Molecular challenges of neuroendocrine tumors (Review)

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Abstract. Neuroendocrine tumors (NETs) are a very heterogeneous group that are thought to originate from the cells of the endocrine and nervous systems. These tumors develop in a number of organs, predominantly in the gastrointestinal and pulmonary systems. Clinical detection and diagnosis are reliable at the late stages when metastatic spread has occurred. However, traditional conventional therapies such as radiation and chemotherapy are not effective. In the majority of cases even surgical resection at that stage is unlikely to produce promising reusults. NETs present a serious clinical challenge, as the survival rates remain low, and as these rare tumors are very difficult to study, novel approaches and therapies are required. This review will highlight the important points of accumulated knowledge covering the molecular aspects of the role of neuroendocrine cells, hormonal peptides, the reasons for ectopic hormone production in NET, neuropeptides and epigenetic regulation as well as the other challenging questions that require further understanding.

Contents

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1. Neuroendocrine tumors, neuroendocrine cells and neuropeptides

Neuroendocrine tumors (NET) are neoplasms originating in the hormone producing cells of the endocrine system, which is combination of hormone producing endocrine and nerve cells, generally from the neural crest, neuroendocrine islets and stem cells. NETs can manifest functional and nonfunctional symptoms and represent a heterogenous group of neoplasm, such as multiple endocrine neoplasia (MEN), type 1 and 2 medullary thyroid carcinoma, pheochromocytomas/paragangliomas (1-3), gastroentheropancreatic NETs (GEP-NETs) and islet cells (4-10), poorly differentiated/small cell/atypical lung carcinoids (11-17), merkel cell carcinoma (18-21).

NETs are sometimes called carcinoid tumors. Surgical resection alone is often curative in patients with early-stage disease. However, patients with advanced disease may suffer from complications of uncontrolled hormone secretion and usually succumb to fatal complications caused by secreted hormones, but mostly due to tumor progression. Patients with advanced NETs has median survival of 33 months (22). The gene expression profiles proved to be extremely helpful to correlate with tumor classification patterns and correspond to WHO nomenclature. Molecular profiling can identify whether it is malignant pancreatic NETs (pan NETs), PNET or gastrointestinal NETs (GI-NET). The progress with molecular profiling also revealed many important gene targets, among them promising platelet derived growth factor receptor (PDGFR) and RET protooncogene (RET) as new therapeutic targets (23).

While there is vast body of literature covering NETs, there is still confusion sometimes when it comes to grading, nomenclature and classification (24-31).

These tumors synthesize and secrete peptide hormones. Listed here are the most common peptide receptors reported in NETs: Somatostatin receptors, vasoactive intestinal peptide/pituitary adenylate cyclase activating peptide family receptors, cholecystokinin/gastrin receptors, bombesin/gastrin releasing peptide receptors, neurotensin receptors, substance P receptors, neuropeptide Y receptors, calcitonin/calcitonin gene-related peptide receptors, atrial natriuretic peptide receptors, glucagon-like-peptide-1 receptors, oxytocin receptors and endothelin receptors 5-hydroxytryptamine, serotonin 5-HT and neuropeptides (32), which can manifest very serious side effects in malignant tumors like heart failure, palpitations and diarrhea. Secretion of the neuropeptides, which can influence metastatic growth and invasiveness is a

Table I. Molecular signaling pathways in neuroendocrine tumors.

