RESEARCH ARTICLE

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Prognostic Role of *Musashi-2* Immunohistochemical Expression and *CD163*+ Tumor- Associated Macrophages in Colorectal Cancer Progression

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Abstract

Background: Preoperative assessment of colorectal cancer requires a multimodal approach including clinical, radiographic, biochemical and colonoscopic assessment with histologic review of biopsies that provides valuable information regarding tumor grading and other significant prognostic factors that aid in determination of the appropriate treatment. The association between *Musashi-2* expression and clinicopathological features and its prognostic significance in CRC patients is still controversial. Our study is designed to investigate the expression and prognostic significance of *MSI2* and *CD163*+Tumor associated macrophages in colorectal cancer patients. **Methods:** Prospective longitudinal study was designed including 114 CRC cases from Tropical Medicine Department and Oncology Center, Mansoura University Hospitals, during the period from July 2021 to October 2024, all cases were diagnosed by colonoscopy then underwent radical surgery with biopsies which were examined by IHC staining for *MSI2* and *CD163*+TAM using tissue microarray technique trying to find out their correlation with clinicopathological parameters and patients prognosis. **Results:** Among 114 CRC cases, high *MSI2* expression was detected in 49 (43%). *MSI2* expression was significantly related to grade of differentiation (P = 0.001*), depth of invasion (P = 0.001*), lymph node metastasis (P = 0.001*), TNM stage (P = 0.001*) and hepatic metastasis (P = 0.009*). Multivariate analysis spotted *MSI2* expression as a predictor for Overall survival in CRC (P = 0.015*) and disease free survival (P = 0.008*). *CD163*+TAM showed a significant correlation to grade of differentiation (P = 0.001*), depth of invasion (P = 0.05*), lymph node metastasis (P = 0.001*), TNM stage (P = 0.001*), and hepatic metastasis (P = 0.007*). Multivariate analysis spotted *CD163*+TAM expression as a predictor for OS in CRC (P = 0.037*) and DFS (P = 0.003*). Conclusion: *MSI2* and *CD163*+TAM are independent prognostic factors for the overall survival and disease free survival and can predict poor prognosis in CRC patients.

Keywords: *Musashi-2- CD163*+TAM- colorectal cancer- prognosis

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Introduction

Colorectal cancer (CRC) is an exceptionally frequent illness worldwide. There are roughly 408,000 new cases with colorectal cancer and 196,000 deaths per year, ranking second in terms of incidence and fourth in terms of mortality [1]. Individuals residing in developed countries are at higher risk of CRC, while the residents of the developing world are at lower risk for this cancer, despite a decrease in CRC cases overall in developed countries, the occurrence of early onset CRC (under 50 years old) is rising in both developed and developing regions. This indicates a need for more research into the risk factors and prevention methods for this type of cancer worldwide [2].

Even with improvements in treatment, colorectal cancer continues to pose a significant healthcare concern and has poor prognosis in advanced stages and carries high risk of recurrence [3]. The musashi (MSI) family is RNA-binding Proteins acts as a posttranslational repressor of target mRNA [4]. *MSI2* seems to be a potential prognostic biomarker and therapeutic target for cancer patients [5].

MSI2 has been proved to be significantly up-regulated in various cancers, such as ovarian carcinoma (OC) [5], non-small cell lung cancer (NSCLC) [6], colorectal cancer (CRC) [7] and cervical cancer (CC) [8]. *MSI2* expression have drug resistance effect; specifically, it boost resistance to tyrosine kinase inhibitors targeting the epidermal growth factor receptor (EGFR), which are useful for patients with EGFR mutations in NSCLC [9]. High levels of *MSI2* expression are linked to negative

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outcomes in various solid tumors and blood cancers [5].

The crucial role of the tumor microenvironment (TME) in tumor development spans from initiation and chronic inflammation to tumor progression and therapy response [10]. Generally, macrophages in cancer have the ability to both promote and inhibit tumor growth depending on various signals such as cytokines, chemokines, antibodies, and myeloid checkpoints [11]. The M2 type of tumor-associated macrophages supports the progression of tumors. *CD163* is expressed in M2 macrophages [12]. *CD163*+tumor associated macrophages (TAM) has great potential to be used as a biomarker to evaluate early tumor recurrence, and patient survival [13].

The value of *Musashi-2* expression in CRC remains indefinite and needs more investigation. Furthermore, the reports on *Musashi-2* are often conflicting. This work aimed to evaluate immunohistochemical expression of Musashi 2 and *CD163*+ TAM and its impact on clinical characteristics and tumor staging as useful biomarkers for tumor aggressiveness in colorectal cancer in Egyptian patients.

Materials and Methods

Observational Prospective longitudinal study, started from July 2021 and continued to October 2024, including 160 recently diagnosed CRC patients by colonoscopy with biopsies collected from Tropical Medicine Department and Oncology Center, Mansoura University Hospitals. Among this patients, 46 cases were excluded (25 cases due to loss their follow up, 10 cases received neoadjuvant chemotherapy and 11 cases were not operable). Finally this study included 114 pathologically confirmed colorectal cancer cases as well as patients with synchronous hepatic metastasis, excluding other distant metastasis. Other extra colonic malignancy patients and patients who received neoadjuvant chemotherapy and/ or radiotherapy were also excluded.

After obtaining a written informed consent from all patients, demographic and clinicopathological data of the enrolled cases including age, sex, medical history (Diabetes Mellitus and smoking), tumor markers including CEA and CA19-9; cut-off values were 5.0 ng/mL and 37 U/mL, respectively [14] and family history of CRC were collected. All of our cases underwent radical surgery with free safety margin then spacimens descripted as regard (site, morphological type, histological type, grade of differentiation, local infiltration, lymph node invasion) then IHC staining was done for these specimens. Then each tumor was assigned a stage according to the latest American Joint Committee on Cancer (AJCC) TNM staging criteria, 8th edition. After surgery 3-Year Follow up data were collected including Disease Free Survival (DFS) that was considered as the period from the date of primary radical surgery to the date of 1st treatment failure in the form of local recurrence or distant metastases. Overall Survival (OS) was calculated from the date of diagnosis to the end of follow up period or death. All data were reported by regular follow up visits of all our patients and by our calling center-follow up for patients' condition and health.

Immunohistochemical Staining

Sections from formalin-fixed, paraffin-embedded (FFPE) tissue blocks were deparaffinized and hydrated by standard approaches using tissue microarray technique to evaluate *Musashi-2* and *CD163*+TAM in colorectal biopsies using the following antibodies: *Musashi-2* antibody: Rabbit monoclonal Antibody at dilution of 1:100, (Catalog No. A19814). *CD163* antibody: Rabbit Polyclonal Antibody, IgG, at dilution of 1:100, (Catalog. No. 400100295).

