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Clinicopathologic significance of high CD133 expression in colorectal carcinomas

Kolorektal kanserlerde yüksek CD133 ekspresyonunun klinikopatolojik önemi

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Öz

Amaç: Kolorektal karsinomlarda (CRC) CD133 ekspresyonunun prognostik öneminin belirlenmesi hedeflendi. Materyal ve Metot: Süleyman Demirel Üniversitesi Patoloji Anabilim Dalı'nda tanı almış sporadik kolorektal kanserli 70 olguya ait CD133 ekspresyonları, parafin bloklarda immunohistokimyasal olarak araştırıldı. Neoplazik bezlerin % 50'sinden azında boyanma düşük pozitif; % 50 ve daha fazlasında boyanma yüksek pozitif imünoreaktivite olarak değerlendirildi. Ayrıca, patolojik parametreler ve sağ kalım oranları ile CD133 immünhistokimyasal ekspresyon düzeyleri arasındaki ilişki araştırıldı. Bulgular: CD133 ekspresyonu incelenen olguların % 82.9'unda tespit edildi ve yüksek CD133 düzeyi anlamlı ($p = 0.004$) derecede kötü hastalıksız sağkalım ile ilişkili bulundu. CD133 ifadesi rektosigmoid bölgede bulunan tümörlerde anlamlı ($p = 0.005$) derecede yüksekti ve histolojik grade ile anlamlı ($p = 0.020$) negatif korelasyon gösterdi. Sonuç: Daha önceden yayınlanmış verilerle uyumlu olarak muhtemelen tümörün kötü diferansiye alanlarındaki luminal bölgelerin eksikliğine bağlı olarak bu çalışmadaki kötü diferensiye tümörlerde yüksek CD133 pozitifliği belirlenmedi. Sonuçlarımız kötü hastalıksız sağkalım ile korelasyon gösteren immünohistokimyasal CD133 ekspresyonunun kolorektal karsinoma için bağımsız bir prognostik belirleyici (markır) olabileceğini göstermektedir. Bu çalışmanın bulguları, kanser kök hücre modelini desteklemekte ve CRC olgularında CD133'ün klinik önemini ve yüksek CD133 ekspresyonu ile hastalıksız sağkalım arasında güçlü bir ilişki olduğunu ortaya koymaktadır.

Anahtar Kelimeler: CD133, kolon kanseri, immünohistokimya, prognoz

Abstract

Background: To investigate the prognostic significance of CD133 expression in colorectal carcinomas (CRC). Material and Method: CD133 expression was investigated immunohistochemically on the paraffin blocks of 70 sporadic colorectal carcinoma cases which were diagnosed at the department of pathology in Suleyman Demirel University. Low positivity was defined as positive immunoreactivity in $< 50\%$ of neoplastic glands, and high positivity was defined as positive immunoreactivity in $\geq 50\%$ of neoplastic glands. Furthermore, the relation of CD133 immunohistochemical expression with pathologic parameters and survival rates were investigated. Results: CD133 expression was detected in 82.9% of the examined cases and high CD133 expression was significantly associated with poorer disease free survival ($p=0.004$). CD133 expression was significantly higher in tumors located in rectosigmoidal region ($p=0.005$) and showed a significant negative correlation with histologic grade ($p=0.020$). Conclusion: In the present study none of the poorly differentiated tumors showed high CD133 positivity probably due to the lack of luminal areas in poorly differentiated areas of the tumor, which is in consistence with the previously published data. Our results indicate that immunohistochemical CD133 expression, which correlates with poorer disease free survival, may be an independent prognostic marker in colorectal carcinoma. The findings of this study support the cancer stem cell model, and reveal the clinical importance of CD133 in CRC cases and present a strong association between high CD133 expression and disease-free survival.

Keywords: CD133, colon cancer, immunohistochemistry, prognosis

Introduction

Colorectal cancer is the second most common type of cancer and a major cause of cancer related morbidity and mortality in western world. It develops in a multifactorial and multistep process, therefore activation of cancer promoting genes and inactivation of tumor suppressor genes are considered as critical molecular mechanisms (1, 2).

Recently described cancer stem cell (CSC) model of tumor development suggests that behavior of a tumor can be determined by a subpopulation of cells that are characterized by the ability to initiate new tumors (3). In colon cancer, the cell surface marker CD133 has recently been investigated in several studies (4, 5). It has been reported that CD133 gene (prominin-1) is located on chromosome 4p15.32 and encodes a cell surface glycoprotein compromising five transmembrane domain and two large glycosylated extracellular loops (6).

