Prognostic significance of SDF-1 in colorectal cancer depends on CD8+ T-cell density

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Abstract

Background: Since colorectal cancer (CRC) remains one of the most common malignancies, a tremendous amount of studies keep taking place in this field. Over the past 25 years, a notable part of the scientific community has focused on the association between the immune system and colorectal cancer. A variety of studies have shown that high densities of infiltrating CD8+ T cells are associated with improved disease-free and overall survival in colorectal cancer (CRC). Stromal cell-derived factor-1 (SDF-1) is a protein that regulates leukocyte trafficking and is variably expressed in several healthy and malignant tissues. There is strong evidence that SDF-1 has a negative prognostic impact on colorectal cancer (CRC). However, based on a significant correlation of SDF-1 and CD8+ T cells in a previous study ($r=0.53$, $p<0.0001$), we hypothesized that the prognostic significance of SDF-1 in CRC could depend on the immune microenvironment. Therefore, we explored the combined prognostic significance of SDF-1 expression and CD8+ T cell density in a large CRC collective.

Methods: We analyzed a tissue microarray (TMA) of 613 patient specimens of primary CRCs by immunohistochemistry (IHC) for the expression of SDF-1 by tumor cells and tumor-infiltrating immune cells (TICs) and CD8+ T-cells. Besides, we analyzed the expression of SDF-1 at the RNA level in The Cancer Genome Atlas cohort (TCGA).

Results: We found that the the combined high expression of SDF-1 and CD8+ T-cell infiltration shows a favorable 5-year overall survival rate (66%; 95%CI=48–79%) compared to tumors showing a high expression of CD8+ T-cells only (55%; 95%CI=45–64%; $p=0.0004$). High expression of SDF-1 and CD8+ T-cells infiltration was significantly associated with a favorable prognosis also in a validation group ($p=0.016$). Univariate and multivariate Hazard Cox regression survival analysis considering the combination of both markers revealed that the combined high expression of SDF-1 and CD8+ T cells was an independent, favorable, prognostic marker for overall survival (HR=0.34, 95%CI=0.17–0.66; $p=0.002$ and HR=0.45, 95%CI=0.23–0.89; $p=0.021$, respectively). In a spearman's correlation analysis from the TCGA cohort, SDF-1 also correlated significantly with CD8+ T cells ($r=0.28$).

Conclusions: SDF-1 high /CD8 high density represents an independent, favorable, prognostic condition in CRC, most likely due to an effective antigen-specific immune response.

Background

Colorectal cancer (CRC) is the third most common malignancy to be diagnosed. Furthermore, CRC remains the second most common cause of death from cancer worldwide, despite the screening and new methods of treatment [1, 2]. These facts explain why so many studies have taken place to identify the mechanisms of CRC development. Understanding these features could lead to new concepts in the approach of diagnosis, prognosis, and even treatment of CRC.

As with the majority of cancers, the Tumor Node Metastasis (TNM) classification serves as the gold standard tool for the staging of CRC [3]. By every newly diagnosed CRC, an interdisciplinary team of specialists in several fields (Visceral Surgery, Oncology, Gastroenterology, Radiology/Nuclear Medicine, Radiation Oncology and Pathology) takes into account the TNM classification to determine the treatment. According to this system, they decide which of the patients are suitable for surgical resection and which of them are candidates for adjuvant chemotherapy following the resection of the primary tumor [4, 5]. However, we repeatedly observe that patients with identical stages and treatments have a completely different outcome in terms of survival and recurrence. This fact indicates that TNM classification alone in the vast majority of cases is not sufficient for the prognosis of colorectal cancer [6, 7]. Subsequently, an enormous amount of studies have investigated and still investigate other features, which could be an excellent additional tool for this classification. The crucial role of the microenvironment in CRC was thoroughly explored and showed that the high

immune cell infiltration by cytotoxic CD8 + cells and memory CD45RO + cells has a favorable prognostic significance [8–12].

CD8 (cluster of differentiation 8) is a well-known protein that serves as a co-receptor for the T-cell receptor (TCR) and binds to the major histocompatibility complex (MHC) molecule [13]. When the cytotoxic T cells are combined with CD8 surface protein, produce the CD8 + T cells and play a vital role in antigen recognition. Naito et al. proved that the infiltration of tumors with CD8 + T-cells has a beneficial prognostic influence in CRC [14]. Since then, a substantial number of studies have also examined the role of CD8 + T-cells, and nowadays, the scientific society has accepted its positive prognostic impact on CRC [15–19]. These results highlight the fact that the infiltration of the tumor with CD8 + T-cells is associated with a better prognosis not only in CRCs but also in other malignancies such as lung [20], renal [21] and endometrial [22]. Following these positive results, various immune cells were investigated with the aim of finding a better and more sufficient way to evaluate the prognosis of CRC. The stromal cell-derived factor 1 (SDF1) is a chemokine protein, which is strongly chemotactic for lymphocytes [23]. SDF-1 and one of its receptors, CXCR4, have been shown to play a crucial role in the tumor-stromal communication affecting cancer growth, angiogenesis, and metastasis formation [24]. Samarenda et al. performed a meta-analysis of 38 studies that evaluated the association between SDF-1 expression and cancer survival. The authors showed that a high SDF-1 expression was associated with significantly reduced overall survival in patients with lung, pancreatic, and esophagus-gastric cancer. Yet, there was no correlation between SDF-1 expression and overall survival in colorectal cancer [25].

