Expression of CD133 in Neuroendocrine Neoplasms of the Digestive Tract: A Detailed Immunohistochemical Analysis

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Gastroenteropancreatic neuroendocrine tumors (GEP-NETs) are potentially malignant with variable biologic behavior that originates from neuroendocrine cells of digestive tract. Recently, the existence of cancer stem cells (CSC) was demonstrated in tumors of gastrointestinal tract. CD133 is a transmembrane glycoprotein that serves as a CSC marker in various malignancies. However, the expression of CD133 in neuroendocrine neoplasms (NEN) of digestive tract has not been studied. We evaluated tissue expression of CD133 by immunohistochemistry in 90 NENs of digestive tract with their matched non-neoplastic mucosa including stomach ($n=15$), small intestine ($n=7$), appendix ($n=3$), colon ($n=8$), rectum ($n=41$), pancreas ($n=2$), gallbladder ($n=4$) and liver ($n=10$). Tumors were divided according to 2010 WHO classification. CD133 expression was detected in 30.3% ($17/56$) of well-differentiated neuroendocrine tumors (NET), 26.1% ($6/23$) of poorly-differentiated neuroendocrine carcinomas (NEC) and 63.6% ($7/11$) of mixed adenoneuroendocrine carcinoma (MANECs). MANEC refers to existence of both adenocarcinoma and NEC together, each one comprising at least 30% of the tumor. CD133 was expressed in cytoplasm, luminal-side of cell membrane, or both and the staining pattern correlated with tumor growth pattern. CD133 expression was not significantly correlated with tumor grade, site, expression of neuroendocrine markers (chromogranin-A and synaptophysin) and patients’ survival. Thus, CD133 expression may lack prognostic significance in GEP-NETs. Importantly, CD133 was not detectable in non-neoplastic neuroendocrine cells of digestive system including pancreatic islets. In conclusion, CD133 is expressed in poorly-differentiated NECs and well-differentiated NETs of the digestive tract.

Keywords: cancer stem cells; CD133; gastroenteropancreatic neuroendocrine tumors; immunohistochemistry; prognosis

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Introduction

Cancer stem cell (CSC) theory explains the biological heterogeneity of human solid tumors, according to which a small fraction of cancer cells is solely responsible for the growth and maintenance of the entire heterogeneous tumors (Pardal et al. 2003). They are resistant to chemotherapy due to their innate ability to escape the cytotoxic effects of conventional therapy by employing drug transporters and enhanced DNA repair mechanisms (Krishnamurthy et al. 2004; Bao et al. 2006). Therefore, CSCs could prove a promising candidate for targeted cancer therapy. However the existence of CSCs in gastroenteropancreatic neuroendocrine tumors (GEP-NETs) remains largely unexplored.

GEP-NETs are rare with an incidence of 1 per 100,000 population for pancreatic and 1.95-2.5 per 100,000 for gastrointestional tumors (Plöckinger et al. 2004; Oberg and Eriksson 2005). However, the incidence has substantially increased in different parts of the world over the last three decades thanks partly to the increased availability of advanced endoscopic and radiologic imaging (Modlin et al. 2008; Cho et al. 2012). GEP-NETs arise at every location, where endocrine precursor cells are located and comprise tumors with variable biologic behavior depending on the characteristic of the cells that the tumor originates from. They were primarily classified according to the site of origin as foregut (respiratory system, stomach, duodenum, proximal jejunum and pancreas), midgut (distal jejunum, ileum, appendix and right hemi-colon) and hindgut (left hemi-colon and rectum) (Williams and Sandler 1963). The term carcinoid was initially used for all neuroendocrine tumors assuming their uncertain potential of malignancy,
but is now replaced by neuroendocrine neoplasms (NENs). Recently NENs are further divided into three main categories: well-differentiated neuroendocrine tumor (NET), poorly differentiated neuroendocrine carcinoma (NEC), and mixed adenoneuroendocrine carcinoma (MANEC). NET refers to NENs composed of cells with similar features to those of the non-neoplastic neuroendocrine cells of the digestive system, with mild to moderate nuclear atypia and a low number of mitosis [< 20 per 10 high power field (HPF)], while NEC is a poorly-differentiated and highly malignant NEN, showing marked nuclear atypia, multifocal necrosis and high number of mitosis (> 20 per 10 HPF). The term NEC is used for neoplasms that were previously classified as small cell carcinoma, large cell neuroendocrine carcinoma and poorly differentiated neuroendocrine carcinoma and has worse prognosis than NETs (Cho et al. 2012). There is no evidence so far to indicate the transformation or progression of a well-differentiated NET to a NEC and neither there has been any report to show the occurrence of metastatic NEC from a well-differentiated NET. MANEC has a phenotype that is morphologically recognizable as both gland-forming epithelial and neuroendocrine, with both components being malignant and each component comprises at least 30% of the tumor. MANECs raise the concept of a “histogenetic tumor typing”. Molecular analysis of MANECs defines that different components in these tumors have a common clonal origin (Reu et al. 2012). Moreover, it is believed that the tumors of the gastrointestinal tract arise from a common stem cell (Thompson et al. 1990).