Immunohistochemical evaluation

Slides were scored in an independent manner by two pathologists who were blinded to the patients' data. MSI-2 was expressed in CRC tissues and mainly localized in the cytoplasm of the cancer cells [15]. The percentage o positive cells was rated as follows: 0, (0%); 1(<10%), 2(10-50%) ,3 (51-80%) and 4(>80%) The intensity of staining was graded semi-quantitatively as follows: 0, no staining; 1, weak staining; 2, moderate staining and 3, strong staining. The staining index (SI) was calculated by multiplying the staining intensity score and the percentage of stained tumor cells. A SI of ≤4 was defined as low *MSI2* expression, whereas a SI of >4 was defined as high *MSI2* expression [16].

CD163 is a macrophage-specific antigen expressed mainly by M2 macrophages. Non-neoplastic cells stained with *CD163* were estimated as TAMs. The positive expression of *CD163* was defined as granular cytoplasmatic or cytoplasmatic and membrane staining pattern [17]. The percentage of positive cells was rated as follows: 0 (0%); 1 (< 10%); 2 (10% to 50%); 3 (51% to 80%); and 4 (> 80%) and defined as staining extension. The staining intensity was scored into the following scale: 0, no staining; 1, weak staining; 2, moderate staining and 3, strong staining [18]. The H-score was calculated as extent x intensity [1]. H score 4 was considered the median, so we defined H score of ≤4 as low *CD163*+TAM expression, whereas H score of >4 as high *CD163*+TAM expression.

Statistical analysis and data interpretation

SPSS software version 25 (SPSS Inc., PASW statistics for Windows version 25) carried out the data analysis. Chicago: SPSS Inc. Qualitative data were described using numbers and percentages. For non-normally distributed data, the median (the middle number between the lowest and highest values) was used to define the data. For normally distributed data, the mean±SD was used after the Kolmogorov-Smirnov test was used to check for normality. The significance of the obtained results was judged at the (≤0.05) level. Chi-Square, Fischer exact test, and Monte Carlo tests were used to compare qualitative data between groups as appropriate. • Kaplan-Meier test: utilized to calculate OS and DFS by utilizing log-rank tests to detect the effect of predisposing factors affecting survival. Cox regression was used to assess predictors of survival with the calculation of the hazard ratio.

Results

Patients' demograghic and clinicopathological

Table 1. Demographic and Clinicopathological Characteristics Studied Cases

	N=114	%
Age / years		
Mean ±SD	52.11±12.84	
(Min-Max)	(22-75)	
≤50	42	36.8
>50	72	63.2
Sex		
Male	61	53.5
Female	53	46.5
Medical history	45	39.5
Family history	18	15.8
Type		
Exophytic	74	64.9
Ulcerative	40	35.1
Tumor site		
Left	51	44.7
Right	42	36.8
Rectum	21	18.4
Grade of differentiation		
Poor	30	26.3
Moderate	75	65.8
Well	9	7.9
Histological type		
Mucinous adenocarcinoma	18	15.8
Adenocarcinoma	96	84.2
Hepatic Mets		
No	97	85.1
Yes	17	14.9
Depth of invasion		
T2	15	13.2
T3	86	75.4
T4	13	11.4
lymph node		
N0	68	59.6
N1	25	21.9
N2	21	18.5
TNM		
I	12	10.5
II	56	49.1
III	45	39.5
IV	1	0.9
CEA		
Low	66	57.9
High	48	42.1
CA19-9		
Low	66	57.9
High	48	42.1
Musashi-2 score		
Negative	9	7.9
Low (≤4)	56	49.1
High (>4)	49	43
CD163 score		
Negative	10	8.8
Low(≤4)	56	49.1
High (>4)	48	42.1

characteristics are described in Table 1.

According to the mentioned criteria for *MSI2* and *CD163*+TAM (Table 2): among 114 CRC cases, high *MSI2* expression was detected in 49/114 (43%) Figure 3. High *MSI 2* expression was significantly related to the grade of differentiation (P = 0.001*), the depth of invasion (P = 0.001*), lymph node metastasis (P = 0.001*), tumor TNM stage (P = 0.001*), hepatic metastases (P = 0.009*) and tumor marker (P = 0.001*) There were no observed associations between *MSI2* expression and other clinicopathological parameters (Table 2). High *CD163*+TAM expression was detected in 48/114 (42.1%) Figure 4 showed a significant correlation the grade of differentiation (P = 0.001*), the depth of invasion (P = 0.05*), lymph node metastasis (P = 0.001*), tumor TNM stage (P = 0.001*), and hepatic metastasis (P = 0.007*) and tumor marker (P = 0.001*) There were no observed associations between *CD163* expression and other clinicopathological parameters (Table 2).

Univariate analysis and Cox regression showed statistically significant relationship between OS and grade of differentiation (P=0.023*), hepatic metastasis (P=0.006*), Lymph node invasion (P=0.009*), TNM staging (P=0.001*), CEA level (P=0.02) and CA19-9 level (P=0.019), Table 3. Univariate analysis and Cox regression showed statistically significant relationship between DFS and grade of differentiation of tumor (P=0.03*), Lymph node invasion (P=0.032*), CEA level (P=0.016*), CA19-9 level (P=0.02*), Table 3.

Multivariate analysis spotted *MSI2* high expression as an independent prognostic predictor for DFS (Figure 1A, P = 0.008*) and OS in CRC (Figure 2A, P = 0.015*). Multivariate analysis spotted *CD163*+TAM high expression as an independent prognostic predictor for DFS (Figure 1B, P = 0.003*) and OS in CRC (Figure 2B, P = 0.037*).

Discussion

Colorectal cancer (CRC), one of the most common human malignancies, accounts about 10% of cancer mortality and incidence worldwide [19]. Despite the significant advances in the diagnosis of CRC, the survival rate decreases for patients diagnosed with metastatic and regional disease. The reported overall median survival time of CRC is only 1.1 years; therefore, understanding about biological factors with impact on CRC is very important [20]. *MSI2* protein regulates cancer invasion, metastasis and development of more aggressive cancer

Table 2. Correlation between *Musashi-2, CD163*+TAM with Clinicopathological Findings among Studied Cases

