GEP- NETS UPDATE

Genetics of neuroendocrine tumors

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Abstract

Neuroendocrine tumors (NETs) are a heterogeneous group of neoplasms, arising from neuroendocrine cells that are dispersed throughout the body. Around 20% of NETs occur in the context of a genetic syndrome. Today there are at least ten recognized NET syndromes. This includes the classical syndromes: multiple endocrine neoplasias types 1 and 2, and von Hippel–Lindau and neurofibromatosis type 1. Additional susceptibility genes associated with a smaller fraction of NETs have also been identified. Recognizing genetic susceptibility has proved essential both to provide genetic counseling and to give the best preventive care. In this review we will also discuss the knowledge of somatic genetic alterations in NETs. At least 24 genes have been implicated as drivers of neuroendocrine tumorigenesis, and the overall rates of genomic instability are relatively low. Genetic intra-tumoral, as well as inter-tumoral heterogeneity in the same patient, have also been identified. Together these data point towards the common pathways in NET evolution, separating early from late disease drivers. Although knowledge of specific mutations in NETs has limited impact on actual patient management, we predict that in the near future genomic profiling of tumors will be included in the clinical arsenal for diagnostics, prognostics and therapeutic decisions.

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Neuroendocrine tumors

Neuroendocrine tumors (NETs) are rare neoplasms originating from neuroendocrine cells most commonly located in the endocrine glands but also in the gastrointestinal and bronchopulmonary systems (Fig. 1) (1). NETs usually display slow proliferation but have a high rate of advanced stage at presentation (2, 3). Neuropeptide hypersecretion is a hallmark of these diseases and often results in distinct hormonal syndromes. These syndromes are associated with both increased morbidity and mortality (2, 4). The median survival of patients with well differentiated NETs is about 10 years, contributing to the relatively high prevalence of these diseases (5, 6).

NET is one of the most heritable groups of neoplasms that feature in at least ten genetic syndromes. Additional susceptibility genes associated with a smaller fraction of NETs have also been identified. In this review we will present relevant genetic information regarding these diagnostic entities. We will also summarize data from tumor sequencing studies that provided comprehensive maps of the genomic landscape of NETs. Finally we comment on the current and future potential of these data as tools for the development of biomarkers as well as novel druggable targets. Below we present syndromes and additional susceptibility genes with increased risk of NET development (Table 1). The present paper reviews genetic profiles, by means of early and late mutations, of relevance for tumorigenesis in NETs.

Genetic syndromes

Multiple endocrine neoplasia type 1

Multiple endocrine neoplasia type 1 (MEN1; OMIM 131100) is one of the first described autosomal dominantly inherited complex endocrine syndromes (7). MEN1

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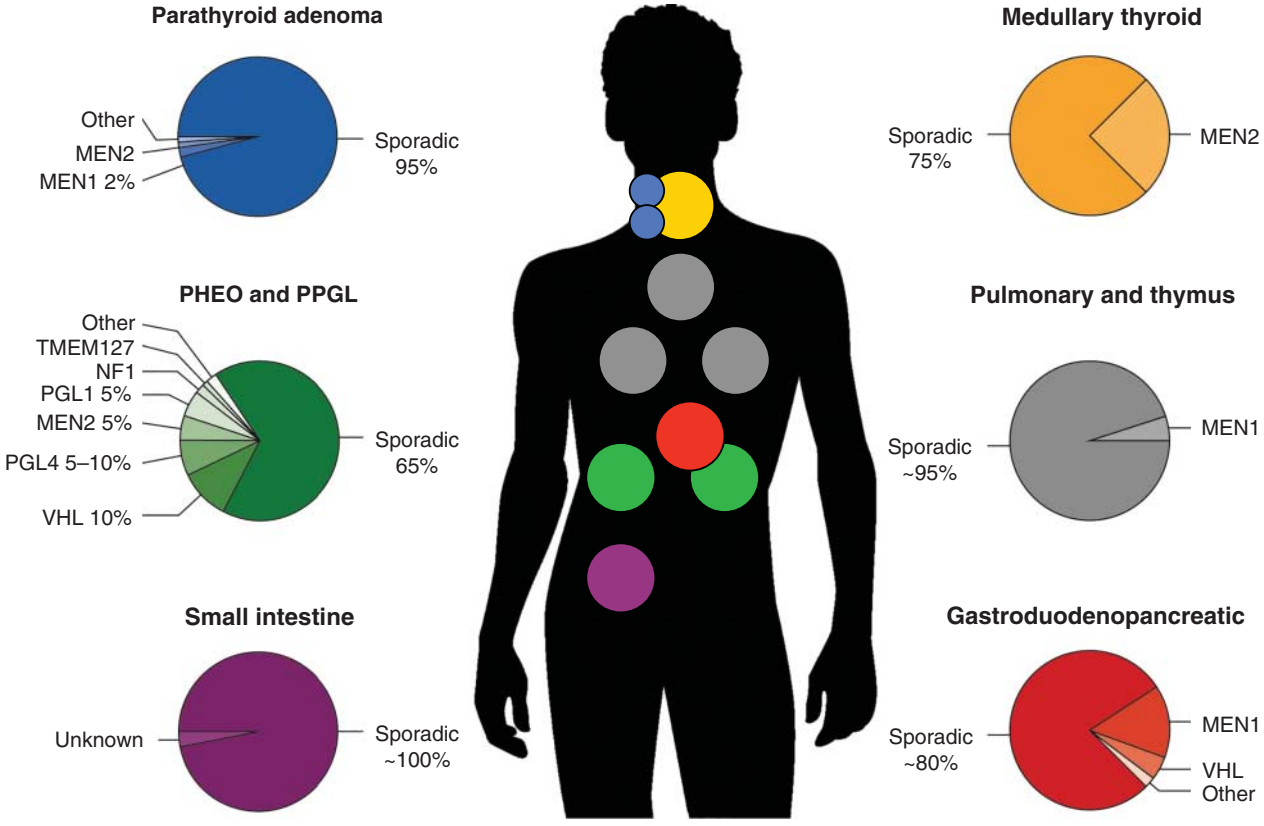

