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Diagnostic Values of a Progenitor Cell Marker CD133 Expression in Various Types of Adenocarcinoma

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Abstract

Background: The role of stem tumor cell involvement in tumorigenesis and partial resistance to chemotherapy has been established in recent years. However, diagnostic value using stem cell markers in various malignant neoplasms is not well addressed. In the current study, we compared CD133, a membranous stem cell marker, in several types of adenocarcinoma originating in colon, breast and kidney tissues, in order to determine whether expression pattern may be useful for diagnosis and differential diagnosis of varying tumors.

Methods: Paraffin embedded control and tumor sections (35 from colon, 40 from breast and 64 from kidneys) were immunohistochemically stained for CD133 using an autostainer. All membranous stains of CD133 were graded from negative (0) to 3+.

Results: In the colon, benign glands showed minimal staining (+/-) for CD133, but CD133 expression was 2+ to 3+ strong in all invasive adenocarcinoma of colon. In the kidney, unremarkable renal tubules were also minimally (+/-) positive for CD133 but the CD133 was strongly expressed in clear cell papillary renal cell carcinoma (CCP-RCC) (24/24) in contrast to its focal and weak expression in conventional clear cell renal cell carcinoma (2/20). In the breast, CD133 showed 2+ to 3+ strong expression in both benign glands and invasive ductal carcinoma.

Conclusion: Our data indicate that CD133 expression patterns can be used for supporting a diagnosis of colonic adenocarcinoma, and differentiate CCP-RCC from conventional clear cell RCC, but is likely not useful in distinguishing breast carcinoma from its benign counterpart.

Keywords: CD133; Pathologic diagnosis; Adenocarcinoma; Colon; Kidney; Breast cancer

Introduction

In addition to the heterozygosity of cells within a tumor, stem cells have been another factor owing to the chemotherapy resistance of cancer [1,2]. The dispute over stem cell's contribution to tumor proliferation has ranged from partial influence to complete attribution. The hope is that if the true malignant potential of stem cells is defined, then attacking this malevolent group of cells will lead to better prognoses and treatments [3,4]. Similar to the thirst of deducing what were the genetic materials in the mid-1900s, a breakthrough here could lead to a number of advancements in its respective field. Although the stem cell theory has been extensively studied and partially approved, the emergence of a range of consistent stem cell markers to assist in diagnosis has not been well established. In order to conduct better research on this subpopulation of cells and come closer to an answer, a dependable way of isolating and recognizing them is necessary.

CD133 is a transmembranous protein and has been found to a stem cell marker [5]. CD133 as a prognostic factor has been found in cancer stem cells of several organs such as pancreas, breast, stomach and gallbladder [6-9]. *In vitro* and *in vivo* studies of colon have shown that small amount of CD133 is normally present at stem cell niche of glandular crypts and presence of CD133 in colon cancer is considered as marker for cancer stem cells [10,11]. In a human colon cancer study, they find that expression of CD133 in colon cancer is associated with worse prognosis [12,13]. It has been demonstrated that it is expressed in benign breast tissue, the crypts of colon mucosa, portions of the renal tubules, and the parietal epithelium of the glomeruli [10,14]. Comparative studies using CD133 in various adenocarcinomas could be helpful in establishing its role in diagnosis and differential diagnosis in tumors. As the prognostic and therapeutic use of CD133 has been partially investigated before, the main goal of the current study was to evaluate whether CD133 had some pathologic diagnostic values in distinguishing several subtypes of invasive adenocarcinoma in the human body.

Patients and Methods

Patients

The study for evaluating diagnostic values of CD133 in colon cancer, renal cell carcinoma and breast carcinoma has been approved by the human investigation committee of William Beaumont health system.

