Colorectal Cancer Stem Cells: Relation to Clinico-pathological Features and Prognosis

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Abstract: Introduction: Colorectal cancer stem cells (CRCSCs) are cells that have the ability of self-renewal, tumour propagation and resistance to systemic anticancer treatment. They are identified by expression of CD44 and CD133 antigens. We aimed to study the expression and prognostic significance of CRCSCs in a cohort of colon cancer patients. **Material and method:** A total of 84 consecutive patients with newly diagnosed stage I-III colon cancer were included in this study between 2005-2010 in the Oncology Center of Mansoura University (OCMU). The resect tissue samples were prepared and stained by Haematoxylin and Eosin as well as immune-histopathological staining for CD133 and CD44. The clinic-pathological features and outcome of those patients were correlated with CD133 and CD44 expression. **Results:** Among the 84 patients; 12 were in stage I (14.3%); 38 in stage II (45.2%) and 34 in stage III clinical stages CRC (40.5%). The median age was 52 years. All patients underwent curative resection surgeries. High grade adenocarcinoma was the most common histology. The median disease free survival (DFS) is 41 months among the studied patients. In multivariate analysis, the relations of DFS to higher grade, advanced stage and CD133 score were statistically significant. Thirty three cases (39.3%) died at the end of follow up. The median overall survival was not reached. **Conclusion:** CD133 score, stage and histological grade are independent prognostic factors of disease free survival in colorectal cancer.
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Key words: colon cancer-stem cells-prognosis.

1. Introduction

Colorectal cancer (CRC) is the fourth most common cancer worldwide and is the second commonest cause of cancer-related mortality (1). CRC is considered as a multistep genetic disorder and has specific mutations in signal transduction pathways. The progression from adenoma to cancer and metastatic disease requires both the failure of tumour suppression mechanisms, e.g.: adenomatous polyposis coli (APC), transforming growth factor b (TGF-b), p53 and the induction of oncogenic pathways, such as RAS (2, 3).

Another concept in the pathogenesis of CRC is the Cancer Stem Cell (CSC) model which proposes that only a small fraction of cells has tumour propagation abilities and self-renewal (4, 5). Colorectal cancer stem cell (CRCSC) markers include the CD133, CD44, CD24, CD166, Lgr-5, and ALDH-1, CD29, Msi-1, Bmi-1, and CDX-2 (10 - 13) (14). Most of the CRCSC cells function as adhesion molecule with a role in colony formation, tumour invasiveness, differentiation, and survival (6-10).

We aimed to study the expression and prognostic significance of CRCSCs in a cohort of patients with newly diagnosed and resected colon cancer.

2. Material and methods

Eighty four consecutive colorectal carcinoma patients who underwent initial curative surgical resection at Gastroentrology Center & Oncology Center of Mansoura University between 2005-2010 were included. Demographic data, clinical characteristics and adjuvant treatment were recorded. Staging of CRC was based on the revised 2010 TNM classification. Overall survival was determined from initial diagnosis till last follow up visit or death. Disease free survival was calculated from the diagnosis till first recurrence.

Haematoxylin and Eosin (H&E) slides were prepared for routine pathological evaluation. Immuno-histochemical staining was carried out on 3 - 4 um section of formalin- fixed paraffin- embedded tissue and placed on positively charged slides, overnight at 58c. The slides were subsequently deparaffinized, rehydrated, and epitope retrieved with a pressure cooker. The staining technique was according to recommendation of Cell Marque´s Trilogy for CD44 using infiltrating duct carcinoma as positive control and for CD133 according to recommendation of Miltenyi Biotec Inc, using kidney cancer as positive control. The cut-off scores to determine positivity of new biomarkers using IHC are often chosen arbitrarily

and vary among various studies. The receiver operating characteristic (ROC) curve analysis was helpful to determine the clinically relevant threshold for IHC positivity (11, 12).