Figure 1

Overview of genes with recurrent mutations in NETs and their distribution accordingly to anatomical location.

has a high penetrance, over 90% by age 40, and is present in about three per 100 000 individuals. Gene carriers most frequently develop tumors in the parathyroid glands (95%), the anterior pituitary (20–40%), and in the endocrine cells of the pancreas/duodenum (40–80%) (8). Any of these can be the presenting lesion, and typically clinically detectable in the young adult, although rare cases of childhood tumors are reported. By definition MEN1 is present if two of these classical target tissues are affected by tumors, and familial MEN1 includes at least one relative with a corresponding tumor. Apart from the above-mentioned organs, MEN1 carriers frequently develop NETs of the foregut, e.g. bronchial, thymic, and gastric ECLomas. Adrenocortical lesions, mostly non-secreting, hyperplasias or adenomas but also rare cases of adrenocortical carcinomas are also present in MEN1 patients. The most lethal of the MEN1 lesions are the pancreatic and the thymic NETs which frequently develop into metastatic disease. Several non-endocrine tumors are also overrepresented in MEN1 patients, such as facial angiofibromas, collagenomas, lipomas and meningeomas. The typical clinical picture of the disease is highly variable between family members and dependent of the affected organs and the pattern of hypersecreted hormones in each case.

In 1988 the *MEN1* gene was linked to chromosome 11q13 and suggested to be a suppressor (9). The gene was finally identified by positional cloning, and heterozygous germline inactivating *MEN1* mutations was revealed in 70% of typical index cases (10). The proportion of MEN1 patients with a causative *MEN1* mutation was later estimated to 75–95% (11). By now more than 1000 mutations have been recognized. The vast majority causes truncation of the protein. The most likely cases to reveal a germline *MEN1* mutation are probands developing lesions at young age and show multiple tumors per organ. There is no convincing genotype–phenotype correlation identified so far. Furthermore if a mutation cannot be found it does not exclude MEN1 and recently alternative genes giving rise to syndromes resembling MEN1 have been suggested, e.g. germline *CDKN1B* (p27kip) mutations resulting in MEN4 (12). It have also been postulated that common genetic variants within *CDKN1B* could act as disease

Table 1 Genetic syndromes with NET manifestation. Overview of genetic syndromes that involve NETs; penetrance of NETs at the specified location is presented in parenthesis; figures on penetrance are presented when available.

Name	Gene	NET manifestation	Other manifestation
MEN1	*MEN1*	Parathyroid (>90%), gastroentero-pancreatic (50%), anterior pituitary (30%), lung and thymus (10%)	Adrenocortical tumors
MEN2A (FMTC)	*RET*	Medullary thyroid (90–100%), adrenal medulla (20–80%), parathyroid (20%)[a]	
MEN2B	*RET*	Parathyroid, medullary thyroid (100%), adrenal medulla (50%)	Marfanoid habitus
MEN4	*CDKN1B*	Parathyroid, pancreas, pituitary	
Neurofibromatosis type 1	*NF1*	Adrenal medulla (1–5%), duodenum	Neurofibroma, cafe-au-lait spots
von Hippel–Lindau	*VHL*	Adrenal medulla and sympathetic ganglia (15%), pancreas (10%)	Hemangioblastoma, renal carcinoma
Familial PGL 1-5	*SDHA-D, SDHAF2*	Sympathetic and parasympathetic paraganglia, Adrenal medulla	GIST, renal carcinoma
Familial PCC and PGL syndromes	*TMEM127, MAX, FH, MDH2*	Adrenal medulla, sympathetic ganglia (*TMEM127* 30%)	
Polycytemia paranganglioma syndrome	*EPAS1*	Sympathetic ganglia, adrenal medulla, duodenum	Polycytemia
Tuberous sclerosis complexes	*TSC1, TSC2*	Pancreas	Hamartoma
HPT-JT syndrome	*HRPT2*	Parathyroid adenoma (80%) and carcinoma (15%)	

[a]Penetrance in MEN2 is specific to the particular mutation.

modifiers in MEN1, possibly contributing to the observed clinical heterogeneity (13).

