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Review

Updates on the role of molecular alterations and NOTCH signaling in the development of neuroendocrine neoplasms

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Abstract: Neuroendocrine neoplasms (NENs) comprise a heterogeneous group of rare malignancies mainly originated from hormones secreting cells, which are widespread in human tissues. The identification of mutations in ATRX/DAXX genes in sporadic NENs, as well as the high burden of mutations scattered throughout MEN-1 gene in both sporadic and inherited syndromes, provided new insights into the molecular biology of tumour development. Other molecular mechanisms, such as the NOTCH signaling pathway, have shown to play an important role in the pathogenesis of NENs. NOTCH receptors are expressed on neuroendocrine cells and generally, act as tumour suppressor proteins, but in some contexts can function as oncogenes. The biological heterogeneity of NENs suggests that to fully understand the roles and the potential therapeutic implications of gene mutations and NOTCH signaling in NENs, a comprehensive analysis of genetic alterations, NOTCH expression patterns and their potential roles across all NEN subtypes is required.

Keywords: Neuroendocrine Neoplasms; NOTCH; cancer driven genes; mutational mechanism; germline mutations.; small cell lung carcinoma; pancreatic NET; small bowel NET; medullary thyroid carcinoma; malignant castration-resistant prostatic cells

1. Introduction

Neuroendocrine cells are sensor cells, which play an important role in the connection between nervous system and endocrine organs. In response to neurogenic stimulation, neuroendocrine cells secrete several molecules, including peptide hormones, which produce slow and long-lasting effects. The neuroendocrine cells are widely scattered throughout the human body, such as the gastro-entero-pancreatic tract, uro-genital apparatus, lung, breast, skin, as well as the central and peripheral

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nervous system. These cells are able to dedifferentiate and transdifferentiate under physiological conditions in response to intracellular metabolic pathways and microenvironmental stress conditions [1].

NOTCH signaling is a highly conserved cell-signaling pathway that is implicated at different stages of development through the regulation of cell proliferation, differentiation and cell death.

At a physiological level, in the neuroendocrine system, NOTCH signaling drives the maturation process of multi-potent cells to become functionally competent cells during the early stage of embryonic neuroendocrine development [2]. For instance, NOTCH signaling regulates the ductal and endocrine differentiation of pancreatic cells during the development of the pancreas [3].

Neuroendocrine neoplasms (NENs) are originated from the neoplastic transformation of neuroendocrine cells at various anatomic locations, being the gastrointestinal tract, the endocrine pancreas and the respiratory tract, the most involved sites. [4].

Little is known about the mechanisms of neoplastic transformation and metastatic dissemination for neuroendocrine cells, but it is known that NENs originating in different organs, despite some common molecular characteristics, have distinct signatures and display significant biological heterogeneity.

In this heterogeneous neoplastic setting, NOTCH pathway has shown to have a role by triggering both tumour suppressor and oncogenic functions in some neuroendocrine cell lines and in different subtypes of NENs [5-10].

The availability of treatments with a modulatory and inhibitory role on NOTCH-dependent pathways, and the possibility to use the molecular alterations as diagnostic and prognostic markers, has highlighted the need a deeper knowledge on the implication of NOTCH pathway and the different molecular signatures in NENs.

This review will summarize the current knowledge on the molecular heterogeneity of NENs and the complex function of NOTCH signaling in different types of NENs, as well as the new therapeutic approaches based on NOTCH pathway modulators.

2. Neuroendocrine Neoplasms and Molecular Heterogeneity

Neuroendocrine neoplasms are genomically and clinically heterogeneous. This heterogeneity occurs between cancers originating from different organs, within the cancers originating in the same organ, and between primary and metastatic lesions [4, 11-13]. For instance, small intestine NETs are genomic stable cancers, with a low mutational load compared with NETs originating from different organs, such as lung and pancreas. Viral-associated Merkel carcinomas have a low mutational burden, in contrast to UV-induced Merkel cell carcinomas [14]. The fully understand of the molecular mechanisms and the clinical significance of this heterogeneity could led to the identification of new hallmarks to target in the neuroendocrine neoplasms treatment.

Current advances in genomic analysis techniques have enabled to identify recurrent mutations and chromosomal aberrations at the base of the molecular landscape of NENs [15,16].

Recurrent mutations have been identified in *MEN1* and *VHL* genes, in chromatin remodelling genes, such as *DAXX* and *ATRX*, in mechanistic target of rapamycin (mTOR) pathway genes, especially in *PTEN*, *TSC2*, and *PIK3CA*, in tumor suppressor gene *CHEK2*, in telomerase maintenance genes, in the cell cycle regulator *CDKN1B* and in the DNA repair gene *MUTYH* [15,16]. These mutations can occur in genetic syndromes, such as multiple endocrine neoplasia 1 (MEN1), tuberous sclerosis complex (TSC1/2), neurofibromatosis type 1 (NF1), and von Hippel–Lindau (VHL) disease, or in sporadic NENS, and can be germline or somatic mutation [15-17].

Genetic syndromes account for 15-20% of NENs, while the remaining 80-85% is sporadic.

Interestingly, as confirmation of the high heterogeneity of NENs, whole exome sequencing analysis performed in different studies has identified only 21 genes in common between the small intestinal NENs samples analysed from different patients [11, 18]. Furthermore, comparing the results of these studies on small bowel NENs with the one on pancreatic NENs, a concordance of only 17 genes with somatic mutations was found [11, 15, 18].

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In addition, some mutations, namely mutations in *MEN1* and *DAXX*/*ATRX* genes, are associated with a better prognosis, and they seem to occur very rarely in poorly differentiated NECs [19]. On the other hand, mutation in *TP53, RB1, PTEN* and *PIK3CA* are more frequent in poorly differentiated NECs [19, 20].

In the following paragraph, we are going to summarise the current knowledge and the clinical significance of the most common genetic alterations in NENs, classifying them in hereditary and sporadic.

3. Common Genetic Alterations and Molecular pathways in the development of Neuroendocrine Neoplasms

3.1 Heritable genetic traits in neuroendocrine neoplasms

NENs comprise at least ten recognized inherited NET syndromes, including multiple endocrine neoplasia type 1 and 2 (MEN-1 and MEN-2), von Hippel-Lindau syndrome (VHL) and neurofibromatosis type 1 (NF1) [21].

The MEN-1 is a rare autosomal dominant syndrome caused by inactivating mutations in the MEN-1 gene, and mostly associated with the appearance of neoplastic lesions in pancreas and duodenum, as well as in pituitary and parathyroid glands [22, 23]. The majority of germline mutations in MEN-1 gene cause the truncation or absence of the menin protein in the cancer cells. Typically, tumour development is associated with the mutation of both MEN-1 alleles, however an incomplete inactivation of this gene has been observed in thymic and duodenal NETs [24, 25]. The menin protein is usually located in the nucleus, cytoplasm and around telomerases. However, its specific biological role has not been described yet [26].

The MEN-2 syndrome is an inherited autosomal dominant disorder comprising MEN-2A (55% of all cases), MEN-2B (5%–10%), and familial medullary thyroid carcinoma (FMTC; 35%–40%) [27]. The MEN-2A and MEN-2B patients have almost 100% risk of developing MTC and about 50% risk of developing pheochromocytoma and parathyroid adenomas. The MEN-2 syndrome is caused by mutations in the RET proto-oncogene, encoding a tyrosine kinase receptor. These mutations cause activation of RAS/MAPK and PI3K/AKT signaling pathways [28], and may occur in two different regions of RET gene, originating two different types of disorders. In addition, the Familial MTC (FMTC) syndrome, which is also caused by RET mutations, is only associated to MTC but is less aggressive than MEN-2 tumours [29].

The MEN-4 is a rare autosomal dominant syndrome predisposing to NETs development, such as parathyroid and pituitary adenomas, associated with the germline mutations in CDKN1B genes encoding p27kip protein [30]. However, more studies are needed to know the penetrance and biological effect of CDKN1B mutations.

