The impact of CD44 immunoreactivity on survival of patients with colorectal cancer

Gabr AG[1], Abdallah AZ[1], Shaban SH[2], Elemary AN[1], Sedik MF[1]

[1] Medical Oncology and Hematological Malignancies Department, South Egypt Cancer Institute, Assiut University, Egypt.
[2] Oncologic Pathology Department, South Egypt Cancer Institute, Assiut University, Egypt.

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Authors Information:
Adel Gomaa Gabr
Medical Oncology and Hematological malignancies Department, South Egypt Cancer Institute, Assiut University, Egypt.
email: adelgabre@yahoo.com
adelgabr@aun.edu.eg

Ashraf Zedan Abdallah
Medical Oncology and Hematological malignancies Department, South Egypt Cancer Institute, Assiut University, Egypt.
email: profzedan@gmail.com

Shimaa Hassan Shaban
Oncologic Pathology Department, South Egypt Cancer Institute, Assiut University, Egypt.
email: Shimaashaban19@yahoo.com
shimaashaban@aun.edu.eg

Amany Nasr Elemary
Medical Oncology and Hematological malignancies Department, South Egypt Cancer Institute, Assiut University, Egypt.
email: amanyelemary89@gmail.com
amany.elemary@aun.edu.eg

Mayada Fawzy Sedik
Medical Oncology and Hematological malignancies Department, South Egypt Cancer Institute, Assiut University, Egypt.
email: mayadaelzohri@gmail.com
mayada@aun.edu.eg

Corresponding Author:
Amany Nasr Elemary
Medical Oncology and Hematological malignancies Department, South Egypt Cancer Institute, Assiut University, Egypt.
email: amanyelemary89@gmail.com
amany.elemary@aun.edu.eg

Abstract:

Background: CD44, a cell hyaluronic acid transmembrane glycoprotein implicated in cell growth, differentiation, survival as well as the metastatic behavior of some cancer cells. Its expression is upregulated in subpopulations of cancer cells and is identified as a molecular marker for cancer stem cells (CSC).
Methodology: This retrospective study investigated CD44 immunoreactivity in 85 cancer colon patient's specimens at diagnosis obtained from the pathology department, South Egypt Cancer Institute. All specimens were stained by Anti CD44 antibody. Patients were followed up for 3 years.
Aim: The study aims at inspecting CD44 immunoreactivity in the epithelium of colorectal cancer specimens and at detecting its association with the patients' survival function by calculating disease free survival and overall survival of patients.
Results: In our current study there was a statistically significant association between low epithelial expression of CD44 marker (H score ≤ 150) and 3-year OS (p=0.009) as well as a statistically significant association between low CD44 epithelial expression and 2-year DFS (P=0.041). In multivariate analysis the high epithelial expression of CD44 is the only independent factor for early recurrence (HR:9.393, P=0.031) concluding that high epithelial expression of CD44 is a bad prognostic factor and the likelihood of death is 9 times more with CD44 overexpression.
Conclusion: CD44 positivity in non-metastatic colorectal cancer specimens at diagnosis is associated with worse overall survival of patients and more likelihood of progression or death.

Keywords: Colorectal Cancer, CD44 Biomarker, Overall survival, Disease free survival.

Introduction:

Colorectal cancer (CRC) is a highly lethal type of cancer. In spite of the declining rates of incidence and mortality from (CRC) in general [1], it still ranks the second-highest mortality of all human cancers globally [2]. In Egypt, Colorectal cancer represents the 7th most common cancer, representing 3.47% of male cancers and 3% of female cancers [3]. Egyptians are diagnosed with CRC at advanced disease stages and have an overall survival of just a couple of years approximately [1]. While studying the pathogenesis of cancers, cancer stem cells (CSCs) hypothesis has been postulated [4].