Classical MEN1 tumorigenesis depends on a second *MEN1* hit, by means of a somatic mutation eliminating also the WT allele. But some NETs in MEN1, such as thymic and duodenal NETs, do not to necessary require a complete inactivation of the gene (14). Somatic homozygous inactivation of *MEN1* is also frequently seen in sporadic tumors of the MEN1 target organs.

The MEN1 protein, named menin, is ubiquitously expressed and preferentially located in the nucleus (10). It has been suggested to be a scaffold protein with more than 40 interacting proteins and thus are involved in a large number of biological functions, such as chromatin modification, DNA repair, transcription, cell division, protein degradation, motility and adhesion (15). Several *Men1* knock-out mouse models mimicking the human syndrome are available.

Multiple endocrine neoplasias type 2 and familial medullary thyroid cancer

Multiple endocrine neoplasias type 2 (MEN2) and familial medullary thyroid carcinoma (FMTC) are autosomal dominantly inherited disorders characterized by development of multiple endocrine MTC, pheochromocytoma (PCC) and parathyroid adenomas (reviewed in Wells *et al.* (16)). MEN2 subtype A (OMIM 171400) accounts for about 80% of cases with an almost complete penetrance for MTC, while PCC and parathyroid adenoma are seen in about 50 and 30% of patients (17). MEN2B (Omim 162300) includes more aggressive MTC, dysmorphic marfanoid features but no parathyroid adenoma (18).

MEN2 and FMTC are caused by gain of function mutations in the rearranged during transfection (*RET*) protooncogene, localized on chromosome 10, which encodes a receptor tyrosine kinase (19, 20, 21). Gain of function mutations in *RET* result in autonomous activation that transduces activating signals through the RAS/MAPK and PI3K/AKT pathways (22). A majority of MEN2 and FMTC cases reveal constitutional mutations in the cysteine-rich extracellular domains (exons 10–11) of the *RET* gene (23), while disease causing variants within *RET* non-cysteine regions (exons 13–16) are less common and the related phenotype is characterized by pronounced heterogeneity (24). Patients with constitutional *RET* mutations show strong genotype–phenotype correlations, e.g. codon 634 transitions linked to MEN2A and codon 918 mutations linked to MEN2B. It is critical to recognize the specific *RET* mutation in order to optimize patient and family management, particularly for the timing of prophylactic thyroidectomy and to provide information

on the risk of developing PCC and parathyroid adenoma (25, 26).

FMTC (OMIM 155240) is characterized exclusively by MTC. It has been suggested that FMTC show less aggressive disease characteristics than MTC occurring in the context of MEN2A and B (16, 27).

Multiple endocrine neoplasia type 4

Multiple endocrine neoplasia type 4 (MEN4; OMIM 610755) is a rare syndrome that is thought to predispose development of NETs, mainly parathyroid and pituitary adenomas. The trait is inherited through germline mutations in *CDKN1B* encoding p27kip (12). MEN4 seems to occur in an autosomal dominant fashion and is linked to loss of function mutations in the cell cycle regulator *CDKN1B* (12). Future studies have to be performed to confirm the phenotype and penetrance of *CDKN1B* mutations.

Neurofibromatosis type 1

Neurofibromatosis type 1 (NF1; OMIM 162200) is an autosomal dominant syndrome that is characterized by multiple endocrinopathies and nervous system manifestations (28). The most common features are fibromatous skin tumors, lichen eye nodules, optic gliomas and café-au-lait spots (29). Endocrinopathies are less common and include PCCs and duodenal NETs (30). Neurofibromatosis type 1 is caused by loss of function mutations in *NF1* that has been linked to deregulation of both rat sarcoma viral oncogene homolog (RAS) proteins and the ERK/MAPK signaling pathway (31, 32).

von Hippel–Lindau syndrome

The von Hippel–Lindau syndrome (VHL; OMIM 193300) syndrome has an incidence of ~1/36 000 individuals (33) and is an autosomal dominant disease. VHL is characterized by increased risk of tumors and cysts; retinal and CNS hemangioblastomas, PCC, paragangliomas (PGLs), renal clear cell carcinomas, renal cysts, pancreatic NETs, pancreatic cysts and endolymphatic sac tumors.

The syndrome is caused by inactivating mutations in the *VHL* tumor suppressor gene, located in 3p25 that is involved in the oxygen-sensing pathway through regulation of hypoxia-inducible factors (34). Truncation of VHL results in decreased ubiquitination of HIF transcription factors resulting in increased expression of target genes such as VEGFA etc. VHL is subclassified into type 1 (truncating mutations) and type 2 (missense mutations) that differ in clinical presentation (35). Subtype 1 presents without PCC whereas type 2 have a high penetrance of PCC. Subtype 2 may be further subclassified into type 2A without renal cell carcinoma or pancreatic cysts that may be present in subtype 2B.