The Von Hippel-Lindau (VHL) syndrome is associated with pheochromocytomas, paragangliomas and pancreatic neoplasia and it is caused by the loss of VHL tumour supressor gene, regulating the HIF and VEGF pathways [31-33]. The VHL protein shuttles between nucleus and cytoplasm binding to elongen C, elongen B, Cul2, and Rbx1 and degrading the alpha subunits of hypoxia-inducible factor (HIF) in an oxygen-dependent manner [32 ,34, 35]. Lack of degradation of this factor due to the absence of the VHL protein results, for instance, in an uncontrolled production of factors promoting blood vessel formation (eg, vascular endothelial growth factor) and implicated in tumour development. The germline mutations in the VHL gene are extremely heterogeneous and are spreaded throughout the coding sequence. They are present in virtually all families with VHL, although the exact molecular mechanism of development of NETs in VHL has still many unknowns [36].

The neurofibromatosis type 1 (NF1) syndrome is another familiar NET disorder, which is associated with duodenal NETs or pheochromocytomas and is linked to RAS and ERK/MAPK pathways' deregulations [37]. Genetic alterations of the NF1 gene include missense, nonsense, and splice site mutations, as well as insertions/deletions (in/dels) and chromosomal rearrangements [38].

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Tuberosclerosis gene TSC1 (9q34) and TSC2 (16p13.3) are regulated by neurofibromin through mTOR activation, linking the three proteins in terms of their potential roles in tumour progression [37].

Loss of function of the NF1 gene causes mTOR activation and tumour development. Disruption of TSC2 in pancreatic beta cells induces beta cell mass expansion in an mTOR-dependent manner [39]. Furthermore, it has recently been demonstrated that patients with PNET, and loss of PTEN protein as well as tuberosclerosis 1 protein, show a significant shorter survival [40].

Familial pheochromocytoma and paraganglioma syndromes are autosomal-dominant disorders caused by mostly germline mutations in the succinate dehydrogenase subunit genes, such as SDHB, SDHC, SDHD, SHDA, and SDHAF2 (Succinate dehydrogenase complex assembly factor 2). These are encoding factors required for the assembly of the mitochondrial complex II [32, 33 41-50]. This mitochondrial complex participates in two main cellular processes: Krebs cycle and the electron-transport chain. The mutations in the key components for the formation of complex II make decrease the enzymatic activity of the rest of the complex. The link between the perturbation in complex II and the tumorigenesis has still many unknowns. SDH deficiency leads to pseudohypoxic conditions in cancer cells. However, this fact alone is probably not sufficient to induce the tumorigenic process so different possibilities seems to be feasible, for instance, the implication of ROS or the possibility of the inhibition of other α-ketoglutaratedependent enzymes.

SDH mutations are commonly associated with multiple pheochromocytomas and paragangliomas, however, gastrointestinal stromal tumours, SDH-deficient renal cell carcinoma and pituitary adenomas can also be associated with these mutations [51, 52].

3.2. Genetic alterations and tumour mutation burden in NENs

Several chromosomal alterations and gene mutations have been consistently identified in different types of sporadic NENs, although the tumour mutational burden is relatively low compared to other tumour's types [21]. In fact, massive parallel sequencing showed that only 24 cancer driver genes are affected by non-synonymous mutations in neuroendocrine neoplasms [53]. Remarkably, cancer driver genes and mutations are unevenly distributed in different tumour types and may contribute to the mechanisms of NENs heterogeneity. The factors encoded by these mutated genes may affect several pathways involved in cell proliferation, metabolism and chromatin modification.

The genetic landscape of GEP-NENs confirmed essential differences of mutational profiles between well-differentiated NETs, including those with high proliferation index, and NECs [54].

Mutations in TP53 and RB1 genes are pivotal drivers in poorly differentiated NECs of any anatomical origin [16, 55-58]. Mutations in TP53 gene have been consistently detected in poorly differentiated GEP-NECs with a frequency ranging from 20% to 73% of the tested patients [20, 55, 59, 60]. The presence of TP53 mutations in GEP-NECs correlate with poor survival [20], and recently Ali et al have demonstrated that p53 immunoexpression, in colorectal NECs, correlates with a poorer response to platinum-based chemotherapy and worse prognosis [61]. These results suggest a potential diagnostic, prognostic and predictive role of p53 immunoexpression in GEP-NECs, this role is currently under investigation in different trials.

The inactivation of RB1 gene product, which occurs mainly by somatic mutations, have been reported in 71% of poorly differentiated pancreatic NECs [54].

KRAS mutations have been identified in gastric, pancreatic and colorectal NECs with frequencies ranging from 8% to 60% [20, 54, 60, 62-66].

On the other hand, BRAF mutations were only found in colorectal NECs with a frequency between 13% and 59% [67], as well as APC affecting some cancer cases [68].

The genetic alterations characterizing poorly differentiated NECs are absent in G3 NETs. This subtype presents typical mutations of G1/G2 NETs. For example, G3 Pancreatic NETs showed high frequency of MEN1, DAXX, and ATRX mutations or protein loss (31-44%, 9-25% and 18-36% respectively). Therefore, the scientific community is proposing these mutations as possible

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biomarkers to distinguish G3 Pancreatic NETs from NECs [69]. It has a particular clinical relevance due to the fact of NECs and G3 NETs are detected at an advanced stage.

The molecular similarities between G1/G2 and G3 NETs suggested a new model for GEP-NENs tumorigenesis in which poorly differentiated NECs and well-differentiated NETs, including G3 NETs, were originated from a common-normal neuroendocrine progenitor through different routes [70]. These foresee the alteration of TP53 and RB1 for all poorly differentiated NECs, and specific alterations for well-differentiated pancreatic NETs and small intestine NETs [71].

Despite the remarkable biological heterogeneity of NETs, the mammalian target of rapamycin (mTOR) molecular pathway has been found to be prominently altered in a vast majority of NETs [72]. The mTOR is a kinase-dependent signaling cascade, formed by the mTORC1 and mTORC2 multiprotein complexes, which main function is related with controlling cell growth. Mutations in NF1, TSC2 or PTEN- encoding for key suppressor's genes of this pathway and altered expression of mTOR pathway components are common hallmarks of a great proportion of NETs, wherein these alterations seem to be directly related with tumour development and progression [72].

4. Structure of NOTCH Receptors and NOTCH Signaling Pathway

NOTCH receptors family in mammalian comprises four transmembrane proteins (NOTCH1-4) which are evolutionary conserved with a high homology between different species. NOTCH receptors are activated by trans-ligands expressed on neighboring cells, whereas cis-ligands within the same cell inhibit the NOTCH signaling [73]

The four NOTCH receptor isoforms in mammals are characterized by an extracellular region of repetitive Epidermal Growth Factor-like (EGF-like) sequences which are involved in the interaction with Delta-like ligands(DLL1, DLL3, DLL4) and Jagged (JAG1, JAG2) proteins, by a single transmembrane portion and by an intra-cytoplasmic tail involved in the signal transduction. The expression of these receptors is in a cell and tissue-type specific manner. The interaction of NOTCH receptors with their ligands causes a structural modification in the protein and the cleavage of the intracellular domain (NICD) that moves into the nucleus where it binds to Centromere Binding Factor – 1 (CBF1) complexes, to activate expression of NOTCH-responsive genes.

Genes regulated by NOTCH signaling pathway include the Hairy-Enhancer of Split (Hes1, Hey1, Hey2) encoding the double-helical transcription factors with negative regulatory function, as well as c-Myc and cyclin D involved in cell cycle regulation [74].

The main roles of NOTCH has been associated to the regulation of homeostasis and cell proliferation as well as development and cell differentiation in a variety of tissues. This regulation can occur during both embryonic stages and postnatal life.

The plethora of ligands regulating NOTCH receptors have been extensively studied in different tumour types for their onco-regulatory effects [75-77]. Depending on the biological microenvironment, the activation of NOTCH signaling seems to have a dual role, showing an oncogenic effects in certain tissues (i.e. small cell lung cancer) [78,79], and tumour-suppressor function in others (i.e. medullary thyroid carcinoma, small cell lung cancer, pancreatic and biliary neuroendocrine tumours) [80-83].

