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ORIGINAL ARTICLE

Expression of protein S100A4 is a predictor of recurrence in colorectal cancer

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Abstract

AIM: To investigate the prognostic significance of S100A4 expression in colorectal cancer and its correlation with expression of E-cadherin and p53.

METHODS: A cohort of archival formalin-fixed paraffin-embedded specimens was selected from 127 patients with colorectal cancer who underwent surgical resection between April 2000 and March 2004 at the Department of Surgery, Korea University Guro Hospital. The expression of protein S100A4 was evaluated according to the proportion of positively stained cancer cells. In each case, three core biopsies with a diameter of 2 mm were punched out and positioned in a recipient paraffin array block. Four-μm sections of these tissue array blocks were used for immunohistochemical analysis of protein S100A4, E-cadherin, and p53. Clinicopathological data were based on the original histopathologic reports and clinical records of patients.

RESULTS: In normal colorectal mucosa, protein S100A4 immunoreactivity was clearly absent in both cytoplasm and nucleus. However, positive immunoreactivity of protein S100A4 was detected in 45 (35.4%) of the tumor cases. There was no significant association between positive immunoreactivity of protein S100A4 and clinicopathological parameters such as tumor differentiation or TNM stage, and also no correlation between the reactivity and E-cadherin or p53 expression. However, positive immunoreactivity of protein S100A4 was found to be associated with tumor recurrence (P = 0.004), and was also associated with significantly worse overall survival in the Kaplan-Meyer survival analysis (P = 0.044). After adjustment for tumor differentiation, tumor depth and nodal status, however, it failed to achieve statistical significance (P = 0.067).

CONCLUSION: The expression of protein S100A4 is associated with tumor recurrence and poor overall survival in patients with colorectal cancer.

Key words: S100A4; E-cadherin; p53; Prognostic factor; Colorectal cancer

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INTRODUCTION

Although the last decade has brought significant improvements in the disease-free and overall survival of colorectal cancer (CRC) patients; achieved largely by more accurate staging of disease, an improved and expanded role of surgery and increased number of available chemotherapeutic options, approximately 20% of advanced CRC patients still die of recurrence of the disease[1]. Invasion and metastasis, which are the most life-threatening properties of malignant tumors, result from the interaction between tumor cells and the surrounding tissues. The invasion and metastasis processes themselves consist of pathogenic sequential steps, such as proliferation and detachment of neoplastic cells, invasion to extracellular matrix, angiogenesis, vascular dissemination, lodging in a distant vascular bed, extravasation into the target organ, and proliferation. The activation of many genes and the expression of their products have been involved in this progression[2]. Current conventional staging has a significant impact on survival of CRC patients. However, there is marked variability in outcome that exists within each stage, and certain populations of patients with early recurrence, resistance to chemotherapy and decreased survival cannot be predicted using conventional histopathologic staging. Thus, the identification of molecular factors that have prognostic significance in CRC is essential to improve treatment and outcome[3-5].

Over the past few years, the S100 family of proteins has emerged as an important group with the capacity to promote invasiveness and metastasis of many human neoplasms. In particular, recent studies have established the mechanisms of action of protein S100A4, and indicate its possible prognostic role in human neoplasia[6-8]. However, studies regarding protein S100A4 have mainly been limited to research laboratories. Moreover, the mechanism of action of protein S100A4 in tumors is not fully understood. Therefore, it would be of great interest to find out whether the detection of protein S100A4 has any predictive value, and also whether it may help select patients who require more extensive diagnostic evaluation to rule out metastatic disease and/or more aggressive treatments.

The aims of this study were to investigate immunohistochemically the prognostic significance of protein S100A4 expression in CRC, compared with clinicopathologic parameters and overall survival, and to investigate the correlation between protein S100A4 expression and E-cadherin and p53, which have been suggested as possible targets of protein S100A4.