PCC and PGL syndromes

There are at least 12 genes that have proved to confer susceptibility to PCCs and/or PGLs: *SDHA* (36), *SDHB* (37), *SDHC* (38), *SDHD* (39), *SDHAF2* (40), *FH* (41), *VHL* (42), *EPAS1* (43), *NF1* (32), *RET* (20), *TMEM127* (44) and *MAX* (45). The classic genetic syndromes MEN2, NF1 and VHL have been presented above. In the following section we will give an overview to more recently described diagnoses that show pronounced heterogeneity both in term of the affected organs, disease penetrance and mode of inheritance.

Familial PGL

Familial PGL syndromes types 1–5 are transmitted in an autosomal dominant manner. These syndromes are caused by the loss of function mutations in *SDHD* (PGL 1), *SDHAF2* (PGL 2), *SDHC* (PGL 3), *SDHB* (PGL 4) and *SDHA* (PGL 5). *SDHx* genes (*SDHA*, *SDHB*, *SDHC* and *SDHD*) encode succinate dehydrogenase subunits that are catalyzing reactions in tricarboxylic acid cycle and in the respiratory electron transfer chain. Due to the disruption of the tricarboxylic acid cycle the associated PGL harbor unique metabolic profiles with accumulation of oncometabolites (46). Although familial PGL syndromes share pathogenic mechanisms, with disruption of the succinate dehydrogenase complex, the phenotype varies between the different subtypes (47). The molecular rationale for this heterogeneity remains to be identified. Paternal transmission has been shown to occur in PGL 1 and 2.

Familial PGL type 1 (OMIM 168000) have an almost complete penetrance for parasympathetic tumors in the head–neck region (48, 49). Furthermore, unilateral PCC and/or sympathetic PGLs are seen in about 25% of patients respectively (50, 51).

Familial PGL type 2 (OMIM 601650) has so far only been detected in a few European families (40, 52, 53, 54), and all reported patients have presented with parasympathetic lesions.

Familial PGL type 3 (605373) is also a rare condition that is mainly manifested by parasympathetic tumors (55, 56, 57).

Familial PGL type 4 (OMIM 115310) is associated with significant morbidity and increased mortality due to the

substantial risk of development of malignant sympathetic PGLs (51, 58, 59). PCC and parasympathetic PGL also occur. In addition, patients with PGL 4 have an increased risk of developing gastrointestinal stromal tumors (GIST) as well as renal cell carcinoma (50).

Familial PGL type 5 (OMIM 614165) is associated with PCC, PGL as well as GIST. Concomitant presentation of two or more of these three tumor types seems to be exceedingly rare (36, 60, 61).

Familial PCC and PGL syndromes

Patients with familial PCC and PGL of the TMEM127 subtype (OMIM 613403) have an estimated penetrance of PCC of 30% whereas abdominal PGL are less frequent (44, 62, 63, 64). No other phenotypes have been described. This disorder presents in patients with loss of function mutations in the *Transmembrane protein 127* gene that is linked to dysinhibition of the mammalian target of rapamycin (mTOR) pathway.

Myc-associated factor X (MAX) associated familial PCC and PGL (OMIM 154950) is an autosomal dominant disorder with a suggested paternal mode of transmission (45, 65). These patients show susceptibility to PCCs and PGLs with unknown penetrance. No other phenotypes have been described. This syndrome is caused by loss of function mutations in MAX that results in deregulation the MYC–MAX–MXD1 pathway (45).

Fumarate hydratase (*FH*) associated PCC and PGL (OMIM 136850) is an autosomal dominant syndrome previously known to result in susceptibility to leimyomatosis and renal call cancer (OMIM 150800 (66)). PCC and PGL was recently recognized as a feature of this syndrome and the penetrance is currently unknown (41, 67, 68). The syndrome is caused by function mutations in *FH* that results in reduced enzymatic activity with fumarate accumulation.

A recent study described germline mutations in *MDH2* as a cause of PGL (69). Although this finding needs to be confirmed, it is indeed remarkable that *MDH2* is the third Krebs cycle gene suggested to be involved in PGL tumorigenesis (69).

Polycytemia and PGL syndrome

Disruption of oxygen sensing is also a recurrent disease mechanism in NETs. The recently recognized polycytemia and PGL syndrome was shown to occur as a result gain of function mutations in *EPAS1* that resulted in a pseudohypoxic state through reduction in HIF2α degradation mediated by disturbed VHL binding (43, 70, 71, 72). The mode of inheritance for the polycytemia and PGL syndrome (OMIM 603349) is currently unknown due to near exclusive presentation in mosaicism. These patients are prone to develop PGL, PCC, polycytemia and somatostatinoma (72). A recent study also suggested ocular manifestations in patients with polycytemia and PGL syndrome (73). A majority of patients with mosaic mutations have had female gender and the underlying mechanism for this phenomenon is unknown (72).

Tuberous sclerosis complex

Tuberous sclerosis complex subtypes 1 and 2 (OMIM 191100 and 613254) are autosomal dominant syndromes that occur in 1:6000–10 000 individuals (74, 75, 76). These patients show multiorgan manifestations; mainly hamartomas but also angiomyolipomas, renal cell carcinoma and pulmonary lymphangioleiomyomatosis (reviewed by Curatolo *et al*. (77)). Pancreatic NET is a less frequent manifestation (78). These syndromes are due to the loss of function mutations in *TSC1* and *TSC2* that encode proteins forming the tuberin–hamartin complex that is essential for mTOR signaling (79). Somatic mutations in TSC2 occur frequently in pancreatic NETs (80).

