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Systematic or Meta-analysis Studies

Unraveling molecular pathways of poorly differentiated neuroendocrine carcinomas of the gastroenteropancreatic system: A systematic review

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ABSTRACT

Background: Poorly differentiated neuroendocrine carcinomas (NECs) are rare and aggressive tumors. Their molecular pathogenesis is still largely unknown, and consequently, the best therapeutic management also remains to be determined. We conducted a systematic review on molecular alterations found in gastroenteropancreatic NECs (GEP-NECs) and discuss potential applications of targeted therapies in setting.

Materials and methods: Systematic review of studies about molecular features in tumor tissues of patients with GEP-NECs. The Medline, Lilacs, Embase, Cochrane, Scopus and Opengrey databases were sought, without time, study design or language restrictions.

Results: Of the 1.564 studies retrieved, 41 were eligible: 33 were retrospective studies and eight were case reports. The studies spanned the years 1997–2017 and involved mostly colorectal, stomach and pancreas primary tumors. Molecular alterations in the *TP53* gene and the p53 protein expression were the most commonly observed, regardless of the primary site. Other consistently found molecular alterations were microsatellite instability (MSI) in approximately 10% of gastric and colorectal NEC, and altered signaling cascades of p16/Rb/cyclin D1, Hedgehog and Notch pathways, and somatic mutations in *KRAS*, *BRAF*, *RB1* and *Bcl2*. In studies of mixed adeno-neuroendocrine carcinomas (MANECs) the molecular features of GEP-NEC largely resemble their carcinoma/adenocarcinomas tumor counterparts.

Conclusions: Despite the paucity of data about the molecular drivers associated with GEP-NEC, some alterations may be potentially targeted with new cancer-directed therapies. Collaborative clinical trials for patients with advanced GEP-NEC are urgently needed.

Introduction

Neuroendocrine tumors encompass a variety of neoplasms with distinct anatomical localizations and clinical behaviors. The WHO-classification from 2010 and the European Neuroendocrine Tumor Society classify NETs according to their proliferative activity into grade 1 and 2 (G1 and G2) well differentiated neuroendocrine tumors (WDNETs) (≤20 mitoses/10 high power fields (HPF); Ki-67 index ≤ 20%) or grade 3 (G3) tumors (>20 mitoses/10 HPF; Ki-67 index > 20%) that can be either well or poorly differentiated neuroendocrine carcinomas (NECs) [1,2]. A new WHO classification is in the process of being published and has subdivided the G3 tumors based on their morphological characteristics in well differentiated NET G3 or poorly differentiated NEC (PDNEC) G3, both with Ki-67 index > 20% or >20 mitoses/10 HPF. PDNEC often

Abbreviations: APC, Adenomatous Polyposis Coli gene; ATM, Ataxia telangiectasia mutated gene; ATRX, Alpha-thalassemia mental retardation syndrome gene; CASP8, Caspase-8 gene; CDKN2A, cyclin-dependent kinase Inhibitor 2A; DAXX, Death-associated protein 6 gene; DCC, Deleted in Colorectal Cancer gene; DPC4, Deleted in Pancreatic Cancer-4 gene; ERBB4, Receptor tyrosine-protein kinase erbB-4 gene; hASH1, human achaete-scute homologue 1; KRAS, Kirsten Rat Sarcoma; Notch1, Notch homolog 1 gene; PDGFRA, Platelet Derived Growth Factor Alpha; PTEN, Phosphatase and Tensin Homolog; RASSF1A, Ras-associated domain family 1A; RB1, retinoblastoma protein gene; SMAD4, Mothers against decapentaplegic homolog 4 gene; TP53, protein 53 gene.

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present Ki-67 higher than 50%, are characterized by small or large cells and up to 40% contain elements of other non-neuroendocrine carcinomas [3]. When both components constitute more than 30% of the tumor, the tumor is classified as mixed adeno-neuroendocrine carcinomas (MANECs) [1], which will be renamed as MINEN/MENEN (mixed endocrine neuroendocrine carcinoma) in the new WHO 2017 classification. The evaluation of Ki-67 index is based on counting 500–2000 cells in areas of higher nuclear labeling (hot spots); mitoses in 50 HPF in areas of higher density and expressed per 10HPF. The final grade should be established on whichever index (mitotic rate or Ki67) places the tumor in the highest grade category [4]. Besides, for assessing Ki67, casual visual estimation ("eyeballing") is not recommended; either manual counting of printed images or digital image analysis is suggested [5].

Very few studies have looked at the carcinogenic mechanisms associated with NECs and therefore, treatment options for this disease are scarce. Indeed, patients with advanced NEC are treated with platinum-based chemotherapy, especially etoposide or irinotecan and platinum, which are exported regimens for advanced small-cell lung carcinoma (SCLC) [6]. Platinum in combination with etoposide have shown response rates (RR) in extrapulmonary NEC ranging from 36% to 67% and median overall survival (OS) between 10 and 19 months [7–10]. Similarly, cisplatin or carboplatin combined with irinotecan offer RR varying from 36.4 to 75% and median OS from 10 to 13 months [11–16]. However, despite using the same treatment regimen, RR in extra-pulmonary NEC are apparently lower than the pulmonary counterpart [6], what suggests that extra-pulmonary NEC are molecularly distinct from SCLC. Additionally predictive factors of RR to chemotherapy in patients with NEC remain unknown [17].

