Expression and Prognostic Significance of Stem Cell Marker CD133 in Survival Rate of Colon Cancer Patients

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Abstract

Background: Today, colon cancer is one of the most common types of gastrointestinal cancers CD133 as a known cancer stem cell marker has been found effective in cell proliferation and differentiation in various cancers, including colon. We aimed to investigate the relationship between the CD133 expression in colon cancer with prognostic factors and the survival rate of colon cancer patients by immunohistochemistry.

Materials and Methods: Formalin- fixed-paraffinembedded (FFPE) tissues was taken from colon patients. Histopathology examination was done using hematoxylin and eosin staining. Immunohistochemistry was performed to determine CD133 expression. An association between CD133 expression and clinic pathological profile was then analyzed.

Results: There was a statistically significant difference between CD133 protein expression and sex, cancer stage and lymphatic invasion (p=0.044, p=0.131 and p=0.002, respectively). However, no significant correlation was identified between the CD133 expression and other factors such as: age of CRC patients (*p*-value=0.267), tumor location (*p*-value=0.494), tumor differentiation grade (*p*-value=0.263), neural tissue invasion and 5 years' survival (p value=0.054).

Conclusion: CD133 is a useful predictive or prognostic biomarker for CRC in clinical assessment and may serve as a potential therapeutic target for CRC.

Introduction

Colon cancer is one of the most prevalent cancers in the world and a major cause of cancer-related mortality [1–3]. In Iran, colon cancer is the third most common cancer in men and fourth common cancer in women [4]. It is suggested that the highly tumorigenic, chemotherapy resistant affect survival rate in colon cancer [5–8]. Targeted therapies have promising potential improvement of survival in colon cancer patients [9]. Recently, cancer stem cells (CSCs) have been known due to their ability of self-renew, self-repair and their role in the tumor initiation, metastasis, therapeutic resistance and recurrence [10–12]. Different markers have been also found to be expressed on the surface of CSCs among which CD133 has been paid the special attention. The CD133/prominin-1 is a 120-kDa 5-transmembrane domain antigen on chromosome 4p15.32 and mainly localized in membrane protrusions [13, 14]. CD133 first discovered in hematopoietic stem and progenitor cells by Yin et al [15]. Previous studies identified CD133 as a putative CSC marker in cancer cells [16–19] with ability to initiate tumor growth [20–24]. This CSC marker now widely used to identify cancer stem cells in many cancers such as brain, colon, and lung cancers [25–28]. CD133 is normally expressed by primitive hematopoietic stem cells from adult blood and bone narrow, human fetal liver and stem cells in cord blood and peripheral blood, endothelial progenitor cells, epithelial cells and neural and glial stem cells, [29–31]. Several studies have suggested that high CD133 expression is associated with the clinic-pathologic characteristics such as poor prognosis, distant metastasis, survival rate and even chemotherapy resistance in colon cancer patients So, as a CRC-

initiating CSC marker, CD133 expression can be used for developing a novel therapeutic approaches in colon cancer [32–40]. In this study, we investigated the association between the expression and prognostic significance of the CSC marker CD133 with clinic-pathologic features of CRC patients including survival rate, using the immunohistochemistry (IHC) method.

Methods And Materials

Tissue samples

In this study, 34 formalin- fixed-paraffinembedded (FFPE) tumor tissues were selected from 34 patients underwent surgical resection from 2008–2019 in Shahid Beheshti Hospital in Kashan, Iran. Written consent was obtained from all subjects and the study was approved by the Research Ethical Committee of Kashan University of Medical Sciences, Iran. All clinic-pathologic data were verified using the hematoxylin and eosin (H&E) stained pathologic slides, patients' medical files and pathology record. Tumors were classified according to the American Joint Committee on Cancer (AJCC) and the International Union Against Cancer (UICC) TNM criteria [93]. Clinic-pathologic parameters were included age, gender, tumor location, tumor stage, tumor differentiation grade, lymphatic and neural tissue invasion, 5 years' survival and tumor recurrence. A colon cancer with detected positive immunostaining for CD133 considered as a positive control. Cases and controls were examined by two expert pathologists. Positive reaction was defined by the presence of at least 10% of cancer cells displaying either membranous and/or cytoplasmic staining. A number of tumor samples were excluded from the study due to inappropriate paraffin blocks, low quality staining and unavailable data. Also, the small size specimens prepared by biopsy which was inadequate for performing histopathological examinations were excluded.