In in vitro studies CD133 subpopulation of colon cancer cells was shown to be quite enriched in tumor-initiating colon CSCs, which have the ability to self-renew and to recapitulate the bulk tumor population (7, 8). The CD133 positive cell population in colon carcinoma has been demonstrated as resistant to the chemotherapy and highly tumorigenic after xenotransplantation in non-obese diabetic / severe combined immunodeficient mice (7-9). It has been proposed that recurrent tumors may comprise of a great number of cancer stem cells and therefore measuring colonic cancer stem cell burden may be a useful prognostic marker (9, 10).

Early evidences have suggested that expression of CD133 is associated with the activation of stemness-related signal pathway, bioenergetic stress and resistance to apoptosis, but the relationship between the percentage of CD133 positive cells and prognosis of colorectal carcinoma is still controversial (4, 5, 11). More evidence is needed to elucidate this relationship. The aims of the present study are to investigate the expression patterns of CD133 in sporadic colorectal cancer, and evaluate the clinicopathologic and prognostic significance of the expression of this marker in colorectal cancer which is one of the most common malignancies around the world.

Material and Methods

Patient population and preparation of the samples

Tissue samples from 70 patients with colorectal carcinoma, which were diagnosed at our institution were included in this study. The clinical records were obtained at admission. Patients with synchronous cancers, complicating familial adenomatous polyposis or inflammatory bowel disease were not included in the study. Hematoxylin and Eosin stained slides of the patients were reviewed for histological classification according to the World Health Organization (WHO) classification system (12). According to the differentiation of the neoplastic cells, histological grades of tumors were subdivided into well-differentiated (grade 1), moderately differentiated (grade 2) and poorly differentiated (grade 3). Tumors were staged according to the 6th edition of Union for International Cancer Control (UICC): TNM Staging System- T for tumor, N for regional lymph node involvement, and M for metastases primary (13). The recurrence-free interval of the patients was calculated as the number of months from the date of primary tumor resection to the date of confirmation of recurrence. The overall survival of the patients was calculated as the number of months from the date of primary tumor resection to death or to the date of the last follow-up evaluation.

Immunohistochemistry

Immunohistochemical analysis for CD133 was performed on formalin fixed, paraffin-embedded archival tissue using the streptavidin-biotin-peroxidase technique. For all cases, 4μm histological section was deparaffinized in xylene and dehydrated in descending dilution of ethanol. For the antigen retrieval, slides were treated by microwave heating in citrate buffer (pH 6.0) for 20 minutes. Endogenous peroxidase activity was blocked by 20 minutes of incubation with 0.3% hydrogen peroxidase. Slides were tested with rabbit polyclonal CD133 antibody (1:100, Clone ab66141, Abcam®, USA). Sections were tested with streptavidin-biotin-peroxidase kit (Ultra Vision Large Volume Detection System Anti- polyvalent, HRP, LabVision ®, USA) and after incubation the reaction product was detected using diaminobenzidine (DAB). Finally, the sections were counterstained with Mayer's hematoxylin and mounted. The positive control was kidney tissue for CD133.

The immunostaining patterns were evaluated independently by three investigators. CD133 immunoreactivity was evaluated by considering the amount of positively stained cells and scored as negative, low (+) positive and high (++) positive. Negative cases had to show no immunoreactivity in any part of the tumor. Low positivity was defined as positive immunoreactivity in < 50% of neoplastic glands and high positivity was defined as positive immunoreactivity in ≥ 50% of neoplastic glands (14).

Statistical Analysis

The associations between the expressions of CD133, and their relationship with clinicopathological features were analyzed by Chi-Square test and Spearman correlation test. The overall survival (OS) curves were analyzed by the Kaplan–Meier method and compared using the log-rank test. Univariate Cox regression analysis was performed for the evaluation of the possible prognostic importance of CD133 expressions. The statistical significance was defined as $p<0.05$.

Results

The study population comprised of 40 (57.1%) males and 30 (42.9%) females. Mean age at the time of surgery was 59.7 years (Range: 29-86 years). Average tumor size was 5.3 cm (range: 0.5-9.4 cm). The distribution of tumors according to the anatomic location was as follows: cecum in 6 (8.6%), right colon in 15 (21.4%), transvers colon in 1 (1.4%), left colon in 7 (10%), sigmoid colon in 19 (27.1%) and rectum in 22 (31.4%) cases. Among 70 colorectal carcinoma patients, 34 (48.5%) cases were diagnosed as well differentiated, 30 (42.9%) cases were moderately differentiated, and 6 cases were (8.6%) poorly differentiated. Thirty five (50%) cases had lymph node metastases, while 28 (40%) cases had perineural invasion. Thirty one (44.3%) cases had vascular invasion. Thirteen (18.5%) cases had distant metastases at the time of diagnosis. Clinical stage distribution at the time of diagnosis was as follows: stage I in 7 (10%) cases, stage II in 25 (35.7%) cases, stage III in 25 (35.7%) cases, stage IV in 13 (18.6%) cases. Clinicopathologic characteristics of the patients are presented in Table 1.

