Jurnal Kedokteran Syiah Kuala
Edisi Khusus Oktober 2022

ISSN: 1412-1026
E-ISSN: 25500112

CD166 as Cancer Stem Cells Marker Based on Colorectal Cancer Location and Individual Characteristic

[1]Imelda Rey, [2]Dharma Lindarto, [3]Fauzi Yusuf, [4]Agung Putra

Medical Faculty, Universitas Sumatera Utara, 20155 Medan, Indonesia
imeldarey@rocketmail.com;imelda.rey@usu.ac.id
Medical Faculty, Universitas Sumatera Utara, 20155 Medan, Indonesia
Medical Faculty, Sultan Agung Islamic University (UNISSULA), 50112Semarang, Indonesia

Abstract. Colorectal cancer stem cells (CR-CSCS) are associated with a sub-population of colorectal cancer (CRC)with stem-cell properties that have s crucial role eith in chemotherapy resitance or in recurrency of CRC. CR0CSCs express high levels of CD166 in the colorectal; however, their expression, based on thespecific location of CRC and individual characteristics, remains unclear. The objecive of this study was to investigate the CD166 exppressionbased on the locationof the proximal colon, distal colon and rectum, and individual profile. This was a cross-sectional study performed at the Internal Medicine Department, Faculty of Medicine, Adam Malik General Hospital and affiliation hospital from September, 2018 to July, 2019. The CRC tissue was taken from colonoscopy biopsy and surgical resection of CRC patients that fulfilled inclusion and exclusion criteria and signed informed consent. We examined immunohistochemistry for analyzing the CD166 expression**.** Of the 118 subjects, 69 (58.5%) them were males, and the expression of CD166 high and low level were 45 (38.1%) and 43 (36.4%), respectively. There were no significant differences in age, sex, and BMI between high and low-level CD166 expression. There was no significant difference in laboratory findings between high and low-level CD166 expression. Based on tumor location, CD166 expression was significantly higher at distal colon than at the rectum (p=0.047). CD166 expression was associated with tumor location, and not with **g**ender, sex, and laboratory parameters.

Keywords: Colorectal cancer stem cell, CD 166, tumor location.

Introduction (Heading 1)

CRC is still one of the most frequent cancers worldwide. In global cancer statistics, new cases of CRC were diagnosed approximately 1,360,600 clinically[1]. In Pirngadi Hospital Medan, Indonesia, 25% (197 of 760) of the patients underwent colonoscopy were diagnosed CRC[2].In Adam Malik hospital, Medan, Indonesia, there were 61.5%, 23.1%, 15.4% patients with rectal cancer, left sided colon cancer, and right sided colon cancer, respectively[3].

The resistance of chemotherapy and recurrences still become a problem in CRC management. Cancer stem cells (CSCs) were considered to be involved, due to the ability to escape from chemotherapy and to differentiate into mature cancer cells[4].

CD166 is one of important CSC markers controlled by gen in human chromosome 3q13.11. CD 166 is functionally involved in cell interaction that is called activated leukocyte cell adhesion (ALCAM). CD166 is also involved in T-cell stimulation and proliferation, hematopoiesis, and angiogenesis[5,6] . Previous studies reported that the molecular mechanisms of colorectal carcinogens might be different in each location of CRC[7,8], and tumor locations have the impact on overall survival in CRC patients underwent surgery [9].These findings showed that location of CRC should have been considered as one factor for the expression of CD166.

Analysis on survival rates showed that CR-CRCs might be one of independent predictors of survival after having been adjusted by ages, races, and genders[10].

However, the expression of CD166 is still unclear. On the other hand, there is still no data about CD 166 expression in CRC and the association with clinical characteristics and tumor location in our region. Our aim was to investigate the CD166 expression and its association with clinicopathological characteristics and tumor location.