In the study we conducted, we hypothesized that the prognostic significance of SDF-1 could depend on the immune microenvironment in CRC [26–29]. To explore this hypothesis, we comparatively investigated the prognostic role of SDF-1 in human CRC in the context of CD8 + T-cell density.

Methods

Tissue microarray construction

In our study, we included 613 patients with unselected, clinically annotated primary CRC specimens in a tissue microarray (TMA). Our study had the approval of the local ethics committee (EKBB). As far as it concerns the technique used for the TMA construction, we have described this in previous studies of our team [30]. Our specialist prepared the tissue cylinders of formalin-fixed, paraffin-embedded tissue blocks according to standard procedures. The tissue cylinders had a diameter of 0.6 mm, and were punched from morphologically representative areas of each donor block and finally brought into one recipient paraffin block (30 × 25 mm), using a semi-automated tissue microarray arrayed. It is crucial to mention that each punch was made from the center of the tumor to enable each TMA spot to include more than 50% of tumor cells.

Clinico-pathological Features

Clinico-pathological data were collected retrospectively in a non-stratified and random manner. Annotation included patient age and gender, tumor diameter in mm, site of the tumor, pT-stage, pN-stage, grade, stage according to TNM-classification, tumor border configuration (infiltrative vs pushing), vascular invasion, overall survival time (months), 5-years survival in % (95%CI) and the presence of peri-tumoral lymphocytic inflammation at the invasive tumor front (Table 1). Tumor border configuration and peri-tumoral lymphocytic inflammation were evaluated using the original hematoxylin-eosin slides of the resection specimens corresponding to each TMA punch.

Table 1

Characteristics of CRC patient cohort (n = 613)*

Characteristics	N or mean	(% or range)
Age, years (median, mean)	70, 68.9	36–96
Tumor size in mm (median, mean)	50, 50.1	4-160
Sex:		
Female	326	53.2
Male	287	46.8
Anatomic site of the tumor:		
Left-sided	428	69.8
Right-sided	183	29.9
T stage:		
T1	31	5.1
T2	83	13.5
T3	406	66.2
T4	79	12.9
N stage:		
N0	317	51.7
N1	160	26.1
N2	119	19.4
Tumor grade:		
G1	14	2.3
G2	552	90.0
G3	33	5.4
UICC TNM classification:		
Stage IA, (T1N0)	24	3.9
Stage IB, (T2N0)	60	9.8
Stage IIA, (T3N0)	204	33.3
Stage IIB-C, (T4N0)	23	3.8
Stage III, (> N0)	279	45.5
Tumor border configuration:		

*Due to missing values of the same variables, percentages may not add to 100%. Age and tumor size were evaluated using the Kruskal-Wallis test. Gender, anatomical site, T stage, N stage, grade, vascular invasion, and tumor border configuration were analyzed using the χ^2 test. Survival analysis was performed using the Kaplan-Meier method.

Characteristics	N or mean	(% or range)
Infiltrative	417	68.0
Pushing	180	29.4
Vascular invasion:		
No	425	69.3
Yes	174	28.4
Microsatellite Stability:		
Proficient	539	87.9
Deficient	74	12.1
Rectal cancers	246	40.1
Rectosigmoid cancers	44	7.2
Overall survival time (months)	58.4	0-152
5-years survival % (95%CI)	0.47	0.43–0.51
CXCR4 histoscore	156.5	0-300
pCXCR4 histoscore	32.2	0-300
CXCR4 TIC	74.2	0-1000
pCXCR4 TIC	4.8	0–79

*Due to missing values of the same variables, percentages may not add to 100%. Age and tumor size were evaluated using the Kruskal-Wallis test. Gender, anatomical site, T stage, N stage, grade, vascular invasion, and tumor border configuration were analyzed using the χ^2 test. Survival analysis was performed using the Kaplan-Meier method.

Immunohistochemistry

We used standard indirect immunoperoxidase procedures (IHC; ABC-Elite, Vector Laboratories, Burlingame, CA) as we have already described previous studies of our team [31]. Our specialists dewaxed and rehydrated slides in distilled water. Afterward, endogenous peroxidase activity was blocked using 0.5% H2O2. Sections were incubated with 10% normal goat serum (DakoCytomation, Carpinteria, CA) for 20 minutes and incubated with primary antibody at room temperature. We used primary antibodies that were specific for CD8 (Ventana 790–4460) and SDF-1 (Abcam ab9797). Subsequently, these parts were incubated with peroxidase-labeled secondary antibody (DakoCytomation) for 30 min at room temperature. For visualization of the antigen, these parts were immersed in 3-amino-9- ethylcarbazole plus substrate-chromogen (DakoCytomation) for 30 minutes and counterstained with Gill's hematoxylin.