5. The role of NOTCH signaling in NENs

Pre-clinical studies showed a heterogeneous expression of NOTCH receptor family in tumoral tissues, and genome sequencing analysis has identified several NOTCH gene mutations in various solid and hematological malignancies [81, 84-86].

The main role attributed to NOTCH signaling pathway is as a mediator of cell differentiation. Depending on the NOTCH receptors expression levels, the cross-talk with other signaling pathways and the cellular context, NOTCH signaling can have a oncogenic or tumor suppressor role [84]. In addition, NOTCH signaling is responsible of the smooth transition from non-neuroendocrine to

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neuroendocrine phenotype, as a result of a coordinated anti-cancer drug response, in pathological cell conditions.

Therefore, to understand completely the impact that NOTCH operates in the development of neuroendocrine tumours, it is required to analyze the NOTCH signaling at different layers of genomic regulation, ranging from gene expression levels to epigenetic alterations, and involving its diverse components as NOTCH receptors and ligands.

In the biggest cancers killers the study of NOTCH pathway was a milestone, and several analyses elucidated its role in pathogenesis. The expression of the different isoforms were exanimated and the presence of mutations was assessed.

In breast cancer tissue,aberrant high levels of NOTCH1 and NOTCH2 was found in comparison with control tissue [87]**.** Moreover, alterations in Notch signaling were also linked to triple-negative breast cancer (TNBC). Mutations were found in NOTCH1-3 at the C-terminal PEST domain and also in the prolyl-isomerase PIN1(Peptidylprolyl Cis/Trans Isomarase, NIMA-Interacting 1) [88], supporting the theory of the involvement of Notch in breast cancer.

In some neoplasms, mutation can contribute to enhance the physiological function of the pathway, as was described in a previous non-small cell lung carcinoma (NSCLC) analysis. In this study it was demonstrated that the presence of a c-terminal mutation in NOTCH-1 gene confers a gain of function, increasing the receptor signaling transduction in NSCLC cancer [89].

In colorectal cancer (CRC), the genomic alteration in NOTCH pathway correlates with clinical outcome: it may lead cells to proliferate without differentiation or to maintain the transcriptional program of normal adult colon cells. A common upregulation of NOTCH-1 gene expression was found in tumoral samples belonging to the three different CRC transcriptional subtypes, characterized by specific transcriptional programs related to normal adult colon, early colon embryonic development, and epithelial mesenchymal transition. This finding is consistent with the critical role of Notch pathway in CRC initiation [90].

Interestingly, a recent study showed that mutation of NOTCH1 in oral squamous cell carcinoma occurs in 15% of Caucasian population, whereas in the Asian population the rate of NOTCH1 mutations was about 50% [91]. This finding emphasizes the need to clarify the NOTCH alteration prevalence in human cancer, even more in rare neoplasms.

In NENs, the NOTCH mutational status assessment has been analyzed only in few studies conducing whole-genome sequencing in specific neuroendocrine neoplasms.

Up-to-date one next generation sequence study was performed on small cell neuroendocrine carcinoma of uterine cervix (SCNEC). Deyin Xing *et al.* found oncogenic driver mutations in KRAS, Erb-B2, c-Myc, BCL6 and NOTCH1 in a cohort of 10 Small cell neuroendocrine carcinomas (SCNEC) of the uterine cervix, a rare but extremely aggressive tumour [92]. In addition, in a cohort of large cells neuroendocrine carcinoma (LCNEC), the most relevant molecular alteration was detected in DLL3, a well-known NOTCH canonical ligand. The DLL3 inhibition in combination with the use of immunotherapy has been also pointed out as therapeutic option for LCNEC [93]**.** And a separate study conducted whole-genome sequencing of Small Cell Lung Cancer (SCLC), identifying inactivating mutations in NOTCH family genes in 25% of cases [58, 94].

In the next paragraphs, we are going to summarize the current knowledges on the epigenetic modifications and NOTCH signaling pathway alteration in different types of NENs.

5.1. NOTCH in NENs : the epigenetic implications

It is conceivable to think in epigenetic changes contributing to the pathological development of the tissue and how these alterations could affect gene expression after stem cell differentiation, as happens in other neoplasms. The epigenetic modifications by definition encompass all the mechanisms that modify the genetic expression and alter the genome stability, without modifying the DNA sequence. These alterations not only can occur at chromatin level, and involve acetylation

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and deacetylation of the histones and the methylation of the cytosine at DNA level, but can also be caused by other molecules like non-coding RNAs, for instance, long non-coding RNAs and microRNAs.

Experimental data suggests that epigenetic alterations are involved in neuroendocrine tumorigenesis [95, 96]. Some pivotal preclinical studies were conducted to explore the role of epigenetic alterations in NETs, obtaining interesting results: silencing regulatory genes (Wnt signaling components) and aberrant mutations in core pathways contributes in NET pathogenesis [96]. Furthermore, missense mutation in the mixed-lineage leukemia protein 3 (MLL3) often trigger aggressive neuroendocrine tumours, medulloblastomas and Merkel cell carcinoma [97] by means of inducing genomic instability.

Moreover, Lysine-specific histone demethylase 1A (LSD1) inhibitor ORY-1001 was described in small cell lung cancer (SCLC) because of its anti-tumorigenic role. This inhibitor activates NOTCH pathway, inhibiting consequently the transcription factor ASCL1 and the repression of the tumorigenesis and the neuroendocrine phenotype in this type of tumours. A complete and long-term tumour regression was obtained after treating with ORY-1001 SCLC patient-derived xenograft (PDX) mice models [98]. Thus, this inhibitor has been suggested as a potential new targeted therapy for SCLC.

Recent findings on transcriptional activation of NOTCH appear to be regulated by means of microRNAs (miRNAs), a small single-stranded RNAs that regulate gene expression post-transcriptionally. Preliminary research about how aberrant miRNA expression can influence neuroendocrine cells behaviors showed a direct post-transcriptional repression of NOTCH2 and RBPJ proteins operated by miR-375 in Merkel cell carcinoma (MCC), a rare cutaneous neuroendocrine malignancy [99]. This small molecule is having an increasing connotation in modern pathology of NET, Arvidsson et al. discovered that miR-375 is highly expressed in small intestinal neuroendocrine tumours and could be used as prognostic biomarker for survival [100].

In the age of precision medicine, the identification of epigenetic biomarkers in a subpopulation of patients could help clinicians to choose the most appropriate therapeutic strategy. Recently, epigenetic drugs are providing promising results in preclinical phases, making attractive the idea of their use in combination with the standard chemotherapy or immunotherapy. However, further validation in clinical trials is needed and side effects have to be assessed for the possible use of these combined strategies [101].

Currently, only few studies are focused on the epigenetic landscape in NET, and even less if we are pointing out to the implications that may occur between these epigenetic factors and one of the main drivers of neuroendocrine differentiation: NOTCH pathway. A coordinated effort between multidisciplinary groups of experts is needed to clarify the role of NOTCH in diverse neuroendocrine neoplasms.

5.2. Role of NOTCH in neuroendocrine tumour of the lung

In lung tissue, the role of NOTCH has been established as driving the differentiation of neuroendocrine cells present in the organ. NOTCH mutation can provoke a dysfunction of its activity and induces neuroendocrine differentiation from no-neuroendocrine progenitors. Clinical data indicate that some neuroendocrine neoplasms of the lung could relapse and present a secondary tumour formation after anticancer therapy.

Recent findings suggest that the presence of inactivating mutations in NOTCH signaling is involved in the pathogenesis of neuroendocrine neoplasms of the lung, being defined as more than a 25% of the cases for small cell lung carcinomas (SCLC) [58, 102]. This fact suggests that NOTCH signaling needs to be inactivated for the development of SCLC. Moreover, NOTCH signaling is involved in the modulation of the neural and neuroendocrine differentiation process, what could mean the implication of mutations in NOTCH in the neuroendocrine features of these tumours, and also in disease progression and relapse.