All NENs are potentially malignant in contrast to the belief held when it was first diagnosed (Modlin et al. 2005). The clinical course of NETs is often indolent but resistant to chemotherapy (Yao et al. 2008). Additionally, patients with NECs are at increased risk of developing synchronous or metachronous non-endocrine malignancies (Modlin et al. 2003).

Recently, the existence of small population (5.8% ± 1.4%) of putative CSCs was demonstrated in the NETs of gastrointestinal tract using Aldefluor assay from 19 primary patients’ samples (Gaur et al. 2011). They also showed that when the neuroendocrine cell line, CNDT96 was positive for ALDH (a putative CSC marker), they revealed higher tumor growth and sphere forming ability. However, these cells failed to show immunoreactivity for CD133. On the other hand, lung small cell carcinoma (NEC) cell lines were reported to express CSC marker, CD133, and these cells revealed stem cell-like features, including self-renewal, differentiation, proliferation and chemoresistance (Cui et al. 2011; Kubo et al. 2013). However, the expression of CD133 in GEP-NETs has not been reported yet.

CD133 or prominin-1 is a membrane glycoprotein that was initially identified in neuroepithelium. Later, the presence of CD133 (+) cells was shown on endothelial progenitor cells. In the last decade, CD133 positivity has served as maker of CSCs in many solid tumors, including brain, lung, stomach, pancreas, colon, endometrium and ovary. CD133 demonstrates poor prognosis and advanced stage (Zhang et al. 2012) in many tumors. It is proposed that CD133 is associated with activation of stemness-related signaling pathway, resistance to apoptosis and bioenergetic stress (Hambardzumyan et al. 2008). However, the exact biologic function of this molecule still remains unknown.

We therefore analyzed the immunohistochemical (IHC) expression of CD133 in the GEP-NETs to identify the CSC population in GEP-NETs.

Material and Methods

Patients and tissue samples

This study includes 90 NENs that were pathologically confirmed at Wonju Severance Christian Hospital from January 2000 to September 2012. Clinicopathological data such as patients’ age, gender, tumor location, invasion depth, grade, and lymph node metastasis were collected from the pathology reports. Follow-up information from national cancer registration center was used for survival analysis. The study used human materials and has been approved by the Institutional Ethics Committee of Yonsei University, Wonju College of Medicine (YWMR-12-4-031).

Pathological evaluation

All slides of cases included in this study were re-evaluated and graded based on WHO 2010 classification for NENs of the digestive system: NET G1, NET G2, NEC and MANEC (Klimstra et al. 2010). The grading of NENs is based on the assessment of proliferation fraction, defined as G1; in which the mitotic count is less than 2 per 10 HPF and/or Ki-67 labeling index is 2% or less, G2; which has mitotic count of 2-20/10 HPF and/or Ki-67 labeling index of 3-20%, and G3 (NEC); in which the mitotic count exceeds 20/10 HPF and/or Ki-67 labeling index is more than 20% (Rindi et al. 2006). The growth pattern of tumor cells at microscopic level was evaluated according to Soga classification. Based on that, NENs are divided into five categories according to the growth pattern of the neuroendocrine tumor cells. Type I refers to the pattern in which tumor cells are arranged in solid, nodular or insular pattern while in type II, tumor cells are arranged in trabecular pattern or in ribbons with frequent anastomosing growth. In type III, tumor cells make tubules, acini and glands or rosette-like pattern and in type IV, tumor cells shows atypical growth and features of poor differentiation, while in type V, a combination of the above-mentioned growth patterns are observed (Soga and Tazawa 1971).

