·Basic Research·

Expression and clinical significance of syndecan-1 mRNA and HPA-1 mRNA in colorectal cancer detected with real-time fluorescent quantitative polymerase chain reaction

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[**Abstract**] **Background and Objective:** Invasion and metastasis are the most common causes of mortality for patients with colorectal neoplasms, and blocking invasion and metastasis in a timely fashion has become a hot research focus. We investigated the expression of the messenger RNA of Syndecan-1 and HPA-1 in colorectal cancer, and their correlation with invasion and metastasis. **Methods:** Real-time fluorescent quantitative polymerase chain reaction (PCR) was used to detect the expression of Syndecan-1 and HPA-1 in specimens from 49 patients with colorectal cancer, 49 paired adjacent colorectal neoplasms (2 cm from the carcinoma), and 49 surgical margins of paired normal colorectal mucosa tissue (5 cm from the carcinoma), to analyze their correlation with clinicopathologic characteristics of colorectal neoplasm. **Results:** The expression of HPA-1 mRNA was significantly higher in colorectal cancer (40.56 ± 11.75) than that in the paired adjacent colorectal neoplasms (18.28 ± 11.33) and normal colorectal mucosa tissue (10.80 ± 10.20) (all $P < 0.001$). The expression of HPA-1 mRNA was significantly higher in paired adjacent colorectal neoplasms than that in normal colorectal mucosa ($P < 0.05$). The expression of Syndecan-1 mRNA was significantly higher in normal colorectal mucosa (61.21 ± 12.96) than in the paired adjacent mucosa (14.35 ± 11.06) or colorectal cancer (10.12 ± 8.58) (all $P < 0.001$). The expression of Syndecan-1 mRNA was significantly higher in the paired adjacent mucosa than that in colorectal cancer ($P < 0.05$). The decreased expression of Syndecan-1 mRNA and the increased expression of HPA-1 were closely associated with the degree of differentiation, the depth of infiltration, lymph node metastasis, vessel metastasis, and TNM staging of colorectal cancer (all $P < 0.05$). Spearman rank correlation analysis demonstrated a significant correlation between Syndecan-1 and HPA-1 ($r = -0.405$, $P < 0.05$). **Conclusions:** The expression of Syndecan-1 mRNA was significantly highest in normal colorectal mucosa and the expression of HPA-1 mRNA was significantly highest in colorectal cancer. At the same time, the decreased expression of Syndecan-1 mRNA and the increased expression of HPA-1 mRNA can promote the invasion and metastasis of colorectal cancer. The determination of Syndecan-1 and HPA-1 may be of value in the treatment as well as in the prognosis of patients with colorectal cancer.

Key words: Syndecan-1, HPA-1, colorectal neoplasm, invasion and metastasis, real-time fluorescent quantitative reverse transcription polymerase chain reaction, RT-PCR

Colon cancer is a common gastrointestinal cancer in China. In recent years, the mortality rate caused by colon cancer showed an upward trend, with invasion and metastasis as the main reasons. Thus, looking for factors triggering tumor cell invasion and metastasis, and effectively blocking them, are a hot research focus and a difficult problem in current research. In the processes of tumor invasion and metastasis, heparan sulfate proteoglycan (HPSG) and its soluble enzyme heparanase (HPA-1) play an important role. Syndecan-1 is a group of transmembrane heparan sulfate proteoglycan (HPSG), and involved in adhesion between the extracellular matrix and cells, the reduction or absence of its expression can promote tumor growth, invasion, and metastasis [1]. HPA-1 is a recently discovered important enzyme functioning in tumors and is the only glucuronic acid incision enzyme that can degrade HPSG in vivo. HPA-1 is capable of degrading HPSG in certain areas, therefore promotes tumor invasion and metastasis [2].

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However, there are rare reports focusing on the Syndecan-1 and HPA-1 expression levels in colon cancer and their relationship to invasion and metastasis of colon cancer. In this study, sensitive real-time fluorescence quantitative PCR was used to detect Syndecan-1 and HPA-1 gene expression in different colon tissue, and the relationship between their expression and clinicopathologic parameters were analyzed to further clarify the relationship between invasion and metastasis of colon cancer and the two genes, in the hopes of providing new solutions for diagnosis and treatment of patients with colon cancer in the future.

Materials and methods

Specimens

Specimens from 49 patients were surgically resected from May 2008 to June 2009 in the Department of General Surgery, Qingdao Municipal Hospital, and these specimens were confirmed by more than 2 senior doctors from the Department of Pathology, that were not subjected to any chemotherapy or radiotherapy before surgery, and all of them included partial lymph node dissection.