MATERIALS AND METHODS

Patients

Formalin-fixed paraffin-embedded specimens were selected from 127 patients with CRC who underwent curative or palliative surgical resection between April 2000 and March 2004 at the Department of Surgery, Korea University Guro Hospital. The 127 patients included 76 males (59.8%) and 51 females (40.2%) with a mean age of 59.3 years (range, 28-88). Clinicopathologic data were based on the histopathologic reports and the clinical records of the patients. Using the American Joint Committee on Cancer (AJCC) TNM system[9], tumors were classified as Stage I in 24 specimens (18.9%), Stage Ⅱ in 49 (38.6%), Stage Ⅲ in 49 (38.6%) and Stage Ⅳ in 5 (3.9%). The Korea University Medical Center Institutional Review Board granted permission for the study.

Preparation of tissue microarray

Paraffin blocks of formalin-fixed surgical specimens were obtained from the Department of Pathology, Korea University Guro Hospital. Pathological evaluation of all blocks was performed by two pathologists who did not know any information about the patients. In each case, three core biopsies were obtained from representative areas of the corresponding paraffin blocks with a precision instrument. These tissue cores from each specimen with a diameter of 2 mm were punched out and positioned in a recipient paraffin array block. Each case also included three internal controls consisting of non-neoplastic colorectal mucosa. Four-μm sections of these tissue array blocks were then cut and used for immunohistochemical analysis.

Immunohistochemistry

Immunohistochemical staining for protein S100A4, E-cadherin, and p53 was performed using a standard avidin-biotin complex (ABC) method. In brief, all sections were deparaffinized by using a series of xylene baths and then hydrated using a graded alcohol series. They were then placed in citric acid buffer (10 mmol/L) and heated in a microwave oven (700 W) for 12 min to retrieve the antigenicity. The sections were then immersed in methanol, containing 0.3% hydrogen peroxide, for 20 min to block endogenous peroxidase activity. The sections were then washed three times in phosphate-buffered saline (PBS) and incubated in 2.5% normal goat serum for 20 min to reduce nonspecific antibody binding. After washing with PBS, the sections were incubated with primary antibodies for 30 min at room temperature. Rabbit polyclonal antibodies against protein S100A4 (Ab-8, Neomarker, 1:100), monoclonal mouse anti-human E-cadherin (NCH-38, Dako, 1:100), and monoclonal mouse anti-human p53 (DO-7, Dako, 1:100) were used. The reaction products were visualized with diaminobenzidine as a chromogen, and counterstained with commercial hematoxylin.

Evaluation of immunohistochemical staining

Evaluation of immunohistochemical staining was performed by two independent pathologists. Any discrepancies in scoring were resolved by simultaneous reassess-