	Musashi 2				CD163+TAM			
	Negative N=9(%)	Low expression N=56(%)	High expression N=49 (%)	Test of significance	Negative N=10(%)	Low expression N=56(%)	High expression N=48(%)	Test of significance
Age / years								
≤50	1 (11.1)	22 (39.3)	19 (38.8)	χ^2=2.78	5 (50)	16 (28.6)	21 (43.8)	χ^2=3.38
>50	8 (88.9)	34 (60.7)	30 (61.2)	P=0.249	5 (50)	40 (71.4)	27 (56.2)	P=0.185
Sex								
Male	5 (55.6)	33 (58.9)	23 (46.9)	χ^2=1.53	4 (40)	33 (58.9)	24 (50)	χ^2=1.63
Female	4 (44.4)	23 (41.1)	26 (53.1)	P=0.466	6 (60)	23 (41.1)	24 (50)	P=0.442
Medical history	2 (22.2)	22 (39.3)	21 (42.9)	χ^2=1.36	4 (40)	23 (41.1)	18 (37.5)	χ^2=0.139
				P=0.507				P=0.933
Family history	1 (11.1)	7 (12.5)	10 (20.4)	χ^2=1.39	3 (30)	9 (16.1)	6 (12.5)	χ^2=1.91
				P=0.499				P=0.384
Type								
Exophytic	5 (55.6)	36 (64.3)	33 (67.3)	χ^2=0.483	9 (90)	35 (62.5)	30 (62.5)	χ^2=3.03
Ulcerative	4 (44.4)	20 (35.7)	16 (32.7)	P=0.785	1 (10)	21 (37.5)	18 (37.5)	P=0.220
Tumor site								
Left	3 (33.3)	27 (48.2)	21 (42.9)	χ^2=4.36	5 (50)	25 (44.6)	21 (43.8)	χ^2=1.30
Right	3 (33.3)	17 (30.4)	22 (44.9)	P=0.359	3 (30)	19 (33.9)	20 (41.7)	P=0.861
Rectum	3 (33.3)	12 (21.4)	6 (12.2)		2 (20)	12 (21.4)	7 (14.6)	
Grade of differentiation								
Poor	0	5 (8.9)	25 (51.0)	χ^2=29.84	0	3 (5.4)	27 (56.2)	χ^2MC=41.6
Moderate	7 (77.8)	45 (80.4)	23 (46.90	P=0.001*	8 (80)	46 (82.1)	21 (43.8)	P=0.001*
Well	2 (22.2)	6 (10.7)	1 (2.0)		2 (20)	7 (12.5)	0	
Histological type								
Mucinous adenocarcinoma	0	6 (10.7)	12 (24.5)	χ^2=5.56	1 (10)	6 (10.7)	11 (22.9)	χ^2=3.17
Adenocarcinoma	9 (100)	50 (89.3)	37 (75.5)	P=0.062	9 (900	50 (89.3)	37 (77.1)	P=0.205
hepatic Mets								
No	9 (100)	52 (92.9)	36 (73.5)	χ^2MC=9.45	10 (100)	52 (92.9)	35 (72.9)	χ^2MC=10.02
Yes	0	4 (7.1)	13 (26.5)	P=0.009*	0	4 (7.1)	13 (27.1)	P=0.007*
Depth of invasion								
T2	5 (55.6)	9 (16.1)	1 (2.0)	χ^2MC=20.28	2 (20)	11 (19.6)	2 (4.20	χ^2MC=9.48
T3	4 (44.4)	41 (73.2)	41 (83.7)	P=0.001*	8 (80)	41 (73.2)	37 (77.1)	P=0.05*
T4	0	6 (10.7)	7 (14.3)		0	4 (7.1)	9 (18.8)	
Lymph node								
N0	8 (88.9)	45 (80.4)	15 (30.6)	χ^2MC=30.79	10 (100)	48 (85.7)	10 (20.8)	χ^2MC=53.67
N1	1 (11.1)	5 (8.9)	19 (38.8)	P=0.001*	0	6 (10.7)	19 (39.6)	P=0.001*
N2	0	6 (10.7)	15 (30.6)		0	2 (3.6)	19 (39.6)	
TNM								
I	4 (44.4)	7 (12.5)	1 (2.0)	χ^2MC=32.7	2 (20)	10 (17.9)	0	χ^2MC=43.63
II	4 (44.4)	36 (64.3)	16 (32.7)	P=0.001*	6 (60)	38 (67.9)	12 (25.0)	P=0.001*
III	1 (11.1)	13 (23.2)	31 (63.3)		2 (20)	8 (14.3)	35 (72.9)	
IV	0	0	1 (2.0)		0	0	1 (2.1)	
CEA								
Low	9 (100)	39 (69.6)	18 (36.7)	χ^2=18.72	9 (90)	44 (78.6)	13 (27.1)	χ^2=32.74
High	0	17 (30.4)	31 (63.3)	P=0.001*	1 (10)	12 (21.4)	35 (72.9)	P=0.001*
CA19-9								
low	9 (100)	42 (75)	10 (20.4)	χ^2=38.79	10 (100)	46 (82.1)	5 (10.4)	χ^2=62.98
high	0	14 (25)	39 (79.6)	P=0.001*	0	10 (17.9)	43 (89.6)	P=0.001*
CD163								
Negative	1 (11.1)	9 (16.1)	0					
Low	8 (88.9)	35 (62.5)	13 (26.5)	χ^2MC=38.71				
High	0	12 (21.4)	36 (73.5)	P=0.001*				

χ^2, Chi-Square test; P, Probability value; *, statistically significant (P<0.05).

