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Evaluation of CD133 expression rate in colon cancers with immunohistochemistry method and its relationship with colon cancer prognosis

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Abstract

Introduction: Colorectal cancer (CRC) is the third most commonly diagnosed cancer and the second leading cause of cancer-related death, worldwide.
Objectives: This study aimed to evaluate the CD133 expression rate in colon cancers and determine its relationship with colon cancer prognosis.
Patients and Methods: This cross-sectional study was conducted in AL-Zahra hospital in Isfahan from April 2008 to April 2014 on 80 patients with CRC. In this study demographic profile such as age and gender, clinicopathologic profile including tumor grade, size, stage, metastasis, 5-year survival and their relation with CD133 expression in form of diffuse, weakly and negative were investigated.
Results: From the 80 investigated patients, 47 (58.8%) were male and rest were female. The most common type of CD133 was diffuse type with the 43.8% of cases. Mean age of patients was 61.4 ± 14.12 years. This study showed a significant difference between type of CD133 in regards of tumour size (from 23.11 mm in negative cases to 38.85 in weakly cases, $P = 0.047$). Moreover, the 5-year survival in the three groups of CD133 were significantly different (from 22.54 months in diffuse to 34.42 months in negative cases, $P = 0.025$).
Conclusion: CD133 may be considered as an important tumor marker and a prognostic and diagnostic marker, as well as a therapeutic approach in patients with CRC.

Introduction

Colorectal cancer (CRC) is the third most commonly diagnosed cancer and the second leading cause of cancer-related death, worldwide. There is a strong association between CRC and environmental risk factors and genetic factors. In recent years, the incidence of CRC gradually decreased except for adults younger than 50 years, which is probably related to the development of cancer screening programs and better diagnosis methods (1). In the united states, CRC affects approximately 135 439 new patients every year (2) and it is the second leading cause of death among cancers with an estimated 50 260 deaths (3). There has been occurred a 51% decline in CRC-related deaths from 1975 to 2014 in the United States, which was attributed to early diagnosis and improved treatment methods. The National Cancer Institute estimates that 65% of treated patients with colon cancer will be survived five years after diagnosis (3,4). The incidence of CRC cancer has increased in developing countries such as Iran over the past three decades. There is additionally a massive difference among the various races and ethnic groups regarding the CRC incidence and mortality (5).

The majority of CRCs are carcinomas, and more than 90% are adenocarcinomas and others such as spindle cell, squamous, adenosquamous and undifferentiated are rare types. Based on the percentage of gland formation; adenocarcinomas are categorized into well differentiated (more than 95%), moderately differentiated (more than 50%) and poorly differentiated (less than 49%), but it further divided by the prognostic significance as two-tier low-grade (well-moderate) and high-grade (poor) (4).

Recently, there is an increasing interest in

Key point

Our cross-sectional study on 80 patients with colorectal cancer (CRC), showed a higher incidence of CD133 expression would be associated with a worse prognosis in these patients.

the cancer stem cell (CSC) hypothesis of carcinogenesis as a small subset of cancer cells with capability of self-renewal (5).

Moreover, numerous cell surface antigens have been identified that express in the normal tissue stem cells, as well as CSCs, but there is no any evidence in related to matured normal cells (6). Due to expression in diverse normal tissues, CD133, a type-I membrane glycoprotein with a molecular weight of 120 kDa, is considered to be the most reliable stem cell marker (7). It is also suggested that CD133 acts as a promising candidate for CSC marker. As an instance, CD133-positive tumor cells compared to CD133-negative tumor cells had higher abilities regarding to self-renewal, tumorigenesis which is resistance to anti-tumor drugs and radiation treatment (8,9). CD133 has expressed in lung, kidney, pancreas, ovarian and brain CSCs while, the higher level of its expression has been reported in relation to the poor prognosis of the patients with colon, pancreas and lung cancers (10,11).

Objectives
To our knowledge, this is the first study to examine the association between CD133 expression and prognosis of colon cancer survival and its metastasis in human specimens in Iran.

Patients and Methods
Tissue sample
In this cross-sectional study, 80 endoscopically resected colon cancer samples obtained from 80 patients undergoing CRC treatment in Al-Zahra hospital affiliated to Isfahan University of Medical Sciences from April 2008 to April 2014 were analyzed. The resected specimens were immediately fixed in 10% buffered formalin and embedded in paraffin. Following hematoxylin and eosin staining, the pathological diagnosis was conducted and 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, the evaluation of the extent of tubular adenoma was assessed (12). Then, the demographic characteristics of the patients, including age, gender, disease grade and 5-year survival of them, were recorded in a researcher-made checklist by examining the archived patients' medical files in the hospital after obtaining the ethics code.

