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Correlation of CD133 Expression with Tumour Location and Lymph Node Involvement in Colorectal Carcinoma

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Introduction
Colorectal carcinoma (CRC) is the third most common cancer worldwide and the fourth most common cause of cancer-related death. Ongoing efforts to investigate new treatment strategies for CRC include understanding CSC (Cancer Stem Cells) involvement. CSC is a group of tumour cells with stem cell characteristics that can play a role in metastasis, recurrence, and therapeutic resistance. This study aims to determine the correlation of CD133 expression as a CSC marker in CRC with tumour location and lymph node involvement in CRC.

Methods
This research is a cross-sectional study. The research's sample was 50 CRC cases with paraffin blocks derived from resection. Immunohistochemical staining was performed to assess CD133 expression. Chi-square test statistical analysis was carried out with p-value <0.05 considered significant.

Results
This study found that CD133 expression was higher in tumour located in the rectum compared to distal and proximal colon by 56%, 28%, and 16%, respectively. Statistically, there is a significant correlation between CD133 expression and lymph node involvement in CRC (p-value=0.048).

Conclusions
There is a significant correlation between CD133 expression and lymph node involvement in CRC. There was no statistically significant correlation between CD133 expression and tumor location.

Keywords: CD133 expression, colorectal carcinoma, lymph node involvement, tumour location

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INTRODUCTION

Colorectal carcinoma (CRC) is the third most common cancer worldwide and the fourth most common cause of cancer-related death. In 2018, there were 19,113 CRC cases in men and 10,904 in women in Indonesia. This high number of cases makes CRC become the fourth most new case in Indonesia after breast, cervical, and lung cancer.[1–3]

CRC originates from the epithelial tissue of the colon or rectum and can develop in the proximal colon, distal colon, or rectum. The tumour behaviour among CRC differs regarding disease progression and overall survival depend on their location.[4]

Survival of CRC patients remains poor despite of the diagnosis, and therapy showed significant improvements. The poor survival is mainly affected by drug resistance, recurrence, and disease progression with metastases.[5] Lymph node metastases are considered a prognostic factor for predicting disease recurrence and survival in CRC patients.[6]

Conventional chemoradiotherapy targets rapidly dividing cells, so it does not eliminate slowly dividing cells such as cancer stem cells (CSC). Ongoing efforts to investigate new treatment strategies for CRC include understanding the involvement of CSC.[1,7]

CSC is a group of tumor cells with stem cell characteristics, including the potential for self-renewal and multi-directional differentiation. CSC may play a role in the abnormal activation of proliferative signaling pathways, neoplasm formation, metastasis, recurrence, and therapeutic resistance.[8,9]

Previous studies have identified markers in CSC populations to differentiate them from most tumor cells. CD133 is now considered the most robust surface marker for identifying CSC in CRC.[1,5,10] Several studies have shown that CD133 expression is correlated with tumor location, lymph node metastases, vascular invasion, nerve invasion, survival, recurrence, and chemotherapy resistance.[11]

CD133 expression is essential in the progression and prognosis of CRC. There are still varying results regarding its expression and correlation with tumor location and lymph node involvement in CRC. Based on this, the authors are interested in researching the correlation between CD133 expression, tumor location, and lymph node involvement in CRC.

METHOD

This research is an observational study with a cross-sectional approach. We examined 50 samples collected at the Anatomic Pathology Laboratory of RSUP dr. M. Djamil Padang from July 2021 to June 2022. The inclusion criteria were all cases of CRC with complete medical record data, underwent surgical resection, and had slides and paraffin blocks that could be re-evaluated.

The tumor's location is based on the patient's medical record. Proximal colon cancer is located in the caecum, ascending colon, hepatic flexure, transverse colon, or splenic flexure. Distal colon cancer is located in the descending or sigmoid colon. Cancer at the rectosigmoid junction or rectum is classified as rectal cancer.[12]

Lymph node involvement is considered positive if there are epithelial cancer cells founded in the lymphoid tissue, at least in one lymph node.[13] CD133 expression is an expression by the reaction of CD133 antigen present on tumour cells in CRC with anti-CD133 antibodies through immunohistochemical staining.