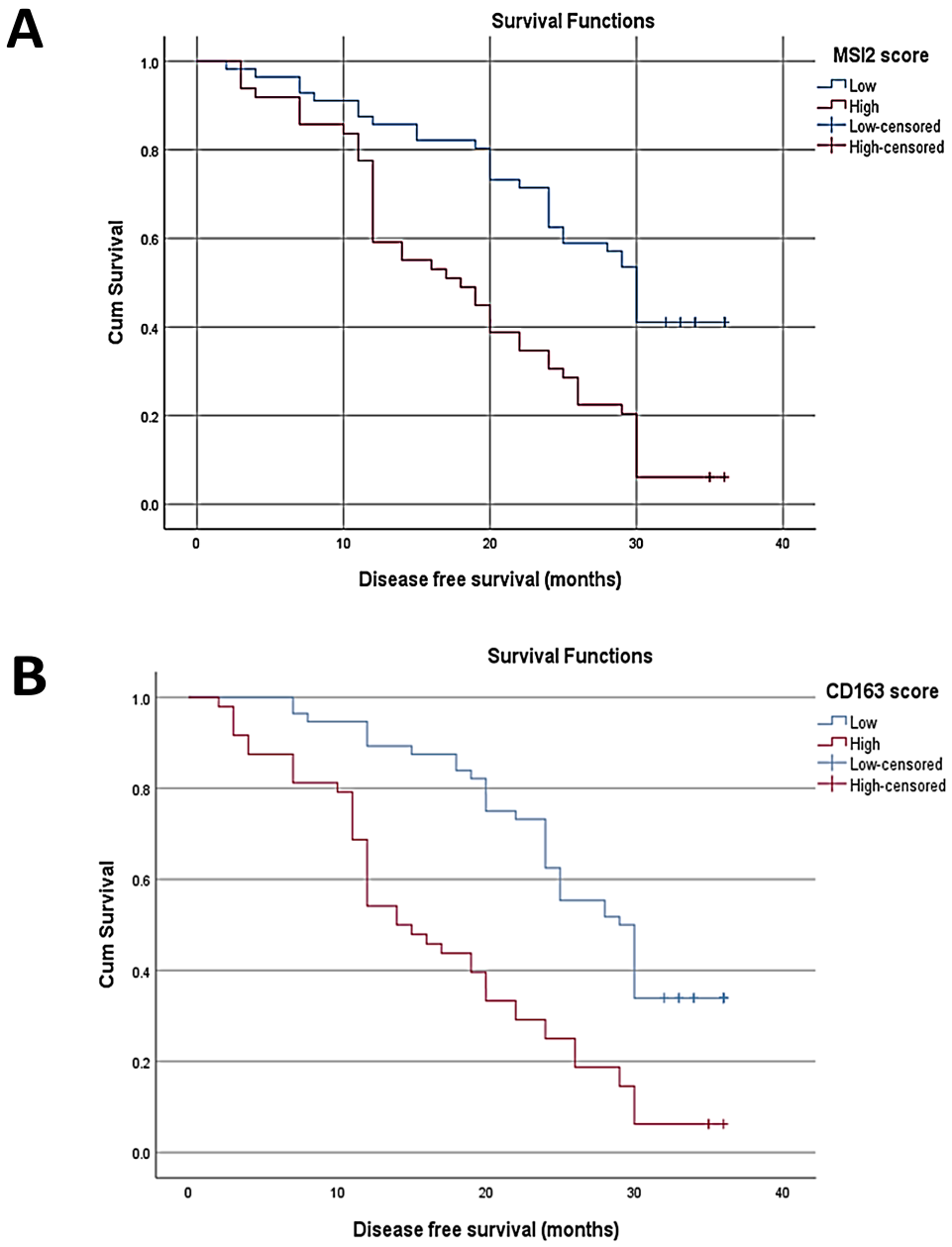

Figure 1. Kaplan-Miere Curve Showing Effect of *MSI2* Score (A) and *CD163* score(B) on disease free survival of studied cases

phenotypes, including drug resistance [21].

Tumor associated macrophages are one of the most dynamic cells in CRC that are associated with cancer development. The function of macrophages depends on with its phenotype and tumor type. M1 is related to the early stage of the tumor that induce inflammatory response and phagocytosis, while M2 polarization inhibits antitumor immune response. In most human cancers, a large number of TAM are significantly related to poor disease prognosis [22].

CD163+TAM has been suggested to be a predictive biomarker in patients with solid tumors. So, it has great potential to be a therapeutic target for solid tumor treatment [23].

We aimed to identify the biomarkers that are most relevant to the prognosis of CRC, so we evaluated immunohistochemical expression of *Musashi-2* and *CD163*+TAM and its effect on clinical characteristics and tumor staging as useful biomarkers for prognosis and aggressiveness of colorectal cancer.

We found that *Musashi-2* has positive correlation with grade of differentiation of tumor, lymph node invasion, hepatic metastasis and TNM staging, in colorectal cancer patients. In agreement to that Huang et al. [24] documented that *MSI2* has been implicated in cancer progression, particularly in the regulation of cell proliferation, metastasis, migration and invasion in pancreatic cancer.

Univariate and multivariate analysis showed that *MSI2* is consider predictor for poor OS and DFS, which was similar to Kharin et al. [25] results who stated that *MSI2* expression is elevated during CRC progression, and associated with poor prognosis. Depletion of *MSI2* reduces CRC cell growth. Additionally, He found that elevated expression of *MSI2* is associated with pre-cancerous

Table 3. Univariate and Multivariate Analysis for Predictors of Both DFS &OS among Studied Cases

	Disease Free Survival				Overall Survival			
	Univariate analysis		Multivariate analysis		Univariate analysis		Multivariate analysis	
	DFS		DFS		OS		OS	
	Median DFS (95%CI)	HR (95%CI)	β	HR (95%CI)	Median OS (95%CI)	P value	β	HR (95%CI)
Age / years								
≤50	25 (19.56-30.4)	R			31.68 (29.57-33.80)	R		
>50	24 (19.39-28.61)	0.995 (0.630-1.57)			32.23 (30.41-34.04)	0.842 (0.428-1.66)		
Sex								
Male	24 (21.13-26.87)	R			32.16 (30.36-33.95)	R		
Female	25 (18.75-31.24)	0.955 (0.617-1.48)			31.88 (29.74-34.03)	0.824 (0.430-1.58)		
Medical history								
No	26 (21.94-30.06)	R			31.81 (30.06-33.56)	R		
Yes	22 (19.26-24.74)	1.23(0.79-1.91)			32.36 (30.11-34.61)	0.873 (0.441-1.72)		
Family history								
-ve	24 (21.12-26.88)	R			31.96 (30.47-33.44)	R		
+ve	25 (20.01-29.99)	0.891(0.491-1.62)			32.38 (28.59-36.18)	0.740 (0.287-1.91)		
Type								
Exophytic	25 (21.26-28.74)	R			32.69 (31.3-34.09)	R		
Ulcerative	24 (17.83-30.17)	1.16(0.741-1.82)			30.78 (27.84-33.72)	1.32 (0.685-2.53)		
Tumor site								
Left	28 (24.5-31.49)	R			31.66 (29.17-34.15)	R		
Right	20 (16.04-23.96)	1.56(0.964-2.51)			31.76 (29.87-33.64)	1.61 (0.79-3.23)		
Rectum	26 (20.39-1.61)	1.08(0.596-1.96)			33.47 (31.18-35.76)	0.963 (0.370-2.51)		
Grade of differentiation								
Poor	12 (11.11-12.88)	5.08(2.08-12.42)	1.1	3.01(1.12-8.14)	24.17 (20.78-27.55)	18.18 (2.45-135.13)	2.42	11.31 (1.4-91.27)
Moderate	30 (27.45-32.55)	1.12(0.482-2.63)	0.175	1.19(0.498-2.85)	34.77 (33.83-35.69)	1.14 (0.145-9.01)	0.461	1.58 (0.197-12.78)
Well	30 (28.61-31.38)	R		R	35.44 (34.42-36.47)	R		R
Histological type								
Mucinous adenocarcinoma	19 (16.92-21.08)	1.89(1.11-3.25)	0.008	1.01(0.529-1.92)	31.76 (29.51-34.02)	1.96 (0.951-4.05)		
Adenocarcinoma	25 (21.16-28.84)	R		R	32.08 (30.48-33.67)	R		

β, regression coefficient; R, reference group; Kaplan-Meier test, used to calculate OS and DFS to detect effect of risk factors on survival. Cox regression was used to assess predictors of survival with calculation of Hazard ratio

