

Recent Advances in Molecular Pathology of Neuroendocrine Neoplasms

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Although grouped as a neoplastic entity (NETs), each neoplasm is derived from distinct cell precursors, produces specific bioactive products, exhibits distinct chromosomal abnormalities and somatic mutation events, and has uniquely dissimilar clinical presentations [1]. In recent clinical trials, VEGF pathway inhibitor sunitinib and the mTOR inhibitor everolimus have shown efficacy in patients with advanced pancreatic NETs [2]. The efficacy of these targeted therapies in NETs suggests that the molecular characterization of NETs may provide an avenue to predict which patients may benefit most from the treatment and how to overcome potential drug resistance [2].

In recent years, a wide range of molecular approaches are being used both for characterization of NETs and also for discovery and validation of novel candidate biomarkers in NETs. Some of these molecular approaches elaborate on the genesis, growth, and progression of these neoplasms, others serve as prognostic markers, and still others have the potential to be used as biomarkers that can predict response or resistance of NETs to various targeted and other emerging therapies, as reviewed in the following sections.

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The Mammalian Target of Rapamycin (mTOR)

The mammalian target of rapamycin (mTOR) is an intracellular serine/threonine kinase that functions as a transduction factor to which a wide variety of physiological and pathologic extra and intracellular signals converge [3]. mTOR is involved in the regulation of cell metabolism, survival, proliferation, and motility, through the regulation of protein translation. Multiple somatic mutations in the mTOR pathway have been identified in pancreatic NETs [4, 5].

Specifically, PTEN was found mutated in 7 %, TSC2 in 8.8 %, and PIK3CA in only 1.4 % [6]. Expression levels of PTEN and TSC2 have also recently been linked to outcome in pancreatic NETs [7]. In other studies, PTEN was mutated or lost in about 10–29 % of sporadic PNETs [8, 9]. The expression of PTEN in PNETs has been positively correlated with longer survival especially when correlated with low expression of p-mTOR [8]. Furthermore, positive p-mTOR expression and PTEN loss may have a synergic effect on tumorigenesis and proliferation of PNETs.

The expression and activity of mTOR have been proved to be higher in PNET tissue than in normal pancreatic islet cells. Expression of mTOR or of its activated downstream target p4EBP1 has also been associated with a higher proliferative index and shorter survival in patients with NETs. The expression of mTOR, p-mTOR, and S6K is significantly related to tumor aggressiveness in the form of higher mitotic count, tumor size, staging, vascular invasion, and metastasis [10]. Also, low levels of 4EBP1 or high levels of eIF4E are thought to confer resistance to rapamycin analogs [11]. Therefore, mTOR and its effectors might be biomarkers of aggressive disease, but are not mutated in PNETs, and most molecular changes occur upstream in the PI3K/Akt/mTOR pathway.

Taken together, the above data suggest that targeted therapy based on mTOR/PTEN signaling pathway and its associated molecular mechanisms may play a role in the medical management of PNETS. However, since biomarkers to select individual patients who would respond to treatment with mTOR inhibitors are still lacking, future analytical and clinical validation studies and evaluation of the clinical utility of the various members of the mTOR pathway in human NETs will be an important molecular advance in the near future. Independent validation of the above findings in prospective studies will further facilitate the development of more personalized approaches to treatment of NET patients.

EGFR Family of Receptors as Biomarkers

Epidermal growth factor receptor (EGFR) is expressed in many cancers and is associated with poor prognosis [12]. Furthermore, co-expression of transforming growth factor alpha (TGF- α) and its receptor, EGFR, is also associated with aggressive biologic behavior and adverse clinical outcome in a variety of tumors, including pancreatic adenocarcinomas [13]. Although in NETs, the expression of EGFR has been disputed as a marker of malignancy [13, 14], a recent study showed significant

correlation between EGFR expression and grade of malignancy in pancreatic NETs with low levels of expression in benign tumors and those of uncertain behavior but high levels of expression in well- and poorly differentiated tumors [15]. Furthermore, patients with pancreatic NETs expressing activated EGFR had significantly worse prognosis than those whose tumors did not express activated EGFR [16]. In another recent study, only rare primary PNETs revealed HER2 amplification and mutations in KIT and ATM genes [5]. On independent validation using IHC staining of 140 human PNETs, EGFR was immunopositive in 18 (13 %), HER2 in 3 (2 %), KIT in 16 (11 %), and PDGFR-alpha in 135 (96 %) [5]. Therefore, a number of studies have shown EGFR expression as a marker of adverse clinical outcome in PNETs.

