNETs.

1. Introduction

Gastrointestinal and pancreatic neuroendocrine tumors (GEPNETs) are rare, and they account for 1-2% of all gastrointestinal malignancies. The incidence of pancreatic neuroendocrine tumors (PNETs) is 0.3 per 100,000 inhabitants per year and for ileal carcinoids the incidence is 0.7 per 100,000 inhabitants per year [1]. Mean age at diagnosis is 59 years for PNETs and 65 years for ileal carcinoids. The clinical presentation may be highly variable with some tumors following an indolent course and other tumors following an aggressive course with rapid tumor dissemination [2]. At diagnosis, there is distant disease in 64% of PNETs and in 30% of ileal carcinoids. The median survival for well-differentiated tumors is 42 months for PNETs and 88 months for ileal carcinoids [1]. Despite there being considerable differences in clinical presentation and biological behaviour, GEPNETs are classified according to a unified histopathological system instigated by the World Health Organisation

[3]. All GEPNETs are regarded as being potentially malignant and they are graded on the basis of mitotic count and Ki67 index into two categories: neuroendocrine tumor (NET) of grades 1-2 and neuroendocrine carcinoma (NEC) of grade 3. However, there is a need for molecular classification of GEPNETs, which could improve the diagnostic accuracy and prognostic value in addition to providing information regarding therapeutic options. The advances in genome technology have greatly improved our understanding of the genetic alterations underlying the development of tumours [4]. A number of high-throughput techniques are available for the analysis of molecular alterations in cancers including array-based comparative genomic hybridisation (aCGH), single nucleotide polymorphism (SNP) arrays [5, 6], next-generation sequencing [4], expression arrays [5, 7], and protein profiling [8]. These technologies have only recently been applied to GEPNETs. A search on PubMed performed in September 2011 revealed 24 publications describing studies

TABLE 1: Summary of genomic profiling studies on pancreatic neuroendocrine tumors (PNETs).

Study	Tumor type (number)	Profiling platform	Main findings	Therapeutic implications
Jonkers et al. [28] Cytogenet Genome Res	Sporadic insulinoma ($n = 30$) (15 benign, 8 uncertain behaviour, 7 malignant)	Array CGH (BAC array, 3.700 clones)	Chromosomal instability (CIN) (≥ 20 chromosomal alterations/tumor) and telomeric losses (≥ 6 losses/tumor) correlate with malignant progression Loss of 22q11.21–13.31 and gain of 7p21.1–11.2 correlate with malignant behaviour	Candidate genes for targeted therapy in regions of chromosomal gains include MAD2L1 , CDC4 , GCK , BRAF , KRAS , KNTC1 , CK19 , BRCA1 , and BCL2
Jonkers et al. [31] J Pathol	Sporadic insulinoma ($n = 27$) (15 benign, 6 uncertain behaviour 6 malignant)	Array CGH (BAC array, and 3.700 clones)	Gain of 9q32 and loss of 22q13.1 are early events in tumorigenesis. Loss of 11q24.1 and 22q13.31 is associated with advanced tumor stage	Candidate genes for targeted therapy in regions of chromosomal gains include ShcC
Nagano et al. [35] Endocr Relat Cancer	PNET ($n = 15$) (14 sporadic, 1 MEN1; 13 NF-PNET, 2 gastrinomas; 9 WDNC, 6 WDNT UMP)	SNP-array (Affymetrix, 50K)	Loss on chromosomes 1, 3, 11, 22. Gains on chromosomes 5, 7, 12, 14, 17, and 20.	Candidate genes for targeted therapy in regions of chromosomal gains include TERT , EGFR , IGFBP-3 , CDK6 , and RET
Hu et al. [39] Genes Cancer	PNET ($n = 55$)	Array CGH (Agilent, 44K)	Loss on chromosomes 1, 2, 7, 10, 11, 16, 17, 19, 20, and 22. Gain on chromosomes 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 13, 14, 17, and 18.	Targeting amplified genes, including MDM2 , MDM4 , and WIP1
Jiao et al. [41] Science	Sporadic PNET ($n = 68$)	NGS (exome) (Illumina)	Mutations in MEN1 (44%), DAXX/ ATRX (43%), PTEN (7%), TSC2 (9%), and PIK3CA (1%). Tumors with mutations in MEN1 and/or DAXX/ATRX are associated with prolonged survival	Targeting mTOR pathway

For genes and proteins in bold, therapeutic agents are available for preclinical or clinical testing. BAC: bacterial artificial chromosome; CGH: comparative genomic hybridisation; F-PNET: functioning PNET; NF-PNET: non-functioning PNET; NGS: next-generation sequencing; PNET: pancreatic neuroendocrine tumor; SNP: single-nucleotide polymorphism; WDNC: well-differentiated neuroendocrine carcinoma; WDNT UMP: well-differentiated neuroendocrine tumor of uncertain malignant potential.

on PNETs or ileal carcinoids that used high-throughput technologies. Nine of these studies used aCGH/SNP arrays, 14 studies used expression arrays, and one study used next-generation sequencing. There were no studies on protein profiling. A summary of the studies on GEPNETs, together with a critical evaluation is given below.