Evaluation Of Immunohistochemistry

Two trained research fellows [A.L. and A.T.] performed immunohistochemical analysis, and an experienced pathologist [L.M.T.] validated the date independently. Histoscores for expression by tumor cells were obtained by multiplying percentages of positive cells by staining intensities (0 = negative, 1 = weak, 2 = moderate, 3 = strong). TICs were counted for each punch (approximately one high power [20×] field).

Public Database Analysis

Correlations using the RNA-sequencing from The Cancer Genome Atlas (TCGA) were performed. Briefly, colorectal cancer clinical information available for 597 patients were retrieved from the Human Protein Atlas database [https://www.proteinatlas.org/ENSG00000132688-NES/pathology/tissue/colorectal+cancer]. Gene Expression levels (FPKM values) for the genes were downloaded using TCGAbiolinks R package.

Statistical analysis

All statistical analyses were made using STATA software version 13 (StataCorp, College Station, TX, USA). We explored associations with survival using the Cox proportional hazard regression model. Cut-off values used to classify CRC with low or high immune cell infiltration were available from previous publications [32] or generated by applying regression tree analysis. Threshold value for CD8 + was 10 cells/TMA-punch. Threshold value for SDF-1 histoscore was 200 (= 75th percentile). Chi-square, Fisher's exact, and Kruskal-Wallis tests were used to determine the association of SDF-1 tumor expression and CD8 + T-cell infiltration and clinical-pathological features.

For survival analysis, the study population was randomly assigned to a test and validation group. Univariate survival analysis was performed by Kaplan-Meier and log-rank tests. Further analysis included all four combinations possible (SDF-1^{high}/CD8 + ^{high}, SDF-1^{high}/CD8 + ^{low}, SDF-1^{low}/CD8 + ^{high}, SDF-1^{low}/CD8 + ^{low}).

The assumption of proportional hazards was verified for all markers by analyzing correlation of Schoenfeld residuals and ranks of individual failure times. Any missing clinicopathological information was assumed to be missing at random. Subsequently, SDF-1 expression and CD8 + cell density data were entered into multivariate Cox regression analysis and hazard ratios (HR) and 95% confidence intervals (CI) were used to determine prognostic effects on survival time. P-values < 0.05 were considered statistically significant.

Results

Patient and tumor characteristics

In our study we included 613 patients with CRC with a median age of 70 years (range 36–96). 53.2% of the patients were female. In 69.8% of the patients CRC was located in the left hemicolon or rectum and in the remaining 29.9% in the right hemicolon. According to the TNM-classification, most of the tumors were of pT3/pT4 stage (n = 485, 79.1%), with the minority of cancers being pT1/pT2 (n = 114, 18.6%). Among the malignancies under evaluation, 317 (51.7%) were pN0, 160 (26.1%) pN1, and 119 (19.4%) pN2 cases. Tumor-grade was G1 in 14 (2.3%) cases, G2 in 552 (90%) cases, and G3 in 33 (5.4%) cases. Furthermore 417 (68%) showed infiltrating tumor border configuration, while in the majority of cases (n = 425, 69.3%) vascular invasion was absent. Most of the tumors belonged to the microsatellite stable subgroup (n = 539, 87.9%). Within the follow-up period, median disease-specific survival was 58.4 months (range 0–152), while mean 5-year-survival was 47% (95% confidence interval: 43–51%). In Table 1, we summarized the clinico-pathological characteristics of the patients. We have to mention, that if the percentages do not add to 100% is due to missing values of same variables.

Association of clinicopathological features with SDF-1 expression and CD8 + T cell density

In Fig. 1, we illustrate representative pictures of low and high expression of SDF-1 in biopsies with high CD8 + T-cell infiltration in CRC. Furthermore, we created a table, that demonstrates the clinicopathological features under investigation and their relation to the four subgroups identified by SDF-1 expression and CD8 + T-cells density (SDF-1^{high}/CD8 + ^{high}, SDF-1^{high}/CD8 + ^{low}, SDF-1^{low}/CD8 + ^{high}, SDF-1^{low}/CD8 + ^{low}) as absolute numbers and percentages. Here we have to mention that during the procedure of TMA preparation, some of the punches may be lost.

