High expression of SDC1 in stromal cells is associated with good prognosis in colorectal cancer

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We have previously reported that patients with high Syndecan 1 (SDC1) expression in colorectal cancer (CRC) cells have a favorable prognosis, and we also found that stromal cells showed upregulation of SDC1, but the clinical significance is unclear. The expression of SDC1 in the stroma cells was assessed by immunohistochemistry using a tissue microarray comprising representative cores from 513 CRC patients. The correlation between the expression of SDC1 in the stroma cells and the clinicopathological features of patients was analyzed. The data showed that the expression of SDC1 in the stroma cells was correlated with the degree of differentiation (P=0.012) and tumor location (up or down) (P=0.005). Also, CRCs patients with high expression of SDC1 in the stromal cells have a good prognosis (P=0.0369).

Accumulating evidence indicates that SDC1, whether in tumor cells or stromal cells, plays a tumor-suppressor role in CRCs. *Anti-Cancer Drugs* 34: 479–482 Copyright © 2022 The Author(s). Published by Wolters Kluwer Health, Inc.

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Introduction

On the cell surface, Syndecan 1 (SDC1) acts as a coreceptor to catalyze the binding of ligands with their respective signaling receptors [1]. Therefore, SDC1 plays an important role in cell-cell and cell-matrix connections [2]. Our previous study has showed that loss expression of SDC1 in the epithelium of colorectal cancer (CRC) was associated with poor prognosis, late clinical stage, poor tumor differentiation and lymph node metastasis [3].

Cancer-associated fibroblasts (CAFs) are involved in tumor metastasis and angiogenesis in different cancer types through their excess production of fibrosis, chemokines and different factors [4]. Studies have shown that SDC1-positive stromal cells that surround the invasive ductal breast carcinoma cells are spindle cells with myofibroblastic differentiation [5].

We also found a high expression of SDC1 in CRC stromal cells, although numerous studies have been conducted on the expression and significance of SDC1 in CRC tumor cells, the relationship between the SDC1 expression in the stroma cells and clinicopathological features and prognosis of CRC patients remains unclear.

Materials and methods

Colorectal cancer specimens

A total of 513 patients with CRC were recruited from the Department of Gastrointestinal Surgery, Affiliated Hospital of Jining Medical University, including 237 females and 276 males, with an average age of 61.5 years. All specimens were fixed with 4% paraformaldehyde and underwent routine treatment. All patients had long-term follow-up results. The study protocol was reviewed and approved by a local ethics committee, and all patients gave written consent to tissue samples.

Immunohistochemistry

Immunohistochemical staining of the SDC1 protein was performed using the streptavidin-peroxidase (S-P) method as previously described [3]. Briefly, each section was deparaffinized and rehydrated, and antigen repair was performed at 95 °C 1×EDTA for 15 mins. Endogenous peroxidase was blocked with 0.3% H_2O_2-methanol for 30 min. Incubate with normal serum at room temperature for 20 min. Then incubated with the primary antibody of anti-human SDC1 (Fuzhou Maixin Biotech Cat# MAB0200) at 4 °C overnight. The EnVision kit was used to detect antibody binding, and 3,3′-Diaminobenzidine Tetrahydrochloride (DAB) was used to incubate for 1 min to observe the immunostaining reaction. SDC1 expression was scored by two independent pathologists without prior knowledge of the patient's clinical information. Plasma cells were selected as the positive control, and the immune response was classified according to the

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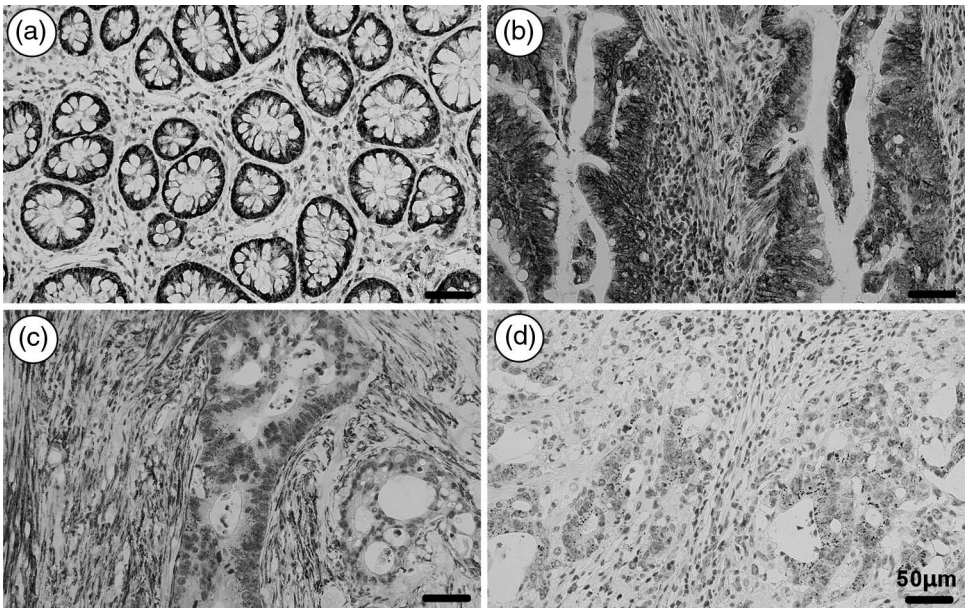
Fig. 1