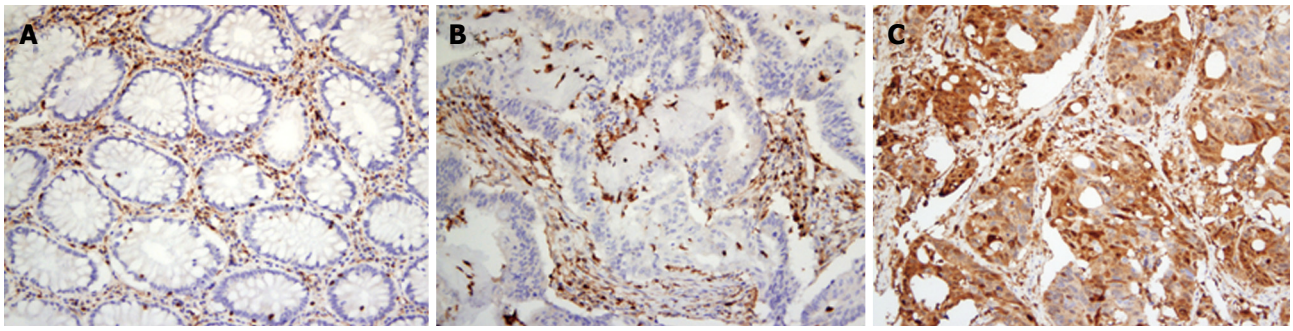

Figure 1 Immunohistochemical expression of protein S100A4. A: Protein S100A4 expression in normal colorectal epithelium. In all normal colonic epithelium, protein S100A4 immunoreactivity was clearly absent at both cytoplasm and nucleus; B: Negative expression of protein S100A4 in colorectal cancer (CRC); C: Positive expression of protein S100A4 in CRC. Cytoplasm of cancer cells was diffusely stained brown (all at × 200 magnification).

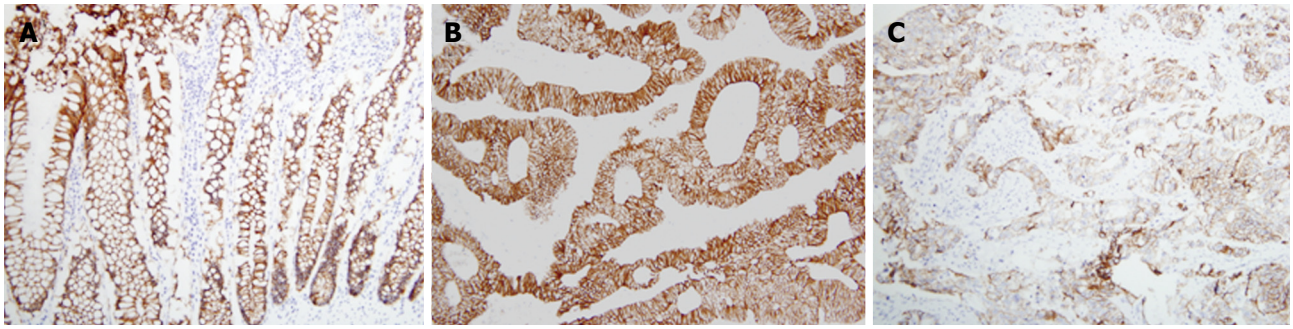

Figure 2 Immunohistochemical expression of E-cadherin. A: E-cadherin expression in normal colorectal epithelium. Normal epithelial cells strongly and homogeneously expressed E-cadherin at intercellular boundaries; B: Preserved expression of E-cadherin in colorectal cancer (CRC); C: Reduced expression of E-cadherin in CRC. Staining of cancer cell at intercellular border was weak and heterogeneous (all at × 200 magnification).

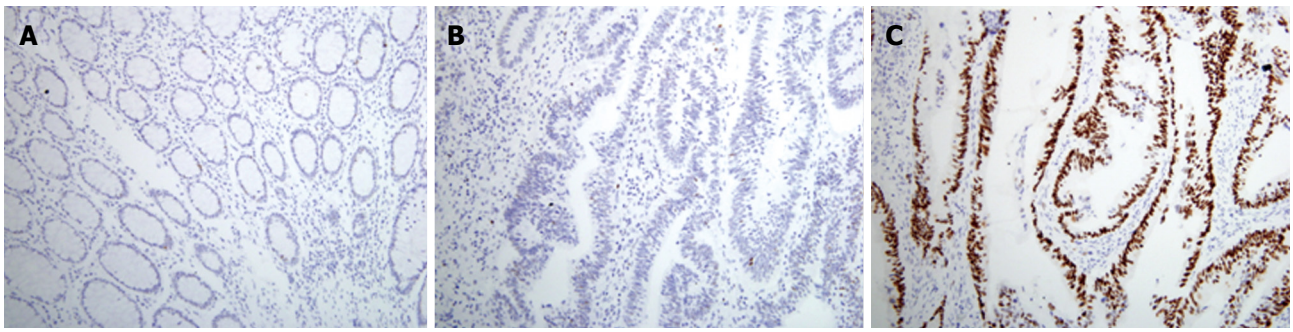

Figure 3 Immunohistochemical expression of p53. A: p53 expression in normal colorectal epithelium. In all normal colonic epithelium, p53 immunoreactivity was clearly absent at nucleus; B: Negative expression of p53 in colorectal cancer (CRC); C: Positive expression of p53 in CRC. More than 10% of cancer cells were stained strongly at their nuclei (all at × 200 magnification).

