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Original contribution

Aberrant Menin expression is an early event in pancreatic neuroendocrine tumorigenesis☆,☆☆

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Summary Pancreatic neuroendocrine tumors (PanNETs) are the second most common pancreatic malignancy and cause significant morbidity and mortality. Neuroendocrine microadenomas have been proposed as a potential precursor lesion for sporadic PanNETs. In this study, we applied telomere-specific fluorescent in situ hybridization (FISH) to a series of well-characterized sporadic neuroendocrine microadenomas and investigated the prevalence of alterations in known PanNET driver genes (*MEN1* and *ATRX*/*DAXX*) in these same tumors using immunohistochemistry for the encoded proteins. We identified aberrant Menin expression in 14 of 19 (74%) microadenomas, suggesting that alterations in Menin, at least a subset of which was likely due to somatic mutation, are early events in pancreatic neuroendocrine tumorigenesis. In contrast, none of the microadenomas met criteria for the alternative lengthening of telomeres phenotype (ALT) based on telomere FISH, a phenotype that is strongly correlated to *ATRX* or *DAXX* mutations. Two of 13 microadenomas (15%) were noted to have very rare abnormal bright telomere foci on FISH, suggestive of early

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ALT, but these lesions did not show loss of ATRX or DAXX protein expression by immunohistochemistry. Overall, these data suggest that loss of Menin is an early event in pancreatic neuroendocrine tumorigenesis and that ATRX/DAXX loss and ALT are relatively late events.

1. Introduction

Pancreatic neuroendocrine tumors (PanNETs) are the second most common malignant neoplasm in the pancreas after ductal adenocarcinoma [1]. Although usually less aggressive than pancreatic ductal adenocarcinoma, the prognosis of PanNETs is still poor, with overall 5-year survival averaging only 40% [2]. Recent studies have elucidated the mutational signature of sporadic PanNET; the driver genes in pancreatic neuroendocrine tumorigenesis are now known, but the timing of these alterations and their functional roles remain to be clarified. As neuroendocrine lesions are fairly common incidental findings [3], it is important to clarify which alterations account for the initiation of early lesions and which fuel the progression to more clinically aggressive neoplasms.

PanNETs usually arise sporadically but can also occur in patients with inherited cancer predisposition syndromes such as multiple endocrine neoplasia type 1 (MEN-1). Patients with MEN-1 syndrome have a germline mutation in the tumor suppressor gene *MEN1* and have a 70% lifetime risk of developing PanNET [4]. Somatic loss of the remaining wild-type allele of *MEN1* (11q13) has been reported in up to 100% of tumors in these syndromic patients [5-7]. In patients without the inherited MEN-1 syndrome, 44% of sporadic PanNETs have somatic mutations in the *MEN1* gene [8], and 19%-44% of sporadic PanNETs have loss of heterozygosity at the *MEN1* locus [5,9].

In addition to frequent somatic mutations in *MEN1*, recent whole-exome and targeted sequencing studies have shown that 43% of sporadic PanNETs have somatic inactivating mutations in *ATRX* or *DAXX* [8]. These genes encode members of a chromatin-remodeling complex, which incorporates the histone variant H3.3 into telomeric DNA. Mutations in *ATRX* and *DAXX* are associated with the alternative lengthening of telomeres (ALT) phenotype, a telomerase-independent mechanism of telomere maintenance [10]. Recent studies have shown that mutations in *ATRX* and *DAXX* (with activation of ALT) in PanNETs correlate with larger tumor size, advanced tumor stage, and chromosomal instability, suggesting that these genetic alterations are late events in sporadic PanNET progression and/or that they confer a more aggressive phenotype [11].

Microadenomas of the pancreas are histologically similar to PanNETs but are by definition smaller than 5 mm [12]. Although not universally accepted, some investigators believe that microadenomas are precursors to larger PanNETs. To date, early tumorigenesis in sporadic PanNETs remains understudied, whereas PanNET development has been well studied in MEN-1 patients. MEN-1 patients have many microadenomas, and it has been shown that 100% of microadenomas in these patients have allelic loss of the *MEN1* locus (which does not occur in surrounding ducts, acinar tissue, and hyperplastic islets) [6,13]. Because of these genetic alterations, as well as the high risk of PanNET development in these patients, microadenomas are considered precursors to PanNETs in MEN-1 patients [14,15]. In contrast to early occurring *MEN1* alterations, ATRX and DAXX expression was reported to be retained in all MEN1-associated microadenomas, demonstrating that ATRX and DAXX loss and therefore ALT are also likely late alterations in PanNET tumorigenesis in MEN-1 patients [16].

Sporadic microadenomas provide insights into the early tumorigenesis of sporadic PanNETs, distinguishing early from late-occurring genetic alterations. In this study, we analyzed 19 well-characterized sporadic microadenomas with fluorescence in situ hybridization (FISH) and immunohistochemistry (IHC) to analyze the ALT phenotype, ATRX and DAXX expression, and Menin expression [10,17].

2. Materials and methods

2.1. Patients and tissue microadenomas

The study was approved by the Institutional Review Board of The Johns Hopkins Hospital and was exempt from the Institutional Review Board of the University of Florida. Microadenomas for this study were identified from 2 sources. First, transplantation-suitable pancreata were recovered from organ donors and processed with identification of nonsyndromic microadenomas in 4 donors as previously described [18]. Second, additional cases of sporadic microadenoma were identified in surgical specimens from the pathology archives of The Johns Hopkins Hospital. For all cases, the following exclusion criteria were used: lesion size >5 mm, synchronous PanNET, and MEN-1 and Von Hippel−Lindau syndrome. In total, 19 cases were included in the study, including 4 cases from organ donors and 15 cases from surgically resected pancreata. Eight 5-μm sections were cut on plus slides (Cardinal Health, Dublin, OH) for IHC. Before and after cutting plus slides, a hematoxylin and eosin–stained slide was made to confirm the presence of microadenoma on slides for IHC. All hematoxylin and eosin slides were reviewed by an expert in pancreatic pathology to confirm the diagnosis of microadenoma.

PanNETs for validation of the Menin antibody were taken from a previously sequenced group of PanNETs by Jiao et al [8]. Six cases with homozygous or heterozygous mutations