Multiple expression pattern of SDC1 in CRC patients. (a) SDC1 was highly expressed in the normal colorectal epithelium. (b) SDC1 was highly expressed in colorectal cancer tumor cells, but poorly expressed in stromal fibroblasts. (c) SDC1 expression was low in colorectal cancer tumor cells but high in stromal fibroblasts. (d) SDC1 expression was low in both colorectal cancer tumor cells and stromal fibroblasts.

intensity. The expression intensity of SDC1 was defined as L when the expression intensity was 0–1 and H when the expression intensity was 2–3.

Statistical analysis

SPSS 13.0 software package (SPSS, Chicago, Illinois, USA) was used to analyze the correlation between SDC1 expression and clinicopathological features using the Pearson χ^2 test. Kaplan–Meier method was used to determine the survival probability, and GraphPad Prism software (Version 6, La Jolla, California, USA) performed a log-rank test on the data. Student's *T*-test and Mann–Whitney test were used for the differences between groups of quantitative variables (nonparametric text, data did not assume gaussian distribution). In the analysis, a P value < 0.05 was considered to be significantly correlated.

Results

Different expression patterns of the SDC1 in colorectal cancer tissues

In the present study, we examined the SDC1 expression pattern in normal colon tissue. The results showed that SDC1 is robustly expressed in colon glandular epithelium, but is not expressed in stromal cells (Fig. 1a). Interestingly, two distinct patterns were observed in CRC tissues with high SDC1 expression. One pattern revealed that SDC1 is expressed at high levels in tumor cells and is absent in stromal cells (Fig. 1b). In another pattern, SDC1 expression is low or nonexistent in tumor cells while highly expressed in adjacent stromal cells (Fig. 1c). Both expression patterns occur in tissues with overall high SDC1 expression, indicating mutually exclusive expression patterns of SDC1 in tumor cells and adjacent stromal cells. Also, we identified the absent expression pattern of SDC1 in CRC tissues with low or no expression in both tumor epithelium and stromal mesenchymal cells (Fig. 1d). These data indicated the existence of multiple expression patterns of SDC1 in CRC patients.

The relationship between the expression of SDC1 in stromal cells and the clinicopathological characteristics of colorectal cancers

Further immunohistochemical analysis and assessment of SDC1 expression were performed to explore potential clinical implications in CRC patients. A total of 488 cases were finally obtained, with exfoliation tissue excluded during the staining procedure. In statistical analysis, the expression intensity of SDC1 was defined as L (low expression) when the expression intensity was 0–1 (301/488, 61.68%) and H (high expression) when the expression intensity was 2–3 (187/488, 38.32%). Next, the relationship between the expression intensity of SDC1 and the clinicopathological characteristics of patients was evaluated. Our results showed that SDC1 expression was not correlated with gender ($P=0.888$), age ($P=0.313$), Ts stage ($P=0.677$), Ns stage ($P=0.927$), Ms stage ($P=0.938$), left and right position ($P=0.179$) and tumor size ($P=0.838$). However, it was closely related to the degree of tumor differentiation ($P=0.012$) and the upper and lower location ($P=0.005$) (Table 1).

SDC1 expression in the stroma cells was correlated with a good prognosis

We defined stroma cells without SDC1 staining as '–' and any intensity staining as '+'. Analysis showed that patients with SDC1 '+' had better overall survival compared with patients assessed as SDC1'–' ($P = 0.0369$) (Fig. 2). The above data suggest that SDC1 expression in stromal cells plays an important role in CRC patient prognosis, which is consistent with the high expression level of SDC1 in the tumor cells [3].

Discussion

Based on a large number of tissue specimens, our study provides evidence for the clinical significance of upregulated SDC1 expression in CRC mesenchymal fibroblasts, and further verifies the relationship between upregulated SDC1 expression in stromal cells and clinicopathological features and prognosis of patients. Previously, we have reported that loss of SDC1 expression in CRC cells is associated with poor prognosis for CRC patients [3,6]. In this study, our results demonstrated that SDC1 expression by stromal fibroblasts in CRC is significantly associated with a favorable prognosis.

Expression of SDC1 in the stroma cells has been found in a variety of different tumors [5]. For example, SDC1 expression by stromal fibroblasts is frequently observed in invasive breast cancer. The extracellular domain of SDC1 bears heparan sulphate chains, which play an important role in the arrangement of the Extracellular matrix (ECM). During ECM production, SDC1 may regulate fibronectin fibrillogenesis and change cell morphology through integrin, thereby mediating the arrangement of ECM, and ultimately leading to targeted invasion and metastasis of breast cancer [7]. However, in CRC, we found that patients with positive SDC1 staining in stroma cells have a relatively good prognosis, which is consistent with the SDC1 high expression in the tumor cells [3].

