Original Article

The association between CD166 detection rate and clinicopathologic parameters of patients with colorectal cancer

Shahriar Shafaei (MD) [1]
Majid Sharbatdaran (MD) [2]
Ghodsieh Kamrani (MD) [*3]
Soraya Khafri (PhD) [4]

1- Infectious Diseases and Tropical Medicine Research Center, Babol University of Medical Sciences, Babol, Iran.
2- Department of Pathology, Babol University of Medical Sciences, Babol, Iran.
3- Babol University of Medical Sciences, Babol, Iran.
4- Department of Social Medicine and Health, Babol University of Medical Sciences, Babol, Iran.

*** Correspondence:**
Ghodsieh Kamrani, Babol University of Medical Sciences, Babol, Iran.

E-mail:
ghodsieh_kamrani@yahoo.com
Tel: 0098 111 2238301-5
Fax: 0098 111 2238284

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Abstract

Background: Metastasis and recurrence of colorectal cancer after treatment is attributed to stem cells. The aim of this study was to determine the relationship between the expression of stem cell marker CD166 in colorectal cancer by immunohistochemistry and clinicopathologic parameters.

Methods: From 2006 to 2012, 121 colectomy specimens of patients with colon cancer that were operated in Babol Medical University in Iran were evaluated. The paraffin blocks were extracted from the archive and the slides were prepared and stained for H&E and Immunohistochemical (IHC) methods. The samples with cytoplasmic and/or membranous staining more than 50% of tumor cells were considered as positive. Pathological parameter including type of tumor, stage and grade, vascular invasion and location of the tumors were recoerded.

Results: The mean age of the patients was 58.7±15.1 years. Sixty-four (54.9%) patients were males. Eighty-six (71.1%) subjects were positive for cytoplasmic and 42 (34.7%) for membranous and 42 (34.7%) for both cytoplasmic and membranous staining. The cytoplasmic expression of marker CD166 marker in mucinous type was 10 (50%) and was lower than non-mucinous type 76 (75.2%) (p=0.031). There was significant relationship between membranous expression of CD166 marker and tumor location (right colon in 23(54.8%), left colon in 18 (24.3%)] (p=0.001). There was no significant difference in the expression of marker with other demographic and clinicopathologic variables.

Conclusion: The results show that CD166 expression was seen in more than two-thirds of the patients. The cytoplasmic expression of CD166 marker was higher in non-mucinous type. The distributions of membranous expression of marker CD166 was related more in right colon with colorectal cancer.

Keywords: Colorectal cancer, Cancer stem cells, CD166, Immunohistochemistry

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Colorectal cancer is one of the most common malignancies and a leading cause of death worldwide (1). One out of the four patients presented with conventionally stages I and II, and over 50% of patients with stage III disease (2, 3). Across all stages, approximately 30% of the patients will develop distant metastases (2). Once metastases become clinically evident, prognosis is often fatal. Moreover, in spite of the fact that modern systemic therapies for colorectal cancer have resulted in the improved overall survival, failure rate in the adjuvant setting is 30% for high-risk stage II and stage III patients, and the overall response rate is only 60% for patients with stage IV colorectal cancer (4-6). Increasing evidence suggests that cancers, including colorectal cancer, may be hierarchically organized, with only a small population of cancer cells, termed cancer stem cells, possessing the potential to initiate and sustain tumor growth and metastasis (7). Cancer stem cell theory explains the biological heterogeneity of human solid tumors, according to which a small fraction of cancer cells is solely responsible for the growth and maintenance of the entire heterogeneous tumors (8).

They are resistant to chemotherapy due to their innate ability to escape the cytotoxic effects of conventional therapy by employing drug transporters and enhanced DNA repair mechanisms (9, 10). A significant advance in the care of patients will be realized by biomarkers that can accurately identify the patients at-risk for disease recurrence and dissemination, and those that fail to respond to systemic therapy. These patients might benefit from early (preventative) treatment, alternative treatment strategies, and/or frequent surveillance for and early detection of disease recurrence (11).

Several studies have identified putative stem cell markers for colorectal cancer, namely CD133, CD44, and CD166, the activated leukocyte adhesion molecule (ALCAM) (12-15). The last is a highly conserved 110-kDa multidomain transmembrane type 1 glycoprotein of the immunoglobulin superfamily. ALCAM plays a role in the development of different tissues during embryogenesis and in adults, and it functions via homotypic and heterotypic interactions between the cells and it also is expressed in various malignant lesions (16, 17) However, inconsistent data exist regarding the prognostic significance of ALCAM expression in colorectal cancer (14, 16-18).