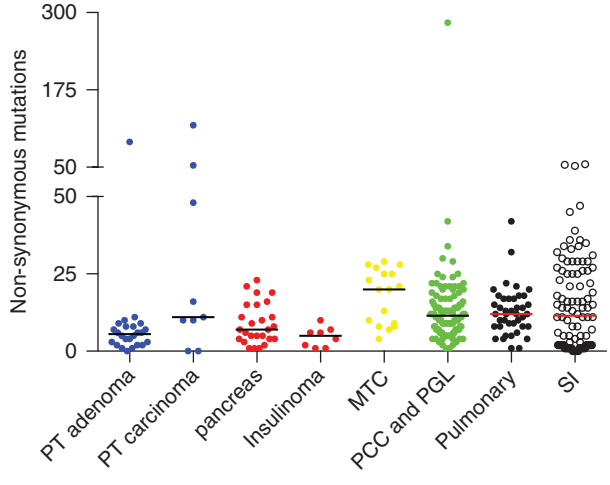

Figure 2
Overview of mutational burden in NETs merged from raw data in (80, 85, 86, 87, 98, 99, 100, 104, 117, 120, 121, 122, 128, 136, 137). Each dot represents a unique tumor, and the line shows the median number of mutations of each category. PT, parathyroid.

Hyperparathyroidism-jaw tumor syndrome

Hyperparathyroidism-jaw tumor syndrome (OMIM 145001) is an autosomal dominant syndrome caused by truncating mutations in the *HRPT2* located on 1q31.2 (81). It is characterized by adenomatous or malignant lesions of the parathyroids, jaw tumors, as well as renal and uterine tumors (82, 83).

Genetic landscape of NETs

Compared with other tumor types the genetic landscape of NETs is characterized by relatively few mutations and chromosomal aberrations per tumor (Fig. 2). So far, around 24 genes have been associated with neuroendocrine tumorigenesis (Table 2). These genes seem to interconnect to important pathways involved in cell metabolism, chromatin modification and growth control (Fig. 3).

Parathyroid adenoma and carcinoma

Constitutional mutations in *CDC73* (81), *MEN1* (84), *RET* (20) and possibly *CDKN1B* (12) and *CASR* (83) cause susceptibility to parathyroid adenoma. Two exome sequencing studies investigated the genomic landscape of parathyroid adenomas and discovered somatic *MEN1* mutations in 35% of cases (85, 86). A third study investigated the exomes of parathyroid carcinomas identified recurrent mutations in the *PRUNE2* gene (87). The median number of somatic amino acid substituting mutations per adenoma was eight (range 2–100) (86). Parathyroid carcinomas harbored more mutations (synonymous average 51, range 3–176) that showed an apolipoprotein B mRNA editing enzyme, catalytic polypeptide-like (APOBEC) like mutational signature (87). APOBEC associated genomic instability is a result of cytosine-to-uracil deamination that is catalyzed by APOBEC3A-H enzymes and have been shown to occur in multiple different types of carcinomas (88).

Gastric, duodenal and pancreatic NETs

Constitutional mutations in *MEN1* (84), *VHL* (42), *NF1* (32), *EPAS1* (somatostatinoma) (43), as well as *TSC1* and *2* (74, 75) cause susceptibility to pancreatic NETs. *CDKN1B* have also been suggested as a susceptibility locus, but limited numbers of cases have been presented (12). In sporadic pancreatic NETs *ATRX*, *DAXX*, *MEN1, TP53*, *ATM* and mTOR pathway related genes are commonly mutated in a somatic fashion (80, 89, 90, 91). Pancreatic NETs harboring somatic mutations in *ATRX* and *DAXX* have

Table 2 List of genes involved in neuroendocrine tumorigenesis. Overview of genes involved in NET tumorigenesis. ALT, alternative lengthening of telomeres.

Gene	Gene function	Chronological classification	Organ specificity
ATM	Chromatin integrity	Unknown	Pancreas
ATRX	ALT	Late	Pancreas, adrenal medulla, paraganglia
CDKN1B	Cell cycle	Unknown	Pancreas, small intestine, parathyroid, anterior pituitary
DAXX	ALT	Late	Pancreas
EPAS1	Cell signaling	Early	Paraganglia, adrenal medulla, duodenum
H-, K-RAS	Cell signaling	Early	Thyroid C-cell, adrenal medulla
FH	Metabolism	Early	Adrenal medulla, paraganglia
KTMD2	Chromatin modification	Unknown	Adrenal medulla
MAX	Cell signaling	Early	Paraganglia, adrenal medulla
MDH2	Metabolism	Unknown	Paraganglia
MEN1	Unknown	Early	Parathyroid, anterior pituitary, endocrine cells in pancreas, duodenum, gastrium, lung, thymus
NF1	Cell signaling	Early	Adrenal medulla, duodenum
RET	Cell signaling	Early	thyroid C-cell, adrenal medulla, parathyroid
SDHx	Metabolism	Early	Paraganglia, adrenal medulla
TERT promoter	Telomere maintenance	Late	Paraganglia
TMEM127	Cell signaling	Early	Paraganglia, adrenal medulla
TP53	Chromatin integrity, Cell signaling	Unknown	Endocrine pancreas, adrenal medulla
TSC1-2	Cell signaling	Unknown	Pancreas
YY1	Transcriptional regulation	Early	Pancreas