c-KIT

In a multivariate analysis of several prognostic factors, only WHO criteria and c-kit expression were identified as independent markers of unfavorable prognosis in pancreatic NETs [17]. Furthermore, based on IHC expression of KIT and CK19, three prognostic groups of PNETs were identified: low risk (KIT-/CK19-), intermediate risk (KIT-/CK19+), and high risk (KIT+/CK19+), with significantly different patient survival, metastases, and recurrence of PNETs among the three groups [17].

MGMT

Emerging data suggest that high methylguanine-methyltransferase (MGMT) expression is associated with therapeutic resistance to temozolomide [18, 19]. The sensitivity of tumor cells to alkylating agents, including temozolomide, has been associated with low MGMT levels, which through its ability to restore DNA to its normal form can prevent chemotherapy-induced cell death [20]. MGMT deficiency seems to be more common in pancreatic NETs than in carcinoid tumors, potentially explaining the greater sensitivity of pancreatic NETs to treatment with the alkylating agents streptozocin and temozolomide and raising the possibility of using MGMT expression as a predictive marker in future studies of these tumors [18]. If confirmed in future prospective studies, low MGMT expression could help select patients for such treatments. Therefore, *quantification of tumor MGMT expression levels is warranted in future studies of alkylating agents in NETs* [19].

Chromogranin A (CgA)

Chromogranin A (CgA) is a 49-kDa protein, found in the neurosecretory vesicles of NET cells, and is commonly detected in the plasma of patients with endocrine neoplasms [21, 22]. Elevated plasma CgA levels have been associated with poor overall

prognosis in patients with NETs [22]. Early decreases in CgA levels have been associated with favorable treatment outcome in some studies [23, 24]. Both plasma CgA and 24-h urinary 5HIAA levels have been evaluated in prior NET studies as surrogate markers of response. Serial measurements of plasma CgA need to be incorporated into prospective clinical trials. Also, further validation of CgA as a prognostic and potentially predictive biomarker study is warranted [19].

In the past decade, knowledge regarding molecular pathology of sporadic pancreatic neuroendocrine tumors (PNETs) has increased substantially, thanks to microarray studies and novel mutational analysis methods [25]. Over the same period of time, a number of targeted agents have been tested in clinical trials in this tumor type. For some of these agents, there is a strong biological rationale. Among them, the mammalian target of rapamycin (mTOR) inhibitor (everolimus) and the antiangiogenic agent sunitinib are approved for the treatment of PNETs. However, there is lack of knowledge regarding biomarkers able to predict their efficacy and about mechanisms of resistance to these targeted agents [26]. It has also been ascertained that the most common mutations in sporadic PNETs are of the multiple endocrine neoplasia type 1 (MEN1) gene and of the genes ATRX and DAXX. Currently, the clinical significance and potential for treatment of these mutations are uncertain. In this chapter, we will review the main molecular changes occurring in GEP-NETs and emphasize as to how they may be linked to various treatment options.

MENIN

MENIN is a nuclear protein, encoded by the MEN1 gene, which regulates gene transcription by coordinating chromatin remodeling. It is involved in the negative modulation of cell cycle inhibitors, such as p27KIP1 and p18INK4c, of transcription factors such as SMAD3 and JUND, and interacts with the DNA repair machinery [27]. MENIN is considered a tumor suppressor, although its exact role is not completely clear and its action is often controversial. In fact, MENIN works as an inhibitor of proliferation, maintaining the promoter activity of CDKN2C (p18) and CDKN1B (p27) through H3K4 methylation, thus regulating the expression of cell cycle progression inhibitors [27, 28]. Nevertheless, under physiological or pathological conditions, such as obesity or pregnancy, MENIN stimulates pancreatic endocrine cell proliferation controlling G1 to S progression [27, 29].