Table 1 - Clinicopathologic characteristics of 70 colorectal carcinoma patients at the time of diagnosis.

Mean Tumor Size (Range)	5.3 cm (0.5-9.4 cm)
Mean Age (Range)	59.7 (29-86)
Age Distribution	
<60	31 (44.3%)
≥60	39 (55.7%)
Gender	
Male	40 (57.1%)
Female	30 (42.9%)
Tumor Location	
Proximal Colon	29 (41.4%)
Rectosigmoid Colon	41 (58.6%)
Histologic Grade	
Well	34 (48.6%)
Moderate	30 (42.9%)
Poor	6 (8.5%)
Lymphovascular Invasion	
Absent	39 (55.7%)
Present	31 (44.3%)
Lymph Node Metastasis	
Absent	35 (50%)
Present	35 (50%)
Perineural Invasion	
Absent	42 (60%)
Present	28 (40%)
TNM Stage	
I	7 (10%)
II	25 (35.7%)
III	25 (35.7%)
IV	13 (18.6%)

CD133 expression was detected in 58 (82.9%) out of 70 cases and was seen as apical staining at the luminal surface of neoplastic glands. Specimens demonstrated low (Figure 1) and high (Figure 2) positivity for CD133 in 26 (37.2%) and 32 (45.7%) cases, respectively. CD133 was negative in 12 (17.1%) cases. There was an increased CD133 expression in tumors, located in rectosigmoid region, compared to the tumors in proximal colon ($p=0.005$).

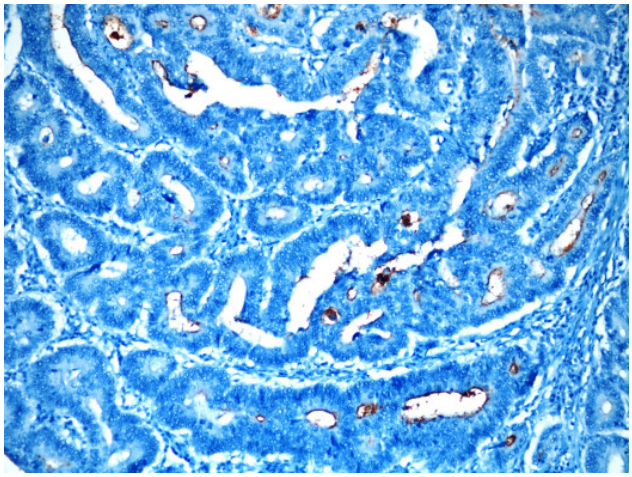

Figure 1 - Immunohistochemical luminal low CD133 expression in colorectal carcinoma (DABx200).

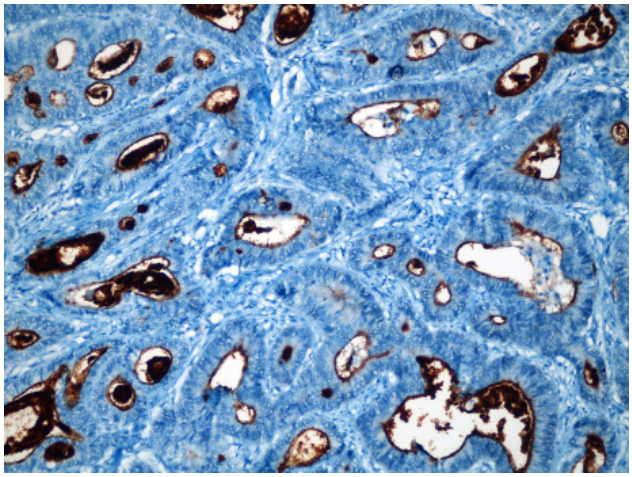

Figure 2 - Immunohistochemical luminal high CD133 expression in colorectal carcinoma (DABx200).

