Human Pathology (2017) 61, 148–157

ELSEVIER

Human PATHOLOGY

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Original contribution

CD133 expression in well-differentiated pancreatic neuroendocrine tumors: a potential predictor of progressive clinical courses☆

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Received 12 July 2016; revised 18 October 2016; accepted 28 October 2016

Keywords:
Neuroendocrine tumor;
CD133;
CK19;
MIB-1;
Prognosis;
Stem cell

Summary The present study aimed to elucidate whether the stemness molecule, CD133, is expressed in well-differentiated pancreatic neuroendocrine tumors (PanNETs; World Health Organization grades 1 and 2) and establish its clinical relevance using 2 separate cohorts. In the first series (n = 178) in which tissue microarrays were available, immunohistochemistry revealed that CD133 was expressed in 14 cases (8%). CD133+ PanNETs had higher TNM stages ($P < .01$), more frequent lymphovascular invasion ($P = .01$), and higher recurrence rates ($P = .01$). In the second cohort (n = 56), the expression of CD133 and CK19 was examined in whole tissue sections. CD133 and CK19 were positive in 10 (18%) and 36 (64%) cases, respectively. CD133 expression correlated with higher pT scores ($P < .01$), the presence of microscopic venous infiltration ($P = .03$), and shorter disease-free periods ($P < .01$). When cases were divided into grade 1 and 2 neoplasms, patients with CD133+ PanNET continued to have shorter disease-free periods than did those with CD133− tumors in both groups ($P < .01$ and $P = .02$, respectively). Although CK19+ cases

☆ Disclosures: None to disclose.

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http://dx.doi.org/10.1016/j.humpath.2016.10.022

had shorter disease-free periods than did CK19− cases in the whole cohort ($P = .02$), this difference was less apparent in subanalyses of grade 1 and 2 cases. CD133 expression also appeared to be an independent predictive factor for tumor recurrence in a multivariate analysis ($P = .018$). The CD133 phenotype was identical between primary and metastatic foci in 17 of 18 cases from which tissues of metastatic deposits were available. In conclusion, the combination of CD133 phenotyping and World Health Organization grading may assist in stratifying patients in terms of the risk of progressive clinical courses.

1. Introduction

Pancreatic neuroendocrine neoplasms are rare and only account for between 1% and 3% of primary pancreatic tumors [1]. They have been classified into pancreatic neuroendocrine tumors (PanNETs) and neuroendocrine carcinomas with distinct pathological features. The former (World Health Organization [WHO] grades 1 and 2 [G1 and G2]) are characterized by relatively uniform tumor cells arranged in a trabecular and solid architecture, whereas the latter (WHO grade 3 [G3]) show high-grade morphologic features resembling small cell carcinomas or large cell neuroendocrine carcinomas of the lungs [1,2]. G1 and G2 neoplasms are also often referred to as well-differentiated PanNETs. One clinical challenge is to predict the biological behavior of well-differentiated PanNETs. The single reproducible histologic predictor is proliferative activity estimated by mitotic counts and Ki-67 indices. G1 neoplasms show less than 2 mitotic counts (per 10 high-power fields) and a Ki-67 labeling index of 3% or less, whereas G2 is defined as having at least 2 to 20 mitotic counts or a Ki-67 index of 3% to 20% [3]. However, because these criteria are not sufficient to predict clinical courses, other standards that may be used in combination with the WHO grades are awaited.

The aims of some pathological studies have been to identify ancillary immunohistochemical markers to grade PanNETs. Among the potential prognostic markers proposed to date (eg, COX2, p27, and CD99) [4-6], CK19 has been the most extensively studied in this aspect [7-13]. CK19 was found to be positive in between 49% and 70% of PanNETs, with its expression being associated with aggressive pathological features (eg, higher mitotic counts, lymphovascular invasion, and higher TNM stages) [7-13]. Cancer-related death occurred in 36% of the CK19+ cases (10/28) and none of the CK19− cases (0/26) examined in an index report [7]. Three studies confirmed that CK19+ cases had a significantly poorer prognosis [9-11], whereas another 2 did not validate this finding [12,13]. Another potential prognostic marker is KIT, which was shown to be expressed in between 8% and 46% of PanNETs, with its relevance to a poor prognosis being indicated in 2 studies [10,13]. CK19 and KIT both appear to be expressed in endocrine cells of the embryonic pancreas, but not in matured islets [14-16], suggesting that an immature or stem cell phenotype in PanNETs is linked to an aggressive biological behavior and poorer prognosis, similar to many other malignant neoplasms of the extrapancreatic organs.

CD133 (also called prominin-1), encoded by the *PROM1* gene (4p15.32), is a widely accepted stem cell marker [17]. It was originally discovered from neural progenitors and hematopoietic stem cells, and its expression was further confirmed in the stem cells of other cellular lineages [18-20]. Because the expression of CD133 has also been detected in malignant neoplasms, it has been used to isolate a principal subset of cells ("cancer stem cells") either alone or in combination with other markers [21]. In the pancreas, ductal adenocarcinomas were mainly investigated from the aspect of CD133 expression [22,23]. However, CD133 expression in PanNETs has not yet been examined. One previous study examined CD133 expression in 90 neuroendocrine neoplasms of the digestive system [24]. One case each of pancreatic neuroendocrine carcinoma (WHO G3) and mixed acinar-neuroendocrine carcinoma was included in the study cohort, but no case of G1 or G2 PanNET was studied.

In the present study, we examined CD133 expression in well-differentiated PanNETs using 2 separate cohorts, and correlated the results obtained with other clinicopathological features to elucidate the prognostic value of CD133 expression in this uncommon pancreatic neoplasm.

2. Materials and methods

2.1. Study cohorts

This study was approved by the ethics committee at Kobe University Graduate School of Medicine (No. 1794). The study consisted of 2 independent cohorts. The first was a consecutive series (n = 178) of well-differentiated PanNETs that were surgically resected at Asan Medical Center in Seoul between 1995 and 2013. In these cases, tissue microarrays had already been constructed and used in previous studies [13,25]. Tissue arrays contained 3 cores (2 mm in diameter) from each tumor. Although clinicopathological features had also been recorded, prognostic data were updated for the present study. Tissue samples and clinical information were already available; therefore, tissue microarrays of this cohort were used for a discovery study to elucidate whether CD133 is expressed in PanNETs and, if so, which clinicopathological features are potentially linked to its expression.

The second cohort was consecutive cases of well-differentiated PanNETs surgically treated at Kobe University Hospital and Hyogo Cancer Center, both belonging to the