The nature of tumor border (pushing vs. infiltrating) has been reported to impact on CRC prognosis [32]. Indeed, infiltrating tumor border, associated with poor survival, was detected with significantly ($p = 0.014$) higher frequency in CRC with SDF-1^{high}/CD8 + ^{low} than in tumors with SDF-1^{high}/CD8 + ^{high} infiltrate, thus suggesting an effect related to CD8 + T-cell infiltration. Furthermore, we observed that cases with SDF-1^{high}/CD8 + ^{high} were characterized by a significantly lower N-stage ($p < 0.001$) in comparison to the cases with SDF-1^{high}/CD8 + ^{low}. In contrast, we demonstrated that T stage, tumor grade and vascular invasion did not significantly differ in CRC with different immune environment profiles.

Interestingly, we detected a strong tendency towards statistical significance ($p = 0.051$) regarding the presence of PTL inflammation, which was higher in the group of SDF-1^{high}/CD8 + ^{high} in contrast to SDF-1^{high}/CD8 + ^{low}. This fact highlights the importance of the microenvironment and supports the hypothesis of an effective antigen-specific immune response. However, this can only be considered as a trend with a $p > 0.05$.

Spearman`s correlation analysis of SDF-1 and markers of the microenvironment

In order to better understand the microenvironment in the context of SDF-1 expression, we performed a Spearman`s correlation analysis with a panel of immune as well as cell signaling markers or growth factors on protein (TMA data; Table 3) and considered correlations above 0.35 to be relatively strong; correlations between 0.15 and 0.35 to be moderate, and those below 0.15 to be weak. Finally, SDF-1 tumor expression moderately correlated with CXCR4 tumor expression. SDF-1 expression by tumor-infiltrating immune cells (TIC) showed a strong correlation with CXCR4 positive TIC and a moderate correlation with CD8 T-cell density as well as pCXCR4 positive TIC on a protein level (Table 3).

Synergistic prognostic significance of SDF-1 tumor expression and CD8 + cell infiltration in the CRC microenvironment

The 5-years survival rates were significantly different depending on the nature of immune infiltrate [Table 2]. Most importantly, Kaplan–Meier plots clearly indicated that 5-year survival rate was significantly better in cases of CRC with high expression on SDF-1 and CD8 + T-cell infiltration compared to tumors showing a high infiltration of CD8 + T-cells only (66% (95%CI = 48–79%) vs 55% (95%CI = 45–64%); $p = 0.0004$; Fig. 2). Interestingly, there was a difference in long-term survival (> 5 years) between the test group (Fig. 2A) and the validation group (Fig. 2B). The long-term survival rate in the test group seems to be better in patients bearing tumors characterized by SDF-1^{low}/CD8 + ^{high} in comparison to the SDF-1^{high}/CD8 + ^{high}. However, the high expression of SDF-1 and CD8 + T-cell infiltration was significantly associated with a favorable prognosis in the validation group ($p = 0.016$).

Table 2

Association of SDF-1 + tumor expression and CD8 + low and high immune cell density with clinicopathological features in CRC (n = 613)*

		SDF-1^{high}/ CD8 + ^{high}		SDF-1^{high}/ CD8 + ^{low}		SDF-1^{low}/ CD8 + ^{high}		SDF-1^{low}/ CD8 + ^{low}		p-value
		N = 35	(100%)	N = 121	(100%)	N = 98	(100%)	N = 359	(100%)	
Age	years, mean ± SD	68.5	± 10.6	69.4	± 10.9	67.6	± 12.0	69.1	± 11.0	0.751
Tumor diameter	mm, mean ± SD	53.2	± 14.2	48.2	± 16.1	52.5	24.4	49.9	± 20.2	0.253
Gender	Female Male	25 10	71.4 28.6	60 61	49.6 50.4	52 46	53.1 46.9	189 170	52.6 47.4	0.146
Tumor location	Left-sided Right-sided	23 12	65.7 34.3	97 24	80.2 19.8	64 33	65.3 33.7	244 114	68 31.8	0.046
Histologic subtype	Mucinous Non-mucinous	2 33	5.7 94.3	3 118	2.5 97.5	3 95	3.1 96.9	22 337	6.1 93.9	0.292
pT stage	pT1-2 pT3-4	9 24	25.7 68.6	23 96	19.0 79.3	23 70	23.5 71.4	59 295	16.4 82.2	0.177
pN stage	pN0 pN1-2	23 12	65.7 34.3	61 57	50.4 42.1	67 27	68.4 27.6	166 183	46.2 51.0	< 0.001
Tumor grade	G1 G2 G3	0 32 1	0 91.4 2.9	2 112 5	1.0 92.6 4.1	2 79 12	2.0 80.6 12.2	10 329 15	2.8 91.6 4.2	0.097
Vascular invasion	Absent Present	26 7	74.3 20.0	88 31	72.7 25.6	67 26	68.4 26.5	244 110	68.0 30.6	0.559
Tumor border	Pushing Infiltrating	13 20	37.1 57.1	37 82	30.6 67.8	39 54	39.8 55.1	91 261	25.3 72.7	0.014
PTL inflammation	Absent Present	20 13	57.1 37.1	94 25	77.7 20.7	67 26	68.4 26.5	282 72	78.6 20.1	0.051
Microsatellite stability	Deficient Proficient	3 32	8.6 91.4	5 116	4.1 95.9	18 80	18.4 81.6	48 311	13.4 86.6	0.004
5-year survival rate	(95%CI)	0.66	(0.48–0.79)	0.40	(0.31–0.48)	0.55	(0.45–0.64)	0.45	(0.40–0.50)	0.0004
CXCR4 histoscore	mean ± SD	203.5	± 100.2	177.3	± 95.5	152.9	± 94.4	143.6	± 99.7	0.001