While the role of MENIN has been extensively investigated in patients with MEN1 syndrome, mutations of the MEN1 gene have also been found in about 25–44 % of sporadic PNETs [6, 30], suggesting a role in the pathogenesis of these tumors. Interestingly, MEN1 mutations have been associated with prolonged survival in patients with metastatic disease [6]. Although mutations of MEN1 are the most frequent alterations in sporadic PNETs, this does not represent a target for treatment yet, although there have been successful attempts of gene therapy to replace the MEN1 gene in PNET models. However, the relation between MENIN and other “druggable” pathways is gaining increasing interest. MENIN is able to

suppress Akt activity reducing its translocation from the cytoplasm to the cell membrane during stimulation with growth factors, therefore reducing Akt-induced proliferation and anti-apoptosis activity [31]. On the other hand, MENIN expression is modulated by PI3K/Akt activity, which leads to phosphorylation of the transcription factor Foxo1, which in turn negatively regulates the expression of MENIN, enhancing proliferation [27, 32].

MENIN has also a role in preventing the RAS-driven activation of the mitogen-activated protein kinase (MAPK) pathway, leaving the RASSFA inhibitory pathway intact. The removal of this mechanism of MAPK pathway blockage could explain why the loss of MENIN causes proliferation in PNETs [33]. However, targeting the RAF or MAPK pathway alone does not seem to be a promising strategy in PNETs. Recently, a novel link between MENIN and the Hedgehog (Hh) signaling pathway has been suggested by Gurung et al. [34]. Furthermore, Fendrich et al. showed how cyclopamine, a Hedgehog inhibitor, decreased tumor cell proliferation, reduced tumor volume, and significantly prolonged median survival in the transgenic mouse model of PNET [35]. The orally available smoothened antagonist LDE225 is therefore under investigation for PNETs. Finally, a valuable target therapy for MENIN could possibly arrive with RNA antagonist (s)-based strategies, as suggested by Luzi et al., but further studies are needed to explore this field [36].

About 15–20 % of NETs are part of inherited genetic syndromes, including multiple endocrine neoplasia type 1 and type 2 (MEN1 and MEN2), von Hippel-Lindau syndrome (vHL), neurofibromatosis type 1 (NF1), and tuberous sclerosis complex (TSC). The genes involved in pancreatic NETs (PNETs) are quite different and fewer than those involved in ductal adenocarcinomas of the pancreas [37]. The genes commonly mutated in PNETs include MEN1 (44 %), DAXX and ATRX (43 %; associated with better prognosis), and those coding for members of the mTOR pathway (15 %) [6, 38–40]. Immunohistochemical assays for DAXX and ATRX proteins demonstrate positive nuclear labeling for DAXX in large cell NEC and absence of nuclear staining for DAXX in a well-differentiated NET (Fig. 1). Similarly, nuclear positivity for ATRX protein has been shown in small cell NEC with absence of nuclear staining for ATRX protein in a well-differentiated NET (Fig. 1). Somatic mutations in DAXX and ATRX appear to be late events in PNETs, since they were absent in microadenomas [4]. On exomic sequence analysis of sporadic pancreatic NETs, an average of 16 nonsynonymous mutations per PNET is far fewer than in ductal adenocarcinoma [6].

The VHL/HIF pathway is also important in PNET pathogenesis [42]. Pancreatic neuroendocrine microadenomas are present in more than 70 % of patients with von Hippel-Lindau syndrome. Sporadic PNETs rarely harbor somatic VHL gene mutation, but promoter hyper-methylation and deletion of VHL occur in up to 25 % sporadic PNETs and are associated with an adverse prognosis [43].

In contrast to pancreatic adenocarcinoma, activation of classical oncogene-mediated pathways does not seem a common event in PNETs [1]. Little is known in respect of pancreatic neuroendocrine oncogenesis and the molecular basis of the progression of sporadic NETs [44]. Mutations in main driver genes like k-Ras, P53,