Since there are differences in predisposition to colorectal cancer between ethnic groups, the aim of this study was to determine the relationship between the expression of stem cell markers CD166 in colorectal cancer by immunohistochemistry and clinicopathological factors.

Methods

The colorectal cancer samples of 121 patients who had undergone colectomy from 2006 to 2012 operated in Babol Medical University in Iran were reviewed. Then, the archival paraffin blocks of the patients were used to prepare slides from tumoral and normal areas in order to stain for hematoxylin-eosin (H & E) and immunohistochemical assays (IHC). The immunohistochemical assays were performed by CD166 mouse monoclonal antibody diagnostic kits (clone: MOG/07, Novo castra). The prepared slides were evaluated in terms of expression of CD166 marker. The cytoplasmic and membranous expression of CD166 marker in tissues was evaluated semi-quantitatively, i.e. the ratio of positive tumor cells to all tumor cells, as well as the intensity of staining. Each sample was compared with an internal control (CD166 positive ganglion cells of neural network). According to literature review, the cut-off point of CD166 marker expression was assumed to be 50%. The positive stains with cell percentages above the cut-off point were considered the over-expression and those below were considered as loss (figure 1).

The spot staining without cytoplasmic or membranous staining was considered negative. The data were collected and analyzed by SPSS Version15. Fisher's exact and chi-square tests were used for the comparison of qualitative variables and t-test for the quantitative variables. A $p<0.05$ was considered as the significance level.

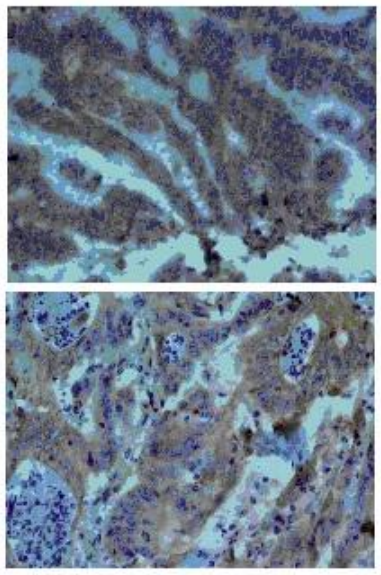

Figure 1. Membranous and cytoplasmic expression of CD166

Results

The mean age of these patients was 58.7±15.1 years. The mean age of patients in men and women was 60.2±16.2 and 56.9±13.7 years, respectively (p=0.221). Sixty four (52.9%) were males. Eighty-six (71.1%) samples had cytoplasmic expression, 42 (34.7%) had cytoplasmic with membranous expression, and the staining intensity was moderate in 58 (66.7%) and severe in 29 (33.3%) patients.

The cytoplasmic expression of CD166 in cancer tissue according to tumor-related factors, and demographic factors are shown in table 1. The distribution of CD166 in mucinous type was significantly lower than the non- mucinous. The membranous expression of CD166 in cancer tissue according to tumor-related factors, and demographic factors are shown in table 2. The membranous expression of CD166 had significant difference for the location of tumor.

Table 1. The cytoplasmic expression of CD166 in cancer tissue according to tumor-related factors, and demographic factors.

Cytoplasmic expression variable		Negative N	Negative %	Positive N	Positive %	p-value
Type of tumor	Non- mucinous	25	24.8	76	75.2	0.031
	mucinus	10	50	10	50	
Stage	T1	0	0	3	100	-
	T2	4	33.3	8	66.7	
	T3	31	30.1	72	69.9	
	T4	0	0	3	100	
	N0	18	26.5	50	73.5	
	N1	11	30.6	25	69.4	0.748
	N2	6	35.3	11	64.7	
Grade	G1	18	23.7	58	76.3	-
	G2	46	26.1	17	73.9	
	G3	1	50	1	50	
Vascular invasion	No	25	27.5	66	72.5	0.643
	yes	10	33.3	20	66.7	
Location	Right	9	21.4	33	78.6	0.285
	Left	24	32.4	50	67.6	
Age	<=65	21	25	63	75	0.192
	>65	14	37.8	23	62.2	
Sex	male	15	23.4	49	76.6	0.167
	Female	20	35.1	37	64.9	

T1: The cancer has grown through the muscularis mucosa and extends into the submucosa; **T2:** The cancer has grown through the submucosa and extends into the muscularis propria (thick outer muscle layer); **T3:** The cancer has grown through the muscularis propria and into the outermost layers of the colon or rectum but not through them. It has not reached any nearby organs or tissues; **T4:** The cancer has grown through the serosa

N0: No cancer in nearby lymph nodes; **N1:** Cancer cells are found in or near 1 to 3 nearby lymph nodes; **N2:** Cancer cells are found in 4 or more nearby lymph nodes, ***G1***-well differentiated; ***G2***-moderately differentiated; G***3***-poorly differentiated

Table 2. The membrane expression of CD166 in cancer tissue according to tumor-related factors, and demographic factors.