Author, year	Description	(Refs.)
Capdevila *et al*, 2014	The importance for tumor stroma interactions is described for NETs along with such pathways as AXX/ATRx, MEN, PI3K/AKT/mTOR pathways.	(66)
Jones *et al*, 2008	Core pathways highlighted for pancreatic cancer GTPase-tumorigenesis: KRAS pathways, TGF-β signaling; integrin and dependent	(67)
Fazio *et al*, 2014	signaling RAF signaling in neuroendocrine neoplasms.	(68)
Gómez *et al*, 2011	RET pathway was proved to be characteristic for medullary thyroid carcinoma, neuroendocrine tumor derived from parafollicular cells of the thyroid gland.	(69)
Cristea and Sage, 2016	This paper presents the evidence that high levels of RAF/MEK/ERK pathway activity may be detrimental to SCLC tumors including in part by interfering with their neuroendocrine fate.	(70)
Jochmanová *et al*, 2014	HIF-alpha signaling has also been shown to be upregulated in neuroendocrine tumors.	(71)
Trobridge *et al*, 2009	A combination of inactivation of the TGF-β signaling pathway intestinal neoplasms through a β-catenin-independent and expression of oncogenic K-Ras led to formation of invasive pathway.	(72)
Brambilla and Gazdar, 2009	The main signaling pathways that could provide roadmaps for therapy include the following: Growth promoting pathways (EGFR/Ras/PI3K) (p53/Rb/P14(ARF), STK11), apoptotic pathways (Bcl- 2/Bax/Fas).	(73)
Vlotides *et al*, 2014	AMPK-dependent and AMPK-independent pathways involvements and mTORC1 signaling is described for neuroendocrine tumors.	(74)
Chen *et al*, 2014	Authors characterize potential involvement of Erk/MAPK signal transduction pathway of NSCLC in the association with neuroendocrine tumors.	(75)
Sriuranpong *et al*, 2001	Active Notch proteins also led to dramatic reduction in hASH1 expression, as well as marked activation of phosphorylated extracellular signal-regulated kinase (ERK)1 and ERK2, findings that have been shown to be associated with cell cycle arrest in SCLC cells.	(76)
Lee *et al*, 2002	Two distinct signal transduction pathways have been identified for IGF-1R. One pathway activates Ras, Raf, and MAPK, the main mitogen-conducting pathway, and the other pathway involves PI3K, which is responsible for antiapoptotic signal transduction.	(77)
Cortez *et al*, 2016	Signaling through the PDGF-DD/PDGFRβ axis is described for pancreatic neuroendocrine tumors.	(78)
Kunnimalaiyaan and Chen, 2007	The review is focused on the tumor suppressor role of Notch-1 signaling in neuroendocrine tumors (NETs) such as carcinoid and medullary thyroid cancers.	(79)
Cakir *et al*, 2010	The antiproliferative action of somatostatins activated phosphotyrosine phosphatase and their action on MAPK and PI3K/Akt pathways is described.	(80)
Zarebczan and Chen, 2010	Signaling mechanisms in neuroendocrine tumors as targets for therapy are discussed.	(81)
de Groot *et al*, 2006	RET proto-oncogene gene encodes a tyrosine kinase receptor, which is a single transmembrane receptor with a cysteine rich extracellular domain and two intracellular tyrosine kinase subdomains. Several previously discussed pathways such as PI3K-Akt and Ras/Raf/ERK/MEK have been known to interact with the RET pathways.	(82)
Shen and Abate-Shen, 2010	FGF signaling was reported to contribute may provide a mechanism for the activation of ERKMAPK pathway activity observed in prostate cancer progression.	(83)
Younes *et al*, 1997	Studies of archival tissues of adenocarcinomas and carcinoid tumors showed that K-ras mutations play an insignificant role in the pathogenesis of jejunal/ileal adenocarcinomas and carcinoid tumors.	(84)
Ravi *et al*, 1998	MAPK activation by DeltaRaf-1:ER, in this study, was shown to activate growth inhibitory pathways leading to cell cycle arrest, suggesting that Raf/MEK/MAPK pathway activation, rather than inhibition, may be a therapeutic target in SCLC and other neuroendocrine tumors.	(85)
Ravi *et al*, 1999	This paper showed DMS53 cells undergo differentiation and G1-specific growth arrest in response to Ras/Raf/Mitogen-activated protein kinase kinase (MEK)/mitogen-activated protein kinase (MAPK) pathway activation.	(86)

Table I. Continued.