This study detected cytoplasmic expression of CD 44 as shown in figures (1-4). For CD44 results were calculated as CD44 score = CD44 intensity+ CD44 percent in tumour cells. 0 stands for negative expression, 1 for minute intensity, 2 for low intensity, 3 for moderate intensity and 4 for strong intensity of CD44. Additionally, 0 for less than 10% of tumour cells, 1 for 10 -24% of tumour cells, 2 for 25 – 49% of tumour cells, 3 for 50- 74% of tumour cells and 4 for 75- 100% of tumour cells expressing CD44.

The expression of CD133 was detected at apical surfaces, the cytoplasm and luminal contents as shown in figures (5-8). CD 133 results were calculated as CD133 score = CD133 intensity + CD 133 percent expressed in tumour cells. 0 stands for negative expression, 1 for low intensity, 2 for moderate intensity and 3 for strong intensity of CD133. Additionally, 0 for negative percent, 1 for less than 10% of tumour cells, 2 for 10% to less than 50% of tumour cells and 3 for ≥ 50% of tumour cells expressed CD133.

For the Statistical Analysis of the study, different statistical methods were used according to the variable types (continuous or categorical variable). The ROC curve was used to determine the significant cut-off points for CD133 & CD44. The probability of survival was determined by the Kaplan-Meier method. The study complied with the guidelines of local ethics committee.

3. Results

Eighty four patients with stage 1-III colon cancer (14.3%, 45.2% and 40.5% respectively), were included into this study. There were 43 (51.2%) females and 41 (48.8%) males. The median age was 52 years (range 44-67); 57.1% were above 50 years old. All patients underwent curative resection surgeries. High grade adenocarcinoma was the most common histology (table 1). Draining lymph nodes involvement was identified in 40.5%. Other risk factors were identified in some of the patients as shown in table 1 such as pT4, lympho-vascular space and perineural invasions. Fifty eight patients (69%) received adjuvant systemic chemotherapy. On last follow up 32 patients (38.1%) had relapsed and 28 had died of metastatic colon cancer (33.3%).

Table 2 describes the relation between CD133 score to some of the clinic-pathological features. Trends of higher expression (4-6 scores) were seen in male patients (51% versus 33% for females) and in association with presence of lympho-vascular space invasion (87.5%). Higher CD44 expression (5-8 score) was detected in relation to male gender, high grade histology, pT3, draining lymph nodes involvement, stage III and the presence of lympho-vascular invasion.

In univariate analysis, disease free survival (DFS) was adversely affected by higher CD44 score, CD133 score, higher grade, advanced stage and the presence of lympho-vascular space invasion (table 4). In multivariate analysis, the relation of DFS to higher grade, advanced stage and CD133 score were statistically significant (5). Figure (11) shows that the median overall survival (OS) was not reached, as only 33 patients (39.3%) died by the end of follow up as illustrated in table (1). Figure (12) shows the median DFS is 41months (35- 47 months) among the studied patients.

Table 1: Clinical Characteristics

Variables		Number	%
Gender	Male	41	48.8
	Female	43	51.2
Performance status	0	10	11.9
	1	68	81
	2	6	7.1
Adjuvant Chemotherapy	Absent	26	31
	Present	58	69
Relapse	No	52	61.9
	Yes	32	38.1
Mortality	Alive	51	60.7
	Died	33	39.3
Histological types	Adenocarcinoma	59	70.3
	Mucinous adenocarcinoma	17	20.2
	Signet adenocarcinoma	8	9.5
Grade	Low	53	63.1
	High	31	36.9
Tumour depth (T)	pT2	21	25.0

	pT3	51	60.7
	pT4	12	14.3
Nodal status (N)	Not involved	50	59.5
	Involved	34	40.5
TNM Staging	I	12	14.3
	II	38	45.2
	III	34	40.5
Lympho-vascular invasion	Absent	73	86.9
	Present	8	9.5
	unknown	3	3.6
Perineual invasion	Absent	72	85.7
	Present	7	8.3
	unknown	5	6

TNM: tumour, node, metastases. P: pathological.