Selection of colon lesions

Three groups of colectomy specimens were searched through our power path system and randomly included in the study. Group 1 was composed to 11 cases with ischemic/infarcted bowel segments. The etiology for the most cases was mainly due to the serosal adhesion of colon and its subsequent infarction of large colon. The selected segments in each case of the Group 1 included a portion of normal colon and a portion of ischemic/infracted segment. Group 2 included 11 cases with colonic resection for inflammatory bowel disease. The patients could have either ulcerative colitis or Crohn's disease. Due to severe activity in their colon, segmental resection of the severe inflamed colon was conducted. In ulcerative colitis, surface ulcer was often noted and severe acute and chronic colitis was usually present at the full layer of colonic mucosa. In Crohn's disease, intense acute and chronic colitis was found similar to ulcerative colitis but the inflammation was transmutably present from the colonic mucosa to the serosa, with additional finding of non-necrotic granulomas in the mucosa or the deep portion of muscularis propria. None of cases in the Group was found to have dysplasia or invasive carcinoma. Sections with both relatively normal colonic mucosa and severely inflamed mucosa were selected for internal comparison between the two components. Group 3 consisted of 13 colonic resections of *in situ* and/or invasive carcinoma of colon. The patients with colonic adenocarcinoma did not have either inflammatory bowel disease or bowel infarction. All selected sections from the Group 3 included a portion of invasive colonic adenocarcinoma, and their adjacent relatively unremarkable colonic mucosa for the internal comparison of CD133 expression.

Selection of renal lesions

The study included two groups. Group 1 as negative controls had twenty (20) nephrectomy sections with unremarkable parenchyma away from any kidney tumors. As the clear cell renal cell carcinoma (RCC) is the most popular RCC, twenty (20) clear cell RCC were randomly selected from our pathology archive as Group 2. The Group 3 was composed of twenty-four (24) clear cell-papillary RCC, collected over 6 years in the department of pathology, William Beaumont hospital, MI. Six of twenty-four (6/24) patients with clear cell-papillary RCC had end stage of renal disease. Five cases had needle core biopsies with diffuse CD133 expression. Clear cell-papillary RCC in four of five (4/5) cases with needle core biopsies were subsequently confirmed in the nephrectomy specimens and 1 case with needle core biopsy had no nephrectomy due to the fact that the patient had only one kidney left.

Selection of breast lesions

There were three groups in this portion of breast study. Group 1 consisted of 15 randomly selected benign breast cases (5 reduction mammoplasty cases with no significant pathology and 10 benign fibroadenoma or fibrocystic changes). Group 2 (malignant group 2) included 13 breast cases with invasive ductal carcinoma (IDC), present in either lumpectomy or mastectomy section. The invasive ductal

carcinomas of the breast primary were graded at 2 or 3. Since the invasive ductal carcinoma was commonly seen in our pathology service, they were randomly selected into the Group 2 (each block contained a portion of benign breast tissue and a portion of cancer). Group 3 (malignant Group 3) was composed of 12 BRCA-mutant breast cases; six cases had IDC whereas the remaining 6 patients did not reveal invasive carcinoma but either ductal carcinoma *in situ* or benign lesions in their breast tissue. In the three groups of breast lesions, all sections from paraffin embedded tissue were immunohistochemically stained for CD133 (an early breast progenitor marker) and CD117 (a later breast progenitor marker) [15]. Expression of the two markers in normal breast glands adjacent to lesions (normal controls), benign lesions (benign controls) and IDC were evaluated and positive rate was calculated.

Antibodies and immunohistochemical stains

Antibodies and dilutions: Monoclonal anti-CD133 (AC133, 1:50) was purchased from Miltenyi Biotec GmbH (Auburn, CA). Monoclonal anti-CD117 (1:100) was purchased from Dakocytomation (Carpinteria, CA).