Immunohistochemical analysis

Paraffin-embedded tissue sections from well representative blocks were deparaffinized with xylene and then rehydrated through graded alcohol solutions. According to manufacturer’s instructions, antigen retrieval comprised of slide warm-up to 75°C and incubation for 4 min followed by the application of cell conditioning solution #2 (Ventana medical system, Roche, Tucson, USA) for 60 min. UV inhibitor was applied for 4 min to block endogenous peroxidase followed by washing slides with reaction buffer. We used the Ultra View Universal DAB Detection Kit for IHC staining; the procedure is briefly described as follows. The primary antibody (CD133/1 (AC133) pure, Human, MACS, Miltenyi Biotec, CA, USA) was applied and incubated for 2 hrs at a 1:100 dilution in Ventana machine.
CD133 Expression in GEP-NET

Results

Clinicopathological characteristics of patients

The 90 patients included in the study comprised of 55 male and 35 female, aged 29 to 87 years old (mean age, 56.9 years). The pathological stages of tumor were T1 \( (n = 58) \), T2 \( (n = 5) \), T3 \( (n = 13) \), T4 \( (n = 4) \), and metastatic \( (n = 10) \). The lymph node metastasis was found in 15 patients \( (N1 = 11 \text{ and } N2 = 4) \). Seven patients with available lymph nodes were free from tumor and the remaining patients did not have submitted lymph nodes. Tumor location consisted of stomach \( (n = 15) \), duodenum \( (n = 6) \), pancreas \( (n = 2) \), gallbladder \( (n = 4) \), liver \( (n = 10) \), ileum \( (n = 1) \), appendix \( (n = 3) \), colon \( (n = 8) \), and rectum \( (n = 41) \). The patients were divided according to 2010 WHO classification into four groups based on the histologic grade of their tumors; NET G1 \( (n = 47) \), NET G2 \( (n = 9) \), NEC \( (n = 23) \), and MANEC \( (n = 11) \). The clinicopathological data of patients are summarized in Tables 1 and 3.

Immunohistochemical expression of CD133 in digestive system

1) Non-neoplastic mucosa of digestive system

The CD133 expression in non-neoplastic mucosa of digestive system (wherever observed) was exclusively localized to the luminal side of the cell membrane. CD133 was not expressed in the cytoplasm of the non-neoplastic neuroendocrine cells that are scattered throughout the gastrointestinal tract. In stomach, there was focal CD133 expression along the luminal side of some non-endocrine cells of normal pyloric glands (Fig. 1A). There was no immunohistochemical expression of CD133 in cardiac or body type glands

| Table 1. The clinicopathological characteristics of patients and their correlation with CD133 immunohistochemical expression level. |
| --- | --- |
| Variables | All patients \( (N = 90) \) | CD133 IHC expression (%) |
| Gender | | Negative | 1+ | 2+ | 3+ |
| Male | 55 | 38.2 | 20 | 23.6 | 18.2 |
| Female | 35 | 54.29 | 28.6 | 11.4 | 5.7 |
| pT stage | | | | | |
| T1 | 58 | 37.9 | 29.3 | 20.7 | 12.1 |
| T2 | 5 | 20 | 20 | 40 | 20 |
| T3 | 13 | 54 | 23 | 23 | 0 |
| T4 | 4 | 0 | 0 | 0 | 75 |
| Metastatic | 10 | 91 | 0 | 0 | 9 |
| pN stage | | | | | |
| N0 | 7 | 42.8 | 14.3 | 14.3 | 28.6 |
| N1 | 11 | 36.4 | 18.2 | 27.2 | 18.2 |
| N2 | 4 | 25 | 25 | 0 | 50 |

IHC, immunohistochemical.
or in glands with intestinal metaplasia. The non-neoplastic mucosa of duodenum showed CD133 positivity in Brunner’s glands (Fig. 1B). No detectable unequivocal CD133 immunoreactivity was observed in non-neoplastic mucosa of ileum, appendix, colon, rectum and gallbladder. The non-neoplastic pancreatic islets which are aggregations of endocrine cells were exclusively non-reactive for CD133 (Fig. 1C). The lining epithelia of large to medium-sized pancreatic ducts were also devoid of CD133 expression. However, the lining epithelia of small ducts, ductules and centroacinar cells showed positivity for CD133 along the luminal side of cell membrane. In the non-neoplastic liver, CD133 was expressed in few small bile ducts and ductules (Fig. 1D). There was no expression of CD133 in the hepatocytes and large bile ducts.