All 49 specimens consisted of primary colon carcinoma, paired adjacent noncancerous tissue (2 cm away from the cancer edge) and matching normal tissue from the surgical margins (more than 5 cm from the edge of the cancer). The resected specimens were immediately placed in a -70℃ refrigerator for real-time fluorescent quantitative reverse transcription-polymerase chain reaction (RT-PCR).

Sources and cultivation of cell lines

Human colon adenocarcinoma cell line SW480, a poorly differentiated colon adenocarcinoma cell line, was provided by the Wuhan University. Cells were cultured by RPMI-1640 medium plus calf serum, in an incubator at 37℃ and 5% CO_2 with saturated humidity as the conventional condition and without any antibiotics.

Main reagents and instruments

We used the Simply P Total RNA Extraction Kit (Hangzhou Bor Technology Co., Ltd.), an RT-PCR Kit (TaKaRa Inc.), and a Quantitative PCR reagent (Rotor-gene 3000 Real-Time PCR System).

Experimental methods

Fluorescence quantitative PCR detection of Syndecan-1 and HPA-1 expression in colon cancer tissue RNA extraction was under strict instructions of the Simply P Total RNA extraction kit. A total of A_{260nm}/A_{280nm} of attained RNA was over 1.7, meeting the purity requirements. The integrity of the RNA was confirmed by agarose gel electrophoresis. The total RNA extracted carried out reverse transcription and PCR amplification according to the instructions of the RT-PCR reaction kit. According to the corresponding sequence in the Gene Bank, primers were designed with Primer Premier 5.0 software and produced by Shanghai Sangon Biological Engineering Technology & Services Co., Ltd (Table 1). The PCR reaction system contained 10 μL SYBR Premix Ex *Taq*™, 0.8 μL of each upstream and downstream primers, 2 μL template cDNA, 6.4 μL ddH_2OL. PCR cycle reaction conditions were 95℃ for 30 s, 95℃ for 5 s, 60℃ for 45 s, with a total of 40 cycles. After PCR, 4 μL of the amplified products were pipetted to run a 2% agarose gel electrophoresis, and then were stained with ethidium bromide for UV observation, all of which was to further validate the specificity of the primers.

Table 1 Sequences of the primers used in real-time flourescent quantitative polymerase chain reaction (RT-PCR)

Gene	Sequence	Fragment length (bp)
Syndecan-1	5′- GGGACTCAGCCTTCAGACAG -3′	128
	5′- CTCGTCAATTTCCAGGAGGA -3′	
HPA-1	5′- CCTTGCCACCTTTAATGGAA -3′	105
	5′-AAGCAGCAACTTTGGCATTT-3′	
GAPDH	5′-TCA TGG GTG TGA ACC ATG AGA A-3′	146
	5′-GGC ATG GAC TGT GGT CAT GAG-3′	

Criteria to determine results The radian of the fluorescence quantitative amplification curve for positively expressed target genes showed an S-type with a specific electrophoresis band, and that of negative samples showed irregular wavy lines without a specific band. △Ct equaled the difference between the Sample Ct Mean value and the Ct Mean value of internal controls, and △△Ct equaled the difference between △Ct and D-value, which equaled the difference between the Ct Mean value of random negative control samples and the mean Ct of the expression of internal controls of this sample. A $2^{-\triangle\triangle\alpha}$ value stood for the relevant level of mRNA expression of a sample. If the Ct Mean value was less than 30, the sample was defined as negative in the expression of the target gene.

Statistical analysis

SPSS16.0 software package was used for statistical analysis, and the expression levels of syndecan-1 mRNA and HPA-1 mRNA were indicated as mean ± standard deviation. A *t*-test was applied to compare measurements between groups and χ^2 test to compare of the counts. The correlation analysis of syndecan-1 and HPA-1 in colon cancer tissue was analyzed by Spearman rank correlation analysis. $P < 0.05$ signified statistical significance.

Results

HPA-1 and Syndecan-1 expressions in different tissues of the colon

Positive expression of HPA-1 mRNA was found in the human colon cancer cell line SW480. Expression of HPA-1 mRNA in colon cancer tissue (40.56 ± 11.75) was significantly higher than that in both adjacent tissue (18.28 ± 11.33) and normal tissue (10.80 ± 10.20). HPA-1 mRNA expression in carcinoma-adjacent tissue was significantly higher than in normal tissue ($P < 0.05$) (Figure 1).

