Expression and Significance of Protein S100A4 in Colorectal Carcinoma

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Abstract **Objective** To investigate the expression and significance of protein S100A4 in colorectal carcinoma. **Methods** The S−P immunohistochemical method is used to detect the expression of S100A4 protein in 68 cases colorectal carcinoma and 20 cases normal colorectal tissues. **Results** The positive expression of S100A4 protein was 61.8% in 68 colorectal carcinoma tissues, but the expression were not observed in all 20 normal colorectal mucosal tissues, there was significant difference between the expression of S100A4 protein (P=0.003) in two groups. There were no significant differences in the expression of S100A4 protein among the age, sex, tumor location, growth style, histological type, differentiation degree of the cancers respectively (P>0.05), but there were significant differences in S100A4 protein expression among the different Dukes stages, with or without lymph node metastasis, and the different survival time (P<0.05), and higher expression of S100A4 protein occurs in the cases with the later stage, lymph node metastasis and shorter survival tim. **Conclusion** Protein S100A4 is closely related with colorectal carcinoma invasion and metastasis; Protein S100A4 might be an important predictor of the clinicopathologic features and prognosis of colorectal carcinoma.

Key words Protein S100A4; Colorectal carcinoma; Immunohistochemistry

Colorectal carcinoma is a common malignant tumor in alimentary tract, its incidence rate has continuously increased in recent twenty years with the change of dietary structure lifestyle and environment in the most countries of the world. It ranks the first in spectrum of malignances in west development countries, while, third to fifth in our country. Invasion and metastasis are two important biological characteristics of malignant tumor. The five−year survival rate of colorectal carcinoma is closely related to the invasive and metastatic ability according to Dukes stage. So, it becomes the hotspot to search for the marker that can reflect the invasive and metastatic potential of colorectal carcinoma cells. S100A4 Protein screened by cDNA library serial analysis of gene expression(SAGE) is an important factor closely related to pathological process such as invasion and metastasis [1]. Our project detect the expression of S100A4 protein in 68 cases with colorectal carcinoma and 20 cases normal colorectal tissues using the S−P immunohistochemical method to investigate its effects on tumorigenesis and development.

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MATERIALS AND METHODS

Cases

Sixty−eight paraffin−embeded samples of colorectal carcinoma diagnosed by pathology were collected in the general surgery of the People´s Hospital of LinYi, Shan Dong Province from Jan. 2004 to Jan. 2005, their ages ranged from 21 to 81 years olds with a mean age of 55.5 years old. Of all the 68 cases, 38 cases were males and 32 cases were females, and 38 cases were colon carcinoma and 30 cases were rectal carcinoma. None of patients underwent radiotherapy or chemotherapy pre−operation or during the operation. All the clinical and pathological data collected, and all the patients were followed up for more than 24 months. 20 normal colorectal mucosal samples which come from the patients with colorectal cancers and were found no abnormities with electronic colonoscopy and biopsy act as compari-

son.

Reagent and methods

Main reagents Primary antibody: rabbit antihuman multiclonal antibody S100A4 (product number RB－1804－AO) purchased from Tianjin Jinmai gene mapping technique limited corporation (product of LabVision corporation). All of the work dilution ratio of antibody is 1:50. S－P immunohistochemical reagent: purchased from Fujian Maijin biological technique explore corporation. Ultrasensitive TM S－P work reagent kit (instant type, product number: KT－9710)

Methods Test by S－P immunohistochemical dyeing technique: Dyeing step follow the instruction of reagent kit, conducting microwave antigen restore before adding the primary antibody, the positive piece provided by Tianjin Jinmai gene mapping technique limited corporation acted as positive comparison, and PBS buffer instead of the primary antibody functions as negative comparison. Result figuration: S100A4 expressed

Table 1 The expression of S100A4 in colorectal carcinoma and normal colorectal tissues

Histological type	n	S100A4				The positive rate	χ^2	*P*
		－	+	++	+++			
Normal tissues	20	20	0	0	0	0%	11.541	0.003
Colorectal carcinoma tissues	68	26	18	15	9	61.8%		