2) Neuroendocrine neoplasms of digestive tract

a) The Pattern of CD133 immunohistochemical expression

CD133 immunohistochemical expression in NENs was observed as cytoplasmic, luminal or a combination of cytoplasmic & luminal (Table 2). The cytoplasmic staining was either diffuse (Fig. 2A) or focal (localized only to the apical or basal side of the cytoplasm) (Fig. 2B). The luminal staining was observed along the apical side of the cell membrane (apical membrane) (Fig. 2C). The combination of cytoplasmic and luminal expression was also observed (Fig. 2D).

b) The pattern of CD133 immunohistochemical expression is related to the histologic growth pattern of tumor

With matched evaluation of CD133 immunohistochemical expression pattern and tumor growth patterns, it was found that tumors with type I (insular or nested) and type IV (poorly differentiated) growth patterns frequently revealed diffuse cytoplasmic expression of CD133, while tumors with type II (trabecular), and type III (glandular or acinar) growth patterns revealed focal & localized cytoplasmic expression and luminal expression of CD133, respectively. MANECs and NETs that revealed solid as well as glandular or acinar growth patterns (type V and type I + III, respectively) showed combination of cytoplasmic and luminal staining for CD133 in each component, respectively. The correlation of the immunohistochemical expression of CD133 according to the growth pattern of tumor cells is summarized in Table 2 and demonstrated in Fig. 2.
c) Immunohistochemical expression of CD133 is not related to tumor location or expression of neuroendocrine markers

CD133 was strongly expressed in NENs of stomach (20%), duodenum (50%), pancreas (50%), liver (10%), gallbladder (25%), appendix (66.6%), colon (50%), and rectum (34.1%). The distribution of CD133 immunohistochemical expression in NENs of different organs included in this study is demonstrated in Table 3. However, no significant difference in the distribution of CD133 immunohistochemical expression according to the location of the tumor was observed. In addition, CD133 immunohistochemical expression showed no significant correlation with the histologic grades of tumors based on WHO classification 2010. The CD133 immunohistochemical expression according to the expression of neuroendocrine markers (Synaptophysin and Chromogranin-A) is shown in Table 4. CD133 expression in NENs was not related to the expression of neuroendocrine markers.

Immunoreactivity for CD133 is not a prognostic factor in NEC

The survival analysis, adjusted for age and tumor stage, was performed for 23 patients with NECs who had available follow-up data. The tumor locations in these patients were as following: stomach \( (n=5) \), duodenum \( (n=2) \), pancreas \( (n=1) \), gallbladder \( (n=2) \), liver \( (n=10) \), colon \( (n=1) \), and rectum \( (n=2) \). The T-stage of the tumors were T1 \( (n=3) \), T2 \( (n=4) \), T3 \( (n=5) \), T4 \( (n=1) \) and metastatic \( (n=10) \). Six out of 23 NECs were positive for CD133. No prognostic significance of CD133 immunohistochemical expression was found in these patients. (P =
Table 3. The distribution of CD133 immunohistochemical expression according to 2010 WHO classification and tumor location.

<table>
<thead>
<tr>
<th>Location</th>
<th>Total No. of cases</th>
<th>No. of CD133 IHC + / No. of total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>NET G1</td>
</tr>
<tr>
<td>Stomach</td>
<td>15</td>
<td>0/5</td>
</tr>
<tr>
<td>Duodenum</td>
<td>6</td>
<td>2/3</td>
</tr>
<tr>
<td>Pancreas</td>
<td>2</td>
<td>–</td>
</tr>
<tr>
<td>Gallbladder</td>
<td>4</td>
<td>–</td>
</tr>
<tr>
<td>Liver</td>
<td>10</td>
<td>–</td>
</tr>
<tr>
<td>Ileum</td>
<td>1</td>
<td>–</td>
</tr>
<tr>
<td>Appendix</td>
<td>3</td>
<td>1/1</td>
</tr>
<tr>
<td>Colon</td>
<td>8</td>
<td>0/3</td>
</tr>
<tr>
<td>Rectum</td>
<td>41</td>
<td>12/35</td>
</tr>
</tbody>
</table>

Total: 90

15/47 (31.9) 2/9 (22.2) 6/23 (26.1) 7/11 (63.6)

(%; CD133 IHC +); NET, neuroendocrine tumor; NEC, neuroendocrine carcinoma; MANEC, mixed adenoneuroendocrine carcinoma; IHC, immunohistochemical.

Table 4. Crosstable of immunohistochemical expression of CD133 according to the expression of neuroendocrine markers.