Methods

A. Patients and clinicopathological data

One hundred eighteen patients with a biopsy-proven colorectal adenocarcinoma who were

operated or by colonoscopy biopsy between September 2018 and July 2019 were enrolled in this study. We analyzed their data recorded in colorectal cancer database. We investigated the clinicopathological factors including gender, age, and location of tumor (proximal colon, distal colon, or rectum) and laboratory parameters.

B. Immunohistochemical staining method

We performed staining for CD166 on primary colorectal cancer tissue from colonoscopy biopsy and surgical resection. We used a Bond polymer detection kit and Bond-max autostainer (Leica Microsystems, Wetzlar, Germany) for the immunohistochemical staining: Deparaffinization by incubation in a dry oven at 65°C for 1 hour and pH 7.4PT Link Dako epitope retrieval solution 1 at 100°C for 15 minutes was used for antigen retrieval and then peroxidase blocking with 0.3% hydrogen peroxide for 5 minutes.

We used the primary anti-CD166 antibody GTX83191 [10F1G12, 1:200-1:1000] (GeneTex International Corporation, California, USA) at room temperature. Tissues were incubated with polymer and developed with 3, 3´-diaminobenzidine tetrahydrochloride-chromogen and counter-stained with Mayer's hematoxylin. The staining procedure was similar for the negative control group, but without using the primary antibody. Two pathologists evaluated the results of Immunohistochemical staining.

The assessment of the CD166 expressions in the primary tumor and included the stronger expression results. Two pathologists that did not have information about the data of patients performed the interpretation of the CD166 expressions. The analysis on immunohistochemical staining of the colorectal cancer samples was performed. The results were scored according to the population score and intensity score of the tumor cells. The total scores ranged from 0 to 6 for CD166.

For CD166 analysis, the immunoreactivity score on the cell membrane and/or cytoplasm was calculated from the sum of both quantitative and qualitative parameters. A total score of 0–3 were considered as low level for CD166 expression, and a total score of 4-6 were considered as high level for CD 166 expression.

For quantitative analysis, the following scores and respective percentages of reactive cells (% of the total area) were adopted as previously described. Briefly, the percentages of reactive cells: 0 (0% of immunoreactive cells); 1 (<10% of immunoreactive cells), 2 (10%–50% of immunoreactivecells), and 3 (more than 50% of immunoreactive cells). For qualitative analysis, the immunoreactive staining intensity was classified according to the scores: 0 (no immunoreactivity), 1 (weak immunoreactivity), 2 (intermediate immunoreactivity), and 3 (strong immunoreactivity). When the total scores of the proportion score and intensity score were less than 4 (range 0 to 6), we considered it as "low-level"[11].

For qualitative analysis of staining intensity, a "strong" staining was considered as the most intense staining observed in thepositive control of respective antibody. The Immunohistochemical detection in colorectal cancer samples with CD 166 staining was showed in Fig. 1 for low level CD166 Expression. The Immunohistochemical detection in colorectal cancer samples with CD 166 staining was showed in Fig. 2 for high level CD166 expression.

C. Statistical analysis

Chi-square test with Kolmogorov-Smirnov test was used to analyze the correlation between clinicopathological factors and the CD166 expressions. Mann-Whitney method was used for the correlation between CD166 and CRC locations. We used $P<0.05$ to be statistically significant.

Results

The patients' mean age was 57.17. Of the 118 patients, 69 (58.5%) of them were males and 49 (41.5%) of them were females. The number of proximal colons was 32 (27.1%), distal colons were 43 (36.4%), and rectums were 43 (36.4%).

The CD166 expressions are shown in Fig. 1 and Fig. 2. The characteristics of subject were presented in Table I.
Table II showed that there was no significant difference of gender, age, and Body Mass Index from CD 166 expressions. There was no significant difference of Hemoglobin, Leucocytes and Platelets from CD 166 expressions.