Table 2: Relations of CD 133 Score to Clinico-pathological Parameters

Variable		0-3		4-6		p
		No	%	No	%	
Gender	Male	20	49	21	**51**	0.083
	Female	29	67	14	33	
Grade	Low Grade	32	60.4	21	59.6	0.39
	High Grade	17	54.8	14	45.2	
pT	2	14	66.7	7	33.3	0.46
	3	27	53	24	47	
	4	8	66.7	4	33.7	
Lymph nodes	Not involved	30	60	20	40	0.88
	Involved	19	56	15	44	
TNM Stage	I-II	31	62	19	38	0.41
	III	18	53	16	47	
lympho-vascular invasion	Absent	45	61.6	28	38.4	0.018
	Present	1	12.5	7	**87.5**	
Perineural invasion	Absent	40	55.6	32	44.4	0.69
	Present	5	71.4	2	28.6	

TNM: tumour, node, metastases. P: pathological.

Table 3: Relations of CD44 Score to Clinico-Pathological Parameters

Variables		0-4		5-8		p
		No	%	No	%	
Gender	Male	19	46.3	22	**53.7**	0.66
	Female	22	51.2	21	48.8	
Grade	Low Grade	27	51	26	49	0.38
	High Grade	14	46.7	16	**53.3**	
pT	2	12	57.1	9	42.9	0.65
	3	23	45.1	28	**54.9**	
	4	6	50	6	50	
Node involvement	0	25	50	25	50	0.97
	present	16	47.1	18	**52.9**	
Stage TNM	I-II	25	50	25	50	0.78
	III	16	47.1	18	**52.9**	
lympho-vascular invasion	Absent	35	47.9	38	52.1	0.77
	Present	3	37.5	5	**62.5**	
Perineural invasion	Absent	31	43.1	41	56.9	0.047
	Present	6	86	1	14	

TNM: tumour, node, metastases. P: pathological.

Table 4: Univariate Analyses of Relations of DFS to Different Prognostic Factors

	Hazard Ratio	95.0% Confidence Interval	P value
CD44socre	1.980	1.031 - 3.804	.040
CD133score	2.979	1.573 - 5.644	.001
Combined Score	1.177	0.98 – 1.414	0.083
Grade	1.840	1.297 - 2.612	.001
Stage	1.674	1.217 - 2.302	.002
Lympho-vascular invasion	2.094	0.876 - 5.005	.097
Perineural invasion	2.062	0.729 - 5.828	.172

Table 5: Multivariate Analyses of Relations of DFS to Different Prognostic Factors

	Hazard Ratio	95.0% Confidence Interval	P value
Grade	2.419	1.176 - 4.975	.016
Stage	2.630	1.383 - 5.003	.003
CD133 score	2.545	1.314 - 4.932	.006

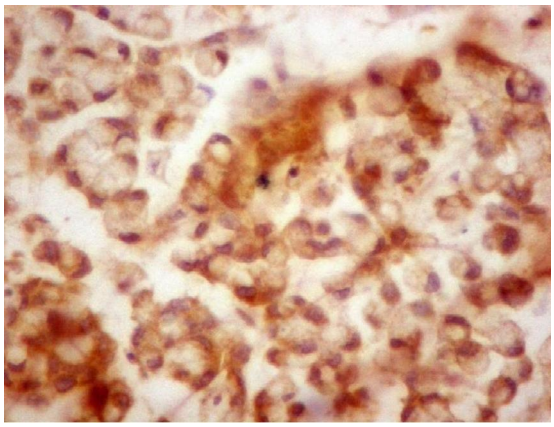

Figure1: Immunohistochemistry for CD44 (× 200 original magnification)

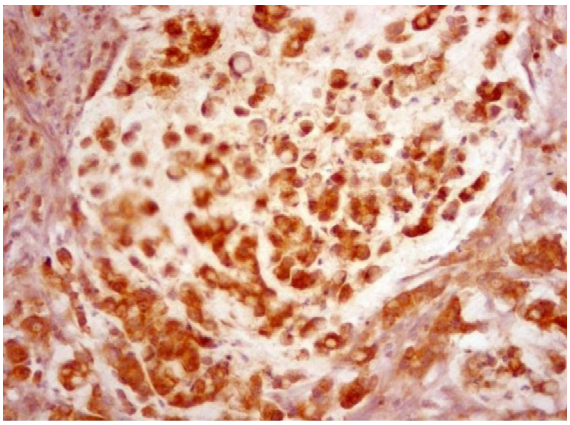

Figure 3: Immunohistochemistry of CD 44 (× 200 original magnification)