* Due to missing values of the same variables, percentages may not add to 100%. Variables are indicated as absolute numbers, %, median or range; age and tumor size were evaluated using the Kruskal-Wallis test. Gender, anatomical site, T stage, N stage, grade, vascular invasion, and tumor border configuration were analyzed using the χ^2 test. Survival analysis was performed using the Kaplan-Meier method.

		SDF-1^{high}/ CD8 + ^{high}		SDF-1^{high}/ CD8 + ^{low}		SDF-1^{low}/ CD8 + ^{high}		SDF-1^{low}/ CD8 + ^{low}		p-value
		N = 35	(100%)	N = 121	(100%)	N = 98	(100%)	N = 359	(100%)	
CXCR4 TIC	mean ± SD	50.7	± 62.3	53.5	± 130.7	121.9	± 208.8	70.7	± 145.8	0.016
pCXCR4 histoscore	mean ± SD	76.3	± 97.3	32.4	± 64.2	54.1	± 81.8	20.9	± 49.2	0.0002
pCXCR4 TIC	mean ± SD	6.2	± 9.1	4.3	± 10.4	7.6	± 14.0	4.0	± 6.8	0.001

* Due to missing values of the same variables, percentages may not add to 100%. Variables are indicated as absolute numbers, %, median or range; age and tumor size were evaluated using the Kruskal-Wallis test. Gender, anatomical site, T stage, N stage, grade, vascular invasion, and tumor border configuration were analyzed using the χ^2 test. Survival analysis was performed using the Kaplan-Meier method.

Table 3

Spearman's correlation analysis of SDF-1 protein expression with CXCR4, pCXCR4 and CD8

	SDF-1+ tumor expression	SDF-1+ TIC	CD8	CXCR4 tumor expression	pCXCR4 tumor expression	CXCR4+ TIC	pCXCR4 TIC
SDF-1+ tumor expression	1.000						
SDF-1+ TIC	-0.1143 0.0149	1.0000					
CD8	-0.0359 0.4455	0.1648 0.0004	1.0000				
CXCR4 tumor expression	0.1864 0.0001	0.0571 0.2252	0.1244 0.0080	1.0000			
pCXCR4 tumor expression	0.0296 0.5291	-0.0245 0.6035	0.2601 0.0000	0.2764 0.0000	1.0000		
CXCR4 + TIC	-0.0957 0.0418	0.3752 0.0000	0.2128 0.0000	0.1174 0.0124	0.1020 0.0300	1.0000	
pCXCR4 + TIC	-0.0806 0.0865	0.1572 0.0008	0.2034 0.0000	0.1131 0.0160	0.2725 0.0000	0.3706 0.0000	1.0000

Univariate and multivariate analysis of SDF-1 expression and CD8 + T-cell infiltration by tumor cells and tumor-infiltrating immune cells

Univariate Cox regression analysis revealed that the combination of high expression of SDF-1 and high CD8 + T cell infiltration is significantly associated with an increased overall survival (HR 0.34; 95%CI = 0.17–0.66; p = 0.002). Age, male gender, tumor grade, T-stage, N-stage, invasive margin and vascular invasion were all significantly associated with a poor prognosis in univariate analyses [Table 4].

Table 4

Uni- and multivariate Hazard Cox regression survival analysis considering the combination of both markers (n = 613 and n = 576, respectively)

	Univariate				Multivariate			
	HR	95%CI		p-value	HR	95%CI		p-value
Age	1.03	1.02	1.04	< 0.001	1.04	1.03	1.05	< 0.001
Gender (male vs female)	1.53	1.23	1.90	< 0.001	1.61	1.28	2.02	< 0.001
pT (high vs low)	3.42	2.32	5.04	< 0.001	2.41	1.57	3.71	< 0.001
pN (high vs low)	3.28	2.61	4.14	< 0.001	2.36	1.84	3.02	< 0.001
Grade (high vs low)	5.31	1.32	21.33	0.019	2.88	0.69	11.95	0.146
Vascular invasion	2.49	1.99	3.12	< 0.001	1.99	1.56	2.53	< 0.001
Invasive margin	1.92	1.48	2.50	< 0.001	1.41	1.06	1.88	0.017
MMR status	1.53	1.07	2.19	0.021	1.32	0.91	1.92	0.149
SDF-1^{high}/CD8 + ^{low}	1.09	0.84	1.42	0.526	1.18	0.89	1.55	0.247
SDF-1^{low}/CD8 + ^{high}	0.66	0.47	0.92	0.015	0.89	0.62	1.29	0.549
SDF-1^{high}/CD8 + ^{high}	0.34	0.17	0.66	0.002	0.45	0.23	0.89	0.021