Table 2 The relationship between the S100A4 expression and the clinical pathological factors of colorectal carcinoma

Clinical pathological factors		n	S100A4				χ^2	*P*
			－	+	++	+++		
Sex	male	36	14	9	8	5	0.001	0.974
	female	32	12	9	7	4		
Age	<55 years	28	10	8	7	3	0.014	0.907
	⩾55 years	40	16	10	8	6		
Location	colon	38	15	9	9	5	0.000	0.990
	rectal	30	11	9	6	4		
Gross style	lump type	18	7	6	3	2	0.360	0.835
	infiltrating type	32	13	7	8	4		
	ulcerative type	18	6	5	4	3		
Histological style	papillary adenocarcinoma	17	7	4	4	2	0.102	0.992
	tubular adenocarcinoma	16	6	4	3	3		
	mucinous adenocarcinoma	23	9	6	5	3		
	signet－ring cell carcinoma	12	4	4	3	1		
Differential degree	high	18	7	6	3	2	0.239	0.887
	middle	28	11	5	8	4		
	low	22	8	7	4	3		
Dukes stage	A+B	33	18	7	6	2	7.096	0.008
	C+D	35	8	11	9	7		
Lymph node metastasis	no	39	20	9	8	2	7.572	0.006
	yes	29	6	9	7	7		
Survival stage	<24 months	26	5	8	7	6	7.115	0.007
	⩾24 months	42	21	10	8	3		

in cytoplasm. If yellow, brown granules appear in cytoplasm of the tumor cell under microscope, the dyeing color of which is higher than the background. Under the circumstance of double blind, two pathological chief doctors observe each slice carefully. Ten high microscope views (400 times) were chosen in each slice randomly, ranked by half quantitative score method depending on positive cell percent and color degree. The standard score follows: ① positive cell percent: none score 0; <25% score 1; 25%−75% score 2; >75% score 3. ② color degree: no color or blurred color score 0; light yellow score 1; yellow score 2; brown score 3. Final result combines the scores from the two parts: negative expression score 0−1 (−), weak positive expression score 2−3(+), positive expression score 4−5(++), strong positive expression score>5(+++).

Statistical analysis of the results

All the data were input into the computer then were sorted with Excel and were treated with SPSS13.0 for

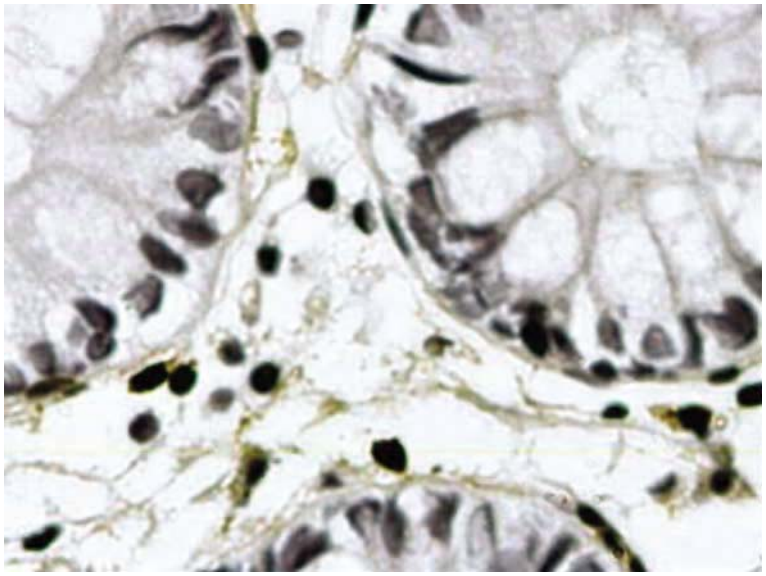

Fig. 1 The expression of S100A4 in normal colorectal mucosal tissues, S100A4(−) S−P×400