2. Genomic Profiling of PNETs

PNETs may be part of hereditary cancer syndromes, for example, multiple endocrine neoplasia type 1 (MEN1), von Hippel-Lindau disease (VHL), neurofibromatosis type 1 (NF1), or tuberous sclerosis (TSC) [9–12]. The tumor suppressor genes responsible for these cancer syndromes have been cloned and characterised [13–15]. However, a majority of PNETs (90%) occur as sporadic tumors without family history. Sporadic tumors may harbour mutations in the *MEN1* gene (15–30%), while mutations in the *VHL*, *NF1*, or *TSC* genes are uncommon [16–20]. Genetic characterisation of sporadic PNETs using LOH and conventional CGH has

demonstrated recurrent chromosomal gains and losses in sporadic PNETs [21–27]. However, introduction of array-based CGH and SNP arrays have greatly enhanced the accuracy by which chromosomal alterations can be determined in tumors. A summary of recent profiling studies on PNETs is given in Table 1.

Jonkers et al., 2006 [28] analysed 30 sporadic insulinomas with array CGH and found that chromosomal instability (CIN), defined as ≥ 20 chromosomal alterations per tumor, and telomeric loss, defined as ≥ 6 losses per tumor, were correlated with tumor progression and metastasis. Loss of 22q11.21–13.31 and gain of 7p21.1–11.2 were the best markers of malignant behaviour. A number of potential targets were identified including *BRAF* and *KRAS*, for which therapeutic agents are available [29, 30]. In a subsequent study, Jonkers et al. [31] analysed 27 sporadic insulinomas by array CGH and identified gain of 9q32 and loss of 22q13.1 as early events in tumor formation, while loss of 11q24.1 and 22q13.31 was a late event during tumor progression. *ShcC* was identified as a candidate gene in regions of chromosomal