Multivariate analyses showing Hazard Ratios and p-value for all CRCs (n = 576 less than 613 due to missing values) conferred by SDF-1 expression and CD8 + cell density, age, sex, tumor size, lymph node involvement, tumor grade, vascular invasion, tumor border configuration and microsatellite stability [33].

Figure 1: Examples of high SDF-1 expression (Picture A) and low SDF-1 expression in CRC biopsies with high CD8 + T-cell infiltration (x200 magnification)

In a multivariate Hazard Cox regression survival analysis the combined high expression of SDF-1 and high CD8 + T-cell infiltration in CRC succeeded to retain its role as an independent prognostic factor for overall survival (HR = 0.45, 95%CI: 0.23–0.89; p = 0.021). Moreover, we found that an increased age (HR = 1.04; 95%CI: 1.03–1.05; p < 0.001), male gender (HR = 1.61; 95%CI: 1.28–2.02; p < 0.001), a higher T-stage (HR = 2.41; 95%CI: 1.57–3.71; p = 0.001), N-stage (HR = 2.36; 95%CI = 1.84–3.02; p < 0.001), vascular invasion (HR = 1.99; 95%CI: 1.56–2.53; p < 0.001) and invasive margin (HR = 1.41; 95%CI: 1.06–1.88; p: 0.017) were independently associated with a poor prognosis [Table 4].

Discussion

A significant amount of studies showed that the infiltration of CRC by CD8 + T-cells represents a favorable prognostic factor for the clinical outcome. Since then, more and more scientists are exploring the role of the microenvironment and immune response in the development of malignancies [11–20]. A great variety of immunocompetent cells, cytokines, and chemokines are currently investigated. Our team has already tested some of these factors in previous studies [34–36]. Furthermore, a study of Pagès et al. from May 2018 showed that the Immunoscore could determine the risk of recurrence in patients with colon cancer [44].

Among other immune markers, several studies have investigated the expression of SDF-1 and its role in tumor immunobiology. However, these studies came to conflicting data. Some of them showed that the high expression of

SDF-1 is associated with reduced overall survival in patients with lung, pancreatic, and esophagus-gastric cancer. In contrast to these results, it was observed that in breast cancer, the high expression of SDF-1 was associated with increased overall survival [37–40]. In the case of colorectal cancer, there is a high heterogeneity across existing studies [26–29].

Some of the cells that produce the SDF-1 are the endothelial and bone marrow cells, mucosal epithelial cells, tumor cells, and T-lymphocytes [41]. SDF-1 expression is increased in tissues characterized by neo-angiogenesis and inflammation, supporting chemotactic gradients attracting immune cells. In a previous study of our team, we investigated the SDF-1-CXCR4 chemokine axis in cell trafficking as well as in tumor progression [42]. In that study, we showed that the activation of CXCR4, which is suggested by the presence of its phosphorylated form (pCXCR4), in CRC tumors and in infiltrating immune cells is associated with a significant favorable prognosis. According to our data, Stanisavljevic et al. have also shown that SDF-1 expression represents a favorable prognostic factor for disease-free survival in CRC [29].

In the presented study we explored if the prognostic role of SDF-1 expression in CRC depends on the tumor microenvironment, especially on CD8 + T-cell infiltration. Finally, we found that a better prognosis characterizes CRC showing SDF-1^{high} tumor expression and CD8 + ^{high} density in contrast to CRC having a SDF-1^{high}/CD8 + ^{low} or SDF-1^{low}/CD8 + ^{high} pattern, most likely due to an effective antigen-specific immune response. Our data provide novel insights into the prognostic relevance of the interaction between the innate and adaptive immune system in CRC microenvironment. Our results could be the reason to design studies that explore the combination of high expression of SDF-1 and CD8 + T-cells infiltration in other types of cancer (for example lung, pancreatic and esophagus-gastric cancer), where the already mentioned current data showed reduced overall survival in the single marker analysis of SDF-1. For instance, Roy et al. showed in an experimental model of pancreatic cancer that SDF-1 expression inhibited tumor growth and cancer cell metastasis formation through cell cycle arrest, resulting in increased overall survival, conflicting the existing data about SDF-1 expression and prognosis in pancreatic cancer [43].