Evaluating of CD133 immunostaining
First, the intensity of staining with ocular lens (×10) was evaluated and it was assigned four numbers from 0 to 3, which indicate negative, weak, medium and strong staining, respectively. Then, to count and express the percentage of cells whose nuclei were stained, the slides were examined with ocular lens (×40) and this time a score of 1 to 4 was given.

For staining less than 25%, number 1, for 25-50%, number 2 was employed. Then for 50-75%, number 3 was used and for more than 75%, number 4 was considered. Finally, the two numbers from the first and second stages were added together and the final number was a score of 7. If the final number is from 0 to 3, the expression CD133 will be considered negative and if it is 4 to 7, it will be considered positive. The CD133 expression was evaluated by the two trained observers in pathological diagnosis independently.

Statistical analysis
The frequency, percentage, mean and standard deviation were used to describe the study population. The normality assumption of the variables was assessed using Kolmogorov-Smirnov test. If the normality assumption were not held, we conducted the Kruskal-Wallis H test as a non-parametric test for between groups comparison of continuous variables. For comparison of categorical variables between groups, chi-square test was used. All statistical calculations were carried out using SPSS (version 20) statistical software. The significant level was considered less than 0.05. The Kaplan-Meier curve was employed to graphical representation of the survival function.

Results
Baseline clinicopathologic characteristic
In this study we evaluated the clinicopathologic characteristics of the 80 patients. The number of 47 (58.8%) patients were male and the rest were female. The most common type of CD133 was diffuse type with the 43.8% of cases. Mean age of patients was 61.4 ± 14.12 years and size of samples varies from 1.5 to 15 mm with the mean of 4.96 ± 2.22 mm. Of these, 26 patients were died during the 5-year follow-up and 29 patients survived for more than five years. The mean of months alive were 39.90 ± 22.46 months (Table 1).

The normality assumption of the variables was assessed using Kolmogorov-Smirnov test. However, the normality assumption for age, alive months and size were not held ($P < 0.05$), therefore we conducted Kruskal-Wallis H test as a non-parametric test for between groups comparison.

As shown in Table 2, there was a significant difference between type of CD133 in regards of tumour size (from 23.11 mm in negative cases to 38.85 in weakly cases, $P = 0.047$). However, three groups were homogenous in regards of age ($P = 0.063$).

According to the obtained results, the 5-year survival in the three groups of CD133 were significantly different (from 22.54 months in diffuse to 34.42 months in negative cases, $P = 0.025$).

There was no significant relationship between different levels of CD133 expression and metastasis ($P = 0.297$), grade ($P = 0.927$) disease stage ($P = 0.338$), gender (0.695), metastasis ($P = 0.297$).

In Table 3, the means survival time according different types of CD133 is presented. The results showed that,

Table 1. The baseline clinicopathologic characteristics of patients

		No. (%)
Gender	Female	33 (41.3)
	Male	47 (58.8)
Type of CD133	Weakly	25 (31.3)
	Negative	20 (25)
	Diffuse	35 (43.8)
Metastasis	PMX	25 (31.3)
	MX	47 (58.8)
	M1a	8 (10)
Grade	Weakly	44 (55)
	Moderate	26 (32.5)
	Poorly	8 (10)
	Missing	2 (2.5)
Stage	I	16 (20)
	IA	2 (2.5)
	IIA	19 (23.8)
	IIB	2 (2.5)
	IIC	1 (1.3)
	IIIA	1 (1.3)
	IIIB	9 (11.3)
	IIIC	3 (3.8)
	IV	4 (5)
	IVA	3 (3.8)
	IVB	4 (5)
5-year survival	Death	26 (32.5)
	Alive	29 (36.3)
	Missing	25 (31.3)

PMX, Distant metastatic status is commonly unconfirmed pathologically; MX, Metastasis cannot be measured; M1a, The cancer has spread to one other part of the body beyond the colon or rectum.