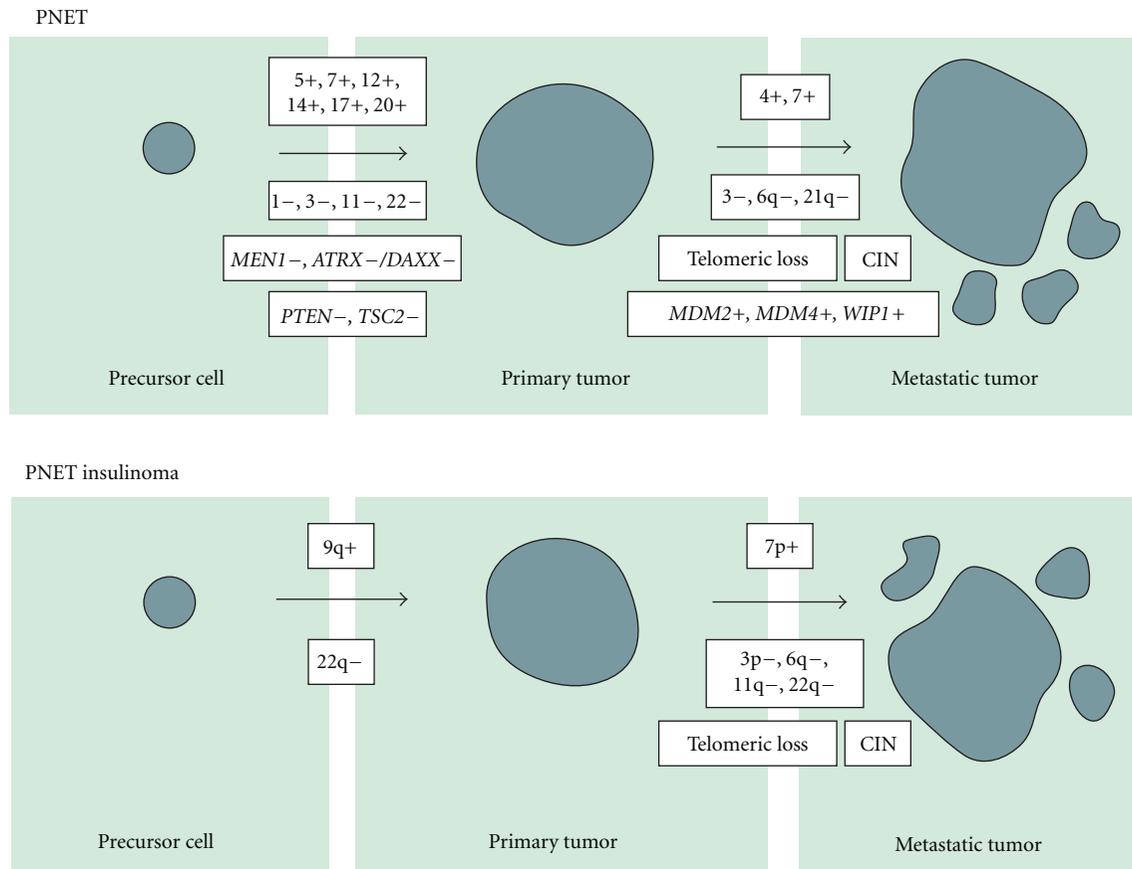


FIGURE 1: Proposed genomic progression model for pancreatic neuroendocrine tumors (PNETs). In sporadic PNETs, losses on chromosomes 1, 3, 11, and 22 and gains on chromosomes 5, 7, 12, 14, 17, and 20 occur early in tumor development. Inactivating mutations in *MEN1* and *ATRX/DAXX* are found in about half of the tumors. A smaller group of tumors harbor inactivating mutations in *PTEN* and *TSC2*, which alter mTOR signaling. With tumor progression, there is an increase in chromosome instability (CIN) accompanied by telomeric losses. Losses on chromosomes 3, 6q, and 21q, and gains on chromosomes 4 and 7 occur late in tumor development and are associated with metastatic behaviour. Syndromic insulin-producing PNETs (insulinomas) are characterised by a different set of genomic alterations. Loss of chromosome 22q and gain of chromosome 9q are early events in tumor development. During tumor progression, there is an increase in chromosome instability (CIN), accompanied by telomeric losses and an increase in *MDM2*, *MDM4*, and *WIP1* expression, attenuating p53 function. Losses on chromosomes 3p, 6q, 11q, and 22q and gains on chromosomes 7p are associated with metastatic behaviour. The model is based on data from references [21–23, 25, 26, 28, 31, 35, 39, 41].

gains (9q22.2–q33.2). ShcC is a substrate for RET suggesting that inhibition of RET signaling is a potential therapeutic principle [32–34].

Nagano et al., 2007 [35] analysed 15 PNETs by SNP-array and reported frequent losses on chromosomes 1, 3, 11, and 22 and gains on chromosomes 5, 7, 12, 14, 17, and 20. A number of putative tumor suppressor genes were located in regions of loss, including *RASSF1A* and *PTEN*. Putative therapeutic targets in regions of gain included *TERT*, *EGFR*, and *RET*, for which inhibitors are available [36–38]. Hu et al. 2010 [39] performed array CGH analysis of 55 PNETs, confirming losses on chromosomes 1, 2, 7, 10, 11, 16, 17, 19, 20, and 22 and gains on chromosomes 1–10, 12–14, 17, and 18. Amplified genes included *MDM2*, *MDM4*, and *WIP1* suggesting attenuation of p53 function in tumors. *MDM2* was suggested as a potential therapeutic target [40].

Jiao et al., 2011 [41] performed whole-exome sequencing of sporadic PNETs and identified mutations in chromatin

remodeling genes, *MEN1* and *DAXX/ATRX*, in 44% and 43% of tumors. Tumors with *DAXX/ATRX* mutations had altered telomeres, which are maintained by a telomerase-independent mechanism (ALT) [42]. PNETs with mutated *MEN1* or *DAXX/ATRX* were associated with prolonged survival compared to tumors without mutations. A subset of PNETs harboured mutations in *PTEN*, *TSC2*, and *PIK3CA*, which are part of the AKT-mTOR signaling pathway. Inhibition of mTOR was suggested as a possible treatment, and clinical trials using mTOR inhibitors for advanced PNETs has shown promising results [43, 44]. The relationship between mutational status in PNETs and response to mTOR inhibition remains to be evaluated.

In general, genomic profiling studies have provided consistent data, which allows construction of genomic progression models for PNETs (Figure 1). However, a detailed analysis of genomic alterations in PNET subgroups still remains to be done. Promising therapeutic targets have been

TABLE 2: Summary of expression profiling studies on pancreatic neuroendocrine tumors (PNETs).