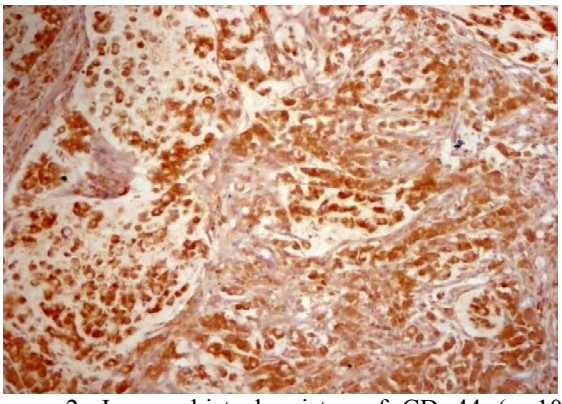

Figure 2: Immunohistochemistry of CD 44 (× 100 original magnification)

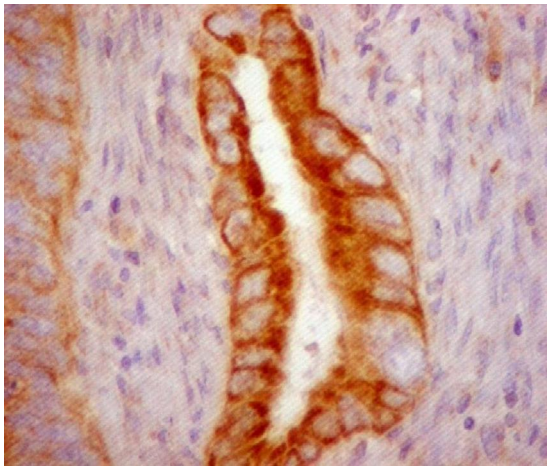

Figure 4: Immunohistochemistry for CD44 in low grade adenocarcinoma (× 400 original magnification).

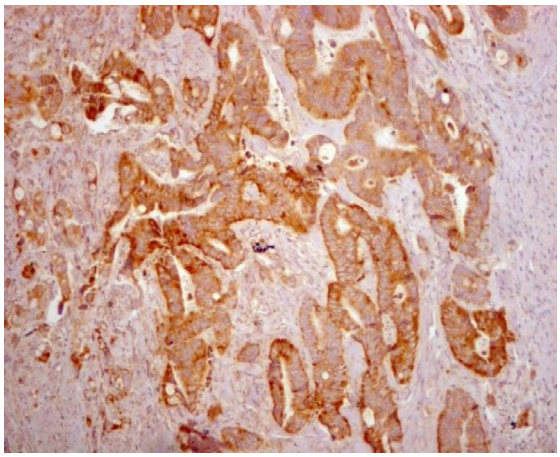

Figure 5: IHC for CD133 shows luminal stain for CD133 in grade II adenocarcinoma (Immunostain ×100 original magnification).

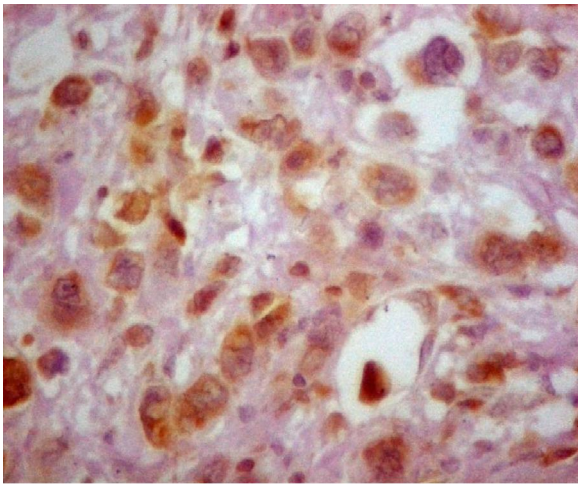

Figure 6: Immunohistochemistry for CD133 shows cytoplasmic staining for CD133 in high grade adenocarcinoma (× 400 original magnification).