Finally, we were able to identify a panel of immune markers with modest to strong correlation on a gene expression level ($r > 0.39$): CD163, MMP2, CD4, CD11b, CD45, CCL21, CD56, CD11c, CD18, MMP9, CD16A, IL-10, CCL7, CCL19, CCL11, CXCR4, FOXP3, CCL8, CCL23, CD14, CCL18, TGF-beta, CCL13 and T-bet and we found a strong upregulation of MMP-2, HLA-DR and CD14 in the SDF-1^{high}/CD8 + ^{high} group indicating possibly an effective antigen-specific immune response in this patient subgroup.

When it comes to limitations, we have to begin with the fact that our study is a retrospective one. Nevertheless, by using the data that emerge from extensive retrospective analyses, we may, in the future, be able to develop prospective studies. Secondary, TMA technology may fail to represent tumor tissue heterogeneity. Yet, the blocks included in our TMA were derived from tumor centers and included more than 50% of cancer cells. Additionally, the large number of individual CRC specimens (> 600) may partly compensate for the heterogeneity of the immune contexture in different tumor areas. Another point to consider is that by analyzing the Kaplan Meier curves of our study, we observe a difference in the long-term survival (> 5 years) rates between the testing and validation group. As the cohort was randomly split; it seems that in the validation group, CD8 + T cells appear to be the dominant marker. However, in the dichotomized cox regression, the combination still had a significantly lower HR. Yet, to overcome this criticism, a prospective validation study is planned on an independent cohort. Finally, the group investigated in this study includes CRC patients that were operated between 1985 and 1998. At that time, the use of neoadjuvant therapy was not part of the treatment of CRC.

Conclusions

Our data show for the first time that the prognostic role of SDF-1 depends on CD8 + T-cell density in CRC and that the combined SDF-1^{high}/CD8 + ^{high} expression represents an independent, favorable, prognostic condition in CRC, thereby shedding new light on the biological role of SDF-1 in colorectal cancer progression. With this side, we provide novel insights into the prognostic role of the immune microenvironment in CRC and raise a number of points, which might have a significant impact on clinical decision-making. To the best of our knowledge there are no data regarding the clinical significance of SDF-1 in the context of the immune microenvironment in CRC. Our finding might help to pave new avenues towards the development of novel treatment modalities by modifying the tumor immune microenvironment in CRC patients, especially in the context of personalized medicine.

Abbreviations

CD8
(cluster of differentiation 8)
CI
confidence intervals
CRC
colorectal cancer
FFPE
formalin-fixed, paraffin-embedded
H&E
hematoxylin-eosin
HR
hazard ratio
IHC
immunohistochemistry
MHC
major histocompatibility complex
SDF-1
Stromal cell-derived factor-1
TCGA
The Cancer Genome Atlas
TCR
T-cell receptor
TICs
tumor-infiltrating immune cells
TMA
tissue microarray
TNM
Tumor Node Metastasis

Declarations

Ethics approval and consent to participate

Our study had the approval of the local ethics committee (EKBB).

Consent for publication

Not applicable, since our manuscript does not contain data from any individual person.

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

R.D. and A.P. had idea and designed the study. A.T. and A.L. performed immunohistochemical analysis independently. Our experienced pathologist, L.M.T., validated the date independently. The Manuscript was written mainly by A.L.. All authors read, suggested corrections and approved the final manuscript.

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All authors have agreed to the submission and have participated in the study to a sufficient extent to be named as authors.

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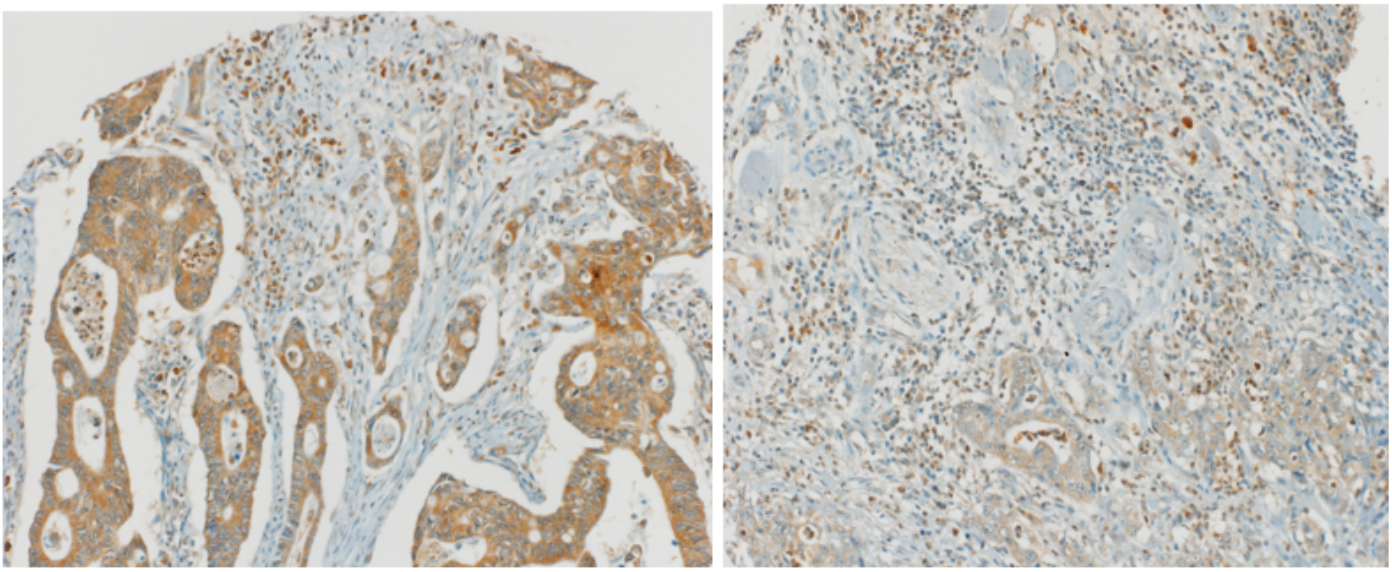
Figures