Table 3. Continued

	Disease Free Survival				Overall Survival			
	Univariate analysis		Multivariate analysis		Univariate analysis		Multivariate analysis	
	DFS		DFS		OS		OS	
	Median DFS (95%CI)	HR (95%CI)	β	HR (95%CI)	Median OS (95%CI)	P value	β	HR (95%CI)
Hepatic Mets								
No	25 (21.14-28.86)	R			32.36 (30.85-33.88)	R		R
Yes	20 (11.93-28.07)	1.53 (0.87-2.68)			30.08 (26.82-33.34)	2.70 (1.34-5.45)	0.994	2.70 (1.34-5.45)
Depth of invasion								
T2	29 (24-33)	R		R	35.60 (34.84-36.36)	R		
T3	24 (19-28)	2.16 (0.993-4.72)	0.439	1.55 (0.695-3.46)	31.76 (30.19-33.34)	6.40 (0.874-46.91)		
T4	22(13.9-30.02)	3.29 (1.27-8.52)	0.736	2.09 (0.763-5.71)	29.64 (23.96-35.32)	8.83 (1.06-73.36)		
LN								
N0	30 (28.22-31.78)	R		R	35.33 (34.42-36.25)	R	2.43	R
N1	20 (15.10-24.89)	2.75 (1.66-4.56)	1.06	2.88 (1.09-7.58)	28.48 (25.04-31.92)	15.12 (5.01-45.68)	2.48	11.39 (2.08-62.53)
N2	12 (11.39-12.61)	4.18 (2.41-7.27)	1.23	3.44 (1.19-9.96)	25.57 (21.81-29.33)	28.29 (9.53-83.97)		11.98 (1.86-77.12)
TNM								
I-II	29 (26.06-31.94)	R		R	35.07 (34.09-36.05)	R	2.5	R
III-IV	18 (11.35-24.65)	2.55 (1.65-3.92)	0.387	1.47 (0.596-3.64)	27.53 (24.91-30.14)	12.2 (5.08-29.33)		12.20 (5.08-29.33)
CEA								
Low	30 (27.15-32.85)	R		R	34.79 (33.68-35.92)	R	0.937	R
High	17 (11.34-22.66)	2.84 (1.83-4.38)	0.627	1.87 (1.12-3.12)	28.22 (25.68-30.76)	7.30 (3.34-15.98)		2.55 (1.15-5.69)
CA19-9								
Low	30 (29.24-30.76)	R		R	35.96 (35.89-36.03)	R		R
High	16 (9.75-22.24)	3.58 (2.28-5.6)	0.853	2.12 (1.11-4.05)	27.49 (25.03-29.96)	68.86 (9.42-503.37)	2.45	11.63 (1.49-90.80)
CD163								2.59 (1.06-6.35)
Low	29 (25.94-32.06)	R		R	35.80 (35.53-36.07)	R	0.954	
High	14 (10.61-17.39)	4.45 (1.07-18.51)	0.805	2.24 (1.32-3.80)	26.78 (24.16-29.42)	35.68 (8.55-148.87)		
MSI-2								
Low	30.0 (28.39-31.60)	R		R	34.66 (323.36-35.96)	R	1.86	R
High	18 (11.14-24.86)	2.88 (1.142-7.26)	0.972	2.64 (1.637-4.16)	28..27 (25.78-30.77)	3.67 (2.55-18.87)		6.4 (1.44-28.40)

β, regression coefficient; R, reference group; Kaplan-Meier test, used to calculate OS and DFS to detect effect of risk factors on survival. Cox regression was used to assess predictors of survival with calculation of Hazard ratio

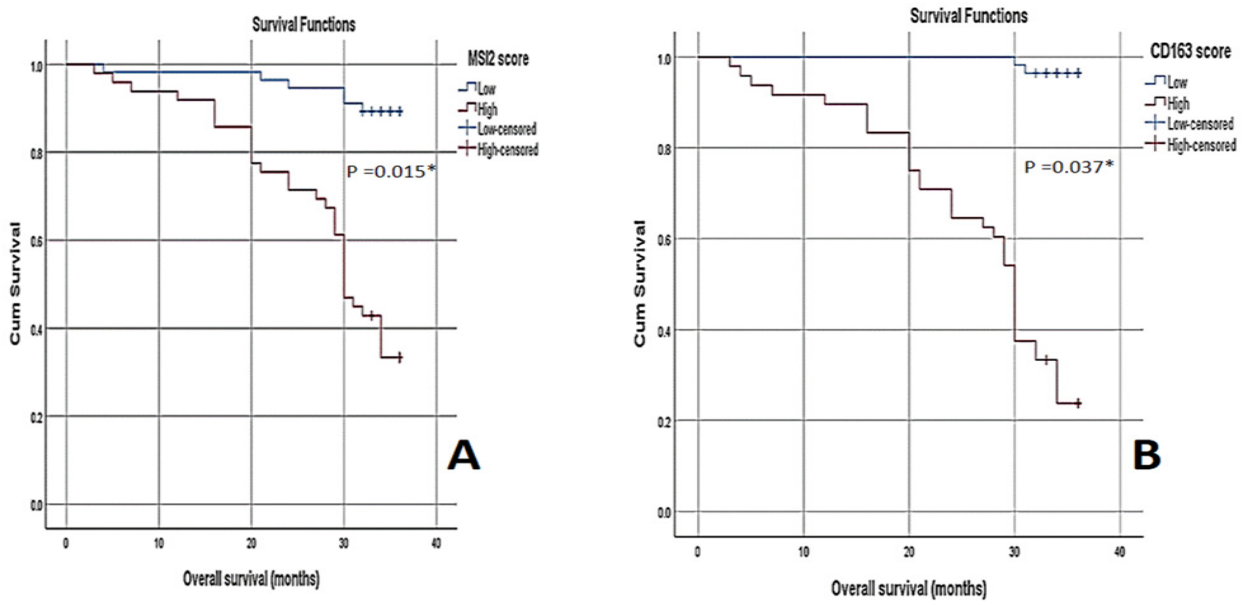

Figure 2. Kaplan-Miere Curve Showing Effect of *MSI-2* score (A) and *CD163* score (B) on overall survival of studied cases

tubulovillous adenoma in the colonic mucosa, suggesting it is an early event in transformation. In agreement to that, Kharin et al. [8] found that high levels of *MSI2* in primary tumors were associated with shorter PFS regardless of tumor stage. Elevated *MSI2* expression in liver metastasis linked to both poor PFS and OS.

In addition, Zong et al. [15] investigated the prognostic values of MSI-2 expression in CRC patients in the group combining stage I, II, III, and IV patients, revealed that the OS of CRC patients with MSI-2 high expression was significantly poorer than those patients with MSI-2 low expression.