Recent research has identified and isolated cancer stem cells (CSCs), which are considered one of the primary causes of resistance to oncological treatments, and contribute to local as well as distant recurrence where CSCs are characterized by their ability to self-renew, to proliferate and contribute to significant tumor progression [5]. For identifying the exact biological features of CSC, the key step is to isolate CSC from cancer cells. Recent research indicated that CD44 expression was elevated in cancer stem-like cells in different types of cancers [6]. A variety of Cancer Stem cells (CSC) have been proposed for CRC [7]. Among which is CD44, which is a multi-structural and multifunctional cell hyaluronic acid transmembrane glycoprotein encoded by the highly conserved CD44 gene on chromosome 11 in humans [8], implicated in cell growth, differentiation, survival as well as the metastatic behavior of some cancer cells [9]. CD44 has different variant isoforms generated through alternative mRNA splicing and these isoforms are reported to be associated with more aggressiveness, higher metastatic potential as well as poorer prognosis in various kinds of cancers including CRC[2].

Patients and Methods:

The study included (85 cases) with non-metastatic colorectal cancer at diagnosis. The selected patients have undergone surgery for primary colorectal cancer at Surgical Oncology Department. Diagnosis of colorectal cancer was confirmed by tissue biopsy and accurate histo-pathological diagnosis of post operative samples.

Demographic data, clinical outcomes as well as survival data of patients were collected form the patients' medical records of South Egypt Cancer Institute for descriptive analyses.

Sample size was calculated using Epi- Info. based on previous study the CD44 was positive in 57% of colorectal cancer cases [10] with a confidence limit of 7% and a confidence level of 80% the minimum number of patients required for this study is 85 patients (we were limited by the availability of CD44 immunohistochemical marker kits).

All patients included in this study were subjected to:

•Full history taking.

•Stage determination using TNM staging.

•Complete laboratory investigation.

•Serum Carcinoembryonic antigen (CEA) level at diagnosis and every 3 months during treatment and every 6 months during follow up.

•Tissue samples for light microscopy were fixed in 10% formaldehyde then installed in paraffin blocks. The paraffin blocks were then cut into sections with thickness five-micrometer and then stained with hematoxylin-eosin.

•Immunohistochemical sections of primary tumor samples of patients with colorectal carcinoma were stained with CD44 antibodies to detect CD44 expression in colorectal cancer cells.

•Patients were followed up through the course of treatment after receiving 5fu based chemotherapy regimens by multi slice computed tomography (MSCT)s. Initially by postoperative CT then every 3 months during treatment and every 6 months during follow up.

•Response to treatment and evaluation of relapse was done by using (RECIST criteria) Response Evaluation Criteria in Solid Tumors.

Immunohistochemical staining and its interpretation:

The formalin-fixed, paraffin-embedded tissue was cut into sections of 5-micron thickness and mounted on positively charged slides. Paraffin is then removed from sections then tissues were rehydrated in graded alcohols to distilled water. Slides were incubated in (Tris EDTA) inside a heated water bath at 97°C for 20 minutes for antigen retrieval process. Primary mouse monoclonal anti- Human CD44, antibody (Catalog #BSB 6238, Bio SB, USA) at optimum dilution of 1/200 concentration was applied for tissue sections and incubated for one hour at room temperature in a humid chamber. Furthermore, tissue sections were washed and stained using a universal staining kit "UltraVision Detection System Anti-Polyvalent, HRP/DAB (Ready-To-Use)" (LAB VISION corporation, catalogue # TP-015-HD, Fremont, California 94539-6406, USA) following the manufacturer's instructions. Lastly, (DAB solution) was added to the slides for about 10 minutes. We used Mayer's hematoxylin as a counter stain. Sections from reactive lymph node were used as positive control. We used sections of tissue specific positive control using the same protocol but with omitting the primary antibody for negative control.

CD44 staining was detected in the membrane of cancer cells in order to assess its expression, our special pathologist inspected the immunostained slides blinded to the clinicopathological data. The histochemical score (H-score) was calculated as the intensity (I) of staining was scored as (0) Absent, (1) weak, (2) Moderate, (3) Strong. Besides, the percentage of positive tumor cells (P) were spotted. (H-score) was calculated by multiplying the intensity with the percentage of positive cells. The H-score values are from 0-300. Cases were categorized as low H score <150, High H score ≥ 150 considering 150 as a cut off value.