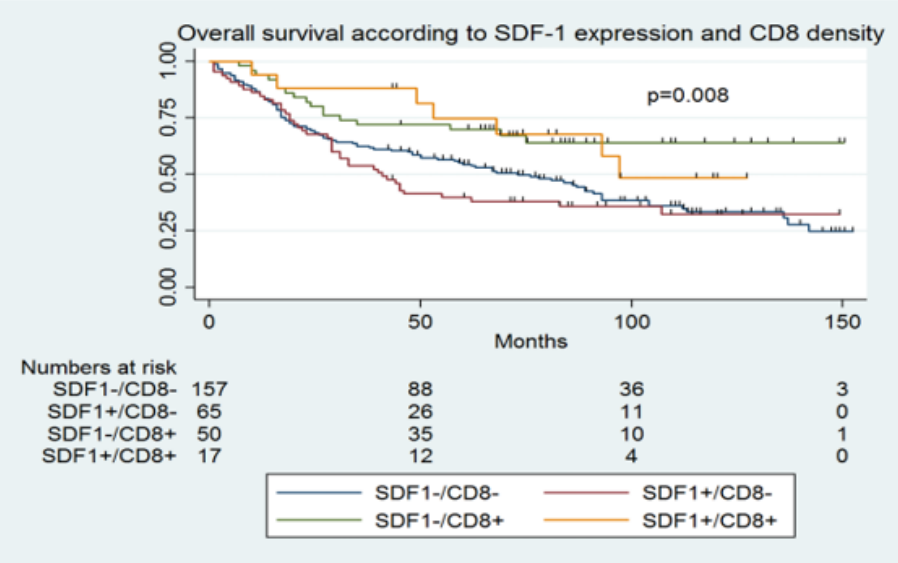


Picture A Picture B

Figure 1

Examples of high SDF-1 expression (Picture A) and low SDF-1 expression in CRC biopsies with high CD8+ T-cell infiltration (x200 magnification)

A



B

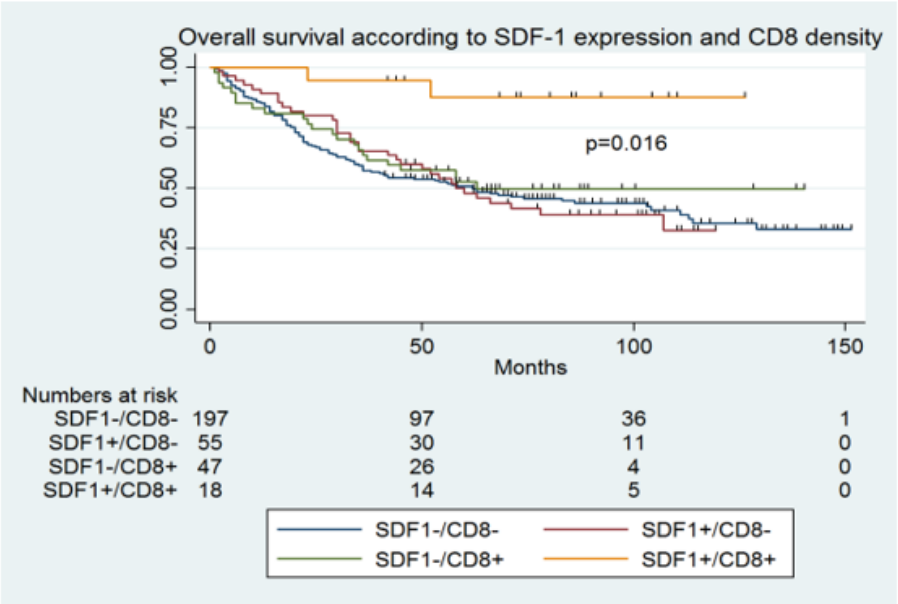


Figure 2

Overall-survival according to SDF-1 expression and CD8+ cell density in a test A (n=289) and validation B group (n=317)