Study	Tumor type (number)	Profiling platform	Main findings	Therapeutic implications
Maitra et al. [45] Clin Cancer Res	PNET ($n = 8$) (all NF-PNET, well-differentiated 6 benign, 2 malignant)	Expression array (Affymetrix U133)	Overexpression of 66 transcripts (≥ 3 -fold versus normal islets) Underexpression of 119 transcripts (≤ 3 -fold versus normal islets) Verified proteins include IGFBP3, Fibronectin, p21, and CD99	Candidate pathways for targeted therapy include IGF signaling
Hansel et al. [46] Clin Cancer Res	PNET ($n = 12$) (all well-differentiated, F-PNET, and NF-PNET-5 benign, 7 malignant)	Expression array (Affymetrix U133)	Overexpression of 65 genes (≥ 3 -fold malignant versus benign) Underexpression of 57 genes (≤ 3 -fold malignant versus benign) Verified proteins include MET and IGFBP3, which are overexpressed in metastatic tumors	Candidates for targeted therapy include IGF signaling and MET tyrosine kinase
Bloomston et al. [47] Annals Surg Oncol	PNET ($n = 9$) (pooled samples)	Expression array (Affymetrix U133)	1340 differentially expressed genes in PNETs versus normal pancreas Overexpressed genes verified by PCR include <i>ANG2</i> , <i>NPDC1</i> , <i>ELOVLA4</i> , and <i>CALCR</i>	Candidates for targeted therapy include ANG2
Dilley et al. [48] Mol Cancer	PNET ($n = 8$) (all MEN1 tumors; 4 F-PNET, 4 NF-PNET; 4 benign, 4 malignant)	Expression array (Affymetrix U95Av2)	Overexpression of 45 genes (PNET versus normal pancreas), underexpression of 148 genes (PNET versus normal pancreas) Apoptosis-related genes frequently downregulated. Verified genes include <i>FGF9</i> , <i>IER3</i> , <i>PHLDA2</i> , <i>IAPP</i> , <i>SST</i>	Candidates for targeted therapy include HGF
Couvelard et al. [52] Gastroenterology	PNET ($n = 24$) (all well-differentiated; 18 sporadic, 5 VHL, 1 MEN1; 20 NF-PNET, 4 F-PNET 12 benign, 12 malignant)	Expression array (Sanger Center)	Overexpression of 72 genes and underexpression of 51 genes (malignant versus benign tumors). Verified proteins include CD34 E-selectin, MKK4, and MDR1	Candidates for targeted therapy include IGF1 , MAP2K4 (MKK4), and DDR1
Capurso et al. [49] Endocr Relat Cancer	PNET ($n = 13$) (all NF-PNET; 10 WDEC, 3PDEC)	Expression array (Affymetrix 133)	Overexpression of 667 genes and underexpression of 323 genes (PNETs versus pancreatic islets) Verified genes include <i>BINI</i> , <i>SERPINA10</i> , <i>LCK</i> , <i>BST2</i> Highly similar expression profile of primaries and metastases.	Candidates for targeted therapy include LCK (Src family of PTKs) and BST2
Roldo et al. [63] J Clin Oncol	PNET ($n = 40$) (all sporadic, well-differentiated; 28 NF-PNET, 12 F-PNET; 22 benign, 18 malignant)	miRNA array (Ohio State University)	Distinct pattern of miRNA distinguish PNETs from normal pancreas. 87 miRNA upregulated and 8 miRNA downregulated in PNET versus normal pancreas Expression of miR-103 and miR-107, and lack of miR-155 distinguish PNET from normal pancreas. miR-204 is primarily expressed in insulinoma. miR-21 is associated with high proliferation and liver metastases.	—

TABLE 2: Continued.

Study	Tumor type (number)	Profiling platform	Main findings	Therapeutic implications
Lowe et al. [50] PLoS ONE	PNET ($n = 6$) (4 NF-PNET, 2 insulinoma)	Expression array (Stanford University)	Overexpression of 311 genes in PNET (PNET versus normal pancreas) including neuroendocrine markers, <i>CHGA</i> , <i>CHGB</i> , <i>SYP</i> , <i>PAM</i> , <i>DDC</i> , and <i>SSTR1</i> . High Expression of <i>AXL</i> , <i>MAFB</i> and <i>AXIN2</i>	Candidates for targeted therapy include <i>AXIN2</i> and Wnt signaling
de Sá et al. [51] Clin Cancer Res	PNET ($n = 10$) (6 benign insulinomas 4 malignant insulinomas)	Expression array (CodeLink)	Overexpression of 110 genes and underexpression of 120 genes (malignant versus benign tumors). Validated genes include <i>PRSS2</i> , <i>CTRB1</i> , <i>SERPINA1</i> , <i>AGT</i> , and <i>CFB</i> . <i>SERPINA1</i> (alpha-1-antitrypsin) is a marker of malignancy in insulinoma	—
Duerr et al. [60] Endocr Relat Cancer	PNET ($n = 24$) (5 benign, 11 low-grade malignant, 8 malignant; 5 NF-PNET, 12 insulinoma, 3 gastrinoma, 2 other)	Expression array (Affymetrix U133)	PNET forms “benign” and “malignant” clusters differing in “transcription regulation” and “binding”. Validated genes upregulated in malignant cluster include <i>FEV</i> , <i>ADCY2</i> , <i>NR4A2</i> , and <i>GADD45β</i> . Expression pattern of PNET is different from GI-NETs. <i>PDGFR-β</i> is frequently activated (phosphorylated) in PNET.	Candidates for targeted therapy include <i>PDGFR-β</i>
Missiaglia et al. [61] J Clin Oncol	PNET ($n = 72$) (39 WDET, 30 WDEC, 3 PDEC; 16 F-PNET, 56 NF-PNET)	Expression array (Ohio State University)	Unsupervised hierarchical clustering separates NF-PNET from insulinoma. <i>TSC2</i> and <i>PTEN</i> are downregulated in PNET and associated with short survival. <i>SSTR2</i> is upregulated in NF-PNET. <i>FGF13</i> expression in PNET correlates with metastatic potential.	Candidates for targeted therapy include <i>AKT-mTOR</i> pathway and <i>SSTR2</i>

For genes and proteins in bold, therapeutic agents are available for preclinical or clinical testing. F-PNET: functioning PNET; GI-NET: gastrointestinal neuroendocrine tumor; MEN1: multiple endocrine neoplasia type 1; NF-PNET: non-functioning PNET; PDEC: poorly differentiated endocrine carcinoma; PNET: pancreatic neuroendocrine tumor; WDET: well-differentiated endocrine tumor; WDEC: well differentiated endocrine carcinoma.

generated, which should be subjected to preclinical or clinical testing.