Additionally, there was a statistically significant negative correlation between CD133 expression and histologic grade (p=0.02, r=-0.220). According to our results the presence of lymphovascular invasion was significantly associated with negative or low CD133 expression (p=0.044). No correlation was noted between CD133 expression and age, gender, tumor size, nodal status, perineural invasion and TNM stage (Table 2).

Table 2 - The relationship of clinicopathologic parameters with CD133 expression.

Parameters	CD133 Expression n (%)		
Age Distribution	None/Low	High	p
<60 years	19 (61.3%)	12 (38.7%)	0.294
≥60 years	19 (48.7%)	20 (51.3%)	
Gender	None/Low	High	p
Female	24 (60%)	16 (40%)	0.268
Male	14 (46.7%)	16 (53.3%)	
Tumor Location	None/Low	High	p
Rectosigmoid	16 (39%)	25 (61%)	0.005
Proximal colon	22 (75.9%)	7 (24.1%)	
Histologic Grade	None/Low	High	p
Well/Moderate	32 (50%)	32 (50%)	0.020
Poor	6 (100%)	0 (0%)	
Lymphovascular Invasion	None/Low	High	p
Absent	17 (43.6%)	22 (56.4%)	0.044
Present	21 (67.7%)	10 (32.3%)	
Lymph Node Metastasis	None/Low	High	p
Absent	17 (48.6%)	18 (51.4%)	0.499
Present	21 (60%)	14 (40%)	
TNM Stage	None/Low	High	p
I	5 (71.4%)	2 (28.6%)	0.363
II	11 (44%)	14 (56%)	
III	16 (64%)	9 (36%)	
IV	6 (46.2%)	7 (53.8%)	

Follow-up information was available for 67 (95.7%) of 70 patients. Mean follow-up time was 52.9 months (range: 1-143 months) in the study population. Nine patients (12.9%) were diagnosed with distant metastasis during the follow-up period. There were four cases with liver metastasis, two cases with lung metastasis, one case with axillary lymph node metastasis, one case with bone metastasis and one case with soft tissue metastasis in inguinal region. At the end of follow up period 37 (55.2%) patients were alive and 30 (44.8%) patients were dead.

Statistical analysis showed that overall survival was shorter in the cases with high CD133 positivity, but this relationship was not statistically significant (long-

rank test, p=0.093) (Figure 3). On the other hand high CD133 expression was significantly associated with shorter disease free survival, compared to the cases with no or low CD133 expression (long-rank test, p=0.004) (Figure 4). Univariate Cox regression analysis revealed that high CD133 expression was significantly correlated with shorter disease free survival (p=0.033), but not correlated with overall survival (p=0.144).

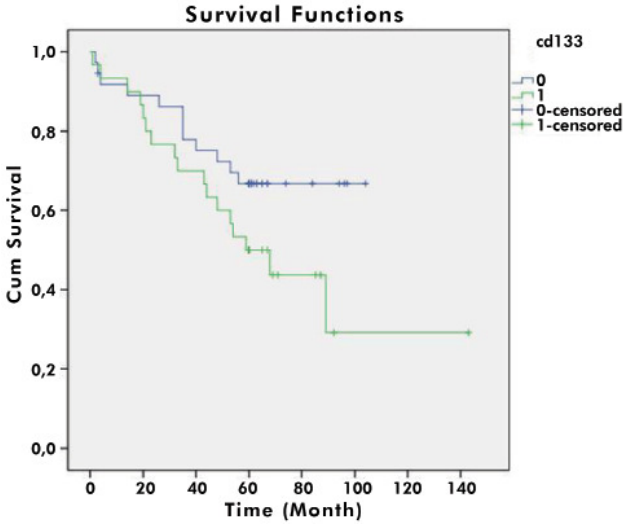

Figure 3 - Overall survival curves of cases with high CD133 expression (1) and cases with no/low CD133 (0) expression. Cases with high CD133 expression showed shorter overall survival than cases with no/low CD133 expression, but the relationship was not significant (long-rank test, p=0.093).

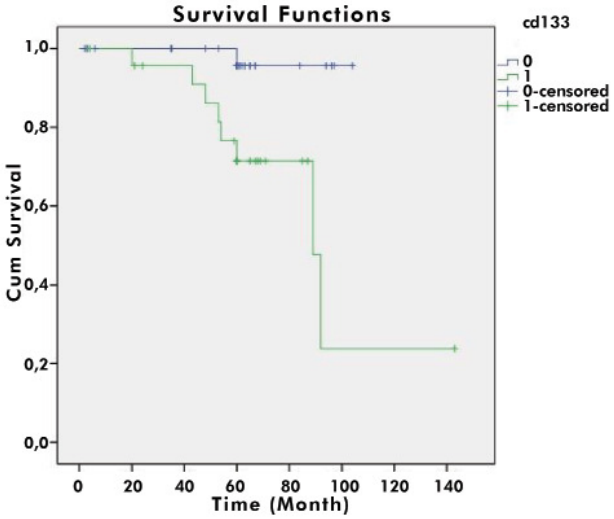

Figure 4 - Disease free survival curves of cases with high CD133 (1) expression and cases with no/low CD133 (0) expression. Cases with high CD133 expression showed significantly shorter disease free survival than cases with no/low CD133 expression by long-rank test (p=0.004).