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NOTCH pathway deregulation has been also pointed out to have a role in chemoresistance in SCLC. The effect of NOTCH in tumorigenesis seems to be done throughout the activation of the delta-like protein 3 (DLL3). Its expression is directly correlated with ASCL1 transcription factor, whose was found expressed in the 85% of SCLCs, in contrast with an absent or minimal expression in normal lung tissue.

In the mixed forms of small cell carcinomas, the modulation of the NOTCH system demonstrated the importance of this pathway in tumorigenesis and response to treatment: the activation of NOTCH reduces the particularly aggressive neuroendocrine subtype by increasing the epithelial component with slower cell proliferation rate, whose growth can be controlled with chemotherapy [79].

Summarizing, NOTCH signaling pathway acquires a tumour suppressive role in neuroendrocrine lung cancers. It could be interesting to explore the possible therapeutic strategies restoring the expression of NOTCH mutated components in SCLC.

5.3. Role of NOTCH in neuroendocrine Gastro-Entero-Pancreatic Neuroendocrine Neoplasms (GEP-NENs).

Gastrointestinal (GI) tract and the pancreas are two of the most common sites of origin for NENs. Tumours arising from these organs are named gastroenteropancreatic NENs (GEP NENs) and they represent almost the 65% of all NENs. Previously GEP-NENs were considered as a unique group of tumours but currently, many studies have highlighted the biological and molecular differences between pancreatic and GI NENs, as well as between the GI NENs originating from different organs of the GI tract [11, 15, 16, 18]. Wang et al have confirmed this heterogeneity also in the NOTCH signalling pathway [103]. They demonstrated a uniform immune-histochemical expression of NOTCH1 and HES1 in well-differentiated rectal NENs, respectively 100% and 64%, whereas only 34% and 10% of well-differentiated pancreatic NENs were positive for NOTCH1 and HES1 at immunohistochemistry, and all ileal NENs were negative to both, suggesting a possible different role of NOTCH1 in the pathogenesis of these cancers. [103].

The majority of the available studies have evaluated the role of NOTCH signaling in pancreatic NENs, thus there is a lack of knowledge on the role of NOTCH signaling in the others GEP-NENs.

In pancreas, endocrine and exocrine cells move from a common pool of multipotent progenitors into differentiated state, coordinately regulated by different mechanisms, forming together a complete and functional adult organ. After the initial developmental phase, the epithelium start to spread pancreatic progenitor cells into different compartments: acinar cells migrated into the tips, and ductal and endocrine cells into the trunk. Endocrine cells leave the adjacent epithelia by delamination, assembling into islets of Langerhans. During the differentiation process, the mechanism of differentiation is not synchronous and it is controlled by several regulatory agents, such as NOTCH receptor that has an important role in early developmental embryologic phase as well as in adult plasticity.

In aggressive tumours of the pancreas like pancreatic ductal adenocarcinomas (PDAC), the tumour is believed to derive from a pancreatic intraperitoneal neoplasia (PanIN). In these cases, Nocth plays a dual role in the tumour initiation and development: NOTCH works as a tumour suppressor in PanIN lessions [104] and later on it has an oncogenic role in PDAC [105]. Even more, these studies indicate not only this dual role of NOTCH pathway in tumorigenesis, but also the implication of several pathway components, revealing a complex fine-tunning regulation of NOTCH pathway.

Moreover, histo-pathological studies had shown that the NOTCH1 is absent or lowly expressed in well-differentiated pancreatic NET (pNET) [8, 103]. However, in MiNEN (mixed neuroendocrine/non neuroendocrine neoplasm), particularly aggressive neoplasms, in the same tissue the expression of NOTCH1 and Hes1 is reduced or absent in the NET cells but present in the adenomatous component [106] what could indicate a possible role of NOTCH as a tumour suppressor

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gene. Further studies are needed characterizing the molecular mechanisms implicated in neuroendocrine tumorigenesis and for understanding the functional differences observed within pancreatic tumours.

In ileal NENs, the low or absent expression of NOTCH and HES1 has led to hypnotize a possible tumor suppressor role of the NOTCH signalling [103]. As confirmation of this hypothesis, Maggi et al. [107] demonstrates that RBP2, a key component of the NOTCH repressor complex, is upregulated in gastrointestinal NENs and in liver metastases. Anyway, further studies are needed to confirm the role of NOTCH signalling in GI-NENs and to drive an effective therapeutic strategy modulating the NOTCH pathway in these tumour.

5.4. Role of NOTCH in medullary thyroid cancer

Medullary thyroid cancer (MTC) is a neuroendocrine tumour that emerges from parafollicular C-cells of thyroid gland. In MTC the proliferation of neuroendocrine cells and tumour growth process appear to be regulated by a common pathway. A major role is played by the achaete-scute complex-like 1 (ASCL1) transcription factor, highly expressed in MTC, that is involved in supporting cell proliferation and embryologic precursors survival, as well as inhibiting apoptosis [108].

The activation of NOTCH signaling directly blocks expression of ASCL1, with an anti-proliferative effect. The stimulation of NOTCH1 and NOTCH3 has demonstrated, in vitro and in vivo, a reduction in tumour growth and a decrease in the production of chromogranin A neuropeptides and specific neuron enolase (NSE), two of the main MTC biomarkers.

More specifically, NOTCH3 expression may be fundamental to the thyroid oncogenesis due to its involvement in the dedifferentiation process suffered by the cells. Thus, activation of NOTCH3 in thyroid cancer cells not only has an antiproliferative effect but also restores the differentiated phenotype, lost in the oncogenic transformation of the cells [109].

Other studies also pointed out to the role of NOTCH1 in the regulation of the aggressiveness of differentiated thyroid carcinomas. This effect could be mediated by the inhibition of SERPIN1 with a possible therapeutic effect [110].

5.5. NOTCH malignant castration-resistant prostatic cells

Prostatic small-cell carcinoma originated from neuroendocrine diffuse cells in prostate is a rare neoplasia with a lack of understanding in tumour development and progression, as well as in useful prognostic factors and genetic biomarkers. More often, in prostatic cancer (PCa), prostatic cells loose the maintenance of tissue identity and by a lineage-plasticity manner transdifferentiate in neuroendocrine phenotype, following androgen deprivation therapy.

The neuroendocrine cells promote the hormone-resistance, secreting peptides that can stimulate androgen-dependent growth, and reducing apoptosis. Neuroendocrine cells do not express androgenic receptors, for which are not sensitive to the therapy of androgenic deprivation, and have poor sensitivity to standard chemotherapeutic agents. Interestingly, this fact seems to be related with the tumour plasticity for the epithelial mesenchymal transition (EMT) process and with the alteration of the signaling pathway regulators involved in cellular proliferation and differentiation.

Currently, there are ongoing studies aimed to explore the role of NOTCH in malignant castration-resistant prostatic cancer models. Preliminary investigations revealed that NOTCH activity is elevated in cancer cells and anti-tumoral effects are achieved with the use of NOTCH pharmacological inhibitors targeting NOTCH pathway [111]. Likewise, it has been recently described the involvement of NOTCH in the resistance of PCa to androgen deprivation therapy, in murine xenografts models, suggesting its use as a possible coadjuvant therapy [112].

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6. Therapeutic Approach Targeting NOTCH in NENs

A pharmacological modulation of NOTCH pathway is an interesting concept to pursue for neuroendocrine tumours treatment. Overall, there are several approaches to modulate NOTCH signaling that are in different stage of development in cancer treatment. Between them neutralizing antibodies, histone deacetylase inhibitors, and γ-secretase NOTCH inhibitors. Positive data were obtained in neuroendocrine neoplasms. *In-vitro* studies observed that the histone deacetylase inhibitors and valproic acid (VA) cause an antineoplastic effect in neuroendocrine cell line, inducing NOTCH1 mRNA expression through binding AP transcription factor [113]. This effect was confirmed in a phase II trial, in which the VA was evaluated in G1/G2 neuroendocrine tumours, with a relative good tumour control [114].