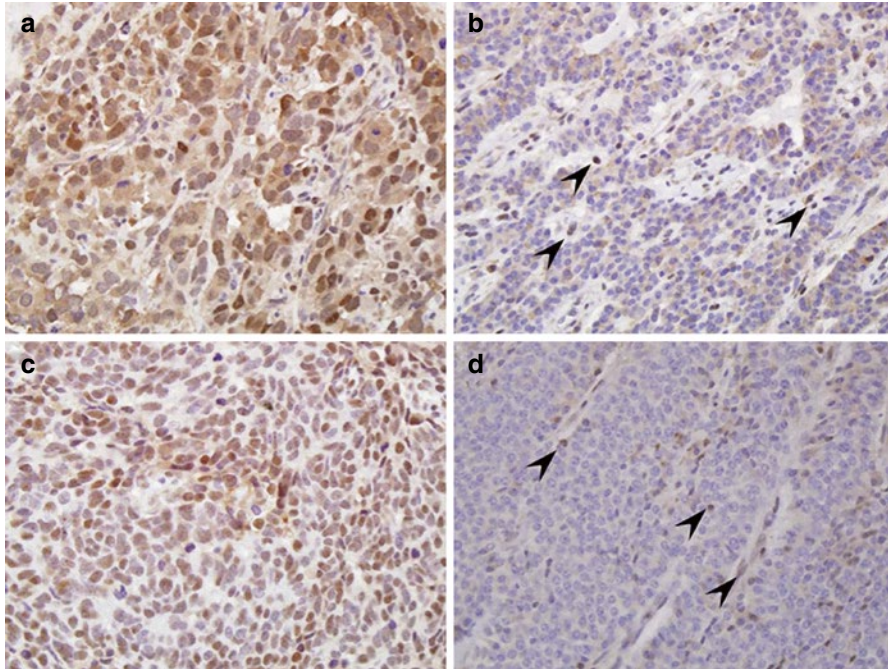


Fig. 1 Immunohistochemical labeling patterns for DAXX and ATRX. (a) Example of positive nuclear labeling for DAXX in a large cell NEC. (b) Loss of nuclear labeling of DAXX in a well-differentiated neuroendocrine tumor. Endothelial cells and lymphocytes within the stroma are positive (indicated by *arrowheads*). (c) Positive nuclear labeling for ATRX in a small cell NEC. (d) Loss of nuclear labeling of ATRX in a well-differentiated neuroendocrine tumor. Similar to that for DAXX shown in (b), endothelial cells and lymphocytes within the stroma are positive (original magnifications $\times 200$) (Reproduced from Yachida et al. [41] with kind permission by Lippincott Williams & Wilkins)

myc, FOS, Jun, src, and Rb have not been specifically implicated [45, 46]. However, copy number alterations in proteins that regulate some of these pathways, MDM2 and P53, have been observed [47].

MDM2 and p53

The tumor suppressor p53 plays a critical role in maintaining genomic stability and tumor prevention [48]. The p53 pathway is tightly regulated by a number of proteins, including the critical negative regulators MDM2, MDM4, and WIP1 [48]. Extra gene copies of MDM2 (22 %), MDM4 (30 %), and WIP1 (51 %) have been reported in PNETs, which may lead to an attenuated p53 function [47]. Thus, development of MDM2 inhibitors and related molecules can restore p53 tumor suppressor function and may provide tangible therapeutic options for PNETs [2].

Molecular Changes in CTCs in NETs

CTCs are associated with progressive NETs and could be used as a prognostic marker. In a prospective study of 120 patients with metastatic NETs, presence of CTCs conferred poorer overall survival, with an HR of 14 [49]. Furthermore, a reduction of CTCs 3–5 weeks after treatment predicted response to therapy in addition to better survival compared with those patients in whom CTCs were increased [50]. Therefore, patients on ineffective or potentially toxic therapies can have their treatment changed appropriately and their management tailored according to CTC changes [2].

CTCs have been reported in blood samples from a number of patients with metastatic NETs, including pancreas, midgut, and bronchial NETs [49] but without any correlation between CTC counts and Ki-67 or CgA levels. In patients with lung cancer treated with anti-EGF receptor (EGFR) therapy, a resistance-associated EGFR mutation has been reported in CTCs [51]. Therefore, molecular characterization of CTCs could potentially assist in understanding NET metastasis and resistance to therapy in addition to their use as biomarkers [2].

Transcription Factors in NETs (PAX8, TTF-1, and CDX2)

PAX8 is a transcription factor expressed in normal adult pancreatic islet cells and is also in primary and metastatic well-differentiated PNETs [52]. Loss of PAX8 may have prognostic significance since PAX8-negative PNETs tend to be larger and are associated with malignant behavior and increased incidence of liver metastases [52].