In times of precision medicine where deciphering the molecular pathways to carcinogenesis could guide treatment decisions through targeted therapies, the pathogenesis of NEC are largely unknown and only a few studies have attempted to characterize the molecular features of this disease. The purpose of this article is to provide a comprehensive and systematic review on the molecular characteristics of NEC of the gastroenteropancreatic (GEP) tract.

Methods

We performed a systematic review of articles that reported molecular features of GEP-NECs. Because NETs comprehend a variety of different anatomical localizations we decided to focus on tumors originated from the gastroenteropancreatic (GEP) system. Eligible studies were clinical trials, prospective or retrospective cohorts, case-controls, case reports or population-based studies reporting any molecular alteration in patients with NECs. NEC were considered as reported by articles, with tumors with ki-67 > 20%, or mitotic count > 20/10 HPF or studies describing poor cell differentiation, including, but not limited to, small cell histology.

Studies were sought in Medline, Embase, Scopus, Lilacs, Cochrane Library of Systematic Reviews and Opengrey databases, using the following strategies, from their date of inception to March 1st 2017 without language restrictions. MEDLINE was assessed through PubMed using the MeSH Terms "neuroendocrine carcinoma" or "neuroendocrine carcinomas" associated with the keywords *molecular alterations OR mutations OR amplification OR epigenetic OR genetic* OR *immunohistochemistry, excluding* articles whose title included the following words: *mammary, lung, breast, merkel, prostate, thyroid, renal, head and neck, pulmonary, medullary, urinary, uterine, mediastinum, orbit* and *cervical*. At EMBASE we used the key words *neuroendocrine carcinoma* OR *poorly differentiated neuroendocrine carcinoma* AND *molecular*. At Scopus we used the key words *neuroendocrine carcinoma* AND *molecular* AND NOT *lung* AND NOT *prostate* AND NOT *cervix* AND NOT *merkel* AND NOT *bladder* AND NOT *thyroid*. At Lilacs we used the registered (mh) terms "neuroendocrine tumors" OR "neuroendocrino" AND "molecular". The key words *neuroendocrine tumors* OR *neuroendocrine carcinoma* were used at Cochrane Library and Opengrey, respectively. We also hand-searched the references of the retrieved articles for further relevant studies.

Molecular alterations or profiles were defined as any study of gene mutations or amplifications, protein over-expression, chromosomal aberrations, loss of heterozygosity (LOH), methylation abnormalities or immunohistochemistry (IHC) hyper- or hypo expressions.

We excluded non-original articles such as reviews, editorials, commentaries, studies that only evaluated molecular alterations of grade 1 and 2 NETs, pre-clinical or pediatric studies, reports of other primary sites of NETs besides the GEP system. In each eligible article, the investigators collected information about tumor primary location, study design, number and characteristics of study populations, and type, methods and results of molecular analyses.

Results

Our initial search at Medline identified 546 potential articles of which 29 were eligible (Fig. 1). Ten articles out of 567 were selected from Embase and two out of 415 studies were identified from Scopus, with a total of 41 eligible studies. Four, thirteen and nineteen potential studies were found, respectively, in Lilacs, Cochrane or Opengrey databases, but no eligible study was identified among them. Thirty-three were retrospective studies and eight were case reports. Due to the rarity of GEP-NEC and the limited number of studies encountered, case reports were included, even though they provide the lowest level of scientific evidence. The studies spanned the years 1997–2017. Twenty-four evaluated patients with colorectal NECs, [18–40] 18 studies included patients with stomach as the primary site, [18–26,41–49] 12 studies in pancreatic NECs [18,20,24–28,41,42,50–52], eight in small bowel [18,20,28,41,42,53–55], seven in esophagus [19–21,41,42,56,57] and two examined NECs of the biliary tract [41,58]. The studies are summarized in Table 1 and detailed below separately by overall molecular features, molecular characteristics particular to certain tumor types and studies describing molecular profiles of MANECs.

Common molecular features in GEP-NEC

The most frequently reported molecular features of NEC were mutations in *TP53*, aberrations in the p16/Rb/cyclin D1 signaling pathway, *RAS* mutations, and microsatellite instability. These and other molecular characteristics of GEP-NECs are described below and summarized in Table 1.

Molecular alterations in the TP53 gene

Mutations in the *TP53* gene by next generation sequencing (NGS) or PCR and/or p53 protein overexpression by IHC were identified in GEP-NEC by several studies [18,23–26,28,35,37,40,45,46,48–50,55]. In GEP-NECs in general, the four studies that evaluate *TP53* mutations showed frequencies of any mutation in 57% (14/23) [28], 92.3% (12/13) [26], 94.1% (16/17) [23] and 100% (4/4) [25] of cases. By immunohistochemistry, p53 protein overexpression was encountered in three out of three cases, confirming the PCR analysis [24]. Exclusively in gastric NECs, *TP53* mutations were detected in 100% (9/9, 6/6 and 6/6) of the cases in three small series [23,26,49] and in 53.3% (8/15) in another retrospective study [48]; reinforcing this finding, two retrospective studies observed p53 overexpression by IHC in 65.4% (34/52) [45] and 100% (5/5)