3. Expression Profiling of PNETs

There have been a number of studies describing expression profiles in PNETs. These studies are summarised in Table 2. Maitra et al. [45], Hansel et al. [46], Bloomston et al. [47], Dilley et al. [48], Capurso et al. [49], Lowe et al. [50] and de Sá et al. [51] have analysed small numbers of PNETs and compared them with normal islets or normal pancreas. Significantly regulated genes were reported, as well as candidate genes for targeted therapy. The IGF-signalling pathway was suggested as a target by Maitra et al. [45], Hansel et al. [46], and Couvelard et al. [52], and HGF/MET signaling was suggested for targeting by Hansel et al. [46] and Dilley et al. [48]. For both of these signalling pathways, inhibitors are

available for preclinical or clinical testing [53–57]. Targeting ANG2 was suggested by Bloomston et al. [47], and inhibitors are available for testing [58]. Capurso et al. [49] suggested LCK (src family member) as target, and src inhibitors are also available for testing [59].

Couvelard et al. [52] analysed a large series ($n = 24$) of benign and malignant PNETs and identified a number of differentially expressed genes, of which *CD34*, *E-selectin*, *MKK4*, and *MDR1* were verified. IGF1 was suggested as a promising target for therapy. Duerr et al. [60] studied a series ($n = 24$) of PNETs and could differentiate the expression profile of benign tumors from those of malignant tumors by hierarchical clustering. Malignant tumors showed high expression of *FEV*, *ADCY2*, *NR4A2*, and *GADD45 β* . *PDGFR β* was shown to be activated by phosphorylation in a high proportion of PNETs, suggesting *PDGFR β* as a target for therapy. Missiaglia et al. [61] have performed expression

TABLE 3: Summary of genomic profiling studies on ileal carcinoids.

Study	Tumor type (number)	Profiling platform	Main findings	Therapeutic implications
Do et al. [68] Genes Chromosomes Cancer	Ileal carcinoids ($n = 15$) (15 WDNC; sporadic)	SNP array (Affymetrix, 50K)	Chromosomal aberrations are less frequent in ileal carcinoids compared to PNET. Loss on chromosomes 9, 16, and 18. Gain on chromosomes 4, 14, 17, and 20.	Candidate genes for targeted therapy in regions of chromosomal gains include <i>ERBB2</i> (<i>HER2</i>)
Kulke et al. [69] Genes Chromosomes Cancer	Ileal carcinoids ($n = 24$) (sporadic)	SNP array (Affymetrix, 50K)	Most frequently occurring chromosomal aberrations comprise large chromosomal regions or whole chromosomes. Loss on chromosomes 9, 16, and 18. Gain on chromosomes 4, 5, 7, and 14. Hierarchical clustering revealed two groups of tumors, a smaller group with clustered gains on chromosomes 4, 5, 7 and 14 and a larger group without clustered gains.	Candidate genes for targeted therapy in regions of chromosomal gains include <i>DAD1</i> , <i>OR4A5</i> , and <i>PRKCA</i>
Andersson et al. [70] Endocr Relat Cancer	Ileal carcinoids ($n = 52$) (sporadic)	Array CGH (Agilent, 44K)	Loss on chromosomes 9, 11, 13, 16 and 18. Loss of chromosome 18 is the most frequent aberration and an early event in tumorigenesis. Gain on chromosomes 4, 5, 14, and 20. Gain of chromosome 14 is associated with short survival. Ileal carcinoids are separated in two groups: tumors with loss on chromosome 18, and tumors with intact chromosome 18 but gain of chromosome 14.	—
Cunningham et al. [71] Genes Chromosomes Cancer	Ileal carcinoids ($n = 61$) (9 familial, 52 sporadic)	Array CGH (BAC array, 32K; SNP, Illumina)	Familial and sporadic tumors have similar chromosomal aberrations. Loss of chromosome 18 is the most frequent alteration occurring early in tumorigenesis. Minimal regions of deletions are 18q21.1-q21.31, 18q22.1-q22.2, and 18q22.3-q23. Gain on chromosome 7 occurred only in metastasis and correlated solid growth pattern.	—