No syndecan-1 mRNA expression was found in SW480. Expression of syndecan-1 mRNA (61.21 ± 12.96) was significantly higher in normal colon tissue than in either adjacent tissue (14.35 ± 11.06) or carcinoma tissue (10.12 ± 8.58). Its expression was

also significantly higher in cancer-adjacent tissue than in cancer tissue ($P < 0.05$) (Figure 1).

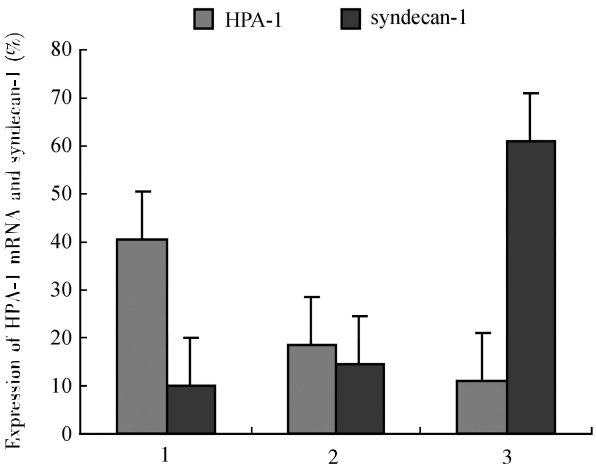

Figure 1 Expression of HPA-1 mRNA and syndecan-1 mRNA in various colorectal tissues

1, colorectal cancer; 2, paired adjacent colorectal neoplasm; 3, paired normal colorectal mucosa tissue.

Curves of fluorescence quantitative amplification of expression of HPA-1 mRNA and Syndecan-1 mRNA

In different colon cancer tissue with expression of HPA-1 mRNA and Syndecan-1 mRNA, the curves of fluorescence quantitative amplification showed a typical S-curve (Figures 2). The melting curves were single-peaked curves, excluding non-pecific amplification or the appearance of a primer dimer (Figures 3). Specific electrophoresis bands were found in these positive samples (Figure 4). While in negative samples, with Ct > 30, there were no peaks but maybe slight rises in the amplification curves; they were irregular wavy lines. In this study, there were 49 cases in the tumor group, and for the expression of syndecan-1 mRNA, 5 of these cases had Ct ⩽ 30 and 44 cases had Ct > 30. For HPA-1 mRNA expression, 42 cases had Ct ⩽ 30 and 7 cases had Ct > 30. In the 49 cases of the cancer-adjacent tissue group, for syndecan-1 mRNA expression, 30 cases had Ct ⩽ 30 and 19 cases had Ct > 30. For HPA-1 mRNA expression, 29 cases had Ct ⩽ 30 and 20 cases had Ct > 30. In the 49 cases in the normal group, for the expression of syndecan-1 mRNA, 45 had Ct ⩽ 30 and 4 cases had Ct > 30 and for HPA-1 mRNA expression, 3 cases had Ct ⩽ 30 and 46 cases had Ct > 30.

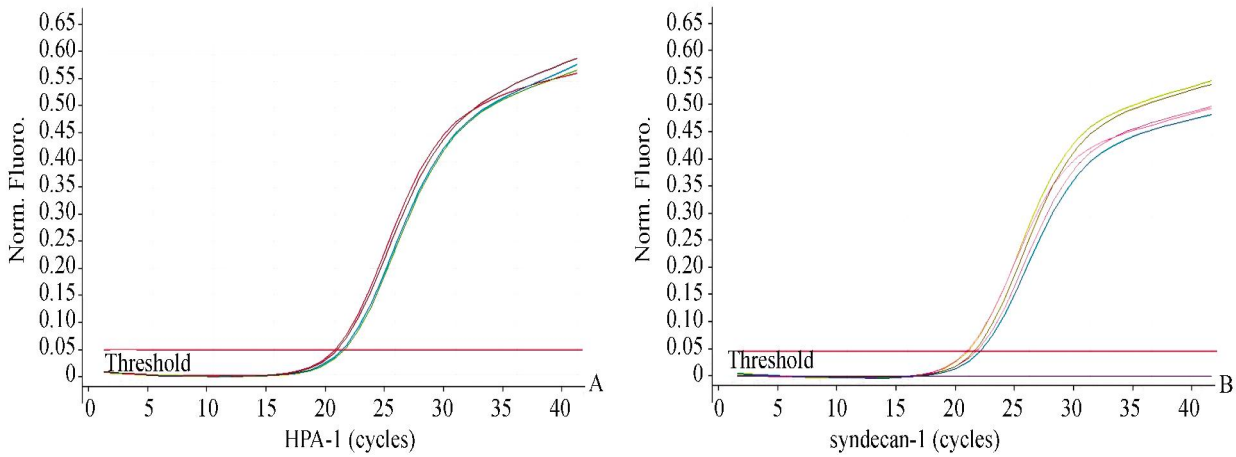

Figure 2 Representative results of real-time flourescent quantitative polymerase chain reaction (RT-PCR) amplification plots for HPA-1 mRNA