ment by both pathologists. The tumor cells whose cytoplasm was stained brown were classified as positive. The protein S100A4 expression of tumor cells was evaluated according to the proportion of positively stained tumor cells. When more than 10% of tumor cells were positively stained, the tumor was considered as "positive expression". On the other hand, the tumor was considered as "negative expression" when less than 10% of tumor cells were positively stained (Figure 1). In the case of E-cadherin, when more than 90% of tumor cells were positively stained, the tumor was considered as "preserved expression". On the other hand, the tumor was considered as "reduced expression" when less than 90% of tumor cells were positively stained (Figure 2). p53 expression was evaluated according to the proportion of tumor cells whose nuclei were positively stained. When more than 10% of tumor cells were positively stained, the tumor was considered as "positive expression". On the other hand, the tumor was considered as "negative expression" when less than 10% of tumor cells were positively stained (Figure 3).

Statistical analysis

Statistical analysis was performed using the SPSS for Windows software package (SPSS, Inc., Chicago, IL, Version 12.0). Correlation between the expression

Table 1 Relationship between expression of protein S100A4, E-cadherin, p53 and clinicopathologic parameters *n* (%)

Parameters	Expression of protein S100A4			Expression of E-cadherin			Expression of p53		
	Negative (*n* = 82)	Positive (*n* = 45)	*P* value	Reduced (*n* = 48)	Preserved (*n* = 79)	*P* value	Negative (*n* = 56)	Positive (*n* = 71)	*P* value
Gender			0.465			0.787			0.364
Male	51 (62.2)	25 (55.6)		28 (58.3)	48 (60.8)		36 (64.3)	40 (56.3)	
Female	31 (37.8)	20 (44.4)		20 (41.7)	31 (39.2)		20 (35.7)	31 (43.7)	
Age (yr)			0.797			0.963			0.344
mean ± SD	58.4 ± 11.1	60.9 ± 11.2		59.3 ± 13.1	59.4 ± 10.0		58.3 ± 9.6	60.2 ± 12.3	
Tumor location			0.352			0.120			0.528
Colon	38 (46.3)	17 (37.8)		25 (52.1)	30 (38.0)		26 (46.4)	29 (40.8)	
Rectum	44 (53.7)	28 (62.2)		23 (47.9)	49 (62.0)		30 (53.6)	42 (59.2)	
Differentiation			0.500[1]			0.001[1]			0.695
Differentiated	75 (91.5)	42 (93.3)		39 (81.3)	78 (98.7)		51 (91.1)	66 (93.0)	
Undifferentiated	7 (8.5)	3 (6.7)		9 (18.8)	1 (1.3)		5 (8.9)	5 (7.0)	
Depth of tumor			0.319			0.615			0.154
T1-2	25 (30.5)	10 (22.2)		12 (25.0)	23 (29.1)		19 (33.9)	16 (22.5)	
T3-4	57 (69.5)	35 (77.8)		36 (75.0)	56 (70.9)		37 (66.1)	55 (77.5)	
Lymph node metastasis			0.282			0.338			0.667
Absent	50 (61.0)	23 (51.1)		25 (52.1)	48 (60.8)		31 (55.4)	42 (59.2)	
Present	32 (39.0)	22 (48.9)		23 (47.9)	31 (39.2)		25 (44.6)	29 (40.8)	
Distant metastasis			0.053[1]			0.365[1]			0.654[1]
Absent	81 (98.8)	41 (91.1)		45 (93.8)	77 (97.5)		53 (94.6)	69 (97.2)	
Present	1 (1.2)	4 (8.9)		3 (6.3)	2 (2.5)		3 (5.4)	2 (2.8)	
pTNM stage			0.175			0.403			0.857
Ⅰ	17 (20.7)	7 (15.6)		6 (12.5)	18 (22.8)		11 (19.6)	13 (18.3)	
Ⅱ	33 (40.2)	16 (35.6)		19 (39.6)	30 (38.0)		20 (35.7)	29 (40.8)	
Ⅲ	31 (37.8)	18 (40.0)		20 (41.7)	29 (36.7)		22 (39.3)	27 (38.0)	
Ⅳ	1 (1.2)	4 (8.9)		3 (6.3)	2 (2.5)		3 (5.4)	2 (2.8)	
Recurrence[2]			0.004			0.269			0.845
Absent	67 (82.7)	24 (58.5)		31 (68.9)	60 (77.9)		40 (75.5)	51 (73.9)	
Present	14 (17.3)	17 (41.5)		14 (31.1)	17 (22.1)		13 (24.5)	18 (26.1)	