For genes and proteins in bold, therapeutic agents are available for preclinical or clinical testing. BAC: bacterial artificial chromosome; CGH: comparative genomic hybridisation; PNET: pancreatic neuroendocrine tumor; SNP: single-nucleotide polymorphism; WDNC: well-differentiated neuroendocrine carcinoma.

profiling on the largest series of PNETs ($n = 72$) and were able to separate insulinomas from nonfunctioning tumors by hierarchical clustering. *PTEN* and *TSC2* was shown to be downregulated in PNETs and found to be correlated with short survival. *PTEN* and *TSC2* are part of the AKT-mTOR signaling pathway and inhibition of mTOR was suggested for therapy. These data are highly concordant with the findings in the whole exome sequencing study by Jiao et al. [41]. *SSTR2* is also highly expressed in non-functioning PNETs and has confirmed the suitability of somatostatin-receptor-mediated therapy for PNETs [62]. Roldo et al. [63] have examined the expression of miRNAs in a large series of PNETs ($n = 40$) and reported miR-204 to be a marker for insulinomas, and miR-21 to be a marker of metastatic behaviour. No therapeutic targets were identified.

In general, expression profiling studies on PNETs have provided less consistent data than genomic studies. This could be due to the low number of tumors studied or to inherent differences in the profiling techniques. Larger

groups of well-characterised tumours should be studied using optimally designed studies, or alternatively by pooling of different studies in a meta-analysis.

4. Genomic Profiling of Ileal Carcinoids

Ileal carcinoids present as sporadic tumors, and familial cases are very rare. Genetic analysis of sporadic ileal carcinoids using LOH and conventional CGH has shown recurrent chromosomal aberrations, with loss of chromosome 18 as a frequent and early event in tumorigenesis [64–67]. High-resolution CGH and SNP arrays have been used to further analyse chromosomal aberrations in ileal carcinoids. A summary of these recent profiling studies is given in Table 3.

Kim et al. [68] analysed 15 ileal carcinoids by SNP arrays and found loss on chromosomes 9, 16, and 18 and gain on chromosomes 4, 14, 17, and 20. *ERBB2* (*HER2*) was suggested as a candidate gene for targeted therapy. Kulke et al. 2008 [69] analysed 24 ileal carcinoids by SNP-array and

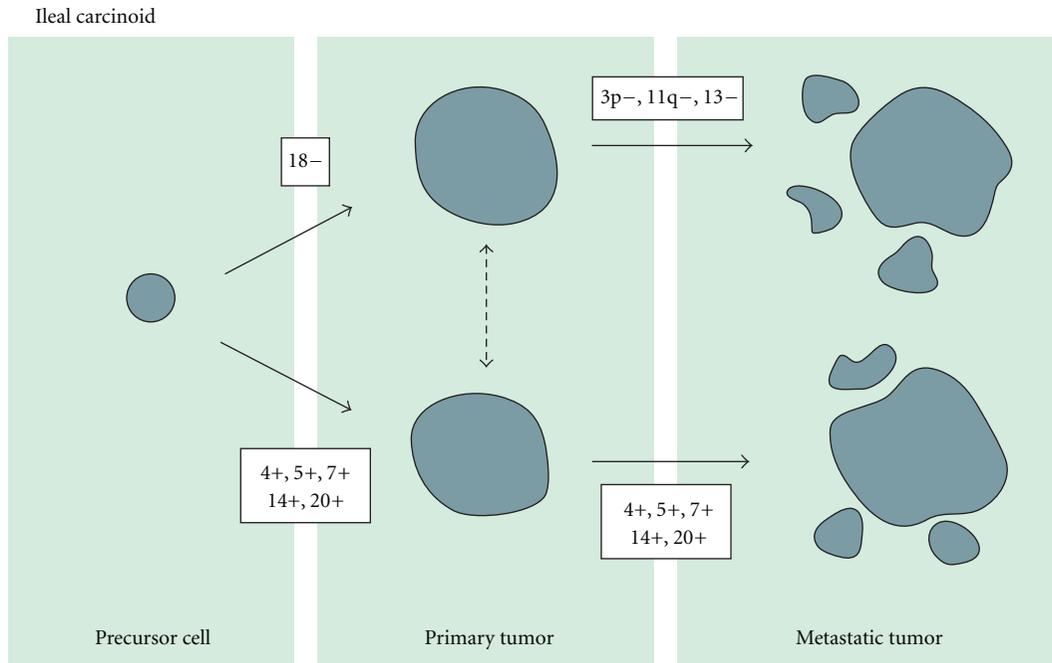


FIGURE 2: Proposed genomic progression model for ileal carcinoids. Two distinct groups of sporadic ileal carcinoids can be identified: a larger group of tumors (65–75%) which is characterised by loss of chromosome 18. Loss of chromosome 18 is an early and pathogenetic event in these tumors. During tumor progression, additional aberrations accumulate, in particular losses on chromosomes 3p, 11q, and 13. A smaller group of tumors (25–35%) is characterised by clustered gains on chromosomes 4, 5, 7, 14, and 20. These tumors frequently have an intact chromosome 18. Gain of chromosome 7 is associated with metastases and solid growth pattern, while gain of chromosome 14 has been associated with shorter patient survival. The model is based on data from references [69–71].