Immunohistochemical stains using one antibody: The tissue was fixed in formalin for 6 to 24 hours, processed according to our standard laboratory procedure, embedded in paraffin, sectioned at 4 μm thickness, and mounted on glass slides. The slides were dried at 60°C for 60 minutes. Slides were then de-waxed in 3 xylene baths for 3 minutes each, dehydrated in 3 100% alcohol baths for 3 minutes each followed by a 30 second rinse under running water. Antigen retrieval was carried out in a Tris EDTA buffer at pH 8.0 and 99°C for 20 minutes followed by a 20 minute cool-down at room temperature and a brief water rinse. Slides were then placed in 3% hydrogen peroxide for 15 minutes followed by a quick water rinse and then placed in Tris buffer pH 7.6. Finally, slides were placed into a programmed Dako Autostainer (DakoCytomation, Carpinteria, CA) using a thermo scientific ultra-vision LP detection system (Kalamazoo, MI). The program consisted of 5 minutes ultra V block, 30 minutes' incubation with a primary antibody, 8 minutes' primary antibody enhancer, 10 minutes HRP polymer (equivalent to secondary antibody) and 5 minutes of the chromagen DAB to develop a brown colored stain.

Quantitation of immunohistochemical staining: Granular staining intensity of CD133 along the cell membranes was graded from 0+ to 3+ as follows: 0, no staining; +/- (0.50), focal weak fine granular staining; 1+, weak fine granular staining along complete luminal surface; 2+, moderate complete granular staining; 3+, strong coarse and complete granular staining.

Results

Evaluation of CD133 in colon lesions

In normal colonic mucosa, CD133 (AC133 clone) was minimally present at the base of glandular crypts, known as the stem cell niche of colon. Deep glandular crypts showed focal and weak upregulation of CD133 (1+) in 5/11 (45%) of IBD, but not in any of ischemic bowels (0/11, 0%) (Figures 1A and 1B). Under reactive condition with either infarction (n=11) or inflammatory bowel disease (n=11), surface reactive epithelium did not express CD133 (0/22, 0%) (Table 1).

However, *in situ* and/or invasive carcinoma of colon revealed much stronger CD133 expression along luminal surface of carcinoma or around individual cells of mucinous carcinoma type, at 2+ to 3+

intensity and ranging from 10% to 90% of tumor areas (13/13, 100%) (Table 1 and Figures 1C, and 1D).

	Total number	CD133 Expression in Surface Epithelium
Group 1 Ischemic bowel	11	0/11 (0%)
Group 2 Inflammatory bowel disease	11	0/11 (0%)
Group 3 Carcinoma *in situ* and adenocarcinoma	13	13/13 (100%)

Table 1: Expression of CD133 in reactive epithelium during bowel ischemia (Group 1) and inflammatory bowel disease (Group 2), and malignant epithelium (Group 3).

Their adjacent low grade dysplasia had either negative or weakly staining (1+) for CD133. The finding indicated that the strong expression of CD133 was diagnostic for indentifying colonic carcinoma cells when compared to no expression of CD133 in reactive colonic epithelium and even low grade dysplasia.
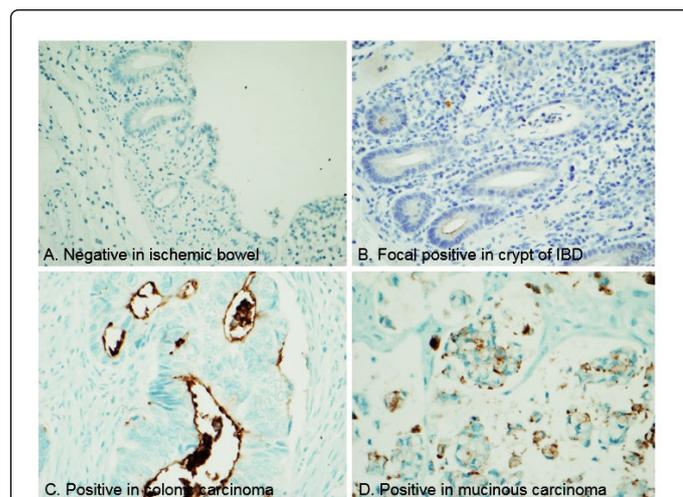
Figure 1: CD133 (AC133 clone monoclonal antibody) expression in reactive epithelium and colonic carcinoma. There is minimal expression of CD133 in reactive colonic epithelium (A). In inflammatory bowel disease, only deep crypt epithelium showed mild expression of CD133, but the surface epithelium stained negatively for CD133 (B). In high grade dysplasia (C) and invasive (D), the CD133 expression was remarkably upregulated. Magnifications x 400 for A-D.