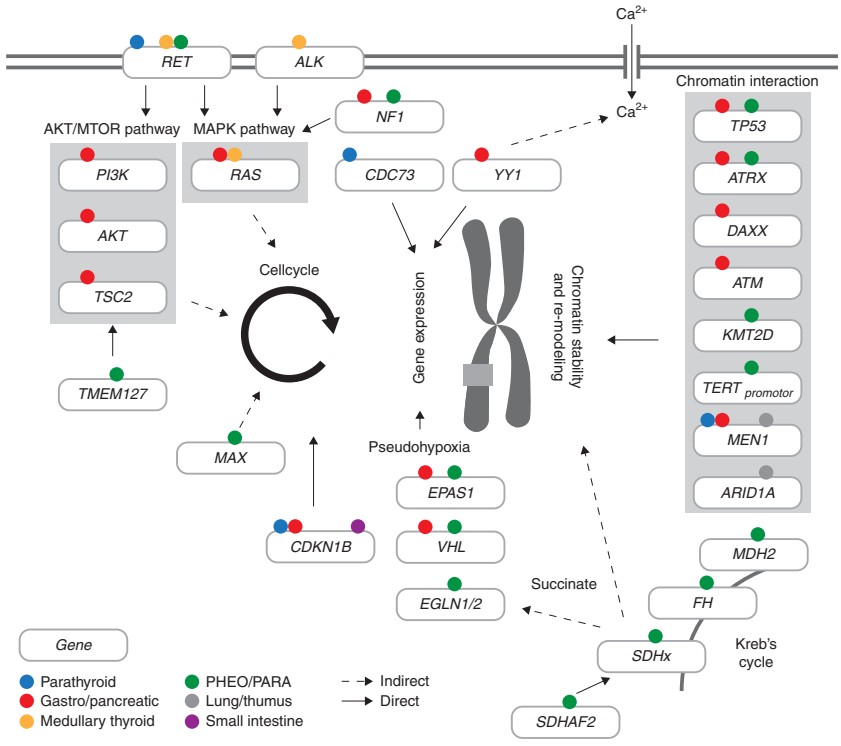

Figure 3

Simplified overview of genes and pathways involved in NET tumorigenesis.

been associated with chromosomal instability and reduced survival (92). It has been suggested that *ATRX* and *DAXX* mutations are not involved in the initial phase of tumorigenesis. Instead these mutations are thought to be late events that result in malignant transformation of PNETs (92, 93). The proposed pathogenic mechanism of *ATRX* and *DAXX* deficiency is the activation of the alternative lengthening of telomeres phenotype (94, 95). This phenotype has been associated with genome rearrangements, defects in the G2/M checkpoint and altered double-strand break repair (95). It have also been suggested that *ATRX*/*DAXX* mutations lead to differential epigenetic deregulation (96). The mean number of amino acid substituting somatic mutations in non-functioning pancreatic NETs were 16 (80). The degree of chromosomal aberrations is more variable and ranges from no somatic copy number alterations to genome-wide aneuploidy (92, 97).

Insulin producing pancreatic NETs with manifest hypoglycemic symptoms, i.e. insulinomas, have been identified with recurrent somatic mutations in *YY1*. This gene encodes a nuclear transcription factor and the described recurrent mutation Thr372Arg has been shown to result in neomorphic effects and altered transcription (98, 99, 100). Insulinomas exhibit a low mutational burden and the average amino acid substituting mutations were four per tumor (98, 99, 100). High chromosomal instability has been recognized in a subset of insulinomas especially in those with malignant features (101).

Although limited studies have been presented, evidence indicates that the genetics of duodenal and gastric NETs overlap with the profile observed in pancreatic NETs (90, 102).

Medullary thyroid carcinoma

Germline mutations in *RET* cause MEN 2 and FMTC; both syndromes showing high penetrance of MTC (20). MTC from MEN2 patients rarely show any other somatic driver mutations (103). Investigating the genomic landscape of sporadic MTCs revealed mutually exclusive oncogenic mutations in *RET* and *RAS* subtypes *K* and *H* in 75 and 15% respectively (104, 105, 106). Furthermore there were an average of 18 amino acid substituting somatic mutations per MTC (104). *ALK* gene fusions were recently described in two out of 98 investigated cases suggesting that ALK inhibition might further be evaluated for treatment of these rare tumors (107, 108).