[1]Calculated by Fisher's exact test; [2]Stage Ⅳ patients were excluded.

of protein S100A4, E-cadherin, and p53 and various clinicopathologic parameters was evaluated using the chi-squared test or Fisher's exact test. Overall survival analysis was done by the Kaplan-Meier method. The difference between the survival curves was analyzed by the log-rank test. Significant variables identified on univariate analysis were subjected to multivariate analysis using the Cox regression model. A *P* value less than 0.05 was considered statistically significant.

RESULTS

Expression of protein S100A4, E-cadherin and p53 in CRC

A wide range of cell types in normal colorectal tissues was stained with polyclonal antibody against protein S100A4. There was a high level of staining of smooth muscle, of the smooth muscle in the walls of vessels, and of infiltrating lymphocytes and macrophages in the stroma. However, in normal colorectal mucosa of all 127 cases, immunoreactivity of protein S100A4 was clearly absent in both cytoplasm and nucleus. Positive immunoreactivity of protein S100A4 was detected in 45 (35.4%) of the tumor specimens. E-cadherin was expressed in cell membranes of all normal colorectal mucosa, and reduced expression of E-cadherin was observed in 48 (37.8%) of the tumor specimens. All normal colorectal mucosa showed negative expression for p53; however, 71 (55.9%) tumors were stained for p53 in their nuclei.

Correlation of protein S100A4, E-cadherin, and p53 expression with clinicopathological parameters

Positive reactivity for protein S100A4 was found to be associated with tumor recurrence ($P = 0.004$). However, there was no significant association between the expression of protein S100A4 and other investigated clinicopathological parameters, including tumor location, differentiation or TNM stage. Reduced expression of E-cadherin was significantly correlated with tumor differentiation ($P = 0.001$). As for p53, there was no significant correlation between expression of p53 and clinicopathological parameters (Table 1).

Correlation between protein S100A4 and E-cadherin/p53 expression

There was no significant correlation in co-expression pattern between protein S100A4 and E-cadherin (Kendall's Tau-b correlation coefficient = 0.068, $P = 0.436$, Table 2). Also, there was no significant correlation between protein S100A4 expression and p53 expression (Kendall's Tau-b correlation coefficient = -0.105, $P = 0.239$, Table 3).

Table 2 Correlations of protein S100A4 and E-cadherin

S100A4	E-cadherin	*n* (%)
Co-expression pattern		
Negative	Negative	55 (39.9)
Negative	Positive	34 (24.6)
Positive	Negative	32 (23.2)
Positive	Positive	17 (12.3)

Kendall's Tau-b correlation coefficient = -0.035, $P = 0.681$.