Survival analysis:

Survival data of the patients were obtained by reviewing the patients records of colorectal cancer patients attending to South Egypt Cancer institute in the time period between 2018-2020.

Survival function curves of patients for calculation of overall survival (OS) and disease-free survival (DFS) were conducted by using the Kaplan-Meier method.

Statistical data:

All statistical calculations were done using SPSS (statistical package for the social science; SPSS Inc., Chicago, IL, USA) version 26. Quantitative data are described in terms of mean ± SD (standard deviation), when not normally distributed as described in terms of median and range. Qualitative data are described in terms of frequencies (number of cases) and relative

frequencies (percentages). Overall and disease-free survival analysis were done using Kaplan-Meier's method with log rank test. Hazard ratio (HR) with 95% Confidence Interval (CI) and COX Regression analysis was calculated to determine significant factors associated with mortality. P-value is always 2 tailed set significant at 0.05 level.

Results:

85 Non metastatic colorectal cancer cases who underwent surgery for primary cancer colon were retrospectively analyzed in the present study. Patient's demographics and clinicopathological data are summarized in Table (1) below.

Table1: Clinicopathological data of the studied colorectal carcinoma cases (n=85)

Patients' characteristics	n	(%)
Age (years)		
• Mean ± SD	41.74 ± 14.43	
• ≤ 45	51	(60.0)
• > 45	34	(40.0)
Gender		
• Male	28	(32.9)
• Female	57	(67.1)
Tumor site		
• Right colon	17	(20.0)
• Left colon	10	(11.8)
• Transverse colon	8	(9.4)
• Sigmoid colon	15	(17.6)
• Ileo-cecal junction	1	(1.2)
• Rectum	28	(32.9)
• Rectosigmoid	6	(7.1)
Histopathology		
• Mucinous	20	(23.5)
• Signet ring	6	(7.1)
• Adenocarcinoma	59	(69.4)
Tumor size (T)		
• T2	10	(11.8)
• T3	64	(75.3)
• T4	11	(12.9)
Lymph Node (N)		
• N0	37	(43.5)
• N1	24	(28.2)
• N2	23	(27.1)
• N3	1	(1.2)
Metastasis (M)		
• M0	85	(100.0)
Stage		
• Stage 1	7	(8.2)
• Stage 2	30	(35.3)
• Stage 3	48	(56.5)
Grade		
• Well differentiated	14	(16.5)
• Moderately differentiated	40	(47.1)
• Poorly differentiated	31	(36.5)
CD44 tumor biomarker		
• Positive	81	(95.3)
• Negative	4	(4.7)

Quantitative data are presented as mean ± SD and median (range), qualitative data are presented as n (%).

The mean age of our patients was 41.7 years where 60% of our cases were ≤ 45 years. 28 of the studied patients were males and 57 were females, we have categorized our patients according to tumor site where the rectum was the most frequent tumor site among our patients accounting for 32.9%. Colorectal adenocarcinoma was the most common histopathological type accounting for 69.4%. All of our patients are non-metastatic with disease stages ranging from stage 1 to stage 3, with stage 3 the most frequently occurring. CD44 showed positivity in 81 of our patients as shown in figure 1 out of 85 patients representing 95.3%.

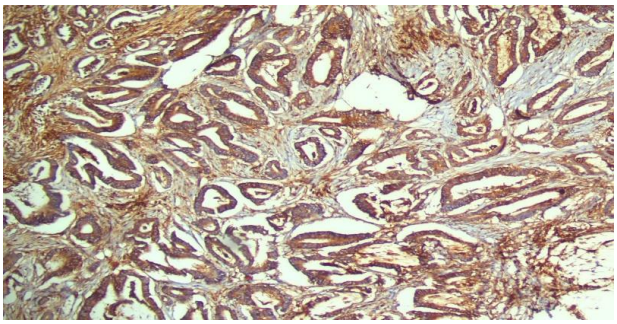

Figure 1: CD44 staining strong positive in tumor cells

CD44 staining positivity in epithelial cancer cells is interpreted by the histochemical score (H-score) which was calculated as the intensity (I) of staining was scored as (0) Absent, (1) weak, (2) Moderate, (3) Strong multiplied by the percentage of positive tumor cells (P). The H-score values are from 0-300. Cases were categorized as low H score <150, High H score ≥ 150 considering 150 as a cut off value. The relation between immunohistochemical expression of CD44 (interpreted as low and high expression) and patient's clinicopathological data is summarized in Table 2. With a highly statistically significant P value between high H score and tumor size (T), lymph node involvement (N) as well as advanced disease stage (P=0.000).