Tissue-specific transcription factors, such as TTF1 and CDX2, will be increasingly utilized to support origin of an occult primary NETs when one is faced with a metastatic neuroendocrine carcinoma (NEC) of unknown primary.

Cyclin-Dependent Kinases and Rb

Cyclin-dependent protein kinases 4 (CDK4) and 6 are involved in phosphorylation of the retinoblastoma (Rb) tumor suppressor gene leading to inactivation [53]. CDK4/CDK6 amplification and expression have been shown in PNETs as well as its activator, cyclin D [54]. Loss of Rb protein via RB1 gene mutation is also frequently observed in poorly differentiated and high-grade NETs (Figs. 2 and 3) [41]. Furthermore, growth of the human PNET cell line QGP1 can be inhibited in a xenograft mouse model by the CDK4/CDK6-specific inhibitor PD 0332991, which reactivates the Rb pathway [55]. Therefore, gene amplification and overexpression of CDK4 and CDK6 suggest that a subset of patients with PNETs may respond favorably to CDK4/CDK6 inhibitors that are currently entering clinical trials [2].

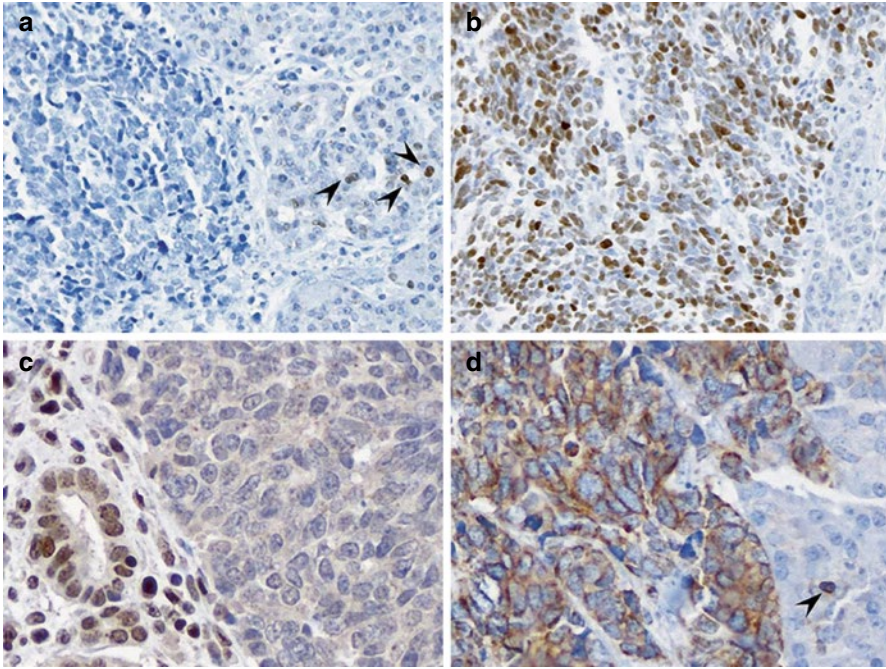


Fig. 2 Immunohistochemical labeling for p53, Rb, and Bcl-2 in small cell neuroendocrine carcinomas. (a) Example of loss of nuclear labeling for p53. Scattered reactive acinar cells with positive nuclear labeling are present in the adjacent normal tissue (*arrowheads*). Sequencing analysis for TP53 in this patient revealed a nonsense mutation (p.E307X). (b) Example of diffusely positive nuclear labeling for p53. (c) Example of loss of nuclear labeling for retinoblastoma (Rb) protein. Adjacent nonneoplastic cells show positive nuclear labeling (*left side*). (d) Diffuse cytoplasmic positivity for Bcl-2 protein. Note the reactive lymphocyte regarded as an internal positive control for this protein (*arrowhead*) (original magnifications $\times 200$) (Reproduced from Yachida et al. [41] with kind permission by Lippincott Williams & Wilkins)