Primary CD133 antibody was used (Genetex; GTX100567;1:200). The expression of CD133 was assessed on the membrane of tumor cells. CD133 intensity was classified as[14] 0: negative expression, 1: weak intensity, 2: moderate intensity, 3: strong intensity. The percentage of CD133 expression at 20x10 magnification from 0-3 with details: 0: no expression, 1: expression <10% of tumour cells, 2: expression of 10% to <50% of tumour cells, 3: expression ≥50% of tumour cells.[15] The assessment was carried out from five different field areas and was averaged.

Receiver Operating Characteristic (ROC) analysis was performed to determine the cut point for CD133 expression. Scores of intensity assessment and expression assessment are summed and grouped. Based on the ROC analysis, a total score of 0-3 indicates a low level, and a total score of 4-6 indicates a high level.[16,17]

The correlation of CD133 expression with tumour location and lymph node involvement using chi-square and p-value <0.05 were considered significant. This research was

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approved by the research ethics committee of RSUP Dr. M. Djamil Padang (LB.02.02/5.7/78/2023).

RESULTS

The clinicopathological characteristics of samples are presented in Table 1. In this table, most cases were found in ≥50 years old group with an average age of 54.52. The youngest patient is 27 years old, and the oldest is 80 years old.

Table 1. Characteristics of colorectal carcinoma.

Characteristic	f (n=50)	Percentage
Age group		
Average	54.52	
≥50 years old	37	74%
<50 years old	13	26%
Gender		
Male	25	50%
Female	25	50%
Tumour location		
Proximal colon	14	28%
Distal colon	15	30%
Rectum	21	42%
Lymph node involvement		
Positive	26	52%
Negative	24	48%
CD133 expression		
High	25	50%
Low	25	50%

The correlation of CD133 expression and tumour location in CRC is shown in Table 2. There was no statistically significant correlation between CD133 expression and tumour location in CRC, with a p-value >0.05.

The correlation between CD133 expression and lymph node involvement in CRC is shown in Table 3. The positive control is the expression of CD133 on the apical membrane of the glandular structures of well-differentiated colorectal adenocarcinoma (Figure 1). The results of CD133 immunohistochemical staining can be seen in Figure 2.

Table 3 shows that CRCs with positive lymph node involvement have high CD133 expression (68%) compared to those without lymph node involvement. Statistically, there is a significant correlation between CD133 expression and lymph node involvement in CRC with a p-value <0.05.

Table 2. The correlation between CD133 expression and tumor location in CRC.

CD133 expression	Tumor location						Total		*P-value*
	Proximal colon		Distal colon		Rectum				
	f	%	f	%	f	%	f	%	
High	4	16%	7	28%	14	56%	25	100%	0.083
Low	10	40%	8	32%	7	28%	25	100%	

Table 3. The correlation between CD133 expression and lymph node involvement in CRC.

CD133 expression	Lymph node involvement				Total		*P-value*	OR	CI
	Positive		Negative						
	f	%	f	%	f	%			
High	17	68%	8	32%	25	100%	0.048	3.778	1.17-12.19
Low	9	36%	16	64%	25	100%			

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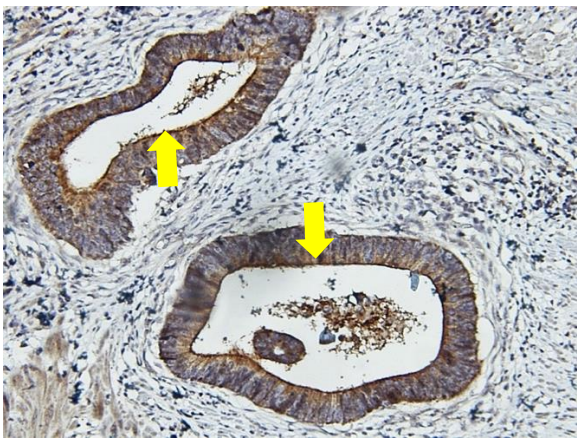

Figure 1. CD133 expression in positive control. Strong positive expression (brown color) is shown in the apical membrane of the glandular structures (arrows) of a well-differentiated colorectal adenocarcinoma. (Original magnification 200 times)