Table 2. Relation between CD133 expression and clinicopathologic patients' characteristics

Characteristics	Type of CD133			P value
	Weakly	Negative	Diffuse	
Age (y)				0.063*
Number	25	20	35	
Mean	43.1	30	44.64	
Size (mm)				0.047*
Number	23	14	31	
Mean	38.85	23.11	36.42	
Survival (mon)				0.025*
Number	16	12	27	
Mean	32.41	34.42	22.54	
Gender				0.695**
Female	12	8	13	
Male	13	12	22	
Metastasis				0.297**
PMX	4	7	14	
Mx	19	11	17	
m1a	2	2	4	
Grade				0.923**
Weakly	14	12	18	
Moderate	8	5	13	
Poorly	3	2	3	
Stage				0.338**
I	5	7	4	
IA	1	0	1	
IIA	7	0	12	
IIB	0	1	1	
IIC	1	0	0	
IIIA	0	0	1	
IIIB	3	1	5	
IIIC	1	2	0	
IV	1	1	2	
IVA	1	0	2	
IVB	1	1	2	
5-Year survival				0.072**
Death	5	4	17	
Alive	11	8	10	

* Kruskal-Wallis H test, ** Chi-square test.

there is a significant difference in the survival time between three groups ($P = 0.038$), and the diffuse group has lower survival rate survive than the other groups. In general, it can be said that a higher CD133 expression will be associated with a greater reduction in life expectancy and a factor of CD133 could indicate a worse prognosis for the patient.

In Figure 1, the survival function of the patients according different types of CD133 graphically presented. As shown with the elapse of time and the addition of the number of months (length axis), the probability of survival decreases more rapidly and from 40 months onwards, the probability of survival for the diffuse group is less than 0.4. While, the probability of survival for other groups was more than 0.65.

Discussion

CD-133 may be considered as an important tumor marker and a prognostic marker as well as a therapeutic approach in patients with CRC. Despite little knowledge about the molecular background of CD133 in cancer, most current research shows that this factor has significant prognostic and predictive value for overall survival, disease-free survival and progression-free survival in many different solid cancers (13). Numerous studies have attempted to assess the clinicopathological relevance of CD133 in CRC and consequently the increasing data suggest its prognostic value. In this study, 80 patients were studied, most of them were male and the most common type of CD133 was diffuse type, which has not been assessed in similar studies.

The present study is the first study that examines the relationship between prognostic variables and expression of CD133 in terms of negative, weak and diffuse expression. In the present study, there was no significant relationship between demographic variables of gender, age and CD133 expression. In other words, CD133 expression

Table 3. Means survival time according different types of CD133

Type	Mean[a]				P value
	Estimate	Standard Error	95% Confidence Interval		
			Lower Bound	Upper Bound	
Weakly	46.250	5.145	36.165	56.335	
Negative	49.500	4.502	40.675	58.325	0.038
Diffuse	31.963	4.400	23.339	40.587	
Overall	39.945	3.002	34.062	45.829	

[a] Estimation is limited to the largest survival time if it is censored.

was not related to the age and gender of the affected patients, while the group with the smallest size had express CD133. Therefore, tumor size can be considered as a prognostic factor for CD133 expression. In a study in 2020 in India, 180 patients with CRC of the relation between CD133 expression and histopathological symptoms were examined. Most of patients were male, and consistent with our findings there was not significance relation between CD133 expression and gender. Moreover, there was no significant difference between age, body mass index (BMI), histopathology and CBC profile (14) that is similar to our study. It seems that expression of CD133 depended on factors other than age and gender, some factors are known to be involved in CRC such as genetic or sex hormones (15) that were not assessed in this study.

Results of an in-vivo study in 2021 in Japan, showed that there is no significance relationship between age, gender, carcinoembryonic antigen level and tumor size (16). Moreover it demonstrated that grade, stage, metastasis and lymph node involvement have no relationship with CD133 expression (16). Our study showed no significant relation between CD133 expression and stage, grade and metastasis. This is consistent with Japanese study.

In line with our findings, in a study in Korea in 2019 on 174 men and 149 women with stage 2 CRC, there was no significant association were observed between the CD133 expression and age, gender, tumour location, stage and tumour grade (17).

Wang et al in their study on of 148 patients with CRC, could not found a significant association between CD133 expression and age, gender, and stage of the disease, which is consistent with the present study (18). In another study in Italy on 137 patients, there were no significant relation between CD133 expression and age, gender grading but there was a significance association between stage and metastasis (19). These results were confirmed by other similar studies (20-22). The difference in the number of samples in this research with the present study is the most obvious indicator that causes differences in the results due to metastasis. It seems better to conduct studies with higher sample sizes to examine this difference in results. Different studies have reported very different results regarding the differences in stage and grade. This may be due to differences in the number of samples, region and race.