CD133 Immuno-histochemical staining

The expression level of CD133 was determined in CRC sections by the Immunohistochemical staining (IHC) method as follows: Formalin-fixed, paraffin-embedded CRC tissue blocks were sectioned at 5-µm thickness for IHC staining. All slides were deparaffinized with xylene, and rehydrated in a graded ethylic alcohol (50%, 70%, 80% and 100%) followed by distilled water. After washing 3 times with phosphate-buffered saline (PBS) buffer every 5 minutes, endogenous peroxidase was inactive by immersing the sections in 1-0.1% H_2O_2 for 5min. Then, for heat-induced antigen unmasking, sample tissues were treated in sodium citrate buffer (pH:6.0) at 95°C for 5 minutes in a microwave oven and allowed to cool for 1 hour at room temperature. CD133 expression was identified and visualized after reaction with primary Anti CD133 antibody (1:100) at 4˚C for one night. After washing the slides three times with PBS, a secondary antibody (peroxides- conjugated) was used to detect the primary antibody, and slides were incubated for 30 minutes in room temperature. Again, after three times washing in PBS for every 5 minutes, samples were incubated with 3, 3´-diaminobenzidine tetrahydrochloride-chromogen (DAB) for 1–5 minutes for visualizing immunostaining and finally counterstained with hematoxylin dye and dehydrated in ethylic alcohol serial dilution (60%,80% and 100%) and cleared in xylene (100%). Our

negative control was the tissue that was covered with just buffer without the primary antibody. Prepared specimens were studied using light microscope. Imaging analysis of immunohistochemical staining of the specimens was evaluated by one pathologist without knowledge about the patients' clinic-pathological characteristics. The CD133 expression was assessed by observing the appearance of yellow to brown color in the cytoplasmic and membrane of glandular epithelial cells. A score of less than 10% CD133 cells (low CD133 expression) considered as negative and more than 10% CD133 cells (high CD133 expression) as positive, respectively. The staining intensity was evaluated according a ranging from non-stained (negative) to strong (positive): 0; non-staining, + 1; weakly staining, + 2; moderate staining, + 3; strong staining.

Statistical analysis

All statistical analyses were performed using SPSS software version 20 (SPSS Inc., Chicago, IL., USA). The results were expressed as the means ± standard deviation. The statistical significance of differences between immunohistochemical CD133 expression levels and clinicopathological features of CRC patients were evaluated using Chi-square and one-way ANOVA, or Mann-Whitney test or Kruskal-Wallis parametric bread tests (based on the results of Kolmogorov-Smirnov test), as appropriate. Survival was evaluated using the Kaplan-Meier method with a log-rank test. *p*-values of < 0.05 were considered statistically significant.

Results

This study included 34 tissue samples from 15 (44.1) male patients and 19 (55.9%) female patients with a median age of 62 (± 15.35) years at the time of diagnosis. The distribution of CRC patients with colon cancer according to demographic features is presented in Table 1.

Table 1
Characteristics of 38 CRC patients and their prognostic factors.

Variable (No.%)	
Gender	
Male	15 (44.1)
Female	19 (55.9)
Age (mean) (± SD) (years)	62 (± 15.35)
Tumor location	
Ascendance colon	14 (429.4%)
Transverse	1(3.0%)
Sekom	6 (18.2%)
Sigmoid 8 (2.2%)	8(24.2%)
Descending	1 (3.0%)
Rectum colon	2 (6.1%)
Dariche ileocecal	2(6.1%)
Histopathology Grade	
Well-differentiated (G1)	21 (61.8%)
Moderately differentiated (G2)	11 (32.4%)
Poorly differentiated (G3)	2(5.8%)
CD133 expression	
High Level	12 (35.3%)
Low Level	9 (26.5%)
5 years' survival	
Positive	26 (76.5%)
Negative	8 (23.5%)
Lymphatic invasion	
Positive	20 (58.8%)
Negative	14 (41.2%0
Tumor stage	