The correlation between the overexpression of *MSI2* and the progression and poor prognosis of patients with other solid tumors studied by Liu et al. [16] who identified that high *MSI2* expression was predictive of poor overall survival in patients with early stage cervical cancer. Also, Topchu et al. [6] stated a significant role for *MSI2* in the processes of carcinogenesis and the progression of the NSCLC. High expression of *MSI2* in late-stage tumors and its correlation with decrease in patient survival suggest a higher aggressiveness of the disease in this subset of patients. Measuring *MSI2* protein expression in primary NSCLC tumors can be used as a novel potential prognostic biomarker in NSCLC patients.

Therefore, Zong et al. [15] stated that the patients with

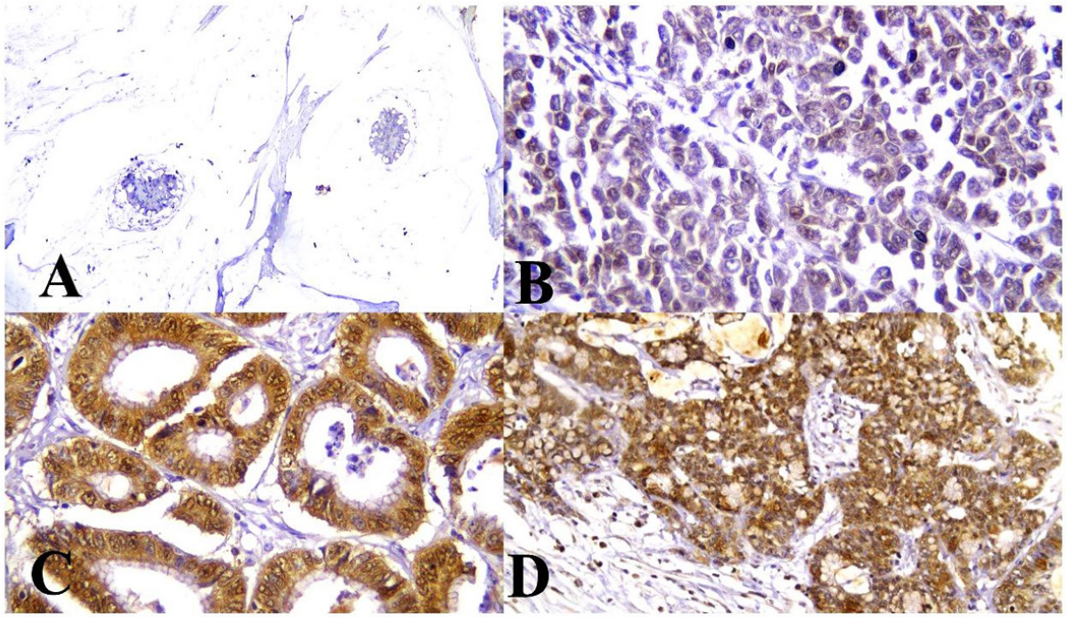

Figure 3. IHC of *Musachi-2* in Different Cases of CRC. Negative expression (A), low Musachi expression (B). High *Musachi* expression (C, D). (Original magnification: A, D 200×; B, C 400×).

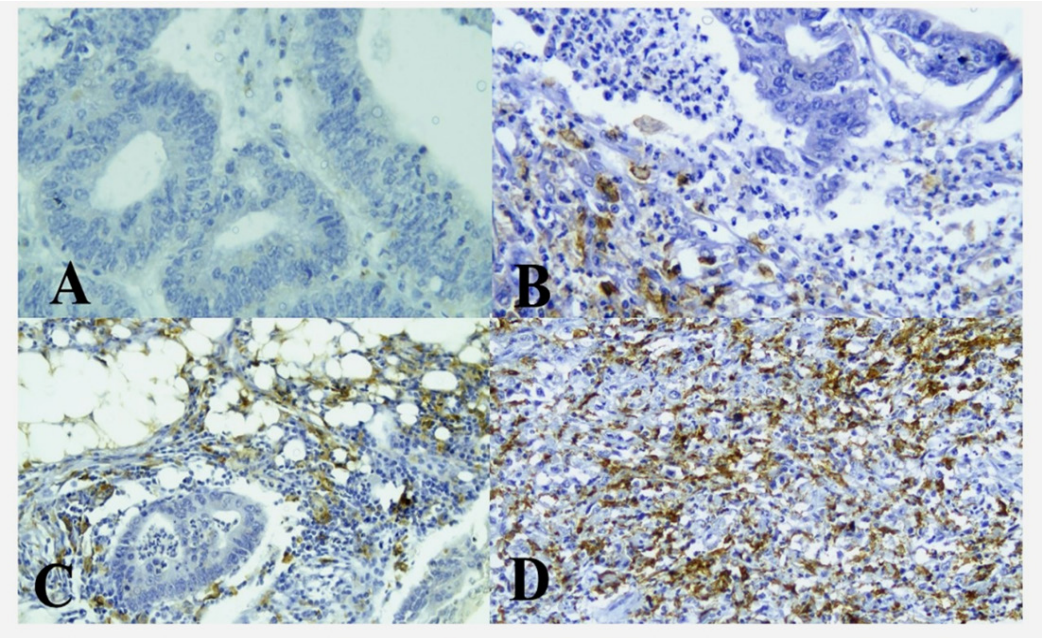

Figure 4. IHC of *CD163* in Different Cases of CRC. Negative expression (A), low *CD163* expression (B). High *CD163* expression (C, D). (Original magnification: A, B 400×; C, D 200×).

MSI2 high expression might require strict surveillance or adjuvant chemotherapy. Furthermore Liu et al. [26] Study which showed that the *MSI2* promote the development of CRC, so *MSI2* maybe a therapeutic target in CRC. In contrast to us, Emadi-Baygi et al. [27] demonstrated that the expression of *MSI2* mRNA was decreased in grade II compared with grade I gastric cancer tissue; however, *MSI2* mRNA expression did not differ significantly between tumor and non tumor tissues and between different tumor types in gastric cancer.

We found that *CD163*+TAM has positive correlation with grade of differentiation of tumor, lymph node invasion, hepatic metastasis, TNM staging, CEA level, CA19-9 level in colorectal cancer patients. This also came hand in hand with the study of Ma et al. [13] that proposed *CD163*+ TAM could be regarded as a biomarker for tumor progression and clinical outcomes in CRC, where a low expression may be seen in the early stages of development, and high expression may suggest invasion, metastasis, and a low survival rate. Up-regulated *CD163*+TAM expression seemed to be a negative prognostic factor for CRC based on the Cox analysis.

Contrary to us, Rey and his colleagues, [28] had proved that there was no relation between *CD163* expression and both histological grade and TNM staging in contrast to our study which found statistically significant relation between *CD163* expression and both grade and stage of cancer this may be due to different ethnicity and including patient received neuadjuvant chemotherapy.