TABLE I. THE CHARACTERISTICS OF SUBJECT

Variable	n = (%)
Gender	
Male	69 (58.5%)
Female	49 (41.5%)
Age (years)	57.30 ± 12.99
Hb(gr/dl)	10.51+2.1
Leucocytes (cells/mm^3)	7420 (1609-34180)
Trombocytes (cells/$mm^{3)}$	286350 (89000-562000)
BMI (kg/m^2)	22.22 (1.22-30.86)
Tumor Location	

Proximal colon	32 (27.1%)
Distal colon	43(36.4%)
Rectum	43(36.4%)
CD166 expression	
High Level	45 (38.1%)
Low Level	73 (61.9%)

TABLE II. CORRELATION BETWEEN CLINICAL CHARACTERISTICS AND CD 166 EXPRESSIONS

Variable	CD166		p
	High level	***Low level***	
Age (years)	57.16 ± 13.69	57.38 ± 12.64	0.927
Gender (Male vs Female)	24 vs 21	45 vs 28	0.374
Body Mass Index (kg/m^2)	21.34 ± 4.52	22.50 ± 3.44	0.156
Hb (gr/dl)	10.37 ±2.10	10.59 ±2.12	0.583
Leucocytes ($cells/mm^3$)	8100 (4100-34180)	8986 (1609-32170)	0.380
Platelets($cells/mm^3$)	312926 ±108243	300557 ±115601	0.564

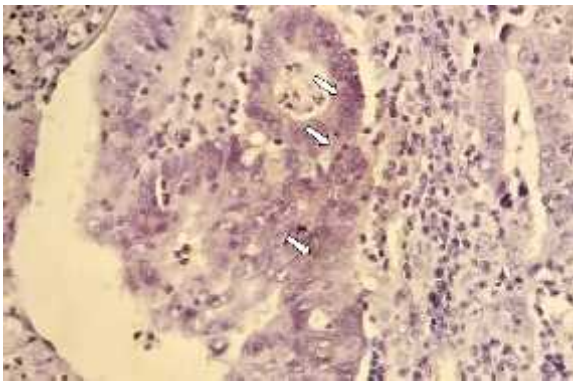

Fig. 1. Immunohistochemical detection in colorectal cancer samples with CD 166 staining. A low level immunoreactivity CD166 is marked by light brown color on cytoplasm (white arrow). (200x magnification).

Table III showed CD166 expressions were significantly higher at distal colon than at rectum. p=0.041.

Discussions

CD166 is one of CS-CRC that was found frequently in colorectal cancer. In this study, we found CD166 expression high level and low level were 45 (38.1) and 73 (61.9%) respectively. Previous study reported that CD 166 was expressed in CRC with the frequency ranging from 30.6% [12] to 76.3%[13].

This study found that there was no difference of age, gender, and Body Mass Index from CD 166 expressions (high level and low level) which was in line with previous studies that also did not found significant difference of gender from CD166 expressions[14, 15].

In this study, we found significant difference of CD166 expressions between distal and rectum (p =0.041). Our study was different from that reported in the literature. In the[14] study, the authors had reported that there was no correlation between CD166 expression and CRC locations. Another study also reported that there was no significant differentiation of CD166 loss vs overexpression between right colon and left colon locations [16] and the mean value of CD166 expressions with tumor locations[17].

We assumed that the difference of our results was due to several reasons. There was variability of cut off levels (5%, 10%, and 65%) in each study. In the future, the cut-off level should be standardized. Another factor was because another study divided the CRC locations into 2 parts: left and right colon. Meanwhile, our study divided the CRC locations into 3 parts: proximal colon, distal colon, and rectum. Race differences might give different results. In addition, previous study reported that inflammation had a role in CRC. The tumor cell regulation included the release of inflammatory mediators by macrophages[18].

TABLE III. CD 166 EXPRESSION BASED ON CRC LOCATIONS.