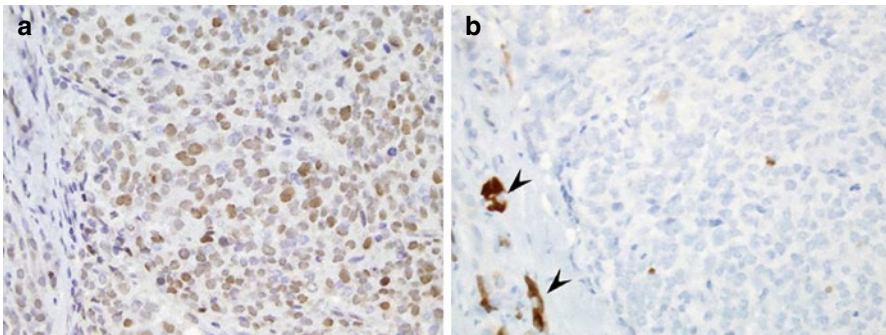


Fig. 3 Immunohistochemical labeling for Rb and p16 in a large cell NEC. (a) Positive nuclear immunolabeling for Rb. (b) Loss of p16 labeling in a serial section of the same large cell NEC. Reactive acinar cells (*arrowheads*) serve as positive internal controls for labeling (original magnifications $\times 200$) (Reproduced from Yachida et al. [41] with kind permission by Lippincott Williams & Wilkins)

Chromosomal Abnormalities

Characteristic allelic imbalances have been observed in sporadic carcinoid and pancreatic NETs [19]. Comparative genomic hybridization (CGH) studies have identified a number of chromosomal alterations in PNETs, among which chromosomal losses are somewhat more frequent than gains, while amplifications are uncommon [56, 57]. In PNETs, the most common chromosomal losses occur on 6, 11, X, and Y, while most common gains include Chr 9, 12, and 17 [1]. Loss of chromosome 18, for example, seems to be a frequent and characteristic feature of small intestinal NETs [1, 58–60], while chromosomes 17 and 19 exhibit the most common gains [1]. It is obvious that pancreatic and small intestinal NETs express significantly different patterns of chromosomal rearrangements; however, the precise implications of the alterations are as still unknown.

Exomic sequence analysis of small intestinal NETs (SI-NETs) showed a much lower rate of somatic single nucleotide variants (SNVs) with an average of 0.1 per 106 nucleotides, suggesting their mutational quiescence [61]. Another study in SI-NETs identified recurrent somatic mutations and deletions in *CDKN1B* [62]. Furthermore, several recurrent somatic copy number alterations (CNAs) were discovered in cancer-related pathways, including PI3K/Akt/mTOR, tumor growth factor (alterations in *SMAD* genes), and *SRC*. Independent large-scale validation of these genetic findings is warranted to confirm these preliminary data, to examine novel epigenetic alterations, and to further determine their value as prognostic or predictive biomarkers in NETs.

Initial proof of concept novel molecular diagnostic studies using comparative genomic hybridization (CGH) and single nucleotide polymorphism (SNP) analyses have shown distinct patterns of allelic alternations in pancreatic and ileal NETs. Most frequent allelic imbalances in WD-NETs were losses of chromosome 18 in 10 tumors (34 %), chromosome 21 or 21q in six (21 %), chromosome 13 or 13q in five (17 %), and chromosome 16 or 16q in four (14 %) tumors and amplification of chromosome 20 or 20p in seven (24 %) tumors. Chromosomal aberrations were significantly less common in WD-NETs from lung and gastrointestinal tract compared to PETs [60]. Also, the observed genetic alterations differed significantly with the anatomical subsite of the NET: allelic loss of chromosome 18 was present in 69 % of ileal carcinoids, 13 % of non-ileal carcinoids, and 6 % of pancreatic NETs [63].

Epigenetic Mechanisms in NETs

Epigenetic mechanisms are an essential component of normal development and gene expression patterns in mammals [2]. Mutations in epigenetic regulators have the potential to lead to misregulation of gene expression that contributes to tumorigenesis, and it has been suggested that epigenetic rather than genetic changes may play a key role in NETs [64]. The long-standing observation that *menin* mutations are associated with NETs supports a role for epigenetic regulation of *menin* in the pathogenesis of NETs [2].