In our study, Univariate and multivariate analysis showed that higher *CD163*+TAM score is considered as a predictor for poor OS and DFS, this is in agreement with Xue et al. [29] who found that infiltration of *CD163*+ TAMs in CRC tissues was an independent risk factor for the prognosis of CRC patients, and high-level TAM infiltration in CRC tissues was associated with poorer OS and DFS.

Similarly, Kou et al. [30] proved that the high expression of *CD163* and CD133+TAM in combination were positively associated with poor prognosis in patients with CRC. Similar to our results on CRC, Yang et al. [31] found that increased *CD163*+ TAMs in tumor stroma in breast cancer were correlated with unfavorable clinicopathological factors, and worse DFS and OS.

Also, Meisel et al. [32] worked on *CD163*+TAM related spatial matrix in breast cancer, discovered that the proximity of and the number of *CD163*+ TAM cells may be seen as separate predictive factors for the cancer prognosis. According to Wei et al. [33] increasing *CD163*+ TAM infiltration at the invasive front of the tumor is significantly related to the poor prognosis of CRC patients and may play a role in promoting the spread and invasion of CRC.

However, a meta-analysis by li et al. [34] showed that high density of TAMs in CRC tissue was significantly associated with favorable 5-year OS but not with DFS. This may be related to the different population and having received chemotherapy before surgery. In contrary to CRC, Zeiner et al. [35] showed that an increased number of *CD163*-positive glioma-associated microglia/ macrophages in the tumor core was related to better survival, However, the study used transcriptome analysis, while we used immunohistochemistry to examine the expressions.

Therefore, both *MSI2* and *CD163*+TAM could be considered a prognostic factors and later on may be a promising therapeutic targets in the CRC. Our study had some limitations as it was single center study and it did not include the type of adjuvant chemotherapy the patients received, which may affect their outcome. So we recommend further multicenter studies including larger number of patients and different ethnicities for longer follow up period.

In conclusion, this study suggests that *MSI2* and

CD163+TAM are independent prognostic factors for the overall survival and disease free survival and can predict poor prognosis in CRC patients.

Author Contribution Statement

Study conception, data collection, analysis and interpretation of results: Dalia Mohamed Hemeda, Ahmed El-Mesery, Mahmoud Abdel-Aziz Soliman; reviewing the pathological diagnosis: Mie Ali Mohamed , Zeinab Mohammed Gawesh; surgical point of view: Waleed Elnahas, All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

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Compliance with Ethical Standards

The present study was carried out after obtaining approval from the committed Institutional Research Board (IRB) at the Faculty of Medicine, Mansoura University, Egypt (Code Number: MD.21.10.541). The study was processed under the ethical standards of the Helsinki Declaration.

Conflict of interest statement

The authors declare no relevant financial affiliations or conflicts of interest.

Availability of data and material

All the clinical, radiological, and pathological data used in this manuscript is available on Mansoura University medical system (Ibn Sina Hospital management system) http://srv137.mans.edu.eg/mus/ newSystem/.

No other scientific organization had approved this research, and it was not a component of any accepted student thesis.

References

1. Zhang H, Zhang N, Wu W, Wang Z, Dai Z, Liang X, et al. Pericyte mediates the infiltration, migration, and polarization of macrophages by *CD163*/MCAM axis in glioblastoma. iScience. 2022;25(9):104918–8. https://doi.org/10.1016/j.isci.2022.104918.
2. Roshandel G, Ghasemi-Kebria F, Malekzadeh R. Colorectal Cancer: Epidemiology, Risk Factors, and Prevention. Cancers. 2024;16(8):1530. https://doi.org/10.3390/cancers16081530.
3. Vabi BW, Gibbs JF, Parker GS. Implications of the growing incidence of global colorectal cancer. J Gastrointest Oncol. 2021;12(Suppl 2):S387–98. https://doi.org/10.21037/jgo-2019-gi-06.
4. Bitaraf A, Razmara E, Bakhshinejad B, Yousefi H, Vatanmakanian M, Garshasbi M, et al. The oncogenic and tumor suppressive roles of RNA-binding proteins in human cancers. J Cell Physiol. 2021;236(9):6200–24. https://doi.org/10.1002/jcp.30311
5. Jiang L, Xue S, Xu J, Fu X, Wei J, Zhang C. Prognostic value of Musashi 2 (*MSI2*) in cancer patients: A systematic review and meta-analysis. Front Oncol. 2022;12:969632. https://doi.org/10.3389/fonc.2022.969632
6. Iuliia Topchu, Nikolai Karnaukhov, Mazitova A, Yugai V, Voloshin M, Mariya Tikhomirova, et al. Musashi 2 (*MSI2*) expression as an independent prognostic biomarker in non-small cell lung cancer (NSCLC). J Thorac Dis. 2021;13(3):1370–9. https://doi.org/10.21037/jtd-20-2787
7. Zhang X, Su K, Liu Y, Zhu D, Pan Y, Ke X, et al. Small Molecule Palmatine Targeting *Musashi-2* in Colorectal Cancer. Front Pharmacol. 2022;12:793449. https://doi.org/10.3389/fphar.2021.793449.
8. Leonid Kharin, Igor Bychkov, Nikolay Karnaukhov, Voloshin M, Rushaniya Fazliyeva, Deneka A, et al. Prognostic role and biologic features of *Musashi-2* expression in colon polyps and during colorectal cancer progression. PLoS ONE. 2021;16(7):e0252132–2 https://doi.org/10.1371/journal.pone.0252132.
9. Reheman Yiming, Takeuchi Y, Nishimura T, Li M, Wang Y, Makiko Meguro-Horike, et al. MUSASHI-2 confers resistance to third-generation EGFR-tyrosine kinase inhibitor osimertinib in lung adenocarcinoma. Cancer Sci. 2021;112(9):3810–21. https://doi.org/10.1111/cas.15036.
10. Kumari S, Advani D, Sharma S, Ambasta RK, Kumar P. Combinatorial therapy in tumor microenvironment: Where do we stand? Biochim Biophys Acta Rev Cancer. 2021;1876(2):188585–5. https://doi.org/10.1016/j.bbcan.2021.188585
11. Locati M, Curtale G, Mantovani A. Diversity, mechanisms, and significance of macrophage plasticity. Annu Rev Pathol. 2020;15:123-47. https://doi.org/10.1146/annurev-pathmechdis-012418-012718.
12. H Hu JM, Liu K, Liu JH, Jiang XL, Wang XL, Chen YZ, et al. Cd163 as a marker of m2 macrophage, contribute to predicte aggressiveness and prognosis of kazakh esophageal squamous cell carcinoma. Oncotarget. 2017;8(13):21526-38. https://doi.org/10.18632/oncotarget.15630.
13. Ma S, Zhao Y, Liu X, Sun Zhang A, Zhang H, Hu G, et al. Cd163 as a potential biomarker in colorectal cancer for tumor microenvironment and cancer prognosis: A swedish study from tissue microarrays to big data analyses. Cancers (Basel). 2022;14(24). https://doi.org/10.3390/cancers14246166.
14. Compton C, Fenoglio-Preiser CM, Pettigrew N, Fielding LP. American joint committee on cancer prognostic factors consensus conference: Colorectal working group. Cancer. 2000;88(7):1739-57. https://doi.org/10.1002/(sici)1097-0142(20000401)88:7<1739::aid-cncr30>3.0.co;2-t.
15. Zong Z, Zhou T, Rao L, Jiang Z, Li Y, Hou Z, et al. Musashi2 as a novel predictive biomarker for liver metastasis and poor prognosis in colorectal cancer. Cancer Med. 2016;5(4):623-30. https://doi.org/10.1002/cam4.624.
16. Liu Y, Fan Y, Wang X, Huang Z, Shi K, Zhou B. *Musashi-2* is a prognostic marker for the survival of patients with cervical cancer. Oncol Lett. 2018;15(4):5425-32. https://doi.org/10.3892/ol.2018.8077.
17. Shabo I, Olsson H, Sun XF, Svanvik J. Expression of the macrophage antigen cd163 in rectal cancer cells is associated with early local recurrence and reduced survival time. Int J Cancer. 2009;125(8):1826-31. https://doi.org/10.1002/ijc.24506.
18. Agarbati S, Mascitti M, Paolucci E, Togni L, Santarelli A, Rubini C, et al. Prognostic relevance of macrophage phenotypes in high-grade oral tongue squamous cell carcinomas. Appl Immunohistochem Mol Morphol. 2021;29(5):359-65. https://doi.org/10.1097/pai.0000000000000867..