	CRC Locations		
	Proximal Colon Med (Min-Max)	***Distal Colon Med (Min-Max)***	***Rectum Med (Min-Max)***
CD166 Expression	3(2-5)	3(2-6) #*	3(2-6)

significantly higher than rectum; *p=0.041 (Mann-Whitney)

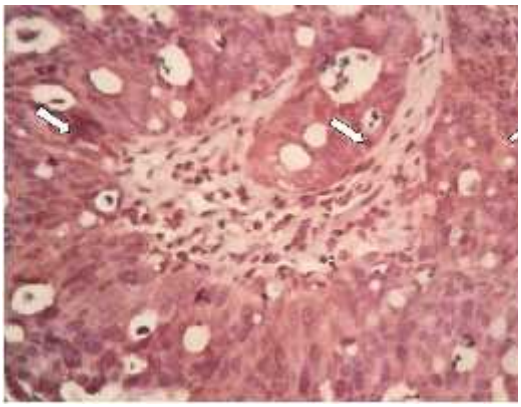

Fig. 2. Immunohistochemical detection in colorectal cancer samples with CD 166 staining. A high level immunoreactivity CD166 is marked by dark brown color on cytoplasm (white arrow). (400x magnification).

Tumor associated macrophages (TAMs) play a role in the regulation of tumor microenvironment and in maintaining the CSCs niche[19]. They can increase tumor growth by angiogenesis, tumor progression, invasion, and metastatic [20]. Previous study found that the carcinogenic pathway involved in CRC as well as molecular background differed in the locations of CRC patients [8]. This study had several limitations. In this study we did not analyze inflammation factors such as TAMs that might influence the CS-CRC. We also did not analyze molecular markers that were involved in genetics and epigenetics such as chromosomal instability (CIN), CpG island methylator phenotype (CIMP) and microsatellite instability (MSI).

Conclusion

We found that CD166 expression had the correlation with tumor location, but not with gender, sex, and laboratory parameters.