Table 3 Correlations of protein S100A4 and p53

S100A4	p53	*n* (%)
Co-expression pattern		
Negative	Negative	35 (25.4)
Negative	Positive	54 (39.1)
Positive	Negative	26 (18.8)
Positive	Positive	23 (16.7)

Kendall's Tau-b correlation coefficient = -0.132, $P = 0.120$.

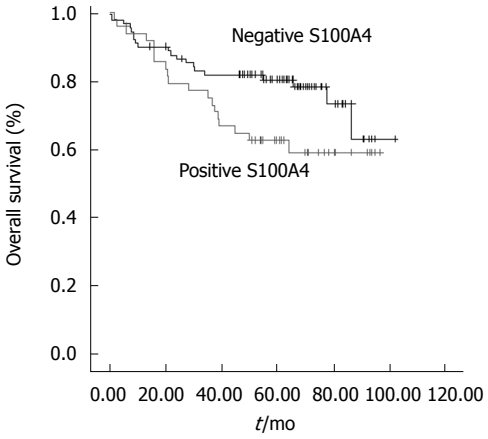

Figure 4 Kaplan-Meier survival curves demonstrating statistically significant differences according to the expression of protein S100A4 (log-rank test, *P* = 0.044). Censored observations are shown as tick marks.

Survival analysis

The median follow-up period for all patients was 58.7 mo (range, 1.1-101.8). The 5-year overall survival rate for the 127 patients was 79.7%. Kaplan-Meier survival analysis showed that tumor differentiation (5-year survival rate 82.3% *vs* 50.0%, $P = 0.001$), depth of tumor (97.1% *vs* 72.9%, $P = 0.001$), lymph node metastasis (88.5% *vs* 68.0%, $P = 0.001$) and positive immunoreactivity of protein S100A4 (86.1% *vs* 68.3%, $P = 0.044$) were associated with poor overall survival (Table 4 and Figure 4).

In a multivariate analysis, however, the positive immunoreactivity of protein S100A4 failed to have association with worse overall survival after adjustment for tumor differentiation, tumor depth and nodal status, which were significant parameters in a univariate analysis (hazard ratio, 1.985; 95% confidence interval: 0.953-4.134; $P = 0.067$, Table 5).

Table 4 Univariate overall survival analysis for seven clinicopathologic parameters

Parameters	*n*	5-yr overall survival rate (%)	*P* value
Gender			0.817
Male	76	80.9	
Female	51	78.0	
Differentiation			0.001
Differentiated	117	82.3	
Undifferentiated	10	50.0	
Depth of tumor			0.001
T1-2	35	97.1	
T3-4	92	72.9	
Lymph node metastasis			0.001
Absent	73	88.5	
Present	54	68.0	
S100A4 expression			0.044
Negative	82	86.1	
Positive	45	68.3	
E-cadherin			0.105
Preserved	79	83.1	
Reduced	48	74.3	
p53			0.218
Negative	56	83.6	
Positive	71	76.6	

Table 5 Cox regression analysis on those parameters shown to significantly influence overall survival in a univariate analysis

Parameters	Hazard ratio	95% CI	*P* value
Lymph node metastasis	2.283	1.014-5.141	0.046
Depth of tumor	10.374	1.391-77.352	0.022
Differentiation	2.748	1.059-7.133	0.038
S100A4 expression	1.985	0.953-4.134	0.067

DISCUSSION

Calcium binding proteins form a large family involved in numerous functions ranging from the control of cell-cycle progression and cell differentiation to enzyme activation and regulation of muscle contraction[10,11]. The S100 proteins represent one of the largest subfamilies of the calcium binding proteins with at least 19 different members; the degree of homology ranges from 25% to 65%. They were initially characterized as low-molecular weight acidic proteins and named by their solubility in 100% ammonium sulfate ("S100"). S100A4, also known as p9Ka, CAPL, or calvasculin, is a member of the S100 family consisting of 101 amino acids and with a molecular weight of about 11.6 kDa. The corresponding gene, cloned by different groups, is known as *mts1* (metastasin), *pEL98, 18A2, 42A*, and *fsp* (fibroblast-specific protein)[10-13].