Author, year	Description	(Refs.)
Sippel *et al*, 2003	Raf-1 induction was shown to suppress a neuroendocrine marker and hormone production in human gastrointestinal carcinoid cells via a pathway dependent on MEK activation.	(87)
Van Gompel *et al*, 2005	Treatment with ZM336372 (a novel Raf-1-activating agent) was shown to reduce bioactive hormone levels and transcription factors within carcinoid tumor cells, as well as, suppress cellular proliferation due to induction of cell cycle inhibitors p18 and p21.	(88)
Kim *et al*, 2002	This study proposes that raptor is a missing component of the mTOR pathway that through its association with mTOR regulates cell size in response to nutrient levels.	(89)
Sancak *et al*, 2007	Two general claims were made: i) the relative strengths of the rheb-and PRAS40-mediated inputs to mTORC1 set overall pathway activity; ii) insulin activates mTORC1 through the coordinated regulation of both.	(90)
Villaume *et al*, 2010	This study points out to the complex regulation of VEGF synthesis and secretion in neoplastic GEP endocrine cells and suggests that the inhibition of VEGF production by octreotide and rapamycin may contribute to their therapeutic effects.	(91)
Couderc *et al*, 2011	Authors of this paper claim that the antitumor efficacy of rapamycin in neuroendocrine tumors results from a ombination of antiproliferative and antiangiogenic effects.	(92)
Vivanco and Sawyers, 2002	A review article on the impact of PI3K on tumor progression and small-molecule therapeutics that affect multiple aspects of tumor cells phenotypes via blocking of PI3K signaling.	(93)
Krystal *et al*, 2002	This paper showed that PI3K-Akt signaling promotes SCLC growth, survival, and chemotherapy resistance.	(94)
Pitt *et al*, 2009	PI3K/Akt signaling performs a critical role in human carcinoid tumor cell survival and neuroendocrine hormone generation	(95)
Hara *et al*, 2002	This paper showed that raptor is an essential scaffold protein for the mTOR-catalyzed phosphorylation of 4EBP1 and mediates TOR action *in vivo*, contributing to mTOR-medicated cell growth.	(96)

AXX-ATXx, ATP dependent DNA translocase, transcriptional regulator; MEN, multiple endocrine neoplasia, menin pathway; PI3K/AKT/mTOR, phosphatidylinositol 3 kinase/protein kinase B/mammalian target of rapamycin; KRAS, Kirsten rat sarcoma viral oncogene; TGF-β, transforming growth factor β; GTPase guanine triphosphatase; RAF, Rapidly accelerated fibrosarcoma serine threonine kinase; RET, Rearranged during transfection pathway; MEK/ERK, mitogen activated kinase, kinase/extracellular; ARF, alteration of p14; STK11, serine threonine kinase 11, BCL-2, B cell lymphoma, 2 pathway; hASH1, achaete scute homolog 1 HIFα, hypoxia inducible factor 1 α; AMPK, -5 Amp activated protein adenosine monophosphate activator; MAPK, mitogen activated protein kinase cascade; FGF, fibroblast growth factor; SCLC, small cell lung cancer; RAS, family of related proteins; NOTCH1, notch homolog 1; PDGF, platelet derived growth factoor; IGF1, insulin growth factor1.

very characteristic feature for NETs. It comes as no surprise that these secreted peptides usually correspond to their normal counterparts. However, in certain tumors like ovarian tumors there are neuroendocrine cells, while in the corresponding normal ovary they are not present (33). The cells of well differentiated NETs produce abundant neurosecretory granules, with diffuse expression of neuroendocrine markers such as chromogranin and synaptophysin. There are differences between well differentiated NETs, which can be either low or intermediate grade, and poorly differentiated NETs that are aggressive and considered high grade, with less resemblance to the normal non transformed cells (31). It is important to understand the difference between differentiation and grade. Differentiation defines how much the neoplastic cells resemble their non-neoplastic counterparts, but the aggressiveness of tumor determines its grade.