Evaluation of CD133 in two types of RCC

In the Group 1 (negative controls in normal kidney parenchyma), CD133 staining was negative in all normal proximal tubules and distal tubules, which are considered the primary cells for developing various of RCC (Table 1). Low grade clear cell RCC is usually composed of clear cytoplasm with small nuclei, which can be morphologically difficult to distinguish from clear cell-papillary RCC (also containing clear cytoplasm with occasional papillary configuration). In the Group 2, CD133 staining was entirely negative in the majority of clear cell RCC (Figure 2A), but two clear cell RCC demonstrated focal and weak staining at 1+ along the cell membranes (Table 2). CD133 expression was diffusely present along the cell membranes of the majority of clear cell papillary renal cell carcinoma (Figure 2B and Table 2).

Evaluation of CD133 in breast lesions

In the current study, both CD133 and CD117 were expressed in normal breast glands (normal controls) of all three groups and benign breast lesions (Figure 3). However, the expression of CD133 was also detected in the majority of invasive ductal carcinoma as compared to low positive percent of CD117 expression in both Groups 2 and 3 (Table 3). Recombined tumor cases, based on the molecular luminal status, also showed 56% to 70% CD133 positivity but 0% to 20% CD117 positivity in invasive carcinoma cells. Thus the CD133 as an early progenitor remained in both benign and invasive carcinoma (either regular cases or BRCA mutated cases), imply no diagnostic value of CD133 to distinguish benign from malignancy in the breast tissue. This CD133 finding had been found very useful for us to distinguish clear cell-papillary RCC (diffuse positive for CD133) from clear cell RCC (negative for CD133 staining).

Discussion

Previous studies of CD133 in colorectal cancer have been focused on whether the CD133 is a marker for cancer stem cell of colon or how it is related to prognosis [10-13] however, utility of CD133 to differentiate colonic cancer from benign colonic lesions has not been well established.

The striking finding of our current study revealed that CD133, using a monoclonal CD133 antibody, was markedly upregulated in both carcinoma *in-situ* and invasive cancer of the colonic primary, which was sharply different from negative stains of CD133 in either normal colonic mucosa or reactive colonic mucosa due to either ischemia or inflammatory bowel disease. This finding was further confirmed using a polyclonal antibody against CD133. Our expanded control studies found that the intensity of CD133 expression was similar between normal and carcinomas from pancreas, thus lacking a differentiating power in pancreas (unpublished data).

	Total number	CD133 expression in surface epithelium
Group 1 Benign renal parenchyma	20	0/20 (0%)
Group 2 Clear cell renal cell carcinoma	20	1/20 (0%)
Group 3 Clear cell papillary renal cell carcinoma	24	24/24 (100%)