An *in-vivo* study showed that tarextumab, a NOTCH 2/3 neutralizing antibody, inhibits tumour growth in SCLC xenograft tumours [115]. In an explorative clinical phase I trial the γ-secretase NOTCH inhibitor RO4929097 was tested in solid malignancies, within this trial a patient affected by colorectal cancer with neuroendocrine feature achieved a partial response [116].

The intensive crosstalk between NOTCH signaling and other biological pathways is an opportunity to exploit, and could be the rationale for combined therapies. For example, in neuroendocrine cells VA showed to stimulate the expression of somatostatin receptor type 2 (SSTR2) that is largely targeted in neuroendocrine anticancer therapy [117], these results could be used to get a sensitization to SSTR2-targeted therapy.

Future steps should be driven to invest more effort in elucidating the possible therapeutic targets within NOTCH pathway susceptible to pharmacological regulation, and how to intervene on NOTCH signaling. In addition, further investigations on the most debated areas concerning the treatment with NOTCH signaling regulator, such as possible side effects of targeting important components of NOTCH pathways due to its biological relevance, and unexpected off targets due to the crosstalk regulated by NOTCH with other different pathways, are also required. In fact, an inappropriate modulation in humans could result in a higher damage of the patient instead of reach the benefit desired in the clinical practice. This is one of the main point to be clarified in the near future.

7. Conclusions

Although several studies have been conducted with the aim of identifying genetic mutations involved in the genesis of neuroendocrine tumours, none of them has shown a substantial mutational percentage in the samples analyzed, revealing a low-abundance of consistent mutations in G1/G2 neoplasms compared to other malignancies. Moreover, the modern sequencing technologies highlighted heterogeneity of mutations depending on the tumour anatomic origin.

For these reasons, there are still many unknowns in the genomic characterization of NETs in comparison with other neoplasia, with the majority of the data covering predominantly pancreatic, lung and small-intestine NET.

Therefore, multi-center collaborations, international databases, biological banks and genome-wide profiling overture should be pursued.

As concerning the role of NOTCH signalling in NETs, it also remains not fully understood.

Interestingly, NOTCH1 and NOTCH3 act as a tumour suppressor in medullary thyroid cancer, with a tumour suppressor role proposed also in SCLC and pancreatic NETs, whereas the NOTCH signalling activation, in particular the expression of NOTCH1, seems to play an important role in the development of docetaxel and castration resistant prostate cancer.

This bivalent action of NOTCH signalling in NETs suggests that separated studies and therapeutic approach for the different NETs subtypes should be undertaken. This approach could lead to a complete understanding of NOTCH signalling role and the potential therapeutic implications in NETs.

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References

1. Merrell AJ, Stanger BZ. Adult cell plasticity in vivo: de-differentiation and transdifferentiation are back in style. *Nat Rev Mol Cell Biol*. 2016, 17(7):413–425. doi:10.1038/nrm.2016.24
2. Artavanis-Tsakonas S, Rand M, et al. NOTCH Signaling: Cell Fate Control and Signal Integration in Development. *Science* 1999, *Vol. 284, Issue 5415,* pp. 770-776. doi: 10.1126/science.284.5415.770
3. Shih HP, Kopp JL, et al. A NOTCH-dependent molecular circuitry initiates pancreatic endocrine and ductal cell differentiation. *Development*. 2012, 139(14):2488–2499. doi:10.1242/dev.078634
4. Patel P, Galoian K. Molecular challenges of neuroendocrine tumors. *Oncol Lett*. 2018;15(3):2715–2725. doi:10.3892/ol.2017.7680
5. Chikara S, Reindl KM. NOTCH signaling: a hero or villain in the war against cancer? *Transl Lung Cancer Res.* 2013, 2:449–451 doi: 10.3978/j.issn.2218-6751.2013.10.15
6. Carter Y, Jaskula-Sztul R,et al. Signaling pathways as specific pharmacologic targets for neuroendocrine tumor therapy: RET, PI3K, MEK, growth factors, and NOTCH. *Neuroendocrinology*.2013,97:57–66. doi: 10.1159/000335136
7. Hassan WA, Yoshida R, et al. NOTCH1 controls cell invasion and metastasis in small cell lung carcinoma cell lines. *Lung Cancer* 2014, 86:304–310 doi: 10.1016/j.lungcan.2014.10.007
8. Krausch M, Kroepil F, et al. NOTCH 1 tumor expression is lacking in highly proliferative pancreatic neuroendocrine tumors. *Endocrine* 2013, 44:182–186 doi: 10.1007/s12020-012-9850-5
9. Kunnimalaiyaan M, Chen H. Tumor suppressor role of NOTCH-1 signaling in neuroendocrine tumors. *Oncologist* 2007, 12:535–542 doi: 10.1634/theoncologist.12-5-535
10. Kunnimalaiyaan M, Yan S, et al. Hairy enhancer of split-1 (HES-1), a NOTCH1 effector, inhibits the growth of carcinoid tumor cells. *Surgery* 2005 138:1137–1142 doi: 10.1016/j.surg.2005.05.027
11. Banck MS, Ames MM, et al. The genomic landscape of small intestine neuroendocrine tumors. *J Clin Invest.* 2013;123(6):2502-2508 doi: 10.1172/JCI67963.
12. Pelosi G, Bianchi F, et al. Recent advances in the molecular landscape of lung neuroendocrine tumors. *Expert Rev Mol Diagn.* 2019, 19(4):281-297. doi: 10.1080/14737159.2019.1595593.
13. Wong HL, Yang KC, et al. Molecular characterization of metastatic pancreatic neuroendocrine tumors (PNETs) using whole-genome and transcriptome sequencing. *Cold Spring Harb Mol Case Stud*. 2018, 4(1):a002329 doi:10.1101/mcs.a002329
14. Rickman DS, Beltran H, et al. Biology and evolution of poorly differentiated neuroendocrine tumors. *Nat. Med*. 2017, 6;23(6):1-10. doi: 10.1038/nm.4341.
15. Jiao Y, Shi C, et al. DAXX/ATRX, MEN1, and mTOR pathway genes are frequently altered in pancreatic neuroendocrine tumors. *Science* 2011, 331: 1199–1203. doi: 10.1126/science.1200609
16. Scarpa A, Chang DK, et al. Whole-genome landscape of pancreatic neuroendocrine tumours. *Nature* 2017, 543: 65–71. doi:10.1038/nature21063
17. Jensen RT, Berna MJ, et al. Inherited pancreatic endocrine tumor syndromes: advances in molecular pathogenesis, diagnosis, management, and controversies. *Cancer* 2008, 113: 1807–1843. doi: 10.1002/cncr.23648.
18. Francis JM, Kiezun A, et al. Somatic mutation of CDKN1B in small intestine neuroendocrine tumors. Nat Genet 2013, 45:1483-6. doi 10.1038/ng.2821