There are three hypotheses explaining the occurrence of NETs (34). The first hypothesis is based on the assumption that these neoplasms derive from mature neuroendocrine cells that undergo a dedifferentiation due to occurrence of mutations. The second hypothesis assumes they derive from the progenitors of the neuroendocrine cells that undergo mutations. The third one states they can derive from non neuroendocrine cells that acquire neuroendocrine characteristics during carcinogenesis due to the loss of certain genes.

2. Paraneoplastic syndromes, ectopic hormone secretion

Paraneoplastic syndromes (PNSs) are syndromes secondary to substances like hormones, growth factors, cytokines secreted from tumors not related to their specific organ or tissue of origin. The term ectopic hormone syndrome defines hormone production by tumors, which in normal conditions do not carry that function. Ectopic hormone production is only associated with endocrine type of secretion and not with any other type, the earlier PNS is recognised, the better, not only for the correct treatment option but also not to consider PNS as metastatic disease (35-37). There are two theories explaining such a phenomenon (38,39). The pluripotentiality concept is the core of the first theory. The genetic derepression of the genetic material capable to synthesise necessary proteins takes place during cancer development. The second, alternative theory based on assumption that these particular tumors arise only from specialized cells, which have the capacity to produce neuropeptides. Most common tumors are those producing corticotropin-releasing hormone (CRH) and adrenocorticotropic hormone (ACTH), accompanied by Cushings syndrome (40). The tumors which produce growth hormone-releasing hormone (GHRH) with acromegaly symptoms are very rare (41,42). Ectopic antidiuretic hormone (ADH) secretion was also reported (42,43). The tumor derived hormones are those found in the central nervous system, gastrointestinal tract anterior pituitary [iACTH, lipotropin, somatostatin, calcitonin, gastrin, human chorionic gonadotropin (hCG), placental lactogen derived from the fetoplacenta]. Their association with certain placental enzymes and fetal proteins (i. e., carcinoembryonic antigen and o-fetal protein have been used to support the concept of arrested differentiation of tumor cells as the basis for hormone production (44,45).

Pan NETs, islet cell tumors, are rare and originate in the pancreas from endocrine tissue can secrete vinsulin, gastrin, glucagon, and vasoactive intestinal peptide (VIP), which can cause multiple clinical syndromes (46-49).

3. Neuropeptides and cancer

Hypothalamic neuropeptides manifest many endocrine, functions in various tissues (50). Growth hormone releasing hormone (GHRH) was isolated from human pancreatic tumors and then identified in human and animal hypothalamus. It was suggested by Nobel Prize laureate Dr Andrew Schally and coworkers that GHRH may function as a growth factor among a large class of mitogens involved in tumorigenesis. This group developed the successful antagonists of GHRH, which were able to inhibit proliferation of number of cancer cell lines (51). GH-RH antagonists, might be beneficial for at least a subset of patients with non small cell lung carcinomas that express GH-RH and insulin growth factor receptor (IGF-I) receptors and are dependent on autocrine stimulation by GH-RH and/or IGF-I (52). Peptide hormones can influence the development and growth of many cancers which are not considered classical hormone-dependent tumors. Analogs of somatostatin, bombesin/gastrin-releasing peptide (GRP), luteinizing hormone-releasing hormone (LH-RH) and GHRH can interfere with receptors on tumor cells or intracellular pathways that are important in cell proliferation and in this way inhibit tumor growth (53). The expression of five subtypes of G-protein-coupled transmembrane somatostatin receptors (SSTRs) is very characteristic feature for NETs (54). Octreotide and lanreotide are somatostatin analogs proved to be useful in alleviation of of flushing and diarrhea which are associated with NETs secretion (55,56). The antitumor effect of somatostatin analogs has been established in many clinical trials (57-59).

Somatostatin analogues comprise a significant part in the therapeutic strategy of metastatic NETs. They exert their inhibitory effect by activating somatostatin receptors (which are expressed in about 80% of well-differentiated NETs. The advantage of these analogs compared to natural somatostatin, is their significantly longer half-life, permitting monthly subcutaneous administration (60). Among the 5 types of SSTRs, SSTR2 is the predominant receptor in NETs (61). The SSTR 2 has the highest density and proved to be associated with overall survival (62-64). Pasireotide (SOM230), a novel somatostatin analog also was reported to have antitumor properties (65).