<table>
<thead>
<tr>
<th>Neuroendocrine markers</th>
<th>CD133 IHC expression level</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Negative 1+ 2+ 3+</td>
<td></td>
</tr>
<tr>
<td>Synaptophysin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>37  20  17  12</td>
<td>85 (94.4)</td>
</tr>
<tr>
<td>Negative</td>
<td>3   1   0   1</td>
<td>5 (5.6)</td>
</tr>
<tr>
<td>Chromogranin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>22  6  12  9</td>
<td>48 (53.3)</td>
</tr>
<tr>
<td>Negative</td>
<td>18  15  5  4</td>
<td>42 (46.7)</td>
</tr>
</tbody>
</table>

(%) IHC, immunohistochemical.

Fig. 3. Survival analysis of 23 patients with NECs according to the immunohistochemical expression of CD133. The Log-rank test comparing the survival curves between the CD133 positive group (n = 6) and CD133 negative group (n = 17) reveals that there is no prognostic significance of CD133 immunohistochemical expression (P = 0.97).
Discussion

In the human embryo, CD133 is expressed in various developing epithelia (Corbeil et al. 2000). CD133 expression seems to be down-regulated in most adult epithelial tissues with few exceptions such as pancreatic ducts that remain to express CD133 in adulthood (Lardon et al. 2008). In adult organ systems, CD133 remains detectable only in very rare cells with stem cell properties. The expression of CD133 as a CSC marker in different solid tumors has been previously reported, although they have been contradictory and controversial. Overall, it is believed that CSCs exist in the CD133 (+) subpopulation of tumors. And CD133 positivity has gained much importance for predicting worse prognosis in several malignancies. We herewith described the pattern and distribution of CSC marker, CD133, in GEP-NET as well as the non-neoplastic mucosa and parenchyma of gastrointestinal tract, pancreas and liver.

CD133 associates with cholesterol-rich membrane microdomains “lipid rafts” and localizes to special subdomains of the plasma membrane of cells (Corbeil et al. 2010). Recent studies have shown the two distinct staining patterns for CD133: apical/endoluminal membrane staining and cytoplasmic staining. It was suggested that membranous expression of CD133 is observed in the malignant glandular epithelia while cytoplasmic positivity is seen in the non-epithelial tumors (Immervoll et al. 2008). Contrary to that, reports from different studies (including our unpublished work) have verified the cytoplasmic expression of CD133 in epithelial malignancies (Fan et al. 2011). The significance of pattern of expression has been controversial. Some studies suggested that cells with cytoplasmic CD133 staining are putative CSCs (Sasaki et al. 2010; Jao et al. 2012). However, majority of studies that claim the CSC marker role for CD133 have reported membranous staining pattern (Kojima et al. 2008; Corbeil et al. 2010).

Localization of this molecule in different cellular compartments by immunohistochemistry has led to the assumption that it may have specific functional role in the membrane and cytoplasm. The cytoplasmic expression is thought to happen when the molecule is transported from the plasma membrane into the cytoplasm during endocytosis (Jao et al. 2012). Membranous localization has been previously described as being important for cell movement, which is associated with important processes such as chemotaxis, embryonic development, mobility and the under-controversy stem cell property (Giebel et al. 2004). Similarly, it was suggested that localized CD133 distribution in the cell membrane is a mechanism of asymmetric CD133 segregation during mitosis in glioblastoma (Lathia et al. 2011). Molecular genetic analysis of autosomal recessive retinal degeneration has demonstrated that the affected individuals had a frame-shift mutation in CD133 with premature termination of translation, and the truncated protein could not be transported to the cell surface. Absence of membranous CD133 expression in a previous study on hepatocellular carcinoma was concluded as the accumulation of such truncated CD133 protein that might result in rapid degradation. Similar phenomenon has been already observed for the epidermal growth factor receptor in cultured cells (Xu et al. 1984). Another possible mechanism discussed for the justification of above-mentioned claim has been pointed out as the failure of transportation and insertion of newly synthesized molecules within the Golgi apparatus into the membranes (Piyathilake et al. 2002). Which pattern of expression represents the thorough nature of CD133 and its functional relevance is yet to be rectified.

In this study, we used monoclonal antibody against the CD133/1 or AC133, one of the two epitopes of the CD133 protein. The other epitope is AC141. Initially generated to target CD133 surface antigen, AC133 has been lately used to identify cancer stem cells in several types of solid tumors. It was demonstrated that the AC133 epitope rather than the CD133 protein seems to be restricted to the stem cells (Miraglia et al. 1997). In a recent paper, using the two widely used monoclonal antibodies (AC133 and Ab19898), it was found that the AC133 epitope expression acted as an adverse prognostic factor in colorectal cancer patients, matching with the CSC characteristics (Ying et al. 2013). Additionally, in our unpublished work on colorectal adenocarcinomas, we have found a significant direct correlation between CD133 immunohistochemical expression (using AC133 monoclonal antibody) and CD133 mRNA expression level (using RT-PCR) in a large number of patients.