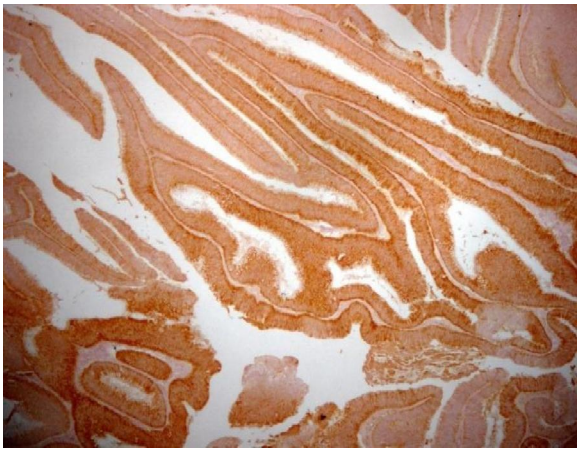

Figure 7: Immunohistochemistry for CD133 shows luminal staining of CD133 in low grade adenocarcinoma, (× 200 original magnification).

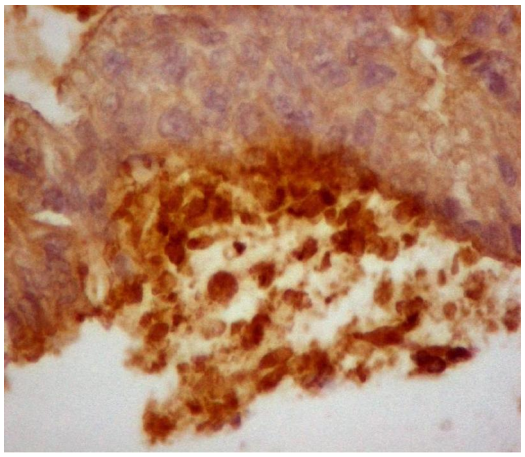

Figure 8: Immunohistochemistry for CD 133 shows luminal, intraluminal staining (× 400 original magnification).

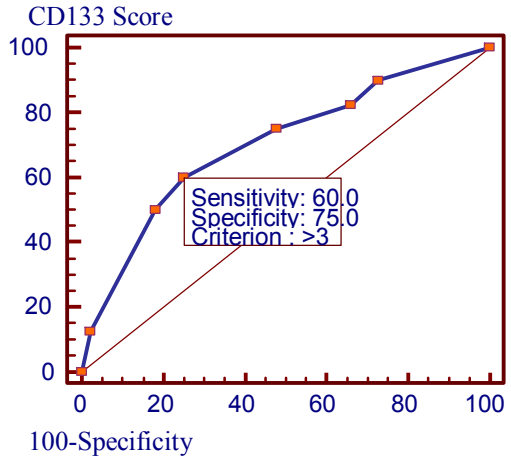

Figure 9: ROC curve for CD133 score cut off.
Area under the ROC curve (AUC) 0.7, 95% CI 0.6-0.8; p=0.0003

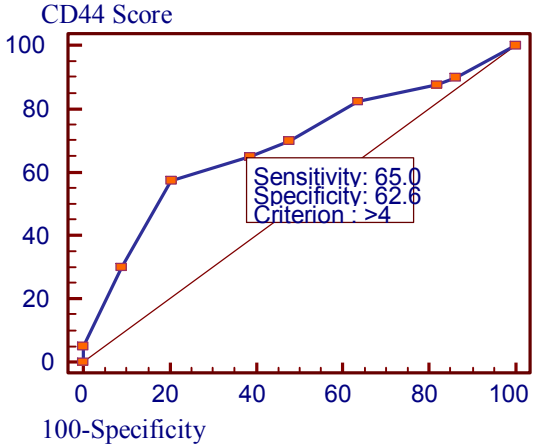

Figure 10: ROC curve for CD44 score cut off
Area under the ROC curve 0.7, 95% CI 0.57-0.78, p=0.002; According to ROC curve, the significant cut off for CD133 score is > 3 & for CD 44 sore is >4 as illustrated above in figures (9) & (10).