confirmed loss on chromosomes 9, 16, and 18 and gain on chromosomes 4, 5, 7, and 14. Hierarchical clustering showed two separate groups of tumors, a smaller group characterised by 2 or more clustered gains on chromosomes 4, 5, 7, and 14 and a larger group without clustered gains. Candidate genes for targeted therapy were from amplified regions on chromosome 14q (*DAD1*), 11p (*OR4A5*), and 17q (*PRKCA*). Andersson et al. [70] analysed 52 ileal carcinoids by array CGH, which showed loss of chromosome 18 as a common and early event in tumor formation. Tumors could be differentiated into two groups, a larger group with loss of chromosome 18 and a smaller group with an intact chromosome 18 but with gain of chromosome 14. Gain of chromosome 14 was also shown to be a strong predictor of short patient survival. Cunningham et al. [71] analysed 61 tumors by array CGH and SNP arrays, and found similar chromosomal alterations in sporadic and familial ileal carcinoids. Again, loss of chromosome 18 was shown to be a frequent and early event in tumor formation with minimal regions of deletions at 18q21.1–q21.31, 18q22.1–q22.2, and 18q22.3–q23.

In general, genomic profiling studies have provided concordant data from large numbers of tumors, allowing construction of a genomic progression model for ileal carcinoids (Figure 2). However, the majority of alterations involved whole chromosomes or large parts of chromosomes, making identification of individual oncogenes or tumor suppressor genes difficult. This is particularly true for

chromosome 18, where identification of a putative tumor suppressor gene is still lacking. Also, for this reason, few candidate genes have been generated for targeted therapy.

5. Expression Profiling of Ileal Carcinoids

A limited number of studies describing expression profiles in ileal carcinoids have been published. These studies are summarised in Table 4. Three studies, Duerr et al. [60], Arvidsson et al. [72], and Leja et al. [73], have analysed small series of ileal carcinoids, generating a limited number of candidate targets for therapy, including RET signaling, Notch signalling, and APLP1. Edfeldt et al. [74] reported a comprehensive study of 42 ileal carcinoids that differentiated tumors as belonging to three different groups by principal component analysis. Genes discriminating the three groups included *TUSC2*, *RUNX1*, *TPH1*, *TGBR2*, and *CDH6*. Reubel et al. [75] have analysed miRNA expression in 16 ileal carcinoids and they were able to show that expression of miRNA-133a is downregulated during tumor progression.

In general, expression profiling studies on ileal carcinoids have provided less consistent data than genomic studies. This is most likely due to the small number of tumors studied in addition to differences in the profiling techniques.

6. Summary

Genomic profiling showed losses on chromosomes 1, 3, 11, and 2 and gains on chromosomes 5, 7, 12, 14, 17, and

TABLE 4: Summary of expression profiling studies on ileal carcinoids.

Study	Tumor type (number)	Profiling platform	Main findings	Therapeutic implications
Duerr et al. [60] Endocr Relat Cancer	GI-NET ($n = 6$) (5 ileal carcinoids, 1 colonic carcinoid; sporadic)	Expression array (Affymetrix U133)	GI-NETs have a different gene expression pattern from PNETs. Frequently upregulated genes in GI-NETs belong to “transporters” and “motor activity.” Validated genes in GI-NETs include <i>ECM1</i> , <i>VMAT1</i> , <i>LGALS4</i> , and <i>RET</i>	Candidates for targeted therapy include RET signaling
Arvidsson et al. [72] Endocr Relat Cancer	Ileal carcinoids ($n = 5$) (sporadic)	Expression array (Swegen, Lund University)	Ileal carcinoid express a large number of neuroendocrine markers including <i>CHGA</i> , <i>SCGN</i> , <i>SYT13</i> , <i>DDC</i> , <i>SYN1</i> , <i>SCG</i> , and <i>VAMP2</i> . Differentially expressed pathways include <i>MAPK</i> , <i>Wnt</i> , <i>Hedgehog</i> , and <i>Notch</i> . Verified genes include <i>APLP1</i> which correlates to tumor progression.	Candidates for targeted therapy include APLP1 Notch signalling
Leja et al. [73] Mod Pathol	Ileal carcinoids ($n = 6$) (sporadic)	Expression array (Affymetrix U133)	Overexpression of 94 genes and underexpression of 276 genes in ileal carcinoids (tumor versus normal ileal mucosa). Upregulated and verified genes include <i>PNMA2</i> , <i>SPOCK1</i> , <i>SERPINA10</i> , <i>GRIA2</i> , <i>GPR112</i> , <i>OR51E1</i> . Downregulation of <i>CXCL14</i> and <i>NKX2-3</i> during tumor progression.	Candidates for targeted therapy include GPR112 and OR51E1
Ruebel et al. [75] Mod Pathol	Ileal carcinoids ($n = 16$) (sporadic)	Expression array (QuantiMir)	Upregulation of miRNA-183, -488, and -19a + b and downregulation of miRNA -133a, -145, -146, -222, and -10b in ileal carcinoids (metastases versus primary). miRNA-133a is expressed in enterochromaffin cells and is downregulated in ileal carcinoids during tumor progression	—
Edfeldt et al. [74] Endocr Relat Cancer	Ileal carcinoids ($n = 42$) (sporadic)	Expression array (KTH Royal Institute of Technology)	Ileal carcinoids cluster in three groups by principal component analysis. Verified genes that were differentially expressed include <i>TUSC2</i> , <i>RUNX1</i> , <i>TPH1</i> , <i>TGFBR2</i> , and <i>CDH6</i> . Downregulation of <i>ACTG2</i> , <i>GREM2</i> , and <i>REG3A</i> during tumor progression.	—