The NENs in different organs arise from the neuroendocrine cells that are located in that area and they reveal the same immunohistochemical profile as the cells that they arise from. However, we did not observe CD133 positivity in the non-neoplastic endocrine cells, neither in those that are scattered throughout the gastrointestinal tract mucosa nor in the pancreatic islets. We further evaluated the expression of CD133 in non-neoplastic parenchyma of pancreas in 15 cases of ductal adenocarcinoma of pancreas, in none of which the pancreatic islet showed immunoreactivity to CD133 (data not shown). These findings indicate that CD133 is not a marker for normal neuroendocrine cells.

On the contrary, the expression of CD133 was observed in NENs of various organs and of different histologic grade. The IHC expression of CD133 in NENs is hence not a mere reflection of the cytoplasmic content of endocrine granules and histologic differentiation. This is the first report to show the expression of CD133 in the GEP-NET and the first report to show that not only poorly differentiated NECs (small cell carcinomas) but also the well differentiated NETs (NET G1 and NET G2) express CD133. The significance of such expression however remains to be clarified in future in-depth and comprehensive studies.

The survival analysis of patients with NENs especially the well-differentiated NETs (G1 and G2) is associated with certain difficulties due to different reasons. Firstly, until
Recent past, NET G1 and NET G2 (previously called carcinoid tumor) were not included in the cancer registry (Cho et al. 2012). The second reason is the indolent course of the NETs, which require long follow up. The third reason is lack of universal acceptance on the nomenclature of NETs and for staging of disease (Edge and Compton 2010), which in turn causes difficulty in evaluating data on a cohort level to specify the prognostic significance of NENs, specially the low grade tumors. In this study, the group of patients with NECs ($n = 23$) had available survival data obtained from cancer registry. The analysis of correlation between CD133 and survival came short of demonstrating significant difference of overall survival between the CD133 (+) and CD133 (−) groups. However, the survival analysis in our study has few short-comings. First, small number of study subjects in this study had available follow up data. Secondly, the tumor locations in these patients were different. And at last, the patients had different T-stage. Therefore, a study with large number of subjects in different categories is needed to elucidate the prognostic significance of CD133 expression. Although it was previously demonstrated that small cell carcinoma cell lines of lung had a subpopulation that were positive for CD133 and these cells showed stem cell like features and chemoresistance (Cui et al. 2011; Kubo et al. 2013), there is no available data in the literature so far on human subjects to show the prognostic significance of CD133 expression in NENs. Our study is the first to shows that patients with NECs do not have significantly different prognosis based on their CD133 IHC expression status. Two things however need to be mentioned here. Firstly, the CD133 expression is not limited to the NENs with high histologic grade and since it is evident that irrespective of CD133 expression, NET G1 and G2 have a far better prognosis than the NECs (G3) and MANECs (Cho et al. 2012), therefore the interpretation of the prognostic role of CD133 as a CSC marker seems questionable in NENs. Similarly it was recently shown that there is no significant difference in the sphere-forming efficiency between the CD133 (+) and CD133 (−) groups in the small cell lung cancer cell line H446 (Qiu et al. 2012). Moreover, Kubo et al have demonstrated that among the two groups of CD133(−) and CD133 (+) small cell cancer cell line SBC-7, the CD133(−) group had more tumorigenic potential than CD133 (+) group, and thereby they referred to CD133 as an “inadequate” marker for CSCs (Kubo et al. 2013). Secondly, as we did not observe the CD133 IHC expression in non-neoplastic neuroendocrine cells in any of the organs in our study, it is of great interest to further study the role and prognostic significance of this molecule in the development of NENs.

Conclusion
Unprecedentedly, we demonstrate the expression of putative CSC marker, CD133, in both well and poorly differentiated GEP-NETs with detailed description of its pattern and distribution. Although the IHC expression for CD133 was not associated with patients’ survival in NECs in our study, the CD133 immunohistochemical expression observed only in tumors (not in normal) of neuroendocrine system is an impelling finding that asks for further exploration.

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Conflict of Interest
We declare that no conflict of interest exists.

References


CD133 Expression in GEP-NET

BMC Cancer, 8, 48.