The biologic functions of several S100 proteins in carcinogenesis have not been fully elucidated to date. Recently, however, much interest has focused on S100A4 and some other S100 family members, such as S100A2, S100A6, and S100B, for their potential roles in invasive growth and metastasis of neoplastic diseases. S100A4 or its corresponding mRNA are found at higher levels in

metastatic relative to non-metastatic rat[14] and mouse[15] tumor cell lines. Transfection experiments further showed that rodent or human S100A4 can induce a metastatic phenotype in previously non-metastatic rat mammary cells[16,17]. Conversely, antisense S100A4 RNA or anti-S100A4 ribozyme suppressed the metastatic potential of highly metastatic cell lines[18,19]. Moreover, in pilot studies of human colorectal adenocarcinoma specimens, elevated levels of immunohistochemically detected S100A4 are associated with the more malignant carcinomatous regions of the primary tumors and with liver metastases[20]. The tight association between S100A4 expression and metastasis observed in these laboratory analyses has led to a number of studies examining the utility of S100A4 expression as a prognostic marker in human cancers. Protein S100A4 has been shown to be a prognostic marker in a number of human cancers, including breast cancer[21], esophageal-squamous cancer[22], non-small cell lung cancer[23], gastric cancer[24], malignant melanoma[25], prostate cancer[26], and pancreatic cancer[27]. The universality of S100A4 expression in a variety of cancers illustrates the potential use of S100A4 as a marker for tumor metastasis and disease progression.

The purpose of this investigation was to establish clinical significance of the calcium-binding protein, S100A4, in CRC. It was found that 35.4% of CRC specimens were stained strongly by the polyclonal antibodies against protein S100A4, in concordance with earlier reports[28,29]. The staining in specimens is not restricted to only carcinoma cells, because highly expressed levels are also detected in normal tissues, in particular, smooth muscle cells, endothelial cells of both arteries and veins, and some reactive fibroblast-like cells and lymphocytes[30]. However, the present study was undertaken on only carcinoma cells.

In this study, positive expression of protein S100A4 was associated with tumor recurrence, in accordance with previous study[28]. This result suggests that the protein S100A4 may play a role in predicting a patient subgroup which would show more unfavorable outcome, thus leading us to substage-oriented tailored therapy with more intensive treatment and more strict follow-up surveillance.

This study showed that the overall survival of CRC patients who had immunohistochemically detectable levels of protein S100A4 was significantly worse than those CRC patients with negative expression of protein S100A4 according to univariate analysis. Because S100A4 was first discovered as a metastasis-inducing protein in experimental models[14-19], and metastasis is the major event responsible for death in patients of CRC, it is quite possible that protein S100A4 causes earlier deaths by its ability to induce metastasis in human CRC. Although it failed to achieve statistical significance in multivariate analysis, the present result suggests a need for further and larger studies to investigate the role of protein S100A4 expression in CRC.

As a typical member of the S100 family, S100A4 exerts dual functions, both intracellular and extracellular. Intracellularly, it interacts with and functionally modifies the tumor suppressor protein p53, non-muscle myosin II, and liprin β1[12,13]. S100A4 interacts with the C terminus of p53 and inhibits protein kinase C (PKC) phosphorylation of the tumor suppressor *in vitro*. Likewise, the interaction between p53 and S100A4 inhibits p53 from binding to its consensus DNA-binding sequence[31]; thus it was expected that S100A4 would be a general inhibitor of p53 function. It has been suggested that a complex of S100A4 with p53 and the sequestration of p53 may result in stimulation of the cells to enter the S phase by abrogating the control functions of p53 at the G1-S checkpoint[8,31,32]. However, as shown in this study, this possibility was difficult to prove by immunohistochemical analysis of these two proteins in CRC. An examination of p53-regulated genes in S100A4-expressing cells indicates that the expression of several genes are up-regulated (e.g. *bax*); other genes are down-regulated initially and then later up-regulated (e.g. *mdm2*), and some genes are inhibited (e.g. *p21*, *thrombospondin-1*)[31]. These opposite effects of S100A4 expression on p53-regulated genes could explain why there was no correlation between S100A4 and p53, notwithstanding the potential interaction of these two proteins.