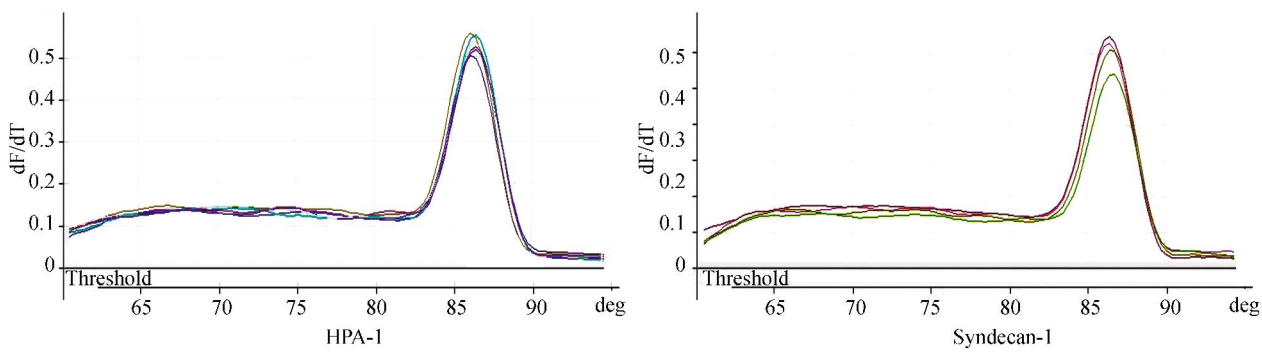

Figure 3 Representative results of RT-PCR amplification plots for syndecan-1 mRNA

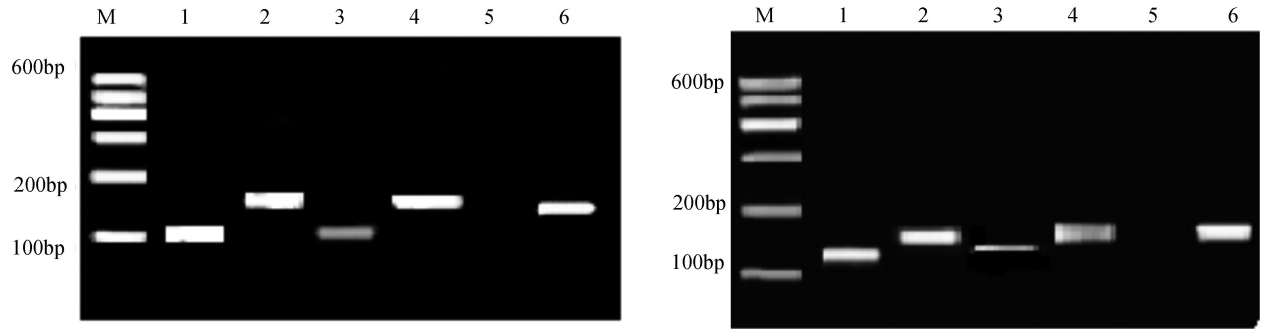

Figure 6 Agarose gel electrophoresis of the sydencan-1 and HPA-1 PCR production in colorectal carcinoma

Left: Lane 1, 3, 5, the expression of HPA–1 mRNA (105 bp) in the carcinoma, adjacent mucosa colorectal, and normal colorectal mucosa; lane 2, 4, 6, the expression of GAPDH mRNA (146 bp) in the carcinoma, adjacent colorectal mucosa, and normal colorectal mucosa.

Right: Lane M, marker 1 (600 bp); lane M, marker 1 (600 bp); lanes 1, 3, 5, the expression of syndecan-1 mRNA (128 bp) in the normal colorectal mucosa, adjacent colorectal mucosa, and carcinoma; lanes 2, 4, 6, the expression of GAPDH mRNA (146bp) in the normal colorectal mucosa, adjacent colorectal mucosa, and carcinoma.