References

1. L. A. Torre, F. Bray, R. L. Siegel, J. Ferlay, J. Lortet Tieulent, A. Jemal, "Global cancer statistics," 2012. CA Cancer J Clin. 2015; 65:87-108.
2. R. Effendi-YS, D. Efendi, L. B. Dairy, M. Sihombing, G. A. Siregar, and L. H. Zain, "Profile of Colorectal Cancer Patients in Endoscopic Unit At Dr. Pirngadi Hospital Medan," in Indonesian Journal of Gastroenterology Hepatology and Digestive Endoscopy, 9(3):pp.75-78, 2008.
3. I.Rey, R. Effendi-YS, and L. H. Zain, "Comparison of CEA level among tumor location and histopathological findings in colorectal cancer," Journal of Gastroenterology and Hepatology; **31** (Suppl. 3): pp. 200, 2016.
4. L. Song, Y. Li, B. He, Y. Gong, "Development of Small Molecules Targeting the Wnt Signaling Pathway in Cancer Stem Cells forthe Treatment of Colorectal Cancer," in Clinical Colorectal Cancer, Vol. 14, No. 3: pp.133-45, 2015
5. A. G. Hansen, G. W. Swart, and A. Zijlstra, ALCAM: Basis Sequence: Mouse. AFCS Nat Mol Pages. 2011.
6. L. C. van Kempen, J. M. Nelissen, W. G. Degen, R. Torensma, U. H. Weidle, H. P. Bloemers, C. G. Figdor, and G. W. Swart, "Molecular basis for the homophilic activated leukocyte cell adhesion molecule (ALCAM)-ALCAM interaction," in J Biol Chem. 2001; 276:25783-25790.
7. M. Watatani, T. Yoshida, K. Kuroda, S. Ieda, and M. Yasutomi, "Allelic Loss of Chromosome 17p, Mutation of The P53 Gene, and Microsatellite Instability in Right and Left-Sided Colorectal Cancer," in Cancer 77 (Suppl), pp. 1688-93, 1996.
8. R. Effendi-YS, L. H. Zain, G. A. Siregar, H. R. Lubis, H. A. Damanik, L. I. Laksmi, and J. Chrestella, "Adenomatous Polyposis Coli, Mismatch Repair, and Microsatellite Instability in Colorectal Cancer Based on Different Locations," in Acta Med Indones-Indones J Intern Med, Vol 45 (4), pp. 275-283, October 2013
9. T. Aoyama, K. Kashiwabara, K. Oba, M. Honda, S. Sadahiro, C. Hamada, H. Maeda, S. Mayanagi, M. Kanda, J. Sakamoto, S. Saji, and T. Yoshikawa, "Clinical impact of tumor location on the colon cancer survival and recurrence: analyses of pooled data from three large phase III randomized clinical trials," in Cancer Medicine. 2017; 6(11): pp.2523-2530.
10. A. G. Hansen, T. J. Freeman, S. A. Arnold, A. Starchenko, C. R. Jones- Paris, M. A. Gilger, M. K. Washington, K. H. Fan, Y. Shyr, R. D. Beauchamp, and A. Zijlstra,"Elevated ALCAM shedding in colorectal cancer correlates with poor patient outcome," in Cancer Res. 2013; 73:2955-2964.
11. Y. Kato, H. Nishihara, H. Mohri, H. Kanno, H. Kobayashi, T. Kimura, M. Tanino, S. Terasaka, and S. Tanaka, "The clinicopathological evaluation of cyclooxygenase-2 expression in meningioma: immunohistochemical analysis of 76 cases of low- and high-grade meningioma," in The Japan Society of Brain Tumor Pathology. 2012, pp. 31: 23-30
12. W.Weichert, T. Knosel, J. Bellach, M. Dietel, and G. Kristiansen, "ALCAM/CD166 is overexpressed in colorectal carcinoma and correlates with shortened patient survival," in J Clin Pathol. 2004; 57:1160-1164.
13. M. Tachezy, H. Zander, F. Gebauer, A. Marx, J. T. Kaifi, J. R. Izbicki, and M. Bockhorn, "Activated leukocyte cell adhesion molecule (CD166)—its prognostic power for colorectal cancer patients," inJ Surg Res. 2012; 177:e15-20.
14. S. Han, W. Yang, S. Zong, H. Li, S. Liu, W. Li, Q, Shi, and F. Hou, "Clinicopathological, prognostic and predictive value of CD166 expression in colorectal cancer: a meta-analysis,"in Oncotarget. 8(38):64373-384, 2017.
15. D. Horst, L. Kriegl, J. Engel, T. Kirchner, and A. Jung,"Prognostic significance of the cancer stem cell markers CD133, CD44, and CD166 in colorectal cancer," in Cancer Invest. 2009; 27:844-850.
16. A. Lugli, G. Iezzi, I. Hostettler, M. G. Muraro, V. Mele, L. Tornillo, V. Carafa, G. Spagnoli, L. Terracciano, and I. Zlobec, "Prognostic impact

of the expression of putative cancer stem cell markers CD133, CD166, CD44s, EpCAM, and ALDH1 in colorectal cancer," in Br J Cancer. 2010; 103:382-390.

17. P. Minoo, I. Zlobec, and M. Peterson, L. Terracciano, and A. Lugli, "Characterization of rectal, proximal and distal colon cancersbased on clinicopathological, molecular and protein profiles," International Journal of Oncology 37: 707-718, 2010
18. A. Jedinak, S. Dudhgaonkara, and D. Slivaa, "Activated macrophages induce metastatic behavior of colon cancer cells," in Immunobiology 215. 2010:pp. 242–249
19. C. Raggi, H. S. Mousa, M. Correnti, A. Sica, and P. Invernizzi, "Cancer stem cells and tumor-associated macrophages: a roadmap for multitargeting strategies," in Oncogene 35. 2015: pp. 671-682.
20. N. Caronni, B. Savino, and R. Bonecchia, "Myeloid cells in cancer-related inflammation," Immunobiology. 2015: pp. 249-253