Variable (No.%)	
1	9 (26.5%)
2a	7 (20.6)
2b	4 (11.8%)
3a	1 (2.9%)
3b	8 (23.5%)
3c	5 (14.7%)
Neuronal invasion	
Positive	4 (11.8%)
Negative	30 (88.2%)

The pathological results showed (as shown at Table 1) that the most tumors were located in ascendance colon 13 (39.4%) and in Sigmoid 8 (2.2%), while lowest tumor location was descending 1 (3.0%) and transverse 1 (3.0%). Also based on tumor differentiation degree, there were 21 (61.8%) well-differentiated (G1), 11 (32.4%) moderated differentiated (G2) and 2(5.8%) poor differentiation (G3) tumors. The highest prevalence of tumor stages was stage I (26.5%) and stage 3b (23.5%), while the lowest frequency was observed at stage 3a (2.9%). Moreover, lymphatic vascular invasion was detected in 20 patients (58.8%), neuronal invasion in 4 cases (11.8%), also 26 (76.5%) patients had five-years survival.

Correlation between CD133 expression and clinicopathological factors features

Based on our pathologic findings, expression of CD133 marker was positive in 25(73.5) patients and negative in 9 (26.5) patients. Associations between CD133 expression rate and clinic-pathological variables are presented in Table 2.

Table 2
Association of CD133 expression with clinic-pathological variables

CD133 expression (%)						
Variables	**No.**	**0**	**1+**	**2+**	**3+**	p-value
Gender						
Male	15	4 (55.6)	0	6(75.0)	4(33.3)	**0.044**
Female	19	8 (66.7)	2 (25.0)	5 (100)	4 (44.4)	
Age	34	55.77 ± 17.15	470 ± 14.47	67.25 ± 10.52	59.83 ± 16.114	**0.267**
Tumor Location						
Colon	16	4(44.4)	2(40.0)	6(75.0)	4(33.3)	**0.182**
Rectum	10	2 (22.3)	3 (60.0)	2 (25.0)	3 (25.0)	
Sekom	8	3(33.3)	0	0	5(41.7)	
Tumor Stage						
I	9	3 (33.3)	4 (80.0)	2 (25.0)	0	**0.011**
II	11	4 (44.4)	1 (20.0)	3 (37.5)	3 (25.0)	
III	14	9 (75.0)	3 (37.5)	0	2 (22.3)	
Tumor grade						
High	4	0	0	0	4 (33.3)	**0.05**
Low	30	8 (66.7)	8 (100.0)	5 (100.0)	9 (100.0)	
Lymphatic invasion						
Positive	20	2 (22.2)	2 (40.0)	4 (50.0)	12 (100.0)	**0.002**
Negative	14	7 (77.8)	3 (60.0)	4(50.00	0	
Neural invasion						
Positive	4	0	0	1 (12.5)	3 (25.0)	**0.273**
Negative	30	9 (75.0)	7 (87.5)	5 (100.0)	9 (100.0)	
5 Years survival						
Positive	26	9 (100.0)	2 (40.0)	7 (87.5)	8 (66.7)	**0.054**
Negative	8	4 (33.3)	1 (12.5)	3 (60.0)	0	

According to the results (shown in table II), there was a significant difference between the expression of CD133 with gender, tumor stage and lymphatic vascular invasion in the studied patients (P = 0.044, P = 0.011, P = 0.002, respectively). The highest CD133 expression was observed at 8 females (66.7%). Also, all cases with high CD133 expression had the detected lymphatic invasion (p = 0.002). In addition, the highest CD133 expression was observed in CRC cases in advanced tumor stages III. However, no significant correlation was found between the CD133 expressions and the other clinical factors such as: age of CRC patients (p = 0.267), tumor location (P = 0.182), tumor differentiation grade (P = 0.05), neural tissue invasion (p = 0.002) and 5 years' survival (P = 0.054). The 5-year survival in 26 (66.7%) patients with high CD13 expression showed no significantly differences with patients without 5-year survival and low CD133 expression (p value = 0.054).