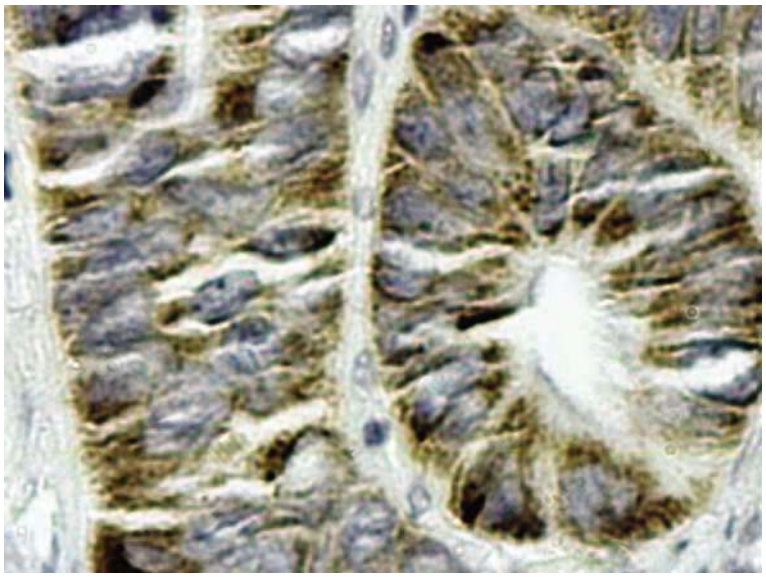

Fig. 2 The expression of S100A4 in colorectal carcinoma tissues, S100A4(+++) S−P×400

windows statistic software. The result was performed by chi-square test in measurement data and by Kruskal-Wallis rank sum test in ranked data. Statistically significant level is considered as "α= 0.05"

RESULTS

The expression of S100A4 Protein in colorectal carcinoma and normal colorectal mucosal tissues

The positive expression of S100A4 protein can not be found in 20 normal colorectal mucosal epithelia cells and staining of tissues is negative, but in the intercellular substance, lymphocyte, plasma of vascular endothelial cell (VEC), different degree of staining can be seen, but no stain in cytoplasm or nucleus (figure 1). The expression of S100A4 protein was mainly detected in plasma of carcinoma cells and mesenchymal cells and in intercellular substance without stain of nucleus. The expression in carcinoma cells is significantly higher than that in mesenchymal (figure 2). The positive expression of S100A4 protein in 68 colorectal carcinoma tissues was 61.8%(42/68), in which 18 cases were weakly positive (26.5%), 15 cases were positive(22.1%) and 9 cases were strong positive (13.2%), the positive expression in colorectal carcinoma tissues was apparently higher than that in normal tissues. There was significant difference between the two groups (P=0.003) (table 1).

The relation between the expression of S100A4 protein and the clinical pathological parameters of colorectal carcinoma

There are no significant differences in S100A4 protein expression among the age, sex, tumor location, growth style, histological type, differentiation degree of the carcinoma tissues respectively, but, there are significant differences among the different Dukes stages, with or without lymph node metastasis, and the different survival time (P<0.05), and higher expression of S100A4 protein occurs in the cases with the later stage, lymph node metastasis and shorter survival tim (table 2).

DISCUSSION

S100A4 protein is one of the 21 members of S100 family which is regarded as a calcium-binding proteins and it can effect on endocellular gene expression, cellular multiplication, cellular adhesive movement and cell apoptosis via calcium signal transduction pathway. S100A4 protein, a 101-amino acid polypeptide, was once called p9Ka, calvasculin, CPL etc. in literature. Its molecular weight is 11.5kDa. Coding gene of human S100A4 protein which once called mtsl (metatasin), pEl98, 18A2, 42A and fsp (fibroblast-specific protein) etc. is located at long arm of chromosome1 (1q21). The area is easy to occur various chromosomal change such as displacement, deletion and overlap, which suggested that S100A4 protein is closely related to the tumorigenesis and development[2].