In a meta-analysis study, CD133 expression was correlated with more T3, and T4 category patients (pooled RR = 1.12, 95% CI 1.01, 1.23, $P = 0.03$). In addition, high expression of CD133 was associated with more N positive and vascular invasion cases. However, there was no significant association between CD133 and other clinicopathological features such as histology, lymphatic invasion or distant metastasis (23).

In another meta-analysis on 37 studies, demonstrated CD133 expression has no significant relation with age, gender and tumor grade, which was similar to our
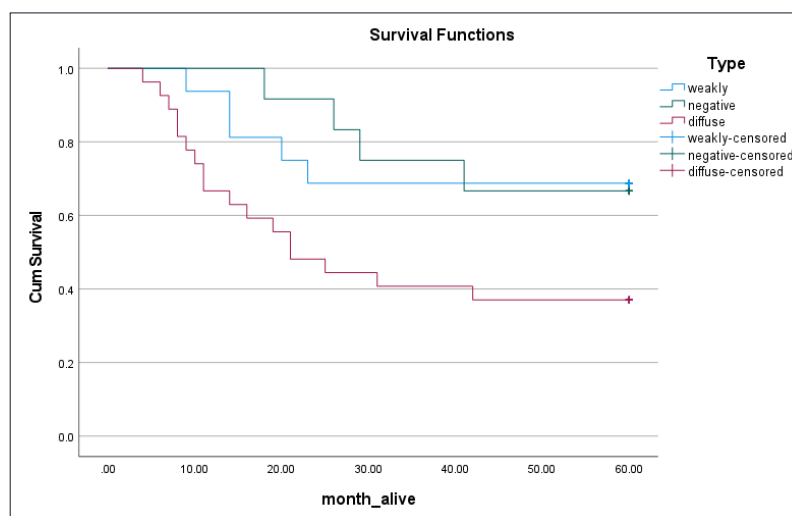

Figure 1. Graphical representation of the survival function.

study. However, in this meta-analysis, tumor size has no statistically significance relation with CD133 expression. This meta-analysis demonstrated that, CD133 expression is in relation with T category, distant metastasis and lymph node involvement (24). This difference in results may be due to the larger sample size in the mentioned studies.

CD133 overexpression was significantly associated with poor 5-year overall survival rate that was 0.67-fold lower in CD133-positive patients ($P=0.01$). In 5-year disease free survival rate there was an slight difference between the groups of CD133-high and CD133-low (23).

In this study, we found that CD133 expression is associated with a lower 5-year survival probability, which is consistent with all the studies mentioned in this study. Furthermore, in this study it was shown that patients with "diffuse" CD133 expression have a lower survival that has not been studied before. It was also shown that over time, the probability of survival will have further reduction and in the diffuse group over time the probability of survival will be less than other groups. In a study conducted in 2021, patients with a high level of CD133 had lower survival rate, and over time, they had further reduction in survival probability (16). These results were confirmed in two conducted meta-analysis in 2013 and 2018 (23,25).

Conclusion

The present study examined the relationship between CD133 and demographic and prognostic factors. We found a significant relationship between CD133 and tumour size and 5-year survival rate, and the number of months of survival, However, more studies are needed in this field with high sample size and enough power to clarify the CD133 expression rate in colon cancers and its relationship with colon cancer prognosis.

Limitations of the study

The small sample size, the retrospective nature of the study, the lack of evaluation of different types of CD133 and the lack of comparison of CD133 expression in healthy tissues can be mentioned as limitations of this study.

Authors' contribution

Conceptualization: Mohammad Hossein Sanei.
Data curation: Tina Foodeh and Maryam Sanei.
Formal analysis: Tina Foodeh.
Funding Acquisition: Mohammad Hossein Sanei.
Investigation: Tina Foodeh.
Methodology: Mohammad Hossein Sanei.
Project Administration: Tina Foodeh.
Resources: Tina Foodeh.
Supervision: Mohammad Hossein Sanei.
Validation: Mohammad Hossein Sanei.
Visualization: Maryam Sanei.
Writing–original draft: Tina Foodeh.
Writing–review & editing: Mohammad Hossein Sanei, Tina Foodeh and Maryam Sanei.

Conflicts of interest

The authors declare that they have no conflict of interest.

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