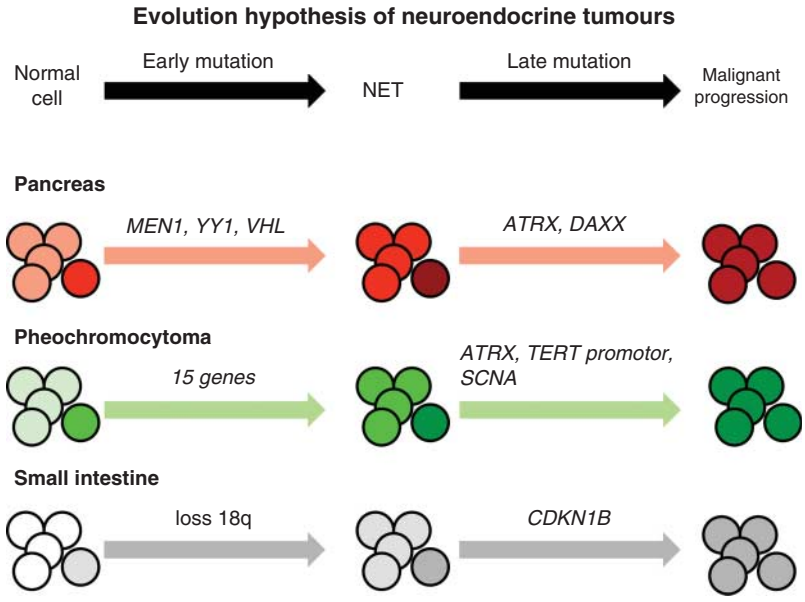

Figure 4

Theory of evolution, with relevant driver genes, in NET tumorigenesis. The 15 genes involved in pheochromocytoma and PGL tumorigenesis are: *SDHx, SDHAF2, MDH2, VHL, EPAS1, NF1, RET, HRAS, TMEM127, MAX, KIF1B* and *KMT2D*.

PCC and PGLs

Mutations in up to 20 genes have been suggested with PCC and PGL tumorigenesis. A total of 12 genes is believed cause of genetic susceptibility to PCC and PGL and a further six loci have been suggested but remains to be validated. These include *KIF1Bβ* (109, 110), *IDH1* (111), *MDH2* (69), *BAP1* (112) as well as *EGLN* subtypes *1* and *2* (113, 114). Somatic mutations in *HRAS* have been shown to cause these diseases (115, 116). Several genes have also been suggested to work as the disease modifiers of PCC and PGL. *ATRX* have been identified in a subset of malignant PCC and PGL having the alternative lengthening of telomeres phenotype (117). TERT promoter mutations have been identified to occur exclusively in succinate dehydrogenase deficient PGL (118, 119). A recent study also described the recurrent somatic mutations in *KMT2D*. Whether this gene acts as a disease driver or modifier remains to be identified (120). The genomic landscape of PCC and PGL is associated with relatively low degree of genomic instability having an average of 19 non-synonymous mutations per tumor (117, 121, 122). Clinical criteria have classified these tumors accordingly to location into PCCs (adrenal medulla) and PGLs (extra adrenal). Accumulating evidence show that it is possible to sub-classify PCC and PGL into at least three distinct molecular clusters (based on methylome, transcriptome and siRNAome) having unique mutational profiles as well as different clinical characteristics (41, 121, 123). Cluster 1a is enriched with noradrenaline producing PGLs that show mutations in genes involved in cell metabolism through the Kreb cycle (*SDHX*, *FH* and *MDH2*) (121). Cluster 1b has noradrenaline producing PCC and PGL with a pseudohypoxic phenotype (*VHL* and *EPAS1*) (43, 123). Cluster 2 has PCC and PGLs with a mixed adrenaline/noradrenaline phenotype having aberrantly activated signaling in the MAPK and PI3K/AKT signaling pathways (*RET*, *NF1*, *TMEM127*, *MAX* and *HRAS*) (45, 115, 124, 125) (Fig. 4).

NETs of the lung

This section describes the genomic findings of well differentiated NETs derived from the broncopulmonary system. These have classically been denoted typical and atypical pulmonary carcinoids based on morphological criteria (126). Pulmonary carcinoids occur in about 5% of MEN1 patients (127). One study examining genome-wide mutation status in 44 sporadic pulmonary carcinoids identified three genes with recurrent mutations: *MEN1*, *PSIP1* and *ARID1A* (128). Network analysis of genes with somatic mutations extended the number of cases with relevant mutations, 40% had covalent histone modifiers

and 22% subunits of the SWI/SNF complex (22%) (128). The occurrence of somatic *MEN1* mutations in sporadic pulmonary carcinoids have previously been highlighted (129). There were the averages of 13 protein-altering mutations per sample (128). Differential expression of miRNAs in typical vs atypical lung carcinoids has also been reported (130).

Thymic carcinoid

NET of the thymus is a part of MEN1 syndrome with a penetrance of <10% (8). CGH analysis found recurrent copy number alterations with the most commonly disturbance identified being gain of 8q24 (*MYC* locus) (131). Mutational screening of tumor tissue has been performed on a per case-basis with the finding of a few somatic *MEN1* mutations (132).