The experimental results showed that transfection of S100A4 gene of rodent and human can induce breast cancer cells of rats which had no metastatic ability to metastasize[1,3]. But transfection of antisense S100A4 protein gene can significantly reduce the transfer rate of cancer clone which has high metastasis[1,2]. Some scholars transfect the neuroblastoma cell using S100A4 protein antisense oligonucleotides, the result showed the expression of S100A4 protein RNA in transfected cells decreased 35.6%, the invasive and metastatic ability of the transfected cell also decreased [4]. While, correlative transfected experiments suggested S100A4 protein can result in metastasis and development of tumor only coordinating with other cancer gene. The transfected rat appeared no risk factors of cancer shows that normal cells have no malign signs after transfection [5]. Some study reveal that the mice whose protein S100A4 gene was knocked-out had no change during 12 to 24 weeks [6]. However, we can find that its offspring has a higher risk of cancer metastasis from breast to lung by hybridizing the protein S100A4 transgenic mice and neu transgenic mice, the same trend by hybridizing the GRS/A mice and the protein S100A4 transgenic mice could be found [7]. According to above study, people conferred that S100A4 protein can promote the invasion and metastasis of tumor cells.

Many results of study showed that S100A4 protein is related to invasion and metastasis of tumor, which has a higher expression in human breast cancer, lung cancer, ovarian cancer, renal cancer, prostate cancer, melanotic

carcinoma, esophageal cancer, etc. The expression in carcinoma is apparently higher than in carcinoid and normal tissues. The mechanism may be: ①increase the transfer ability of tumor cells; ②decrease the inter-adhesion between tumor cells and enhance the tumor cells adhesion to matrix; ③degrade and remold extracellular matrix; ④inhibit apoptosis of p53-dependent cell by chelating the wild p53 and accelerate cell canceration ⑤ promote the formation of newborn blood vessels.

The study on the relationship between S100A4 protein and colorectal carcinoma has been carried out one after another. The expression of S100A4 protein in 709 pathological specimens of colorectal carcinoma was observed with immunohistochemical method by Gongle *et al.*[8], results showed that 16% was high expression, 31% low expression and 55% no expression, so they considered that S100A4 protein was an independent prognostic-related parameter and it was better than others such as lymph node status, p53 etc. in classification of subtypes as a prognostic-related parameter of colorectal carcinoma. Flatmark *et al.*[9] found S100A4 protein in 178 cases cytoplasm stained(64%) and 88 cases nucleolus stained (32%) in 277 paraffin samples of colorectal carcinoma with immunohistochemical method. Further statistical analysis showed that the expression of S100A4 protein in nucleus is related to the clinical stages of colorectal carcinoma, and the expresion in cytoplasm was not, therefore, he conferred the expression of S100A4 gene can regulate metastasis. Cho *et al.*[10] analyzed 124 pathological slices of patients with immunohistochemical method and found S100A4 protein in normal mucosal tissues had no or weak expression, 69 cases (55.6%)were positive expression in tumor tissues and 43 cases with lymph nodes metastasis(69.4%)positive. Statistical analysis showed that the expression of S100A4 protein is closely related to tumor stage and lymph node metastasis, but not related to the location or size of tumor. The study of Hemandas *et al.*[11] showed that the expression of S100A4 protein is opposite to clinical prognosis, it is helpful to judge the prognosis of colorectal carcinoma by detecting S100A4 protein state.

The results of this study showed that the expression of S100A4 protein in colorectal carcinoma tissues (61.8%) is significantly higher than that in normal mucosal tissues. There are no significant differences in S100A4 protein expression among the age, sex, tumor location, growth style, histological type, differential degree of the patients respectively($P>0.05$). There are significant differences in S100A4 protein expression according to Dukes stage, lymph node metastasis, and survival time ($P<0.05$), and higher expression of S100A4 protein occurs in the cancer tissues with later stage, lymph node metastasis and shorter survival time sufferers. It suggested that detecting expression of S100A4 protein with immunohistochemical method can evaluate the invasive and metastatic potential of colorectal carcinoma cells, judge the prognosis of patients and provide pathology evidence for further study and clinical therapy.

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