19. Singhi AD, Liu T, et al. Alternative lengthening of telomeres and loss of DAXX/ATRX expression predicts metastatic disease and poor survival in patients with pancreatic neuroendocrine tumors. *Clin Cancer Res* 2017, 23: 600–609. doi: 10.1158/1078-0432.CCR-16-1113
20. Vijayvergia N, Boland PM, et al. Molecular profiling of neuroendocrine malignancies to identify prognostic and therapeutic markers: a Fox Chase Cancer Center Pilot Study. *Br J Cancer.* 2016, 115(5):564-70. doi: 10.1038/bjc.2016.229.
21. Öberg K. The genetics of neuroendocrine tumors. *Semin Oncol.* 2013 Feb;40(1):37-44. doi: 10.1053/j.seminoncol.2012.11.005.
22. Marx SJ. Recent Topics Around Multiple Endocrine Neoplasia Type 1. *J Clin Endocrinol Metab.* 2018, 103(4):1296-1301. doi: 10.1210/jc.2017-02340.
23. Frost M, Lines K E, et al. Current and emerging therapies for PNETs in patients with or without MEN1. *Nat Rev Endocrinol*. 2018, 14(4):216-227. doi: 10.1038/nrendo.2018.3.
24. Pepe S, Korbonits M, et al. Germline and mosaic mutations causing pituitary tumours: genetic and molecular aspects. *J Endocrinol.* 2019, 240(2):R21-R45. doi: 10.1530/JOE-18-0446.
25. Khatami F, Tavangar S M. Multiple Endocrine Neoplasia Syndromes from Genetic and Epigenetic Perspectives. *Biomark Insights.* 2018 , 13:1177271918785129. doi: 10.1177/1177271918785129.
26. Ren F, Xu HW, et al. Expression and subcellular localization of menin in human cancer cells. *Exp Ther Med.* 2012, 3(6):1087-1091.
27. Petr EJ, Else T. Genetic predisposition to endocrine tumors: Diagnosis, surveillance and challenges in care. *Semin Oncol.* 2016, 43(5):582-590. doi: 10.1053/j.seminoncol.2016.08.007.
28. Plaza-Menacho I. Structure and function of RET in multiple endocrine neoplasia type 2. *Endocr Relat Cancer.* 2018, 25(2):T79-T90. doi: 10.1530/ERC-17-0354.
29. Wells SA Jr, Pacini F, et al. Multiple endocrine neoplasia type 2 and familial medullary thyroid carcinoma: an update. *J Clin Endocrinol Metab.* 2013, 98(8):3149-64. doi: 10.1210/jc.2013-1204.
30. Pellegata NS, Quintanilla-Martinez L, et al. Germ-line mutations in p27Kip1 cause a multiple endocrine neoplasia syndrome in rats and humans. *Proc Natl Acad Sci U S A*. 2006, 103(42):15558-63.
31. Varshney N, Kebede A A,et al. A Review of Von Hippel-Lindau Syndrome. *J Kidney Cancer VHL*. 2017, 4(3):20-29. doi: 10.15586/jkcvhl.2017.88.
32. López-Jiménez E, Gómez-López G, et al. Research resource: Transcriptional profiling reveals different pseudohypoxic signatures in SDHB and VHL-related pheochromocytomas. *Mol Endocrinol.* 2010, 24(12):2382-91. doi: 10.1210/me.2010-0256.
33. Dahia PL, Ross KN, et al. A HIF1alpha regulatory loop links hypoxia and mitochondrial signals in pheochromocytomas. *PLoS Genet.* 2005, 1(1):72-80.
34. Maeda Y, Suzuki T, et al. . CUL2 is required for the activity of hypoxia-inducible factor and vasculogenesis. *J Biol Chem*. 2008, 283(23):16084-92. doi: 10.1074/jbc.M710223200.
35. Miyauchi Y, Kato M, et al. The COP9/signalosome increases the efficiency of von Hippel-Lindau protein ubiquitin ligase-mediated hypoxia-inducible factor-alpha ubiquitination. *J Biol Chem.* 2008, 283(24):16622-31. doi: 10.1074/jbc.M710599200.
36. Ku YH, Ahn CH, et al. .A novel mutation in the von hippel-lindau tumor suppressor gene identified in a patient presenting with gestational diabetes mellitus. *Endocrinol Metab (Seoul).* 2013, 28(4):320-5. doi: 10.3803/EnM.2013.28.4.320.
37. Lodish MB, Stratakis CA. Endocrine tumours in neurofibromatosis type 1, tuberous sclerosis and related syndromes. *Best Pract Res Clin Endocrinol Metab.* 2010, 24(3):439-49. doi: 10.1016/j.beem.2010.02.002.
38. De Luca A, Bottillo I, et al. Deletions of NF1 gene and exons detected by multiplex ligation-dependent probe amplification. *J Med Genet.* 2007, 44(12):800-8.
39. Rachdi L, Balcazar N, et al. Disruption of Tsc2 in pancreatic beta cells induces beta cell mass expansion and improved glucose tolerance in a TORC1-dependent manner. *Proc Natl Acad Sci U S A.* 2008,105(27):9250-5. doi: 10.1073/pnas.0803047105.
40. Krausch M, Raffel A, et al. Loss of PTEN expression in neuroendocrine pancreatic tumors. *Horm Metab Res.* 2011, 43(12):865-71. doi: 10.1055/s-0031-1291333. Epub 2011 Nov 21.
41. Lefebvre M, Foulkes WD. Pheochromocytoma and paraganglioma syndromes: genetics and management update. *Curr Oncol*. 2014, 21(1):e8-e17. doi: 10.3747/co.21.1579.
42. Ricketts CJ, Forman JR, et al. Tumor risks and genotype-phenotype-proteotype analysis in 358 patients with germline mutations in SDHB and SDHD. *Hum Mutat.* 2010, 31(1):41-51. doi: 10.1002/humu.21136.

43. Guzy RD, Sharma B, et al. Loss of the SdhB, but Not the SdhA, subunit of complex II triggers reactive oxygen species-dependent hypoxia-inducible factor activation and tumorigenesis. *Mol Cell Biol.* 2008, 28(2):718-31.
44. Burnichon N, Brière JJ, et al. SDHA is a tumor suppressor gene causing paraganglioma. *Hum Mol Genet.* 2010, 19(15):3011-20. doi: 10.1093/hmg/ddq206.
45. Gimenez-Roqueplo AP, Favier J, at al. Mutations in the SDHB gene are associated with extra-adrenal and/or malignant phaeochromocytomas. *Cancer Res.* 2003, 63(17):5615-21.
46. Hao HX, Khalimonchuk O, et al. SDH5, a gene required for flavination of succinate dehydrogenase, is mutated in paraganglioma. *Science.* 2009,325(5944):1139-42. doi: 10.1126/science.1175689.
47. Astuti D, Latif F, at al. Gene mutations in the succinate dehydrogenase subunit SDHB cause susceptibility to familial pheochromocytoma and to familial paraganglioma. *Am J Hum Genet.* 2001,69(1):49-54.
48. Niemann S, Müller U. Mutations in SDHC cause autosomal dominant paraganglioma, type 3. *Nat Genet.* 2000, 26(3):268-70.
49. Niemann S, Müller U, et al. Autosomal dominant malignant and catecholamine-producing paraganglioma caused by a splice donor site mutation in SDHC. *Hum Genet.* 2003, 113(1):92-4.
50. Baysal BE, Ferrell RE, et al. Mutations in SDHD, a mitochondrial complex II gene, in hereditary paraganglioma. Science. 2000, 287(5454):848-51.
51. Ugarte-Camara M, Fernandez-Prado R, et al. Positive/retained SDHB immunostaining in renal cell carcinomas associated to germline SDHB-deficiency: case report. *Diagn Pathol.* 2019, 14(1):42. doi: 10.1186/s13000-019-0812-6.
52. Eijkelenkamp K, Osinga TE, et al. Clinical implications of the oncometabolite succinate in SDHx-mutation carriers. *Clin Genet.* 2019 doi: 10.1111/cge.13553.
53. Crona J, Skogseid B. GEP- NETS UPDATE: Genetics of neuroendocrine tumors. *Eur J Endocrinol.* 2016,174(6):R275-90. doi: 10.1530/EJE-15-0972.
54. Yachida S, Vakiani E, et al. Small cell and large cell neuroendocrine carcinomas of the pancreas are genetically similar and distinct from well-differentiated pancreatic neuroendocrine tumors. *Am J Surg Pathol.* 2012, 36(2):173-84. doi: 10.1097/PAS.0b013e3182417d36.
55. Basturk O, Yang Z, et al. The high-grade (WHO G3) pancreatic neuroendocrine tumor category is morphologically and biologically heterogenous and includes both well differentiated and poorly differentiated neoplasms. *Am J Surg Pathol.* 2015, 39(5):683-90. doi: 10.1097/PAS.0000000000000408.
56. Scarpa A, Mantovani W, et al. Pancreatic endocrine tumors: improved TNM staging and histopathological grading permit a clinically efficient prognostic stratification of patients. *Mod Pathol.* 2010, 23(6):824-33. doi: 10.1038/modpathol.2010.58.
57. Vélayoudom-Céphise FL, Duvillard P, at al. Are G3 ENETS neuroendocrine neoplasms heterogeneous? *Endocr Relat Cancer.* 2013, 20(5):649-57. doi: 10.1530/ERC-13-0027.
58. George J, Lim JS, et al.Comprehensive genomic profiles of small cell lung cancer. *Nature.* 2015, 524(7563):47-53. doi: 10.1038/nature14664.
59. Makuuchi R, Terashima M, et al. Comprehensive analysis of gene mutation and expression profiles in neuroendocrine carcinomas of the stomach. *Biomed Res.* 2017;38(1):19-27. doi: 10.2220/biomedres.38.19.
60. Takizawa N, Ohishi Y, et al. Molecular characteristics of colorectal neuroendocrine carcinoma; similarities with adenocarcinoma rather than neuroendocrine tumor. *Hum Pathol.* 2015, 46(12):1890-900. doi: 10.1016/j.humpath.2015.08.006.
61. Ali AS, Grönberg M, et al. Expression of p53 protein in high-grade gastroenteropancreatic neuroendocrine carcinoma. *PLoS One.* 2017, 12(11):e0187667. doi: 10.1371/journal.pone.0187667.
62. Sahnane N, Furlan D, et al. Microsatellite unstable gastrointestinal neuroendocrine carcinomas: a new clinicopathologic entity. *Endocr Relat Cancer.* 2015, 22(1):35-45. doi: 10.1530/ERC-14-0410. Epub 2014 Dec 2.
63. Olevian DC, Nikiforova MN, et al. Colorectal poorly differentiated neuroendocrine carcinomas frequently exhibit BRAF mutations and are associated with poor overall survival. *Hum Pathol.* 2016, 49:124-34. doi: 10.1016/j.humpath.2015.11.004.
64. Jesinghaus M, Konukiewitz B, at al. Colorectal mixed adenoneuroendocrine carcinomas and neuroendocrine carcinomas are genetically closely related to colorectal adenocarcinomas. *Mod Pathol.* 2017, 30(4):610-619. doi: 10.1038/modpathol.2016.220.
65. Hijioka S, Hosoda W, et al. Does the WHO 2010 classification of pancreatic neuroendocrine neoplasms accurately characterize pancreatic neuroendocrine carcinomas? *J Gastroenterol.* 2015, 50(5):564-72. doi: 10.1007/s00535-014-0987-2.