Another possible mechanism of action of S100A4 in carcinogenesis is cytoskeletal dysregulation by down-regulation of E-cadherin induced by protein S100A4. E-cadherin is a member of the large cadherin superfamily. It is the predominant intercellular adhesion molecule expressed by intestinal epithelial cells, and functions to mediate epithelial cell-cell adhesion and maintain the integrity of the epithelium[33-35]. The expressions of E-cadherin and protein S100A4 in two mouse tumor cell lines were found to be inversely regulated, and transfection experiments showed a reciprocal down-regulation of both molecules, suggesting that the invasiveness of tumors expressing protein S100A4 may be at least partially induced by the abrogation of E-cadherin expression[36]. A similar mechanism has also been postulated in humans, on the basis of immunohistochemical analysis of both proteins in a series of non-small cell lung cancer[23] and gastric cancer[24]; an inverse correlation of E-cadherin and protein S100A4 expression was demonstrated. In this study, we attempted to immunohistochemically establish an inverse correlation between the expression of protein S100A4 and E-cadherin in CRC; however, data failed to prove the relationship (Kendall's Tau-b correlation coefficient = -0.035, P = 0.681). Nevertheless, it is quite possible that different antibodies against protein S100A4, different cancer tissue, and small numbers enrolled in this study might have contributed to this difference.

In conclusion, in the present retrospective study, positive immunoreactivity of protein S100A4 is closely associated with cancer recurrence. However, there is no correlation between the expression of protein S100A4 and E-cadherin or p53. The overall survival for patients with CRC expressing immunohistochemically detectable levels of protein S100A4 is significantly worse than for those patients with CRC considered negative for S100A4. Furthermore, protein S100A4 shows borderline tendency toward being a prognostic marker in multivariate regres-

sion analysis. Although these results suggest that protein S100A4 could be a useful biologic predictor of cancer recurrence and poor outcome, subsequent prospective, large-scale studies are required to confirm its importance and relevance in the invasive potential of human CRC.

COMMENTS

Background

Although current conventional staging has a significant impact on survival of colorectal cancer patients, there is marked variability in outcome within each stage. As the protein S100A4 has been known to promote invasiveness and metastasis of many human neoplasms, the question is raised as to whether this protein represents a useful prognostic marker in clinical practice.

Research frontiers

Studies regarding the protein S100A4 have mainly been limited to research laboratories and clinical data are extremely limited. Therefore, it would be of great interest to find out whether the expression of protein S100A4 has any predictive value and may help select patients who require more extensive diagnostic evaluation to rule out metastasis and/or more aggressive treatments.

Innovations and breakthroughs

The results of this study showed that positive immunoreactivity of protein S100A4 was associated with tumor recurrence and worse overall survival.

Applications

Since there is an association between protein S100A4 expression, tumor recurrence and poor overall survival, this can lead to substage-oriented tailored therapy with more intensive treatment and more strict follow-up surveillance in colorectal cancer patients.

Terminology

The protein S100A4 was first discovered as a metastasis-inducing protein in experimental models. It is a polypeptide of 101 amino acids with a molecular mass of 11.5 kDa. The evidence gathered throughout the past few years demonstrates that protein S100A4 is involved in the regulation of invasiveness and metastasis in many human cancers.

Peer review

This paper is very well written and has a strong message about expression of protein S100A4 in 127 cases of colorectal cancer, showing a statistically significant association with tumor recurrence and overall survival.

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