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ORIGINAL ARTICLE

Expression of the stem cell marker CD133 is related to tumor development in colorectal carcinogenesis

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Summary *Background/Objective:* CD133 is currently considered the most robust surface marker for colorectal cancer stem cells. Two meta-analysis reports have suggested that CD133 expression is significantly associated with shorter survival, and CD133 may play an important role in the progression of colorectal cancer. However, the role of CD133 in colorectal adenoma has not been fully elucidated.
Methods: We used immunohistochemistry to evaluate CD133 expression in 200 endoscopically resected colorectal polyps from 200 patients and 20 normal mucosae between January 1993 and December 1996.
Results: CD133 staining was positive in 17.9% of the colorectal adenomas. Moreover, CD133 expression was associated with differentiation status ($p = 0.003$) and tumor size ($p = 0.03$).
Conclusion: CD133 might play an important role in tumor development.

1. Introduction

The cancer stem cell (CSC) theory suggests that cancers may be hierarchically organized, with only a small population of cancer cells maintained as CSCs, allowing them to both self-renew and differentiate. The first evidence of CSCs was reported from a study of human acute myeloid leukemia in 1997.[1] Following this report, several CSC cell surface markers have been identified in the past few years both in hematological disorders[2] and solid tumors. Among these markers, CD133 is now thought to be the most robust surface marker for colorectal CSCs.

The cell surface marker CD133 (also known as prominin-1) is a five-transmembrane glycoprotein with a molecular

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weight of 120 kDa, and is localized in membrane protrusions.[3] Although its cellular function is not clear, CD133 has been identified as a candidate marker for CSCs in various cancers, including brain tumors,[4] ovarian cancer,[5] hepatocellular carcinoma,[6] prostate cancer,[7] and pancreatic cancer.[8] In colorectal cancer, two studies showed that CD133 was a candidate marker for CSCs in 2007.[9,10] In one of these studies, CD133-positive tumor initiating cells were able to maintain themselves as well as differentiate and re-establish tumor heterogeneity upon serial transplantation.[9] In the second study, CD133-positive cells, but not CD133-negative cells, were shown to form tumors when injected into immunodeficient mice.[10] These results suggested that CD133-positive cells have a much higher capacity for initiating tumors than their CD133-negative counterparts. Following this initial work, there have been many further studies to evaluate the relationship between CD133 expression and clinicopathological parameters in colorectal cancer.[11–23] Moreover, several studies have reported that CD133+ colorectal cancers might be more resistant to chemotherapy or chemoradiotherapy,[24–27] although the clinical and prognostic significance of CD133 expression in colorectal cancer remains unclear. In 2012 and 2013, two meta-analysis reports suggested that CD133 expression is significantly related to shorter patient survival, and may play an important role in the progression of colorectal cancer.[28,29] According to the adenoma-carcinoma sequence model, almost all colorectal cancers arise from adenomas.[30] However, the role of CD133 in colorectal carcinogenesis has not been fully elucidated. Therefore, the aim of this study was to examine the expression for CD133 in the adenoma-carcinoma sequence of the human colon using immunohistochemistry, and to clarify the stage at which CD133 expression increases.

2. Methods

2.1. Tissue samples

We examined 200 endoscopically resected colorectal polyps (20 hyperplastic polyps, 145 adenomas, and 35 cancerous polyps), and 20 normal mucosae, all of which were obtained from another 20 patients treated for colorectal cancer at the University of Tokyo Hospital, Tokyo, Japan between January 1993 and December 1996. The resected specimens were immediately fixed in 10% buffered formalin and embedded in paraffin. Pathological diagnosis was performed using hematoxylin-eosin staining, and the evaluation of the extent of tubular adenomas was assessed according to the General Rules for Clinical and Pathological Studies on Cancer of the Colon, Rectum and Anus from the Japanese Research Society for Cancer of the Colon and Rectum.[31]

2.2. CD133 immunohistochemical staining

A single 4-μm-thick section was used for immunohistochemical staining as described below. Tissues were treated with xylene and ethanol, and then washed with phosphate-buffered saline (PBS). Endogenous peroxidase was blocked with 3% hydrogen peroxidase solution in methanol for 15 minutes. After washing with PBS, heat-induced antigen retrieval was performed in 0.01M sodium citrate buffer at pH 6.0. Tissues were washed with PBS and incubated with 5% bovine serum albumin (BSA) for 30 minutes to block nonspecific proteins. Each slide was incubated overnight at 4°C with primary anti-CD133 antibody (AC133; Miltenyi Biotec, Auburn, CA, USA) at a dilution of 1:100. After washing the slides three times with PBS, they were incubated with a Dako Envision Kit (Dako, Carpinteria, CA, USA) following the manufacturer's recommended protocol. After three washes with PBS, each slide was incubated for 3 minutes in 2% 3,3′-diaminobenzidine tetrahydrochloride and 50mM tris-buffer (pH 7.6) containing 0.3% hydrogen peroxidase as a chromogen. Mayer's/Lillie-Mayer's hematoxylin was used for counterstaining. Renal tubules were used as a positive control, and for a negative control the antibody was replaced with PBS.

2.3. Evaluation of CD133 immunostaining

The evaluation of CD133 immunostaining was performed following the method reported by Maeda et al.[32] Slides were examined under a microscope at low power (from 40× to 200×) to identify the region containing the highest percentage of CD133-positive cells (hot spot) in the cancer nest. Ten hot spot fields inside the tumor tissue were selected, and CD133 expression was evaluated in 1000 tumor cells (100 cells/field) under high power (400×). Expression of CD133 was defined as positive when CD133 staining was found in $>$ 5% of the entire tumor. The CD133 expression was independently evaluated by two observers who had received training in pathological diagnosis (S.K. and J.K.), and who were unaware of the clinical findings. Discrepancies between their findings were resolved by discussion. Their interobserver agreement was calculated using κ-statistics. The correlation between CD133 expression and clinicopathological features, tumor recurrence, and overall survival was analyzed.

2.4. Statistical analysis

The statistical significance of differences was evaluated using the Chi-square, Fisher's exact, or nonpaired Student *t* test, as appropriate. All statistical calculations were carried out using JMP Pro 11.0.0 statistical software (SAS Institute, Cary, NJ, USA). An association was considered significant when the *p* value was $<$ 0.05.

3. Results

3.1. Polyp characteristics and histological examination

The characteristics of the patients and polyps are summarized in Table 1. The patients (164 men and 38 women) had a mean age of 59.4 years (range, 18–90 years). The polyps were located in the cecum (n = 7), ascending colon (n = 26), transverse colon (n = 33), descending colon (n = 24), sigmoid colon (n = 71), and rectum (n = 39). Their mean size was 10.3 mm (range, 2–35 mm). The histological types were adenocarcinoma with submucosal invasion (n = 10), intramucosal adenocarcinoma (n = 25), adenoma with severe atypia (n = 10), adenoma with

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