Discovery and Validation of Novel Molecular Biomarkers in PNETs

Gene Expression Profiling

In addition to clinic-pathologic criteria, the discovery of novel molecular markers of metastases may allow development of newer objective criteria to determine patient prognosis and may improve our understanding of the biology of clinical progression in PETs [65]. In recent years, we [66–70] and others [71–77] have carried out gene expression profiling analyses in PNETs. To identify candidate progression genes in PNETs, we used Affymetrix 2.0 gene chip on fresh-frozen metastatic primary PECAs (MP PECAs) that already had metastasized to the liver at the time of resection of the pancreatic primary and a set of frozen non-MP PETs that remained free of clinically detectable metastases until the last patient follow-up. All of the pancreatic endocrine primaries used in this experiment were nonfunctional. Using rigorous thresholds for *P*-values and fold changes in the differentially expressed genes, including gene function/class and pathway analysis, we selected our leading “candidate progression genes” that were differentially expressed between the two sets of pancreatic primaries (metastatic and nonmetastatic). Among these, CD24 antigen, insulin receptor, TMPRSS6, SERPINA1, SMURF1, RNF43, and AKR1C2 were notably upregulated, whereas RUNX1T1, protocadherin 9, RASSF5, RERG, ST14, glucagon, and PDGFRL were notably downregulated [66–68, 78, 79].

From our “candidate progression gene list,” we validated under-expression of several leading candidate progression genes including RUNX1T1 and overexpression of TMPRSS6, SERPINA1, and others on an independent test set of archival PECAs (with liver metastases) using quantitative real-time polymerase chain reaction [70]. We then went on to further validate several of our novel genes, including RUNX1T1 and palladin as progression genes at the protein level using immunohistochemistry on independent test sets of metastatic and non-MP pancreatic endocrine neoplasms [65, 70]. Because IHC expression of RUNX1T1 protein showed a striking difference between the two sets of primaries (metastatic and nonmetastatic), we used ROC curve analysis to further elucidate the relationship between sensitivity and specificity of RUNX1T1 as a prognostic test to predict the presence of liver metastases. Using a cutoff of RUNX1T1 score of four or less in the primary tumor tissue as a predictor of liver metastases, we found low RUNX1T1 expression to have a sensitivity of 85 % and specificity of 96 %.

Based on these data, decreased IHC expression of RUNX1T1 protein in the primary PNET tissue appears to be a surrogate end point for prediction of risk of liver metastases in patients with surgically resected primary PETs [70]. Under-expression of this novel prognostic marker may identify patients with resected primary PETs who are at high risk for metastatic recurrence and may be candidates for closer follow-up for early detection of metastatic disease. Furthermore, RUNX1T1 protein expression in the primary tumor tissue emerges as a stronger predictor of liver

metastases as compared with the other conventional pathological criteria, such as tumor size, grade, and Ki-67 index. A genome-wide screen to detect somatic mutations of genes in colorectal cancer also identified RUNX1T1 as 1 of the 189 new candidate cancer genes, related to TGF-alpha signaling [80]. Another leading gene that was validated as progression gene in PNETs was palladin [65, 69] which was a known metastasis-associated gene with a defined role in cell motility and cell-cell interactions [81, 82]. It is possible that palladin protein may be contributing to liver metastases in case on PNETs by intensifying the invasive properties of the tumor cells.

Using a liquid chromatography-mass spectrometry (LC-MS)-based proteomic approach to identify proteins that can be used as biomarkers for malignant (metastatic) vs. benign (nonmetastatic) insulinomas, 16 (out of 3000) proteins were found differentially expressed between the two insulinoma subsets [83]. IHC validation on tissue microarrays containing 62 insulinomas revealed ALDH1A1, VDAC1, and TPD52 to fulfill the requirements for biologic markers that can be used beyond ENETS and WHO criteria for prognostic stratification of resected insulinomas [83]. Based on these data, additional investigations using proteomic biomarker discovery approach and further validation of these and other promising protein biomarkers in other types of PNETs and non-pancreatic NETs are warranted.