66. Woischke C, Schaaf CW, et al. In-depth mutational analyses of colorectal neuroendocrine carcinomas with adenoma or adenocarcinoma components. *Mod Pathol.* 2017, 30(1):95-103. doi: 10.1038/modpathol.2016.150.
67. Karkouche R, Bachet JB, et al. Colorectal neuroendocrine carcinomas and adenocarcinomas share oncogenic pathways. A clinico-pathologic study of 12 cases. *Eur J Gastroenterol Hepatol.* 2012, 24(12):1430-7. doi: 10.1097/MEG.0b013e3283583c87.
68. Vortmeyer AO, Lubensky IA, et al. Concordance of genetic alterations in poorly differentiated colorectal neuroendocrine carcinomas and associated adenocarcinomas. *J Natl Cancer Inst.* 1997 Oct 1;89(19):1448-53.
69. Tang LH, Basturk O, et al. A Practical Approach to the Classification of WHO Grade 3 (G3) Well-differentiated Neuroendocrine Tumor (WD-NET) and Poorly Differentiated Neuroendocrine Carcinoma (PD-NEC) of the Pancreas. *Am J Surg Pathol.* 2016, 40(9):1192-202. doi: 10.1097/PAS.0000000000000662.
70. Mafficini A, Scarpa A. Genetics and Epigenetics of Gastroenteropancreatic Neuroendocrine Neoplasms. *Endocr Rev.* 2019, 40(2):506-536. doi: 10.1210/er.2018-00160.
71. Ohmoto A, Rokutan H, et al. Pancreatic Neuroendocrine Neoplasms: Basic Biology, Current Treatment Strategies and Prospects for the Future. *Int J Mol Sci.* 2017, 18(1). pii: E143. doi: 10.3390/ijms18010143.
72. Lamberti G, Brighi N, et al. The Role of mTOR in Neuroendocrine Tumors: Future Cornerstone of a Winning Strategy?. *Int J Mol Sci*. 2018, 19(3):747. doi:10.3390/ijms19030747
73. Palmer WH, Jia D, et al. Cis-interactions between Notch and its ligands block ligand-independent Notch activity. *Elife.* 2014 Dec, 3. doi: 10.7554/eLife.04415.
74. Wöltje K, Jabs M, et al. Serum induces transcription of Hey1 and Hey2 genes by Alk1 but not Notch signaling in endothelial cells. *PLoS One*. 2015, 10(3):e0120547. doi: 10.1371/journal.pone.0120547.
75. Brzozowa-Zasada M, Piecuch A et al. Notch and its oncogenic activity in human malignancies. *Eur Surg.* 2017;49(5):199-209. doi: 10.1007/s10353-017-0491-z.
76. Zou B, Zhou XL, et al. Notch signaling and non-small cell lung cancer. *Oncol Lett.* 2018 Mar;15(3):3415-3421. doi: 10.3892/ol.2018.7738.
77. Fukusumi T, Califano JA. The NOTCH Pathway in Head and Neck Squamous Cell Carcinoma. *J Dent Res.* 2018, 97(6):645-653. doi: 10.1177/0022034518760297
78. Furuta M, Kikuchi H, et al. DLL3 regulates the migration and invasion of small cell lung cancer by modulating Snail. *Cancer Sci.* 2019, 110(5):1599-1608. doi: 10.1111/cas.13997.
79. Lim JS, Ibaseta A, et al. Intratumoural heterogeneity generated by Notch signalling promotes small-cell lung cancer. *Nature*. 2017, 545(7654):360-364. doi: 10.1038/nature22323.
80. Jaskula-Sztul R, Eide J, et al. Tumor-suppressor role of Notch3 in medullary thyroid carcinoma revealed by genetic and pharmacological induction. *Mol Cancer Ther.* 2015, 14(2):499-512. doi: 10.1158/1535-7163.MCT-14-0073.
81. Lobry C, Oh P, et al. Notch signaling: switching an oncogene to a tumor suppressor. *Blood.* 2014, ;123(16):2451-9. doi: 10.1182/blood-2013-08-355818.
82. Crabtree JS, Singleton CS, et al. Notch Signaling in Neuroendocrine Tumors. *Front Oncol.* 2016, 6:94. doi: 10.3389/fonc.2016.00094.
83. Harada K, Sato Y, et al. Notch1-Hes1 signalling axis in the tumourigenesis of biliary neuroendocrine tumours. *J Clin Pathol.* 2013, 66(5):386-91. doi: 10.1136/jclinpath-2012-201273.
84. Aster JC, Pear WS, et al. The Varied Roles of Notch in Cancer. *Annu Rev Pathol.* 2017 Jan 24;12:245-275. doi: 10.1146/annurev-pathol-052016-100127.
85. Robinson DR, Kalyana-Sundaram S et al. Functionally recurrent rearrangements of the MAST kinase and Notch gene families in breast cancer. *Nat Med.* 2011, 17(12):1646-51. doi: 10.1038/nm.2580.
86. Stoeck A, Lejnine S, et al. Discovery of biomarkers predictive of GSI response in triple-negative breast cancer and adenoid cystic carcinoma. *Cancer Discov.* 2014, 4(10):1154-67. doi: 10.1158/2159-8290.CD-13-0830.
87. Parr C, Watkins G, et al. The possible correlation of Notch-1 and Notch-2 with clinical outcome and tumour clinicopathological parameters in human breast cancer. *Int J Mol Med.* 2004 Nov;14(5):779-86.
88. Wang K, Zhang Q, et al. PEST domain mutations in Notch receptors comprise an oncogenic driver segment in triple-negative breast cancer sensitive to a γ-secretase inhibitor. *Clin Cancer Res.* 2015, 21(6):1487-96. doi: 10.1158/1078-0432.CCR-14-1348.
89. Westhoff B, Colaluca IN, et al. Alterations of the Notch pathway in lung cancer. *Proc Natl Acad Sci U S A*. 2009, 106(52):22293-8.
90. Zhu J, Wang J, et al. Deciphering genomic alterations in colorectal cancer through transcriptional subtype-based network analysis. *PLoS One.* 2013, 8(11):e79282. doi: 10.1371/journal.pone.0079282.