For genes and proteins in bold, therapeutic agents are available for preclinical or clinical testing. GI-NET: gastrointestinal neuroendocrine tumor; PNET: pancreatic neuroendocrine tumor.

20 in the early development of PNETs. Telomeric losses, attenuation of p53 function, and chromosomal instability (CIN) occurred during tumor progression. Losses on chromosomes 3, 6q, and 21q and gains on chromosomes 4 and 7 characterised metastatic tumors. Inactivating mutations of *MEN1* and *ATRX/DAXX* were common, while inactivating mutations of genes in the mTOR pathway (*PTEN*, *TSC2*, and *PIK3CA*) occurred in a subset of tumors. PNETs of B-cell phenotype (insulinoma) showed different genetic alterations compared to the majority of PNETS, with loss on chromosome 22 and gain on chromosome 9q as early

events in tumorigenesis. Telomeric losses, chromosomal instability, loss on chromosome 3p, 6q, 11q, and 22q, and gain on chromosome 7p occurred during malignant progression. Expression profiling of PNETs showed different subgroups by hierarchical clustering, differentiating benign PNETs from malignant PNETs, and non-functioning PNETs from functioning PNETs (insulinoma). Genes associated with malignant PNETs included *MET*, *IGFBP3*, *CD34*, *E-selectin*, *MKK4*, *MDR1*, *FEV*, *ADCY*, *NR4A2*, *GADD45 β* , and *FGF13*. Expression of miRNA-21 was also associated with metastatic behaviour. In functioning PNETs (insulinoma),

expression of *SERPINA1* (alpha-1-antitrypsin) was correlated with malignancy. Profiling studies have suggested a number of candidates to be evaluated for targeted therapy of PNETs. These targets and signaling pathways include BRAF, KRAS, TERT, EGFR, RET, MDM2, IGF, MET/HGF, ANG2, LCK, PDGFRB, AKT-mTOR, and SSTR2.

Genomic profiling of ileal carcinoids showed alterations mainly involving large parts of chromosomes or whole chromosomes. The number of alterations per tumor was lower than that for PNETs. Ileal carcinoids could be separated into two groups by hierarchical clustering. The majority of ileal carcinoids showed loss on chromosome 18, which is an early and most likely pathogenetic event in this group of tumors. Minimal regions of deletions on chromosome 18 included 18q21.1–q21.31, 18q22.1–q22.2, and 18q22.3–q23. No mutations have so far been detected in genes located in these regions. During tumor progression, chromosomal alterations accumulate, most frequently loss on chromosomes 3p, 11q, and 13. A small proportions of ileal carcinoids show clustered gains on chromosomes 4, 5, 7, 14, and 20. These alterations may be present early in tumor development. Chromosome 18 was usually intact in this group of tumors. Gain of chromosome 7 was associated with solid growth pattern and metastatic behaviour, while gain of chromosome 14 was correlated with shorter patient survival. Expression profiles of ileal carcinoids were different from those of PNETs and allowed differentiation of carcinoid tumors into three groups by principal component analysis. During tumor progression, there was downregulation of expression of *CXCL14*, *NKX2-3*, *ACTG2*, *GREM2*, *REG3A*, and miRNA133a. Profiling studies have suggested a limited number of candidates for targeted therapy including ERBB2 (HER2), RET, APLP1, and Notch.

7. Conclusion

High-throughput technologies have greatly facilitated the molecular characterisation of GEPNETs. Recent studies applying genomic and expression profiling to GEPNETs have permitted molecular classification of tumors that goes beyond the traditional histopathological classification. However, data from different studies, notably expression studies, are not always concordant and provide limited information on candidates for targeted therapy. Thus, additional high-quality studies are needed, which should include large numbers of clinically well-characterised tumors, stringent study design and bioinformatics analysis. Future studies must also address stromal cells, cancer stem cells, and bulk cells separately. The recent identification of cancer stem cells by Gaur et al. [76] in intestinal carcinoids will stimulate such studies.

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