Small intestinal NETs

Small intestinal NETs (SI-NETs) have also been described to occur with familial aggregation in a small fraction of patients (133, 134). Despite substantial efforts no definitive genetic basis for this phenomenon has been described. A recent study used linkage analysis and exome sequencing that revealed *IPMK* germline mutations in a single family (135). Although experimental investigations seem to indicate a pathogenic effect of the *IPMK* mutation a validation effort that included 32 additional families did not reveal the presence of any additional mutations (135). Thus *IPMK* inactivation is not likely to be a significant cause of familial SI-NET. The genomic landscape of SI-NETs has been investigated with exome coverage in close to 100 tumor samples (136, 137). Recurrent mutations were only identified in the *CDKN1B* gene with a mutation prevalence of 9% of SI-NET patients (136, 137, 138). Instead, the most frequent genomic alteration is hemizygous deletions affecting chromosome 18q (133, 136, 137, 139). Recent data have shown that SI-NETs can be sub-classified based on tumor methylome into three distinct clusters (140, 141). Sub classification of SI-NETs based on gene expression may also provide relevant information (142).

Genetic heterogeneity and tumor evolution

As clinicians seek to select treatments based on genetic biomarkers, knowledge of spatial and temporal heterogeneity could be important and should be researched further. Indeed genetic heterogeneity within and between paired tumors has been described in most NET types. Consistent with the theoretical assumption that MEN1 patients experience parallel development of independent NET clones, studies using loss of heterozygosity (LOH) LOH markers were able to show that paired tumors from MEN1 patients have unique genetic compositions (143, 144). In contrast, X-chromosome inactivation studies of sporadic parathyroid adenomas, gastrinomas, gastric-NETs and MTCs indicate that these tumors develop in a monoclonal fashion (145, 146, 147, 148). Multiple intestinal tumor lesions have been observed in about 20% of SI-NET patients (149). Whether these are the consequences of independent tumor formation or metastatic spread remain to be settled (144, 145). Recent studies highlighted that a subset of SI-NETs show pronounced genetic heterogeneity within and between tumor lesions (136, 138). Korpershoek *et al*. (150) determined, by using LOH markers, that there is genetic heterogeneity within different areas of selected PCCs and PGLs. Furthermore they recognized that adrenal medullary hyperplasia in MEN2 patients is in fact a precursor lesion for PPGL (151). Widespread genetic heterogeneity within these tumors as well as and between paired lesions has been recently shown (152). Of particular notice was the observed differences between paired primary and metastatic tumor lesions as well as indication of parallel evolution of different metastatic clones (152).

Authors perspectives and future implications

Precision medicine, where the unique properties of patients and tumors are used to tailor diagnostic and therapeutic procedures is now the standard of care of selected cancer types. For instance in carcinomas of the lung and gastrointestinal system knowledge of tumor mutation status is used to predict the response of treatment and detect the emergence of resistance during treatment with kinase signaling inhibitors (153, 154). The concept of precision medicine has also been useful in the management of patients with NETs, mainly for identification of those with genetic syndromes, enabling genetic counseling followed by appropriate diagnostic measures. However, knowledge of tumor mutation status is not yet used on a routine basis in NETs. Ongoing research is currently investigating whether specific *RET* mutations could predict the success of treatment with receptor kinase inhibitors (NCT01945762) in MTC. Another hypothesis that needs to be tested is that mutations in genes involved in mTOR signaling could be used as biomarkers of rapalogue response (155, 156).

Recent times also saw the publication of experimental findings in NETs that could be important for the

development of future therapies: both combination therapy with *FAK* and mTOR inhibitors (157) as well as inhibition of B-catenin in MEN1 deficient pancreatic NETs (158). Immunotherapy is also an emerging branch in cancer treatment where the knowledge of tumor genetics and biology has proved to be important for prediction. Through determination of the degree of genomic instability and the neo-antigen immunoreactivity the success of PD-1 and CTLA-4 blockade may be estimated (159, 160). On the experimental level mapping the unique somatic mutations present in a particular tumor can also be used to design personalized tumor vaccination protocols (161). Furthermore, genetic instability could be targeted in *ATRX* or *DAXX* mutated tumors as they harbor the unique alternative lengthening of telomeres phenotype. Recent experimental data suggest that such tumors are sensitive to ATR inhibition (162).

However hypotheses based on extrapolation of data from basic NET research needs to be thoroughly tested in a clinical setting. There is an on-going debate whether currently available NET models are representative for human NET disease, supported by the discrepancies of the genomic landscape between NETs and commonly used cell lines (163, 164). Interpretation of basic science data must therefore be made with caution.

In order to screen for genetic disturbances in the established NET genes in a cost effective manner, reliable protocols for novel deep sequencing techniques should be implemented (165, 166). These techniques could also facilitate improvement of diagnostic yield of patients with suspicion of heritable disease. It has also been proposed that deep sequencing can improve the sensitivity in the detection of somatic mutations, especially in scenarios with a low tumor cell fraction (167). Current strategies to analyze tumor genomes are limited by the need of sufficient amount of good quality tumor tissue. In the future peripheral blood samples (liquid biopsies) containing either circulating tumor cells or circulating cell free DNA might instead be used for recognition of the genomic landscape of the individual tumors (168, 169).

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the review.

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