Table 2: Expression of CD133 in benign renal parenchyma (Group 1), clear cell renal cell carcinoma (Group 2), and clear cell papillary renal cell carcinoma (Group 3).
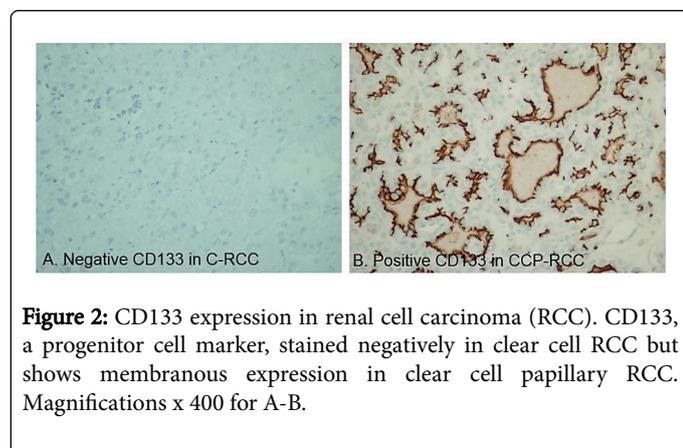
Figure 2: CD133 expression in renal cell carcinoma (RCC). CD133, a progenitor cell marker, stained negatively in clear cell RCC but shows membranous expression in clear cell papillary RCC. Magnifications x 400 for A-B.
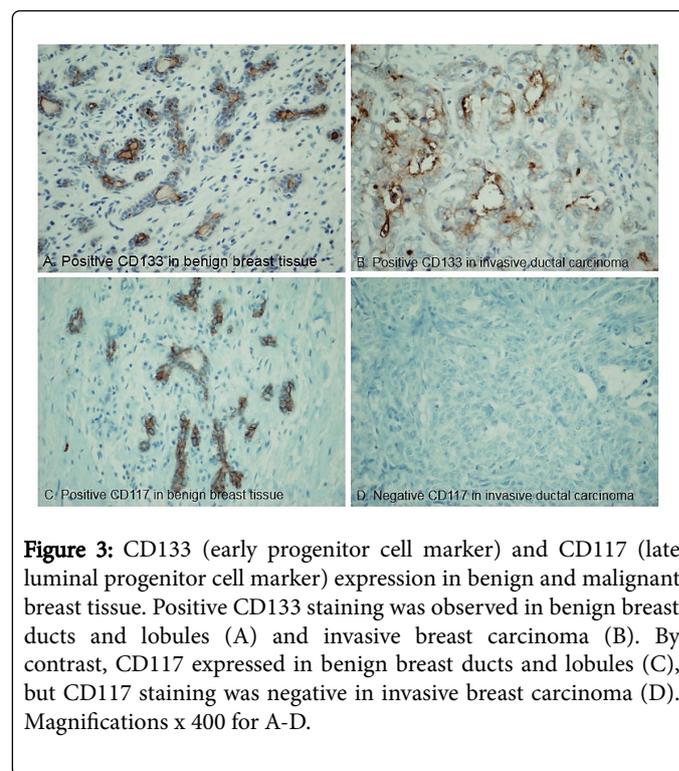
CD133 expression in colonic benign and malignant lesions shines a unique contrast, which allows a potential clinic application for early tumor detection in colon. As the CD133 was positive in carcinoma in-situ (also called high grade dysplasia) but only weakly or negative in tubular adenoma (also called low grade dysplasia), in addition to the morphologic difference between high grade dysplasia and low grade dysplasia, positive CD133 would provide another marker support for favoring high grade dysplasia (carcinoma *in situ*). In inflammatory bowel disease, the overwhelming reactive changes of colonic epithelium irritated by extensive inflammation can be hard to morphologically differentiate dysplastic changes toward the tumor development direction. Under such a circumstance, CD133 staining may provide pathologists with a powerful maker to resolve the dilemma-negative in reactive but positive in high grade dysplasia.

market potential and enormous financial impact on our healthcare system.

Figure 3: CD133 (early progenitor cell marker) and CD117 (late luminal progenitor cell marker) expression in benign and malignant breast tissue. Positive CD133 staining was observed in benign breast ducts and lobules (A) and invasive breast carcinoma (B). By contrast, CD117 expressed in benign breast ducts and lobules (C), but CD117 staining was negative in invasive breast carcinoma (D). Magnifications x 400 for A-D.

	Group 1, Total	Group 2, Total	Group 3, Total
CD133+ in normal	15/15 (100%)	13/13 (100%)	12/12 (100%)
CD117+ in normal	15/15 (100%)	13/13 (100%)	11/12 (91%)
	Group 1, Benign Lesions	Group 2, Invasive cancer	Group 3 Invasive cancer
CD133+ in lesions	10/10 (100%)	8/13 (61%)	4/6 (67%)
CD117+ in lesions	9/10 (90%)	1/13 (8%)	1/6 (17%)

Table 3: CD113 and CD117 Expression in benign breast lesion and invasive ductal carcinoma.