4. Signaling pathways and NET

There are several pathways like mTOR, PI3K-Akt, Ras/Raf/MEK/ERK, Notch pathway and others, which regulate the proliferation of neuroendocrine cancers (Table I) (66-96). Targeting the mTOR pathway, downstream from PI3K-Akt and the Ras/Raf/MEK/ERK pathways has emerged as an effective treatment strategy in the management of advanced NETs. Treatment of carcinoid cells with the mTOR inhibitor, rapamycin, has been shown to decrease tumor growth both *in vitro* and *in vivo* (97). Two rapamycin derivatives, temsirolimus and everolimus, have been tested in multicenter, phase II clinical trials on patients with NETs with some promising

results. The everolimus plus octreotide combined therapeutic treatment demonstrated antitumor effects which is capable to target upstream and downstream key players of mTOR pathway (83-86,98-103). The mTOR complex 1 (mTORC1) inhibitor everolimus and the multikinase (including vascular endothelial growth factor receptor (VEGFR) inhibitor sunitinib were approved by FDA for the treatment of metastatic pNET [Food and Drug Administration, *SUTENT®* (sunitinib malate) prescribing information: http: //www. accessdata. fda. gov/drugsatfda_docs/label/2011/021938s13s17s18lbl. pdf].

Combination strategy of dual RAS, PI3K/mTOR and MEK inhibition proves to be as an effective treatment for NETs, thus securing the occurrence feedback loops (104). Imatinib is an orally available phenylaminopyrimidine analog which specifically inhibits tyrosine kinase activity associated with c-kit, PDGFR-α, PDGFR-β, and BCR-ABL (105). In laboratory setting Imatinib inhibited cell proliferation and induced apoptosis in both c-kit-positive and c-kit-negative neuroendocrine cells, however it failed in clinical trials (106-108). Experience with the small molecule EGFR tyrosine kinase inhibitor (TKI) gefitinib is an example of thwarted rational target choice. EGFR is over-expressed in NETs, and EGFR inhibitors reduce growth in carcinoid cell lines. Gefitinib in phase II study demonstrated only one positive radiological response among forty patients with carcinoid tumors (109).

Immunoblot analysis revealed that tyrosine kinase target PDGFR-α and -β were expressed in PNETs regardless of stage. More importantly, PDGFR-β was activated by phosphorylation in the majority of PNETs. Others have reported high levels of expression of PDGFR-α, PDGFR-β, and c-Kit in PNETs, but no assessment of receptor activation has been previously performed (110). In recent years there is body of data supporting important role of targeting Hedgehog, TGF-β, Notch pathway when considering options for pan NETs treatment. NOTCH1/Achaete-Scute Complex-Like 1 (ASCL1) conserved pathway plays an important role in embryonic development. Its primary role is to ensure proper stem cell maintenance and terminal differentiation. Notch proteins are comprised of four 300 kDa transmembrane receptors and five ligands. Notch induced apoptosis in NETs as it was not the case for epithelial tumors. Indeed, in several publications the tumor suppressor function of Notch, its proapoptotic or its minimal activity is indicated for NETS (111). The phosphatidylinositol 3-kinase (PI3K)/Akt/mammalian target of rapamycin (mTOR) pathway plays very important role in the pathogenesis of pNETs.

NVP-BEZ235, OSI-027 and AZD2014 are novel drugs that target PI3K-AKT-mTOR pathway. BEZ235 is a potent oral multitargeted inhibitor PI3K isoforms and the downstream, mTORC1 and mTORC2 proteins. This drug had much higher activity in NETs than everolimus or its combinations (112). NVP-BEZ235 docks in the active pocket of both molecules and reduces kinase activity of PI3K and mTOR by competing with ATP-binding. Dual PI3K-mTOR blocker NVP-BEZ235 and AZD2014, which is ATP-competitive mTOR blocker were able to overcome this rapalogue resistance (113). NVP-BEZ235 binds to the active pocket of of both molecules and by competing with ATP binding inhibit PI3K and mTOR.