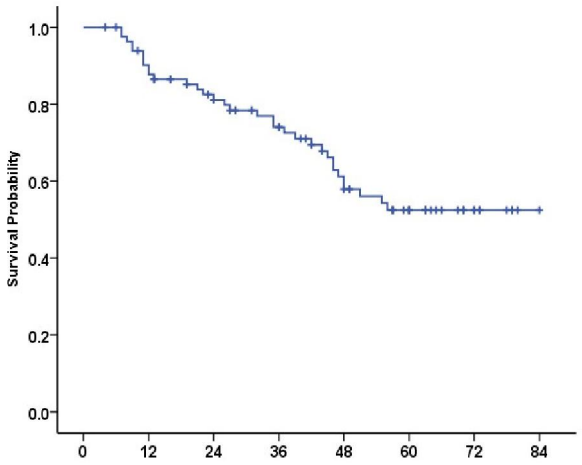

Figure 11: The median OS of the studied patients.

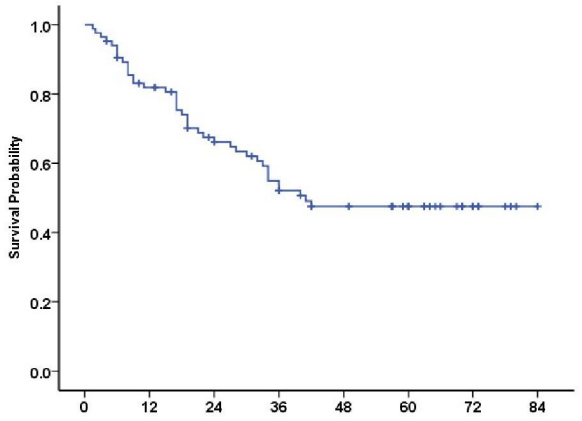

Figure 12: The median DFS of studied patients.

4. Discussion

In our study, no statistically significant correlations between clinico-pathological features and CD44 score or CD133 score were identified, although there were trends of higher CD44 score in male patients, high grade tumours, pT3, lymph node involvement and the presence of lympho-vascular space invasion. Higher CD133 score was associated with the presence of lympho-vascular space invasion in the resected tumour tissue.

This study found that advanced clinical stage, higher grade and CD 133 score are independent adverse prognostic factors for disease free survival. However, the median overall survival was not reached.

The results of our study agree with the findings of several studies which proposed that patients with high CD133 expression had a worse disease free survival (10-13). Therefore CD133 expression can be considered as an independent negative prognostic factor in colorectal cancer patients according to our study and as well as indicated in a meta-analysis by Chen and colleagues (14).

However discrepancy in results among various studies can be partly attributed to site of expression of CD133 in these studies; membranous CD133 expression was linked to survival, recurrence free survival and chemo-resistance, while cytoplasmic expression of CD133 was not an independent prognostic marker for survival or recurrence. Alternation from cytoplasmic to membranous expression of CD133 was correlated to the transition of epithelial cells to a more invasive phenotype (14). In our study, the used IHC monoclonal antibody detected CD 133 expression at both the apical/ endoluminal surfaces and the cytoplasm.

Furthermore, both the proliferative cells of colonic mucosa, and the cells in the basal segment of differentiation zone are able to express CD44, while differentiated mucosal cells don't (15).

On conclusion, CD 133 score, clinical stage, histological grade are independent prognostic factors of disease free survival in colorectal cancer patients. Larger in vivo clinical studies considering the tumour heterogeneity are needed for better understanding of the interplay between genetic and molecular factors aiming for better prevention and treatment of CRC.

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