Membrane expression variable		Negative N	Negative %	Positive N	Positive %	p-value
Type of tumor	Non- mucinous	56	61.5	35	38.5	0.184
	mucinous	23	76.7	7	23.3	
Stage	T1	2	66.7	1	33.3	-
	T2	8	66.7	4	33.3	
	T3	68	66	35	34	
	T4	1	33.3	2	66.7	
	N0	48	70.6	20	29.4	
	N1	21	58.3	15	41.7	0.382
	N2	10	58.8	7	41.2	
Grade	G1	51	67.1	25	32.9	-
	G2	5	15	8	34.8	
	G3	2	2	0	0	
Invasion	No	56	61.5	35	38.5	0.184
	yes	23	76.7	7	23.3	
location	Right	19	45.2	23	54.8	0.001
	Left	56	75.7	18	24.3	
Age	<=65	53	63.1	31	36.9	0.536
	>65	26	70.3	11	29.7	
Sex	male	41	64.1	23	35.9	0.489
	Female	38	66.7	19	33.3	

Discussion

The present study was carried out through immunohistochemical method with clinicopathologic factors aiming to determine the relationship between CD166 marker of colorectal cancer stem cells. In this study, we found that 86 (71.1%) cases were positive cytoplasmic expression, 42 (34.7%) had membranous expression and 42 (34.7%) patients had simultaneous cytoplasmic expression and membranous CD166 marker. In 2012, Tachezy et al. studied the expression of CD166 marker through immunohistochemical method in tissue samples of 299 patients with colon cancer. This marker was found in 76% of the primary lesions and in 62% of the secondary lesions of cancer, which was similar to the results of the present study (19). Weichert et al. studied the expression pattern of CD166 in 111 patients with colon cancer through IHC method. The severe cytoplasmic and membranous expression of CD166 in colorectal cancer were 58.6% and 30.6%, respectively (20).

Other markers like CD44 and CD133 with different frequencies were reported by other researchers (21). Dangho et al. also reported CD44, CD24, and CD133 on 523 colorectal adenocarcinoma samples through IHC method with the order of frequency of 40%, 50.5% and 24.5% of their patients respectively (1). In this study, we found no significant relationship among cytoplasmic expression of CD166 marker and the tumor-related factors such as grade, stage, and vascular invasion. Tachezy et al. examined the expression of CD166 marker in 2012 through immunohistochemical method in tissue samples of 299 patients with colon cancer. There was a reversed significant relationship between marker expression rate and tumor grade; however, there was no significant relationship between marker expression and the rest of clinical and histopathological characteristics of tumor (19). Weichert et al. examined CD166 expression in 111 patients with colon cancer through IHC method and showed no considerable relationship among expression of this marker and tumor grade, stage of illness and involvement of lymph nodes which is similar to the findings of our study (20).

Horst et al. evaluated 110 patients with colon cancer and they found no significant relationship between CD166 marker expression and tumor characteristics like the finding of our study (22). With respect to the role of the stem cells and the role of CD166 as an adhesion molecule, it seems that they may involve in the disease prognosis. It might also be due to the fact that these markers may have appeared after cancer development and do not involve much in tumor invasion. To approve involvement of the markers in invasion and prognosis of a disease, the results obtained from the study of Lugli et al. showed that there was a relationship between lack of expression of CD44, CD166, and EPCAM and invasive cases of colorectal tumor. Lack of expression of CD166 and CD44 markers accompanied with a higher pathologic T stage, lymph node metastasis, and worse survival (23). Moreover, in 2011 Sanders et al. expressed markers of colon cancer stem cells as a prognostic factor in colon cancer survival. They expressed that it is necessary to develop some therapies focusing on these cells (24).

In our study, we found that the expression of CD166 in mucinous type was significantly lower than non- mucinous. More researches are needed to confirm our finding. The weakness of this study was the retrospective design of this work as well as the lack of the survival of the patients with expression of CD166.

In conclusion, the results of our study show that CD166 expression was seen in more than two-thirds of the patients. The cytoplasmic expression of CD166 marker was higher in non-mucinous type. The distribution of membranous expression of marker CD166 was related more in the right colon with colorectal cancer.

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