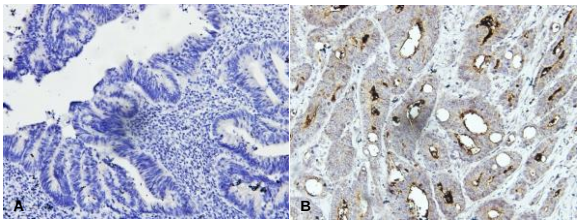

Figure 2. A. Low expression of CD133 in CRC with a score of 0. B. An overview of CD133 expression with a score of 5. Expression with a score of 2 (10% to <50% of tumor cells) and strong intensity with a score of 3 (A, B. Original magnification 200 times)

The difference in results could be due to several reasons. Cut-off variability was used to determine low and high CD133 levels between studies. Different cut-offs can make different results. The studies were conducted in several countries, differences may be due to different geographic regions or races. Other studies divide the location of the CRC into two parts, the proximal and distal colon or the colon with the rectum. This study divided the location of the CRC into three parts, the proximal colon, distal colon, and rectum.[18,19]

The variation in the incidence of CRC also differs among racial and ethnic groups. Non-Hispanic blacks had the highest percentage of proximal colon cancer (46.1%), followed by non-Hispanic whites (41.1%), American Indians

DISCUSSION

There was no statistically significant correlation between CD133 expression and tumor location in this study. A study by Kazama et al found that the most common locations for CRC were in the rectum (29.7%), followed by the sigmoid colon (29%). The highest expression of CD133 was found in the colon, with 68.8%, and in the rectum, with 31.2% (p >0.05).[18]

The distal colon (35%) was the most common location for CRC in the study by Czeczko et al. The highest positive expression of CD133 was found in the proximal colon, which was 44% (p >0.05).[19] Park et al examined a sample of 303 CRC. The tumor location was found most often in the colon region, with 175 cases and 128 cases in the rectum. Most of the high expression of CD133 in the colon is 55% (p >0.05).[20]

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(37.2%), and Hispanics (37.2%). Asians showed the lowest incidence of proximal colon cancer (30.1%). Of the racial and ethnic groups examined, Asians and Hispanics had a higher percentage of rectal cancer (35.6% and 34.9%, respectively).[21]

This study found a significant correlation between CD133 expression and lymph node involvement in CRC. Lymph node metastases are a prognostic factor for predicting disease recurrence and survival in patients with CRC.[6] Rezaee et al found high expression of CD133 in 79.4% of tumor samples with lymph node involvement in 483 CRC patients. Based on this study, positive lymph node involvement was more common in cases with higher CD133 expression than in cases with negative or low CD133

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expression, which was statistically significant ($p<0.05$).[11]

Consistent with the findings of Rezaee et al, a meta-analysis based on 37 studies also reported increased expression of CD133 in CRC as a poor prognostic factor in CRC patients and positively correlated with lymphatic and vascular invasion, distant metastases and the T category. Li et al indicate that an increased percentage of CD133-positive tumor cells is associated with poor prognosis in patients with higher-stage CRC.[11]

Meta-analysis was carried out by Chen et al based on 15 studies. This study suggests that high CD133 expression in primary tumors is associated with higher T category, amount of lymph node involvement, and vascular invasion. This study states that high CD133 expression is significantly associated with a worse prognosis in CRC patients.[22]

Most human malignancies are carcinomas, and EMT (Epithelial-Mesenchymal Transition) causes the transformation of non-CSC carcinoma cells into CSC. EMT-related phenotypic modulation alters gene expression profiles, cell metabolism, cell polarity, and cell-cell and cell-matrix interactions, thus promoting increased CSC motility or migration and formation of cell clusters, perivascular niches, and distant metastases by CSCs.[23]

After tumor cells undergo EMT, CSCs produce large amounts of angiogenic factors, accelerating tumor vascularization and tumor expansion by carrying out metastases. Integrin 1 expression and activation of extracellular proteinases facilitate the migration of CSCs across the extracellular matrix, invasion of surrounding normal tissue and nearby organs, and penetration into blood vessels and lymphatic vessels to disseminate metastases. All of these phenotypic changes are associated with increased tumorigenic potential.[23–25]

CONCLUSION

There is a significant correlation between CD133 expression and lymph node involvement in CRC. Otherwise, there was no statistically significant correlation between CD133 expression and tumor location. This study is expected to become an input for clinicians in considering the risk of recurrence and prognosis of patients with CRC and become a basis for the literature for other researchers to carry out further research related to CD133 expression in CRC.

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