Discussion

Colon cancer, as the leading cause of cancer- related death is a significant public health concern due to its increasing rate in Iran [41–44]. Many specific immunohistochemical and PCR markers have been identified for detecting colon primary and metastatic tumors. CD133 is one of the immunohistochemical markers which was first observed in stem cells [45–48]. This marker has been suggested to be effective in cell proliferation and differentiation of various cancers including colon [49–51]. Therefore, in this study, we purposed to evaluate the expressions of C D133 using immunohistochemically staining, also its relationship to clinic pathological features as well as the prognostic indicator in colon cancer. Our study demonstrated that the CD133 expression was significantly correlated with CRC histopathology such as gender tumor, stage and lymphatic vascular invasion (P = 0.044, P = 0.011, P = 0.002, respectively). The higher CD133 expression was found at advance tumor stage (III). But it did not affect other factors such as age, five-year survival and tumor location in this study. In the line with our study, there was no significant difference between positive and negative CD133 and tumor location in several previous studies [16, 31, 34, 52–54]. In contrary, our study result was different from a study in which CD133 expression was higher in the rectum colon [45]. Also, similar to our result, in a study done by Kojima on samples from 189 patients with different stages of CRC using IHC, they concluded that CD133 overexpression occurred mainly in well- to moderately-differentiated tumors and was not correlated with recurrence-free survival [34]. However, there was conflicting results with our study regarding the expression of CD133 IHC staining in CRC and it relationship with the clinic pathological factors [68,55]. Many studies have demonstrated that CD133 expression is correlated with survival, recurrence, metastases and chemotherapy resistance, and most studies support the hypothesis that high D133 expression is a poor prognostic marker. [56–57]. In addition, in contrast to our results, Pitule et al. showed that patients with high CD133 expression had longer disease-free survival interval [40]. While our study as well as some other studies reported that CD133 was not significantly correlated with the survival time (5 years) [58–60]. Another study performed by Park on CRC 303 patients using immunhistchemical staining, showed that CD133 expression in CRC was significantly associated with five-year survival and tumor stage, but no with other clinical factors such as gender which was significantly related in our study. Moreover, they suggested Cd133 expression may consider as a more potential biomarker for prognosis or

a high-risk feature in the stage II CRC patients [61]. This result was not consistent with our results. Contrary to our results, the results of a meta-analysis of 37 studies done by Huang in 2018 demonstrated that higher CD133 expression was positively correlated with shorter overall survival, lymphatic vascular invasion, distant metastasis and poorer prognosis in CRC patients. They also assumed that this low 5-year survival rate that might play an important role in the progression of colorectal cancer in CRC patients, was due to sex hormone, genetic and epigenetic factors that is affected by the environment and lifestyle and a variety of therapies [37]. In Rey study on 118 patients in 2020, CD133 expression was significantly associated with the tumor location ($p = 0.002$), but not with other clinic pathological factors such as gender, age, body mass. Also, the tumor location had impact on the survival of CRC patients. This was in line with two previous studies [45, 49, 51] but no with our study. In immunohistochemistry study of Kazama on 200 endoscopically resected colorectal polyps and 20 normal mucosae, they demonstrated that CD133 expression was associated with only the degree of tumor differentiation and tumor size but not with gender, age, tumor location. Apart from two factors including age and tumor location, the statistical result of the gender parameter was consistent with our results. So, CD133 might play an important role in tumor development. Finally, the difference of our results with other researches may be due to different geographical area or race.

Conclusions

In Conclusion, based on our findings there were a statistically significant difference between CD133 expression with gender, tumor stage III and lymph vascular invasion, but no other factors, although they showed some non-significantly differences. Therefore, the CD133 expression is potential to be used as a CRC prognostic stem cell marker for colon cancer. There was a limitation in our study. A larger number of cases is needed to achieve higher statistical power to detect significant differences.

Declarations

Ethics approval and consent to participate

This study was approved by Ethical Committee of Kashan University of Medical Sciences, All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and national research committee and with the 1964 Helsinki Declaration and its later amendments.

Consent for publication

All study process was presented to patients and they were reassured about confidentiality of their records, they were requested to present their written consent of participation in the study.

Availability of data and material

The primary data for this study is available from the authors on direct request.

Competing interests

The authors declare no conflict of interest.

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Authors' contributions

MT and HE were responsible for the study conception and design. MT and HE performed data collection. HE and HHK preparing the first draft of the manuscript. HHK made critical revisions to the paper for important intellectual content.

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