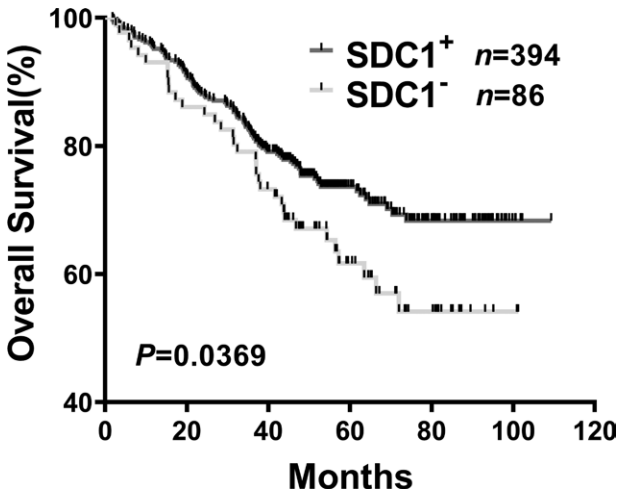
CAF are a heterogeneous group of cells known to be a key component of the tumor microenvironment [4]. It is generally thought that CAFs are recruited from mesenchymal cells in the bone marrow. CAF can secrete Fibroblast Activation Protein Alpha (FAP), which plays a role in cancer-associated fibroblasts in CRC. FAP expression is a prognostic marker in CRC, and higher expression of FAP is associated with a favorable prognosis [4]. CAF can also express a disintegrin and metalloproteinases, which play a role in promoting fibrous connective tissue and participating in the metastasis of CRC. CAF regulates the ability of cancer cells to locally invade or form secondary tumors at distant sites of metastasis. Studies have shown that CAF has the ability to restrict tumor growth and promote the progression of apoptosis of cancer cells [4]. Compared with normal fibroblasts, CAF also produces a variety of ECM interpretation enzymes, releasing a variety of angiogenic factors that bind to receptors on vascular endothelial cells [4]. Yet in our study, the SDC1-positive fibroblasts appear to play a tumor-suppressor role in CRCs, and the SDC1-negative fibroblasts may be CAF. Thus, investigating the protein expression of stromal cells around tumors may help to distinguish between CAF or non-CAF in different tumors.

Table 1 The correlation between SDC1 expression in stromal cells and clinical characteristics of colorectal cancer patients, 488 cases

	SDC1 mesenchymal (L) case (%)	SDC1 mesenchymal (H) case (%)		X^2	*P* value
Gender					
Female	142 (62)	87 (38)	229	0.02	0.888
Male	159 (61)	100 (39)	259		
Age, years					
<60.5	126 (59)	87 (41)	213	1.02	0.313
>60.5	175 (64)	100 (36)	275		
Tumor size, cm					
≤4	192 (61)	121 (39)	313	0.042	0.838
>4	109 (62)	66 (38)	175		
Ts					
1–2	46 (64)	26 (36)	72	0.174	0.677
3–4	255 (61)	161 (39)	416		
Ns					
N_0	183 (61)	115 (39)	298	0.152	0.927
N_1	69 (61)	44 (3)	113		
N_2	49 (64)	28 (36)	77		
Ms					
M_0	298 (61)	185 (39)	483	0.006	0.938
M_1	3 (60)	2 (40)	5		
Location					
Left	36 (73)	13 (27)	49	3.444	0.179
Right	92 (64)	53 (36)	145		
Location					
Up	27 (82)	6 (18)	33	7.941	0.005
Down	206 (57)	158 (43)	364		
Histological grade					
Well	156 (68)	75 (32)	231	6.346	0.012
Moderately or poorly	135 (56)	105 (44)	240		

H, high expression; L, low expression; χ^2,chi-square test.

Fig. 2



Survival curves of different groups. Overall survival of CRC patients stratified in accordance with SDC1 protein expression in stromal cells. CRC, colorectal cancer.

In pancreatic cancer, SDC1 is located on the cell surface and regulates macropinocytosis, which is an important pathway to promote the growth and metabolism of pancreatic cancer cells [8]. Extracellular vesicles play an important role in the occurrence and development of cancer [9]. SDC1 can be shed into plasma as a soluble component, vesicle localization of SDC1 was confirmed by a combination of SDC1 and vesicle labeling, and plasma vesicle SDC1 can be used as a diagnostic tool for differentiating low-grade gliomas from high-grade gliomas [10]. Tumor cells and the surrounding stromal cells are relatively close, and appear as a repulsive expression pattern. Whether SDC1 shed by tumor cells is transferred into stromal cells? As the same cells that stain positive for SDC1 protein can also be detected at the mRNA level, it is most likely that the proteins are generated by the stromal cells themselves [5]. The SDC1 expression in breast cancer is also presented as the pattern mentioned before: low expression of tumor cells and high expression in around stromal cells [7]. So, this mutual exclusion may be widespread. But the mechanisms that promote the expression of SDC1 in tumor stromal fibroblasts still need to be further studied.

Conclusion

In conclusion, high expression of SDC1 in stromal cells is associated with good prognosis in CRC.

Acknowledgements

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Conflicts of interest

There are no conflicts of interest.

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