Expression of HPA-1 and Syndecan-1 in colon cancer and their relationships with clinicopathologic parameters

A total of 49 cases of colon cancer were classified according to tumor diameter, degree of differentiation, depth of invasion, lymph node metastasis, hematogenous metastasis, and tumor stage, and the expression of syndecan-1 mRNA and the HPA-1 mRNA was detected. Table 2 shows that the expression of syndecan-1 mRNA in colon cancer was negatively correlated with tumor diameter, degree of differentiation, depth of invasion, lymph node metastasis, hematogenous metastasis, and tumor stage, and the expression of HPA-1 mRNA was positively correlated with these parameters. The differences were significant ($P < 0.05$).

Table 2 Correlation of HPA-1 and Syndecan-1 expression with clinicopathologic features in colorectal cancer

Clinicopathologic features	Patient No.	HPA-1 mRNA		Syndecan-1 mRNA	
		Expression	*P*	Expression	*P*
Tumor size					
< 5 cm	26	33.83 ± 21.35	0.036	13.49 ± 6.60	0.001
⩾ 5 cm	23	48.16 ± 25.14		6.31 ± 6.34	
Differentiation					
Well	18	29.64 ± 24.93	0.032	13.37 ± 6.01	0.004
Poor	31	46.91 ± 27.24		8.23 ± 5.48	
Depth of infiltration					
T1, T2	24	35.62 ± 15.09	0.041	12.43 ± 4.30	0.003
T3, T4	25	45.29 ± 17.05		7.90 ± 5.63	
Lymph node metastasis					
No	23	28.18 ± 29.76	0.005	12.56 ± 3.96	0.001
Yes	26	51.49 ± 25.19		7.96 ± 4.64	
Vessel invasion					
No	19	33.35 ± 18.40	0.034	14.91 ± 4.44	0.000
Yes	30	45.12 ± 18.39		7.08 ± 5.89	
TNM stage					
I+II	20	30.19 ± 21.92	0.015	10.94 ± 2.12	0.037
III+IV	29	46.48 ± 22.39		9.55 ± 2.30	

Correlations between syndecan-1 and HPA-1 expression in colon cancer tissue

The results of Spearman rank correlation showed that the expressions of syndecan-1 and HPA-1 in colon cancer tissue were negatively correlated ($r = -0.405$, $P = 0.005$).

Discussion

Syndecan-1, a member of the transmembrane heparan sulfate proteoglycan family, composed of 5 exons, is a newly discovered adhesion molecule expressed mainly in epithelial cells, and it also expresses in fibroblasts and plasma cells. Through their heparan sulfate chains, this family can combine covalently with a variety of extracellular ligands, thus to regulate growth, differentiation, adhesion, and migration behaviors of tissues. By promoting cell-matrix and cell-cell adhesion to prevent cell detachment and invasion, syndecan-1 can limit the malignant phenotype of tumors. It has been found that, syndecan-1

expression was reduced in a majority of malignant tumors [3]. Studies by Zhou *et al.*[4] have shown that in normal colorectal mucosa, syndecan-1 expression was strongly positive, however, in colorectal cancer tissue, it was missing or existed at a reduced level. With the in-depth studies, it was found that when normal cells become malignant, the cell surface expression of syndecan-1 decreased significantly, or was even missing, which might lead to the loss of the function of contact inhibition of growth, then a large number of tumor cells proliferated, with strong active invasion and metastasis. According to domestic and international studies [5-6], syndecan-1 expression is reduced in digestive tract cancer, suggesting that syndecan-1 deficiencies may be related to tumorigenesis, invasion, and metastasis.

In this study, we used sensitive real-time quantitative RT-PCR combined with the melting curve analysis, and we found that the level of syndecan-1 mRNA expression in normal colon tissue was significantly higher than in the paired-adjacent tissue and cancer tissue, and cancer tissue had the weakest expression level. Those differences were statistically significant ($P < 0.05$). No syndecan-1 mRNA expression was detected in human colon cancer cell line SW480. With increased in tumor malignancy, syndecan-1 expression gradually decreased. In tumor tissue that is poorly differentiated, strongly invasive, or with distant metastasis with advanced TNM staging, the expression of syndecan-1 reduced or even was absent. This was consistent with the results of Fujiya *et al.*[7] study of the abnormal expression of syndecan-1 in colorectal adenoma, tumors, and invasive cancer. Our results provided further evidence that syndecan-1 was involved in tumor invasion and metastasis processes. In addition to its relationship with malignancies of the tumor, the level of syndecan-1 expression was also associated with the prognosis of patients with colon cancer to certain degree. Ru *et al.* [8] showed that the 5-year survival rate for patients with gastric cancer with reduced syndecan-1 expression was significantly lower than those with high expression, suggesting that the abnormal expression of this gene may be a better indicator for prognosis, which needs to be further explored. In summary, syndecan-1 is involved in the processes of tumor invasion and metastasis and it may become an important indicator for tumor diagnosis, development, and prognosis.