19. Ciardiello F, Ciardiello D, Martini G, Napolitano S, Tabernero J, Cervantes A. Clinical management of metastatic colorectal cancer in the era of precision medicine. CA: a cancer journal for clinicians. 2022;72(4):372-401. https://doi.org/10.3322/caac.21728.
20. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: Globocan estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2018;68(6):394-424. https://doi.org/10.3322/caac.21492.
21. Yang Y, Zhao M, Ding T, Ni C, Zheng Q, Li X. [advances in research of musashi2 in solid tumors]. Nan Fang Yi Ke Da Xue Xue Bao. 2022;42(3):448-56. https://doi.org/10.12122/j.issn.1673-4254.2022.03.20.
22. Liu C, Yao Z, Wang J, Zhang W, Yang Y, Zhang Y, et al. Macrophage-derived ccl5 facilitates immune escape of colorectal cancer cells via the p65/stat3-csn5-pd-l1 pathway. Cell Death Differ. 2020;27(6):1765-81. https://doi.org/10.1038/s41418-019-0460-0.
23. Ding D, Yao Y, Yang C, Zhang S. Identification of mannose receptor and cd163 as novel biomarkers for colorectal cancer. Cancer Biomark. 2018;21(3):689-700. https://doi.org/10.3233/cbm-170796.
24. Huang L, Sun J, Ma Y, Chen H, Tian C, Dong M. Msi2 regulates nlk-mediated emt and pi3k/akt/mtor pathway to promote pancreatic cancer progression. Cancer Cell Int. 2024;24(1):273. https://doi.org/10.1186/s12935-024-03444-9.
25. Leonid Kharin, Karnaukhov NS, Voloshin M, Milakin AG, Igor Bychkov, Deneka A, et al. Prognostic significance of Musashi 2 (*MSI2*) RNA-binding protein expression in precancerous polyps and during colorectal cancer (CRC) progression. J Clin Oncol. 2020;38(15_suppl):e16009–9. https://doi.org/10.1200/JCO.2020.38.15_suppl.e16009
26. Liu G, Zhou J, Wang X, Gao W, Tang J, Dong M. Musashi2 promotes the proliferation and migration of colorectal cancer by down-regulating Numb protein. Res Sq. 2023. https://doi.org/10.21203/rs.3.rs-2413378/v1
27. Emadi-Baygi M, Nikpour P, Mohammad-Hashem F, Maracy MR, Haghjooy-Javanmard S. Msi2 expression is decreased in grade ii of gastric carcinoma. Pathol Res Pract. 2013;209(11):689-91. https://doi.org/10.1016/j.prp.2013.07.008.
28. Rey I, Putra A, Lindarto D, Yusuf F. Relationship between CD 163 Tumor-Associated Macrophages and Colorectal-Cancer Stem Cell Markers. Open Access Maced J Med Sci. 2021;9(B):1381–6. https://doi.org/10.3889/oamjms.2021.7188
29. Xue T, Yan K, Cai Y, Sun J, Chen Z, Chen X, et al. Prognostic significance of cd163+ tumor-associated macrophages in colorectal cancer. World J Surg Oncol. 2021;19(1):186. https://doi.org/10.1186/s12957-021-02299-y.
30. Kou Y, Shui M, Tang H, Gu F, Zhu R, Wang Y, Han B. Prognostic value and predictive biomarkers of tumor-associated macrophages and cancer stem cells cooperatively in colorectal cancer. 2024.
31. Yang M, Li Z, Ren M, Li S, Zhang L, Zhang X, et al. Stromal infiltration of tumor-associated macrophages conferring poor prognosis of patients with basal-like breast carcinoma. Journal of Cancer. 2018;9(13):2308-16. https://doi.org/10.7150/jca.25155
32. Maisel BA, Yi M, Peck AR, Sun Y, Hooke JA, Kovatich AJ, et al. Spatial metrics of interaction between cd163-positive macrophages and cancer cells and progression-free survival in chemo-treated breast cancer. Cancers (Basel). 2022;14(2). https://doi.org/10.3390/cancers14020308.
33. Wei C, Yang C, Wang S, Shi D, Zhang C, Lin X, et al. Crosstalk between cancer cells and tumor associated macrophages is required for mesenchymal circulating tumor cell-mediated colorectal cancer metastasis. Mol Cancer. 2019;18(1):64. https://doi.org/10.1186/s12943-019-0976-4.
34. Li J, Li L, Li Y, Long Y, Zhao Q, Ouyang Y, et al. Tumor-associated macrophage infiltration and prognosis in colorectal cancer: Systematic review and meta-analysis. Int J Colorectal Dis. 2020;35(7):1203-10. https://doi.org/10.1007/s00384-020-03593-z.
35. Zeiner PS, Preusse C, Golebiewska A, Zinke J, Iriondo A, Muller A, et al. Distribution and prognostic impact of microglia/macrophage subpopulations in gliomas. Brain Pathol. 2019;29(4):513-29. https://doi.org/10.1111/bpa.12690.