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Original contribution

Pancreatic neuroendocrine carcinomas reveal a closer relationship to ductal adenocarcinomas than to neuroendocrine tumors G3☆

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Summary Pancreatic neuroendocrine carcinoma is a rare aggressive tumor commonly harboring *TP53* and *RB1* alterations and lacking neuroendocrine-related genetic changes such as mutations in *MEN1* and *ATRX/DAXX*. Little is known about its genetic profile with regard to that of pancreatic ductal adenocarcinoma. We therefore conducted a detailed genetic study in 12 pancreatic neuroendocrine carcinomas of large cell (n = 9) and small cell type (n = 3) using massive parallel sequencing applying a 409-gene panel on an Ion Torrent system. The genetic data were compared with known data of pancreatic ductal adenocarcinoma and correlated with exocrine lineage marker expression. A similar analysis was performed in 11 pancreatic neuroendocrine tumors G3. Neuroendocrine carcinomas harbored 63 somatic mutations in 45 different genes, affecting most commonly *TP53* (8/12 cases), *KRAS* (5/12 cases), and *RB1* (loss of expression with or without deletion in 4/12 cases). Five carcinomas had both *TP53* and *KRAS* mutations. Neuroendocrine tumors G3 only shared singular mutations in 5 different genes with neuroendocrine carcinomas, including *TP53*, *CDKN2A*, *ARID1A*, *LRP1B*, and *APC*, affecting 5 different cases. Most *KRAS*-positive neuroendocrine carcinomas also expressed MUC1 (4/5) and carcinoembryonic antigen (3/5) as markers of ductal differentiation. Our data indicate that almost half of the pancreatic neuroendocrine carcinomas are genetically and phenotypically related to pancreatic ductal adenocarcinoma, and might therefore respond to chemotherapies targeting the latter carcinomas.

1. Introduction

The G3 category of the 2017 World Health Organization (WHO) classification of pancreatic neuroendocrine neoplasms

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defines tumors with a Ki-67 index greater than 20% that are either well or poorly differentiated. The well-differentiated neuroendocrine neoplasms are called neuroendocrine tumors G3, and the poorly differentiated ones are called neuroendocrine carcinomas, either of small cell or of large cell type [1]. Neuroendocrine tumors G3 often evolve from G1/2 tumors after metastasizing to the liver during the course of the disease, but can also present as primary tumor in the pancreas [1,2]. They show an organoid solid or trabecular endocrine growth pattern, and usually express hormones and the somatostatin receptor 2A. In contrast, pancreatic neuroendocrine carcinomas display irregular sheets of cells or solid structures, either of small cell or of large cell cytology, and are rarely somatostatin receptor 2A and hormone positive. In addition, they commonly show an abnormal nuclear expression of p53 and Rb1 that is usually lacking in neuroendocrine tumors [2]. These data suggest that neuroendocrine tumors and neuroendocrine carcinomas may be fundamentally different neoplasms, a distinction that has a strong impact on treatment strategies [1,3].

It is not known which cells give rise to neuroendocrine tumors and neuroendocrine carcinomas in the pancreas. Candidates are cells belonging to either the neuroendocrine or the ductal-acinar cell lineage. Although the neuroendocrine tumors may be related to the neuroendocrine cell lineage, the neuroendocrine carcinomas could derive from the neuroendocrine cell lineage, the ductal-acinar cell lineage, or a still uncommitted cell lineage. In view of these possibilities, it is of interest to see whether there is a genetic relationship between neuroendocrine carcinomas and conventional ductal adenocarcinomas. Such an assumption is fostered by data from genetic studies in colorectal neuroendocrine carcinomas, suggesting that these special neoplasms are closely related to conventional colorectal adenocarcinomas [4-7].

In this study, we conducted a detailed molecular profiling of a relatively large cohort of these exceedingly rare neuroendocrine carcinomas of the pancreas and correlated the results with an immunohistochemical panel including exocrine lineage markers such as MUC1, MUC2, and carcinoembryonic antigen (CEA) [8]. The following questions were addressed: First, are neuroendocrine carcinomas genetically related to ductal adenocarcinomas? Second, are there also genetic similarities between neuroendocrine tumors G3 and ductal adenocarcinomas? Third, are neuroendocrine carcinomas homogeneous in their genetic profile or are there differences between small cell and large cell types?

2. Materials and methods

2.1. Tissue recruitment

Formalin-fixed and paraffin embedded tissue blocks from 23 surgical resection specimens of primary pancreatic neuroendocrine neoplasms (12 neuroendocrine carcinomas and 11 neuroendocrine tumors G3) were retrieved from the files of the Consultation Center for Pancreatic and Endocrine Tumors, Technical University of Munich, as well as from the Department of Pathology, Technical University of Munich, Germany. Clinicopathological information was obtained from the medical charts and pathology reports. Twenty of the 23 cases were already included in a previous study that focused on the expression of somatostatin receptor 2A, Rb1, ATRX, DAXX, and the immunohistochemical and genetic status of *TP53* [2]. The study was approved by the local ethic committee (document number 503/16s).

2.2. Histologic analysis

Hematoxylin and eosin and periodic acid–Schiff (PAS)–stained slides were reassessed using the diagnostic and grading criteria of the 2017 WHO classification of neuroendocrine neoplasms of the pancreas [1]. Poorly differentiated neuroendocrine neoplasms (neuroendocrine carcinomas) showed a diffuse, nonorganoid growth pattern, and either a large cell or small cell–type cytology. Well-differentiated neuroendocrine neoplasms (neuroendocrine tumors G3) presented with an organoid trabecular or solid growth pattern, as previously described [1,2].

2.3. Immunohistochemistry

Formalin-fixed and paraffin-embedded tissue sections from all cases were immunostained using a fully automated stainer ("Benchmark XT"; Ventana Medical Systems, Tuscon, AZ). Immunohistochemical analysis was performed using antibodies against synaptophysin (Ventana Medical Systems; MRQ-40, 1:1), chromogranin A (Boehringer Mannheim, Germany; LK2H10, 1:500), Ki-67 (DakoCytomation, Glostrup, Denmark; MIB1, 1:50), p53 (DakoCytomation; DO-7, 1:200), Rb1 (BD Biosciences, Heidelberg, Germany; G 3-245, 1:200), ATRX (Sigma-Aldrich, Munich, Germany; polyclonal, 1:250), DAXX (Sigma-Aldrich; polyclonal, 1:100), MUC1 (Leica Biosystems, Nussloch, Germany; Ma695, 1:50), MUC2 (Santa Cruz, Dallas, TX; Ccp58, 1:150), CEA (Zytomed Systems GmbH, Berlin, Germany; Col-1, 1:600), p16 (Ventana Medical System; E6H4, ready to use), ISLET1 (Abcam, Cambridge, UK; 1H9, 1:500), and PDX1 (Epitomics, Burlingame, CA, EP139, 1:2000).

Synaptophysin, chromogranin A, Ki-67, p53, Rb1, ATRX, and DAXX were evaluated as previously described [2]. MUC1, MUC2, and CEA showed apical or full cytoplasmic positivity; PDX1 and ISLET1 showed a nuclear positivity; and p16 exhibited cytoplasmic and nuclear positivity. The latter marker was evaluated using a 3-tired scoring system (weak/moderate/strong positivity).

2.4. DNA preparation

Molecular analysis was performed on extracted DNA from manually microdissected formalin-fixed and paraffin-embedded