HPA-1 (heparanase) is the only nucleoside endonuclease with the capacity to crack heparan sulfate (HS) and side chains of heparan sulfate proteoglycan (HSPG) in extracellular matrix. It is able cleave the extracellular matrix and the basement membrane, thus promoting tumor cell invasion and metastasis. In human normal tissue, HPA-1 is mainly distributed in the placenta, spleen, lymph nodes, thymus and other immune organs, and cells, and does not show up in other non-immune organs such as the heart, lungs, liver, kidney, or pancreas. However, in a variety of human malignant tumors it expresses at a elevated level. Colon, stomach, liver, esophageal, and other gastrointestinal tumors express high levels of HPA-1, and its expression is especially high in a number of malignant tumors with strong invasive and metatastic abilities[9]. Using RT-PCR, Wang *et al.* [10] analyzed the expression of HPA in metastatic and nonmetastatic gastric cancer, and normal gastric tissue, and found that among 30 gastric cancer samples, the positive rate of HPA-1 expression in primary foci were 47% and metatastic foci were 95%, but no expression was found in epithelial cells of normal tissue, showing that HPA-1 expressed at a higher level in gastric cancer metastatic foci than in the primary tumors. Yasu *et al.*[11] found that high expressions of heparanase were strongly related to the degree of malignancy in colon cancer. Altogether, HPA-1 plays a very important role in tumor invasion and metastasis. By using immunohistochemistry, Liu *et al.*[12] showed that, in normal breast tissue, HPA-1 expression was negative in breast cancer, and it increased with the level of tumor malignancy. In poorly differentiated tumors and highly invasive tissue, its expression was positive and increased significantly with the diameter of the primary foci.

In this study, with combination of highly sensitive and specific fluorescence quantitative PCR and the corresponding solubility curve analysis, it was found that human colon cancer cell line SW480 expressed HPA-1 m RNA, and the expression of HPA-1 mRNA in colon cancer tissue was higher than in paired adjacent tissue (2 cm from the cancer edge) and matching normal tissue surgical margins (more than 5 cm from the cancer edge). Moreover, HPA-1 mRNA expression in cancer-adjacent tissue was significantly higher than in normal tissue ($P < 0.05$).

In the correlation analysis between HPA-1 mRNA and clinicopathologic parameters, the expression of HPA-1 mRNA in colon cancer had a positive correlation with tumor diameter, degree of differentiation, depth of invasion, lymph node metastasis, hematogenous metastasis, and tumor stage. HPA-1 was involved in tumor invasion and metastasis, and the expression of HPA-1 mRNA gradually increased as the degree of malignancy increased. Tang *et al.* [13] from Japan detected the expression of HPA-1 in gastric cancer and evaluated its correlation with clinicopathologic parameters by in situ hybridization. The difference was found to be more significant in advanced tumors than in low-stage tumors and also more significant in large-sized tumors than in small-sized tumors. Further, the prognosis was worse in patients with positive expressions of HPA-1. As Sato *et al.*[14] reported, 5-year survival rates of patients with colorectal cancer with HPA-negative and HPA-positive expression were 84.6% and 47.7%, respectively, and HPA was suggested to be closely related to 5-year survival rates of patients with colorectal cancer and an independent risk factor of prognosis. For the close relationship between HPA-1 and tumor prognosis, many researchers suggest that HPA-1 is a new target for cancer treatment, and offer new ideas and methods about HPA-1 in tumor diagnosis, treatment, prognosis evaluation, anticancer drug screening, and so on.

In conclusion, this study explored syndecan-1 and HPA-1 expression in different colon tissue by fluorescence quantitative PCR, and clinicopathologic analysis was performed. The results confirmed that the two markers were involved in the occurrence and development of colon tumors, and may be related to invasion and metastasis of tumor cells. The expression of the two markers had negative correlations ($r = -0.405$, $P < 0.05$). Therefore, diagnosis, evaluation of metastatic potential, and prognosis would benefit from the combined detection of syndecan-1 and HPA-1 for

patients with colon cancer, which may provide a new method of treatment.

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