91. Mao L. NOTCH mutations: multiple faces in human malignancies. *Cancer Prev Res (Phila).* 2015, 8(4):259-61. doi: 10.1158/1940-6207.CAPR-15-0063.
92. Xing D, Zheng G, et al. Next-generation Sequencing Reveals Recurrent Somatic Mutations in Small Cell Neuroendocrine Carcinoma of the Uterine Cervix. *Am J Surg Pathol.* 2018, 42(6):750-760. doi: 10.1097/PAS.0000000000001042.
93. Derks JL, Leblay N, et al. New Insights into the Molecular Characteristics of Pulmonary Carcinoids and Large Cell Neuroendocrine Carcinomas, and the Impact on Their Clinical Management. *J Thorac Oncol.* 2018, 13(8):1229. doi: 10.1016/j.jtho.2018.05.025.
94. Fernandez-Cuesta L, Peifer M, et al. Frequent mutations in chromatin-remodelling genes in pulmonary carcinoids. *Nat Commun.* 2014 Mar 27;5:3518. doi: 10.1038/ncomms4518.
95. Cives M, Simone V, et al. NETs: organ-related epigenetic derangements and potential clinical applications. *Oncotarget.* 2016, 7(35):57414-57429. doi: 10.18632/oncotarget.10598
96. Kim JT, Li J, et al. Deregulation of Wnt/β-catenin signaling through genetic or epigenetic alterations in human neuroendocrine tumors. *Carcinogenesis.* 2013, 34(5):953-61. doi: 10.1093/carcin/bgt018. Epub 2013 Jan 25.
97. Graves CA, Jones A, et al. Neuroendocrine Merkel cell carcinoma is associated with mutations in key DNA repair, epigenetic and apoptosis pathways: a case-based study using targeted massively parallel sequencing. *Neuroendocrinology.* 2015;101(2):112-9. doi: 10.1159/000370310.
98. Augert A, Eastwood E, et al. Targeting NOTCH activation in small cell lung cancer through LSD1 inhibition. *Sci Signal.* 2019,12(567). pii: eaau2922. doi: 10.1126/scisignal.aau2922.
99. Abraham KJ, Zhang X, et al. Roles for miR-375 in Neuroendocrine Differentiation and Tumor Suppression via Notch Pathway Suppression in Merkel Cell Carcinoma. *Am J Pathol.* 2016, 186(4):1025-35. doi: 10.1016/j.ajpath.2015.11.020.
100. Arvidsson Y, Rehammar A, et al. miRNA profiling of small intestinal neuroendocrine tumors defines novel molecular subtypes and identifies miR-375 as a biomarker of patient survival. *Mod Pathol.* 2018, 31(8):1302-1317. doi: 10.1038/s41379-018-0010-1
101. Chiappinelli KB, Zahnow CA, et al. Combining Epigenetic and Immunotherapy to Combat Cancer. *Cancer Res.* 2016,, 76(7):1683-9. doi: 10.1038/s41379-018-0010-1.
102. Meder L, König K, et al. NOTCH, ASCL1, p53 and RB alterations define an alternative pathway driving neuroendocrine and small cell lung carcinomas. *Int J Cancer.* 2016, 38(4):927-38. doi: 10.1002/ijc.29835.
103. Wang H, Chen Y, et al. Heterogeneity in signaling pathways of gastroenteropancreatic neuroendocrine tumors: a critical look at notch signaling pathway. *Mod Pathol.* 2013, 26(1):139-47. doi: 10.1038/modpathol.2012.143.
104. Hanlon L, Avila JL, et al. Notch1 functions as a tumor suppressor in a model of K-ras-induced pancreatic ductal adenocarcinoma. *Cancer Res.* 2010 Jun 1;70(11):4280-6. doi: 10.1158/0008-5472.CAN-09-4645.
105. Miyamoto Y, Maitra A, et al. Notch mediates TGF alpha-induced changes in epithelial differentiation during pancreatic tumorigenesis. *Cancer Cell.* 2003 Jun;3(6):565-76.
106. Harada K, Sato Y, et al. Notch1-Hes1 signalling axis in the tumourigenesis of biliary neuroendocrine tumours. *J Clin Pathol.* 2013,66(5):386-91. doi: 10.1136/jclinpath-2012-201273.
107. Maggi EC, Trillo-Tinoco J, Retinoblastoma-binding protein 2 (RBP2) is frequently expressed in neuroendocrine tumors and promotes the neoplastic phenotype. *Oncogenesis.* 2016, 5(8):e257. doi:10.1038/oncsis.2016.58
108. Sippel RS, Carpenter JE, et al. The role of human achaete-scute homolog-1 in medullary thyroid cancer cells. *Surgery.* 2003 Dec, 134(6):866-71.
109. Somnay YR, Yu XM, et al. Notch3 expression correlates with thyroid cancer differentiation, induces apoptosis, and predicts disease prognosis. *Cancer.* 2017,123(5):769-782. doi: 10.1002/cncr.30403.
110. Yu XM1, Jaskula-Sztul R, at al. Notch1 Signaling Regulates the Aggressiveness of Differentiated Thyroid Cancer and Inhibits SERPINE1 Expression. *Clin Cancer Res.* 2016 Jul 15;22(14):3582-92. doi: 10.1158/1078-0432.CCR-15-1749.
111. Du Z, Li L, at al. HepaCAM inhibits the malignant behavior of castration-resistant prostate cancer cells by downregulating Notch signaling and PF-3084014 (a γ-secretase inhibitor) partly reverses the resistance of refractory prostate cancer to docetaxel and enzalutamide in vitro. *Int J Oncol.* 2018, 53(1):99-112. doi: 10.3892/ijo.2018.4370.
112. Cui J, Wang Y, at al. Pharmacological inhibition of the Notch pathway enhances the efficacy of androgen deprivation therapy for prostate cancer. *Int J Cancer.* 2018, 1;143(3):645-656. doi: 10.1002/ijc.31346.

113. Jang S, Jin H, et al. Antineoplastic effects of histone deacetylase inhibitors in neuroendocrine cancer cells are mediated through transcriptional regulation of Notch1 by activator protein 1. *Cancer Med.* 2017, 6(9):2142-2152. doi: 10.1002/cam4.1151.
114. Mohammed TA, Holen KD, et al. A pilot phase II study of valproic acid for treatment of low-grade neuroendocrine carcinoma. *Oncologist.* 2011, 16(6):835-43. doi: 10.1634/theoncologist.2011-0031.
115. Yen WC, Fischer MM, et al. Targeting Notch signaling with a Notch2/Notch3 antagonist (tarextumab) inhibits tumor growth and decreases tumor-initiating cell frequency. *Clin Cancer Res* 2015, 21:2084–95. doi: 10.1158/1078-0432.CCR-14-2808
116. Tolcher AW, Messersmith WA, et al. Phase I study of RO4929097, a gamma secretase inhibitor of Notch signaling, in patients with refractory metastatic or locally advanced solid tumors. *J Clin Oncol.* 2012, 1;30(19):2348-53. doi: 10.1200/JCO.2011.36.8282.
117. Sun L, Qian Q, et al. Valproic acid induces NET cell growth arrest and enhances tumor suppression of the receptor-targeted peptide-drug conjugate via activating somatostatin receptor type II. J *Drug Target.* 2016,24(2):169-77. doi: 10.3109/1061186X.2015.1066794.