Table 3 illustrates the 3 years overall survival and 2 years disease-free survival in relation to the clinicopathological details of the studied colorectal cancer cases as well as in relation to H score. Where low H score a high statistically significant p value with the overall survival (OS) and disease-free survival (DFS) of patients with P values (P=0.009) and (P= 0.041) respectively.

Kaplan Meier analysis of overall survival of patients and disease-free survival according to H score is demonstrated in figure 2 below.

On the left side the cumulative survival is represented in relation to H score either high expression or low expression where longer cumulative survival occurs with low H score expression with significant P value (P=0.009). On the right-side Kaplan Meier survival curve shows the relation between disease free survival and H score expression with significant P value

with low H score expression (P=0.041) as shown in Figure 2.

Cox regression analysis was calculated for predicting the likelihood of death in relation to the patients clinicopathological characteristics as well as H score as shown in table 3 below. Multivariate analysis showed that the high epithelial expression of CD44 which is represented by high H score is the only independent factor for early recurrence (HR:9.393, P=0.031) concluding that high epithelial expression of CD44 is a bad prognostic factor and the likelihood of death is 9 time more than that with low CD44 expression.

Table 2: Relationship between immunohistochemical expression of CD44 and clinic-pathological details of studied colorectal carcinoma (n=85)

Variable name	**Low expression (n=28)**		**High expression (n=57)**		**P value**
Age					
• Mean ± SD	46.82 ± 13.32		39.25 ± 14.40		**0.019***
• ≤ 45	13	(25.5)	38	(74.5)	0.073
• > 45	15	(44.1)	19	(55.9)	
Gender					0.703
• Male	10	(35.7)	18	(64.3)	
• Female	18	(31.6)	39	(68.4)	
Tumor site					0.367
• Right colon	7	(26.9)	19	(73.1)	
• Left colon	11	(44.0)	14	(56.0)	
• Rectosigmoid	10	(29.4)	24	(70.6)	
Histopathology					0.593
• Mucinous	8	(40.0)	12	(60.0)	
• Signet ring	1	(16.7)	5	(83.3)	
• Adenocarcinoma	19	(32.2)	40	(67.8)	
T					**0.009***
• T2	7	(70.0)	3	(30.0)	
• T3	20	(31.3)	44	(68.8)	
• T4	1	(9.1)	10	(90.9)	
N					**0.000***
• Negative	27	(73.0)	10	(27.0)	
• Positive	1	(2.1)	47	(97.9)	
M					----
• M0	28	(32.9)	57	(67.1)	
Stage					**0.000***
• Stage 1	7	(100.0)	0	(0.0)	
• Stage 2	20	(66.7)	10	(33.3)	
• Stage 3	1	(2.1)	47	(97.9)	
Grade					0.657
• Well differentiated	6	(42.9)	8	(57.1)	
• Moderately differentiated	13	(32.5)	27	(67.5)	
• Poorly differentiated	9	(29.0)	22	(71.0)	

Table 3: Represents the Overall survival (OS) and disease-free survival (DFS) according to clinic-pathological details of the studied colorectal cancer cases.