Discussion

CD133 is known as a glycosylated form of human prominin-1, which is a member of the pentaspan transmembrane glycoprotein family (6). It has been reported that CD133 expression is located in normal neonatal and adult precursor cells (5, 15, 16). In the present study we did not observe CD133 expression in normal colon mucosa, which is in agreement with previously published data (4). However, some of the authors have reported CD133 expression in normal colonic epithelium, especially at the basis of the crypts, where stem cells are more likely to reside (7, 8, 17).

In our study none of the poorly differentiated tumors showed high CD133 positivity, which was consistence with the results of Kojima et al (5). According to our results, well differentiated tumors showed significantly higher CD133 expression, which lends support to findings of Kojima et al (5), reporting that immunohistochemical CD133 expression was reported to be restricted in tumor areas exhibiting ductal structures. This finding may also be related to the diminished number of CD133 positive cells in poorly differentiated areas of the tumor.

The relationship between the expression of CD133 and prognosis in colorectal carcinoma is still controversial.

Horst et al (4, 14) reported that CD133 expression was an independent prognostic marker whereas this kind of correlation was not confirmed by Kojima et al (5) and Mia-Jan et al (18). Mia-Jan et al (18) reported that CD133 expression was a good prognostic marker in patients with adjuvant therapy. However, Zhang et al (19) suggest that the co-expression of the CD133 proteins is closely associated with poor prognosis in patients with colon cancer. Additionally, Wang et al (17) reported an increased CD133 expression in tumors with high T stage and found CD133 expression to be an independent prognostic marker in colorectal cancers. Oliver et al (20) stated that CD133 may be useful for determining the prognosis of colorectal cancer patients. In the present study, high CD133 expression was significantly associated with poorer disease free survival, suggesting that it may be an independent prognostic marker. Patients with high CD133 expression also had shorter overall survival, but the relationship was not significant. However this potential relationship might be revealed by the studies assessing larger number of cases in the future.

Colorectal tumors spread with direct invasion to adjacent structures. They perform remote metastases through the lymphatics and blood vessels. The most common metastatic tissues of colorectal cancers are the liver and regional lymph nodes, and lymph node metastasis is more common in the tumor areas with poor differentiation (19, 21, 22). In the present study, levels of lymph node metastases were 18.5% for well-differentiated tumors, 21.4% for the moderately differentiated tumors and 8.5% for poorly differentiated tumors. As expected, it was determined that moderately differentiated tumors, exhibited more lymph node metastases than well-differentiated tumors. Surprisingly, lymph node metastasis was observed to be significantly lower in patients with poorly differentiated cases. This situation is thought to be caused by the lack of poorly differentiated cases in our study.

The accepted therapy for colon cancer is resectional treatment with the addition of postoperative chemotherapy for lymph node-positive cancers and those with poor pathologic features such as lymphovascular invasion (23, 24). Regrettably, in spite of suitable surgery and standard chemotherapeutic treatments, up to 50% of these cancers will recur, making chemotherapeutic resistance and local recurrence or distant organ metastasis the major causes of cancer mortality (25, 26). By the evaluation of the data in our study, it can be suggest that the significant relationship ($p = 0.044$) between the expression of CD133 and the presence of lymphovascular invasion showed agreement with the results of Wang et al (17). In parallel with our data, Wang et al (17) also have reported higher CD133 expression in patients without lymphovascular invasion. Also a significant association between CD133 mRNA expression and lymphovascular invasion was reported by Huh et al (27).

In the present study, high CD133 expression was observed in 45.7% of colorectal carcinoma cases. CD133 expression was significantly low in well differentiated tumors and tumors with lymphovascular invasion. In addition patients with high CD133 expressing tumors had a poorer disease free survival. Our findings suggest that immunohistochemical CD133 expression may be a predictor for poor prognosis and it is an independent prognostic marker from other clinicopathologic features in colorectal cancers.

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