Conclusion

With increasing recognition of the differences in biology and clinical behavior among various NETs, advances in molecular pathology of NETs will facilitate development, validation, and qualification of robust prognostic and predictive biomarkers that can play important roles in pathologic classification, prognostication, and optimal management of NETs. The rigorous analysis of molecular aberrations and clinical outcomes in annotated bio-specimens would represent a useful and feasible first step in defining clinically relevant molecular subgroups of pancreatic and non-pancreatic NETs. A more in-depth understanding of the molecular abnormalities in NETs will be increasingly relevant as additional molecularly targeted therapies are developed. Although large-scale, prospective studies enrolling patients with specific molecular NET subtypes may not be entirely feasible at this point, useful information regarding the activity of specific drugs in molecular subtypes can be gained from retrospective/exploratory analyses of relevant human cancer tissues and annotated clinical data either from clinical trials or through academic-industry collaborations. This will create more and more opportunities for diagnostic pathologists in the academia and community to build and advance molecular pathology expertise using the gold standard and newer molecular methodologies, as part of the implementation of personalized NET oncology model in the twenty-first century.

Abbreviations

4EBP1	Eukaryotic translational initiation factor 4E-binding protein 1
AKR1C2	Candidate progression aldo-keto reductase family 1, member C2 gene
Akt	Protein kinase B
ALDH1A1	Aldehyde dehydrogenase 1 family member A1 gene
ATM	Ataxia Telangiectasia Mutated gene
ATRX	Alpha thalassemia/mental retardation syndrome X-linked gene
CAN	Copy number alterations
CDKN1B	Cyclin-dependent kinase inhibitor 1B gene
CDKN2C	Cyclin-dependent kinase inhibitor 2C gene
CDX2	Caudal type homeobox 2 gene (a transcription factor)
CGH	Comparative genomic hybridization
CK19	Cytokeratin 19
c-Kit	Stem cell growth factor receptor
DAXX	Death-domain associated protein
EGFR	Epidermal growth factor receptor
eIF4E	Eukaryotic translation initiation factor 4E gene
Foxo1	Forkhead box O1 gene
GEP	Gastroenteropancreatic
HER2	Human epidermal growth factor receptor 2
HIF	Hypoxia-inducible factor
IHC	Immunohistochemical
JUND	Jun D proto-oncogene
KIT	c-Kit encoding gene
LC-MS	Liquid chromatography-mass spectrometry
LDE225	Signaling pathway inhibitor
MAPK	Mitogen-activated protein kinase
MEN1	Multiple endocrine neoplasia type 1 gene
MEN2	Multiple endocrine neoplasia type 2 gene
MENIN	Protein encoded by MEN1 gene
MGMT	Methylguanine-methyltransferase
MP	Metastatic primary
mTOR	Mechanistic target of rapamycin
NET	Neuroendocrine tumor
p18INK4c	Cell cycle inhibitor
p27KIP1	Cell cycle inhibitor
p4EBP1	Phosphorylated 4EBP1
PDGFR-alpha	Platelet-derived growth factor receptor alpha
PDGFRL	Platelet derived growth factor receptor like gene candidate progression gene
PECA	Pancreatic endocrine carcinoma
PET	Pancreatic endocrine tumor
PI3K	Phosphoinositide 3-kinase

PIK3CA	Akt/mTOR pathway gene
p-mTOR	Phosphorylated mechanistic target of rapamycin
PNET	Primitive neuroendocrine tumor
PTEN	Phosphatase and tensin homolog
RASSF5	Candidate progression gene
RERG	Candidate progression gene
RNF43	Candidate progression gene
ROC	Receiver operating characteristic
RUNX1T1	Candidate progression gene
S6K	Ribosomal protein S6 kinase
SERPINA1	Candidate progression gene
SI-NET	Small intestinal neuroendocrine tumors
SMAD	Homologs of both the <i>Drosophila</i> protein, mothers against decapentaplegic (MAD) and the <i>Caenorhabditis elegans</i> protein SMA
SMAD3	Transcription factor
SMURF1	Candidate progression gene
SNP	Single nucleotide polymorphism
SNV	Single nucleotide variants
SRC	SRC proto-oncogene, non-receptor tyrosine kinase
ST14	Candidate progression gene
TGF	Transforming growth factor
TGF-alpha	Transforming growth factor alpha
TMPRSS6	Candidate progression gene
TPD52	Tumor protein D52
TSC	Tuberous sclerosis complex
TSC2	Tuberous sclerosis complex 2 gene
TTF1	Transcription termination factor, RNA polymerase I
VDAC1	Voltage dependent anion channel 1 gene
vHL	von Hippel-Lindau syndrome
WD-NET	Well-differentiated neuroendocrine tumors
WHO	World Health Organization

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