In xEric Baudin. The Ras/Raf/MEK/ERK mitogen pathway can act as, an oncogene, and or tumor suppressor in NETs (114-116). Important to mention the impact of antiangiogenesis inhibitors on NET treatment, for example, VEGFR significantly reduce pancreatic tumor growth or cause regression of established tumors in treated mice, compared with controls, and also disrupt tumor vasculature (117,118). Interestingly, the mutation rate of NET is significantly lower than for other types of cancer, suggesting that they are more genetically stable. Though frequency of mutations had tendency to increase with higher grade, the classical tumor suppressors (like p53, Rb) implicated for tumor development in other tumors, do not play significant role in NET pathogenesis (119,120).

Although mTOR-inhibition leads to significant improvement of progression-free survival in advanced pan NETs, the drug resistance to mTOR inhibition continue to dominate as a major clinical challenge like in many other types of tumors.

5. Epigenetics in neuroendocrine tumors

Experimental data suggests that epigenetic programs, such as chromatin and DNA modifications, pre- and posttranscriptional gene regulations by noncoding RNAs are actively involved in changes in gene expressions as a result of stem cell differentiation. Usually self renewal genes are silenced in the differentiating cells, while cell specific genes are very transcriptionally active.

The hypermethylation of RASSF1A promoter was demonstrated to be increased in metastatic tumors (121). DNA methylation at RASSF1A was correlated to worse prognosis in NETs (122,123). Rather than having K-RAS or BRAF mutations, well differentiated NETs have methylation in RASSF1A gene, observed in pancreatic, pulmonary and gastrointestinal tumors (122,123). MEN1 and Daxx/ATRX are part of chromatin modifying complexes the most frequently mutated genes in NETs and mutations of menin and Daxx/ATRX are not mutually exclusive in the same tumor. Tumor suppressors, menin and Daxx, reported to suppress NETs by interacting with each other and epigenetically inhibiting a pro-proliferative gene in endocrine tumors, Mme, via enhancing H3K9me3 modification. MEN1 can interact with histone deacethylases (HDACs) and histone methyltransferases including PRMT5 and SUV39H1, and depending on that act either as activator or supressor of gene transcriptional activity (124-127). Menins role as the regulators in Hox gene expression was well documented (128,129). On the other hand more data is needed to understand the effect of such regulation.

MEN1 was capable of inducing epigenetic modification in pancreas and insulin-like growth factor 2 mRNA binding protein 2 (*IGF2BP2*) gene was identified as a target subjected to MEN1 regulation. One of the existing theories stating that the loss of MEN1 can play a role in pNET pathogenesis (130). Menin recruits the H3K4me3 histone methyltransferase mixed lineage leukaemia (MLL1) complex, which is utmost important for chromatin remodeling and gene expression regulation (131-133).

In tumors with poor prognosis the promoter hypermethylation was found for such genes as *DAPK1*, *TIMP3*, *PAX5*, *HIC1*, *CADM1*, and many others (134).

It was postulated that chromatin-remodeling pathways was sufficient to drive early oncologic event, given the absence of any other type of cancer mutations and presence of mutations

of mutations of SWI/SNF complex members, such as ARID1/2 and SMARCA1/2 (135). DNA damage repair, chromatin remodelling, telomere maintenance and mTOR signalling (136,137) were reported as common mutated pathways for PanNET based on genome sequencing analysis. Like in many other tumors hyperactivation of the Wnt/β-catenin signaling contributes pNETs progression. The epigenetic silencing via promoter methylation of Wnt inhibitors like Axin 2 and secreted Frizzled-related proteins (*SFRPs*), Wnt inhibitory factor-1 (*WIF-1*) and DICKKOPFs (*DKKs*) were reported, wheras donwregulation of others, such as *WIF-1*, *DKK-1* and *DKK-3* were caused by H3K9me1 increased levels at the promoter (138).