As colonic mucosa is a fast turn-over tissue, many epithelial cells from colonic mucosa are sloughed into fecal materials and many new colonic epithelial cells are generated from their stem cells each day. CD133, also called prominin, is considered as an "organizer" in cell membranes, [16,17] and small amount of CD133 is found in human saliva, seminal fluid and urine [18]. Conceivably, many colonic epithelial cells containing small amount of CD133 in each individual and injured epithelial cells from patients with benign bowel lesions are sloughed into stool, while large amount of CD133 sloughed from colon tumor cells in cancer patients are dumped into stool daily. Our idea to use this marker as a stool screening test is primary and patenting this carries a strong commercial potential in early detection of colonic cancer. Given the fact that six million people in the United States undergo colonoscopies each year, this simple test will have great

One leading group performing cancer stem cell research in the human kidneys has provided some base evidence that RCC cells, at least those derived from proximal tubules, contain some cancer stem cells [19,20]. The authors isolate CD133+ stem/progenitor cells from the interstitial or peritubular population of human kidneys [21]. Then they isolate CD133 positive cancer stem cells from human RCC, mainly composed of clear cell and papillary types, which represented 1% of all RCC cells [19]. CD133+ cancer stem cells can support other existing RCC cells in transplant masses in mice but cannot grow into a tumor by themselves, indicating that CD133 cancer stem cells may provide a support system for the tumor to grow [19]. Clear cell-papillary RCC is a low-grade RCC and found in patients with or without end-stage renal disease [22-25]. Clear cell-papillary RCC carry no metastatic potential in recent 10 years of studies since its discovery in 2006, [24,25] while clear cell RCC is well known to be associated with distant metastasis and worse prognosis [26-28]. However, a morphologic distinction between the two entities remains challenging despite some staining difference [29]. The clear cell papillary RCC demonstrate diffuse and strong membranous CD133 expression in all cases, but CD133 expression was focal and weak in the clear type of renal cell carcinoma. In other words, diffuse membrane staining for CD133 is a definite diagnosis for clear cell-papillary RCC to stand out from CD133 negative clear cell RCC. A recent study has cited our previous observation for the diffuse CD133 expression in clear cell-papillary RCC and reveals a consistent finding in this tumor [30]. We have had at least two patients who had bilateral kidney tumors. When a renal biopsy diagnosis of clear cell-papillary RCC was made, a cryoablation or a partial nephrectomy was considered adequate for removing the clear cell-papillary RCC due to its good prognosis (unpublished data). We notice that CD133 can be focally positive in papillary RCC and patchy positive in acquired cystic disease associated

RCC, but only the clear cell papillary RCC demonstrates diffuse membranous positivity around the luminal surface (a pattern of "Michigan lakes" in Figure 2B).

CD133 and CD117 represent markers of human breast tissue differentiation. CD133 is an early progenitor marker, while CD117 represents late luminal progenitor marker [15]. We showed that normal breast glands expressed both CD133 and CD117, compatible with the presence of stem cell niche in normal breast ducts and acini. However, our data suggest that in invasive ductal carcinoma, there is presence of early progenitor cells (CD133 positive) but not late luminal progenitor cells (CD117 negative), regardless of the molecular luminal status, supporting the view that cancer stem cells exist in the invasive breast carcinoma [3,4]. In term of the pathologic diagnostic value, CD133 does not help differentiating benign from malignant breast tumor, in contrast to a previous study showing that CD133 is strongly positive in breast malignancy but much weaker in benign breast lesions [31]. CD117 may have a value in differentiating benign breast tissue/lesions (negative) from in situ and invasive breast carcinoma (positive), supporting the view that CD117 is partially useful in differentiating ductal carcinoma in situ (negative) from usually ductal hyperplasia (positive) in the breast tissue [32-34].

In summary, the progenitor cell marker CD133 exhibits various values in tumor diagnosis. Its expression helps differentiating invasive colonic adenocarcinoma (CD133 positive) from benign colonic tissue (CD133 minimally positive in crypts), and differentiating the clear cell papillary renal cell carcinoma (diffuse membranous positivity of CD133) from conventional clear cell carcinoma (focal and weak positive CD133 staining). However, positive staining of CD133 in both benign breast glands and malignant breast carcinoma denies a differentiation role of CD133 in the breast malignancy.

Acknowledgement

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