	OS (3 years)		DFS (2 years)	
	Estimate ± SE	**P value**	**Estimate ± SE**	**P value**
Age		0.972		0.511
• ≤ 45	77.4 ± 7.5%		75.2 ± 9.8%	
• > 45	76.4 ± 8.7%		93.3 ± 6.4%	
Gender		0.934		0.202
• Male	74.3 ± 10.7%		74.7 ± 17.5%	
• Female	77.6 ± 6.9%		84.0 ± 7.1%	
Tumor site		0.067		0.243
• Right colon	60.2 ± 13.7%		91.7 ± 8.0%	
• Left colon	100.0 ± 0.0%		93.8 ± 6.1%	
• Rectosigmoid	72.1 ± 8.7%		57.8 ± 18.6%	
T		0.201		0.203
• T2	100.0 ± 0.0%		100.0 ± 0.0%	
• T3	73.1 ± 7.2%		73.7 ± 10.7%	
• T4	72.0 ± 17.8%		100.0 ± 0.0%	
N		0.059		0.125
• Negative	87.7 ± 6.7%		87.5 ± 11.7%	
• Positive	66.6 ± 9.3%		76.9 ± 9.1%	
Stage		0.059		0.125
• Early	87.7 ± 9.3%		87.5 ± 11.7%	
• Advanced	66.6 ± 9.3%		76.9 ± 9.1%	
H score		**0.009***		**0.041***
• Low expression	94.1 ± 5.7%		83.3 ± 15.2%	
• High expression	67.5 ± 8.2%		79.6 ± 8.1%	

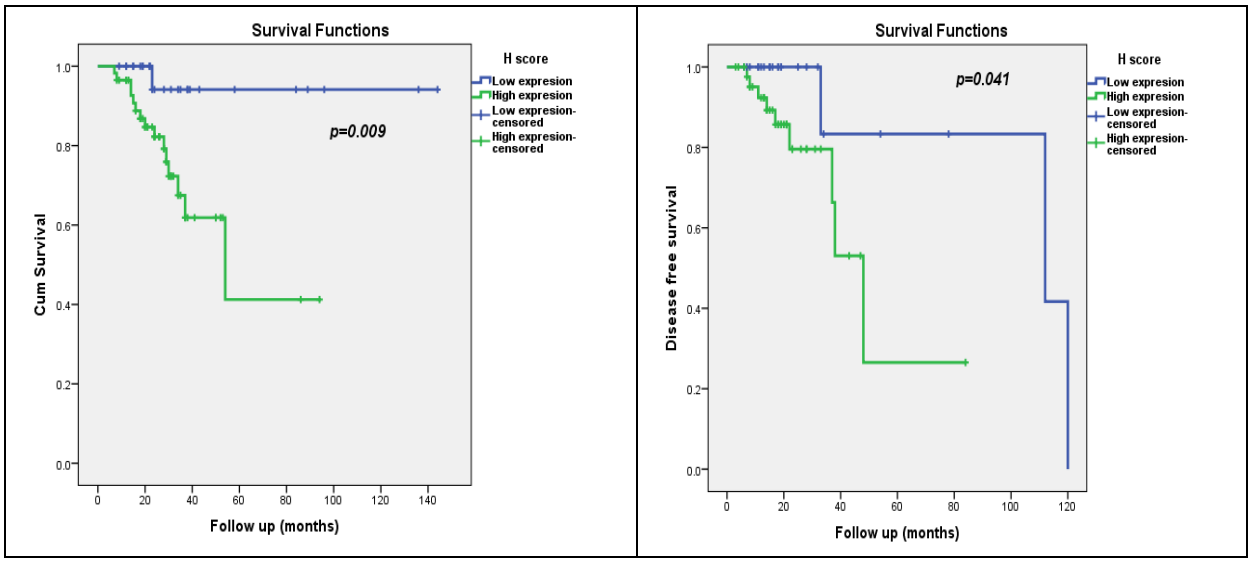

Figure 2: Kaplan Meier analysis of Survival function according to H score

Table 4: Results of COX regression analysis for predicting likelihood of death according to clinic-pathological characteristics of the study participants (n=85)

Variable name	n	B	S.E.	P value	HR	95% C.I. for OR
Tumor site						
• Right colon	26	-0.137	0.528	0.795	0.872	0.310 – 2.451
• Left colon	25	-2.079	1.056	**0.049***	0.125	0.016 – 0.990
• Rectosigmoid	34				ref	
N						
• Negative	37				ref	
• Positive	48	1.064	0.589	0.071	2.897	0.913 – 9.188
Stage						
• Early	37				ref	
• Advanced	48	1.064	0.589	0.071	2.897	0.913 – 9.188
H score						
• Low expression	28				ref	
• High expression	57	2.240	1.041	**0.031***	9.393	1.220 – 72.310