MicroRNAs signatures and histone modifications can be very helpful when it comes to diagnostic uncertainty, whether to identify the subtypes of NETs. One of the attractive features of epigenetic changes is their reversibility, which makes it very appealing as therapeutic targets. miRNAs can serve as biomarkers to distinguish between normal and diseased tissue, miRNAs 103/107/155, for example, can distinguish pan NET from normal pancreatic tissue. Some of miRNAs miRNA-21 and -155 can be upregulated in high grade tumors and not in low grade, others can be detected in metastatic low grade tumors and not normal tissues (139).

Differential gene expression of miRNAs is not a stranger for many tumors, and NETs are not constituting exception. Indeed, miR-183, -488, and 19a+b were upregulated while miR-133a, -145, 146, -222 and -10b were downregulated in metastatic tissue with respect to primary tumors in study with ileal carcinoid tumors (140). Intestinal NETs had different expression profiles of miRNA during different stages of the disease (141). Literature data suggests that miR-129-5p may have an anti-proliferative and anti-metastatic effect in midgut carcinoid tumors (142).

6. Conclusions

NETs are arising from neuroendocrine cells. This process should not be confused with phenomenon of occurrence of neuroendocrine cells in non- neuroendocrine neoplasm as a reflection of heterogenous neuroendocrine differentiation in neoplasms. Neuroendocrine cells occur also in tumors which developed in tissues where neuroendocrine cells are not found (143,144). The picture cannot be complete without understanding the involvement of key signal transduction pathways in pathogenesis and therapeutic response of these tumors. One of the predominant pathways is mTOR pathway that was highlighted in this review. The presence of the feedback loops though has to be seriously considered when it comes to combination treatment of somatostatin analogues and mTOR inhibitors (145,146). There still obvious challenges when it comes to drug resistance in clinical trials setting. Somatostatin analogues did not overcome everolimus-induced Akt upregulation. As it is known mTORC inhibitors like everolimus can trigger feedback loops. When used alone, they were able to induce apoptosis, but that effect was lost in combined treatments. Thus, based on the evidence there is no indication of beneficial effect in NETs for cotreatment with everolimus (146). The resistance of treatments also can be explained by presence of the cancer stem cells, which is very controvercial issue for NETs and there is not much evidence for the presence of CSC in these tumors, however some of dual targeted therapies reported to prevent drug resistance (147-151). There is evident lack of reliable biomarkers for correct treatment selection, although there are some for specific NETs, like CDX-2, for example, which is highly specific for metastatic and ileal NETS (14,152). Ki-67 was identified as prognostic biomarker for pancreatic tumors (153-155). There are many open questions and challenges concerning pathogenesis and molecular events leading to NETs and PNS. One of the challenging and unresolved questions is whether ectopic hormone syndrome occurs as a consequence of hormonal secretion gene activation because of malignant trasformation or can be attributed to the intrinsic ability of the primitive cell of origin for this function that was arrested in differentiation process (37-157). The aspect requiring thorough investigation is the modulation of tumor cell behavior by neurohormonal peptides, secreted by neuroendocrine cell population. It still remains to be elucidated how much genetic, epigenetic and chromosomal alterations, can affect the expression or function of the neuropeptides receptors. Unveiling receptor dynamics, density, metabolism or trafficking may help to better understand and predict the effects of analogs in diagnostics and therapy (32). Estimation of neurokinin A levels is assumed to be very useful for more aggressive NETs in their ealy stages. Poor short term survival was reported with neurokinin A concentrations (>50 pg/ml) (158). Uniform expression of Angiopoietin-2 (Ang-2) messenger RNA (mRNA) described in endothelial cells of both nontransformed pancreatic tissue and pan NET tissue (159).

The management of neuroendocrine neoplasia is challenging difficult problem, the molecular pathways involved in the pathology of NETs waiting to be explored further and to develop new synergistic treatments impacting prognosis and patients well being (160-162).

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