B = regression coefficient, SE = standard error, HR= hazard ratio, CI =confidence interval, p value is significant ≤0.05

Discussion:

CD44 is an important membrane receptor for hyaluronic acid (HA) [11] [12]. It is accepted as a cancer stem cell (CSC) marker in many solid tumors [13]. In several studies, it has been proved that CD44 is associated with aggressive biological behavior of the cancers, including proliferation, metastasis, recurrence and even resistance to therapy [14]. CD44 expression has been described as a putative CSC marker in CRC patients [15]. However, there are still controversies about its prognostic role and its effect on patients' survival [16].

Our current study showed a statistically significant association between CD44 expression and tumor size (T), lymph node metastasis (N) as well as advanced disease stage with P values (P=0.009), (P=0.000) and (P=0.000), respectively in agreement with Zhao et al.,2015 [17] who found a statistically significant association between CD44 expression and stages II and III. This relationship was is in contrary with Chen et al., 2011 [18] where CD44 expression showed no significant relationship with disease stage.

H score of CD44 (high expression) had no statistically significant relationship with older age group (age > 45 years) in accordance with Hong et al., 2015 [19], Liu et al., 2014 [20] and Zhao et al., 2015 [17] who found that there was no significant relationship between CD44 and age of studied CRC patients. This may be due to rapid increase in the incidence of CRC in general under the age of 50 years [21]. This result was in disagreement with Holah et al., 2017 [10] who reported a statistically significant association between CD44 epithelial expression and older age group (P = 0.04).

Survival analysis was performed for CD44 expression and there was a statistically significant association between low epithelial expression (H score) and 3-year OS (p=0.009) as well as a statistically significant association between low epithelial expression and 2-year DFS (p=0.041). In multivariate analysis the high epithelial expression of CD44 is the only independent factor for early recurrence (HR:9.393, P=0.031) concluding that high epithelial expression of CD44 is a bad prognostic factor and the likelihood of death is 9 times higher than that with low CD44 expression. Our results agreed with Fernandez et al and Huh et al [22] [23] who similarly found that CD44 overexpression was associated with lower cancer related survival. Also, a study done by Yan et al [24] concluded that overexpression of CD44 is associated with short disease-free survival. However, our results disagreed with Galizia et al and Holah et al [25] [10] who found no significant association between CD44 and overall survival. This difference could be attributed to the difference in the number of the studied cases and different techniques of CD44 detection.

Our study has a limitation of small number of cases due to the limited availability of CD44 antibody kits and the limitation of the organizational financial support. Besides, evaluation of CD44 by other methods as measurement of its m- RNA by real time PCR Real time polymerase chain reaction.

Conclusion:

CD44 positivity in non-metastatic colorectal cancer specimens at diagnosis is associated with worse overall survival of patients and more likelihood of progression or death.

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References:

1. Nawwar A, Brand Bateman L, Khamess S, et al. Using Intervention Mapping to Develop a Theory-Based Intervention to Promote Colorectal Cancer Screening in Egypt. Asian Pac J Cancer Prev. 2022 Jun 1;23(6):1975-1981.
2. Zhang Y, Chen S, Zhu J, et al. Overexpression of CBS/H2S inhibits proliferation and metastasis of colon cancer cells through downregulation of CD44. Cancer Cell Int. 2022 Feb 16;22(1):85.
3. Ibrahim AS, Khaled HM, Mikhail NN, et al. Cancer incidence in Egypt: results of the national population-based cancer registry program. J Cancer Epidemiol. 2014;2014:437971.
4. Avgustinova A and Benitah SA. The epigenetics of tumour initiation: cancer stem cells and their chromatin. Curr Opin Genet Dev. 2016 Feb;36:8-15.
5. Aramini B, Masciale V, Grisendi G, et al. Dissecting tumor growth: the role of cancer stem cells in drug resistance and recurrence. Cancers (Basel). 2022 Feb 15;14(4):976.
6. Xia P and Xu X-Y. Prognostic significance of CD44 in human colon cancer and gastric cancer: Evidence from bioinformatic analyses. Oncotarget. 2016 Jul 19;7(29):45538-45546.
7. Hatano Y, Fukuda S, Hisamatsu K, et al. Multifaceted interpretation of colon cancer stem cells. Int J Mol Sci. 2017 Jul 5;18(7):1446.
8. Goldstein LA, Zhou DF, Picker LJ, et al. A human lymphocyte homing receptor, the hermes antigen, is related to cartilage proteoglycan core and link proteins. Cell. 1989 Mar 24;56(6):1063-72.
9. Zöller M. CD44: can a cancer-initiating cell profit from an abundantly expressed molecule? Nat Rev Cancer. 2011 Apr;11(4):254-67.
10. Holah NS, Aiad HA, Asaad NY, et al. Evaluation of the role of CD44 as a cancer stem cell marker in colorectal carcinoma: Immunohistochemical study. Menoufia Med J 2017;30(1):174-83.
11. Nagano O and Saya H. Mechanism and biological significance of CD44 cleavage. Cancer Sci. 2004 Dec;95(12):930-5.
12. Cheng C and Sharp PA. Regulation of CD44 alternative splicing by SRm160 and its potential role in tumor cell invasion. Mol Cell Biol. 2006 Jan;26(1):362-70.
13. Hurt EM, Kawasaki BT, Klarmann GJ, et al. CD44+ CD24− prostate cells are early cancer progenitor/stem cells that provide a model for patients with poor prognosis. Br J Cancer. 2008 Feb 26;98(4):756-65.
14. Wang Z, Tang Y, Xie L, et al. The prognostic and clinical value of CD44 in colorectal cancer: a meta-analysis. Front Oncol. 2019 Apr 30;9:309.
15. Visvader JE and Lindeman GJ. Cancer stem cells in solid tumours: accumulating evidence and unresolved questions. Nat Rev Cancer. 2008 Oct;8(10):755-68.
16. Nour El Hoda SI, Sharaf WM, Helmy DO, et al. Detection of cancer stem cells in colorectal cancer: histopathological and immunohistochemical study. Open Access Maced J Med Sci. 2016 Dec 15;4(4):543-547..
17. Zhao L, Lin Q, Wei J, et al. CD44v6 expression in patients with stage II or stage III sporadic colorectal cancer is superior to CD44 expression for predicting progression. Int J Clin Exp Pathol. 2015 Jan 1;8(1):692-701.
18. Chen S, Song X, Chen Z, et al. Correlation analysis of CD133/CD44 expression in colorectal cancer tissues and 5-year survival rate in colorectal cancer patients. Chin J Pathophysiol. 2011;27(5):883-889.
19. Hong I, Hong SW, Chang YG, et al. Expression of the cancer stem cell markers CD44 and CD133 in colorectal cancer: an immunohistochemical staining analysis. Ann Coloproctol. 2015 Jun;31(3):84-91.
20. Liu D, Sun J, Zhu J, et al. Expression and clinical significance of colorectal cancer stem cell marker EpCAM(high)/CD44(+) in colorectal cancer. Oncol Lett. 2014 May;7(5):1544-1548.
21. Patel SG and Ahnen DJ. Colorectal cancer in the young. Curr Gastroenterol Rep. 2018 Mar 28;20(4):15.
22. Fernández JC, Vizoso FJ, Corte MD, et al. CD44s expression in resectable colorectal carcinomas and surrounding mucosa. Cancer Invest. 2004;22(6):878-85.
23. Huh JW, Kim HR, Kim YJ, et al. Expression of standard CD44 in human colorectal carcinoma: association with prognosis. Pathol Int. 2009 Apr;59(4):241-6.
24. Yan X, Han D, Chen Z, et al. RUNX2 interacts with BRG1 to target CD44 for promoting invasion and migration of colorectal cancer cells. Cancer Cell Int. 2020 Oct 15;20:505.
25. Galizia G, Gemei M, Del Vecchio L, et al. Combined CD133/CD44 expression as a prognostic indicator of disease-free survival in patients with colorectal cancer. Arch Surg. 2012 Jan;147(1):18-24.