Loss of S100A14 Expression Is Associated with the Progression of Adenocarcinomas of the Small Intestine

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Key Words

Adenocarcinoma · p21 · p53 · S100A14 · Small intestine

Abstract

Objectives: Small intestinal adenocarcinoma (SIAC) is an exceedingly rare human malignant tumor, and its association with the S100A14 gene is not known yet. We aimed to investigate the clinicopathological correlations between S100A14 expression and SIAC. **Methods:** Immunohistochemical analyses of S100A14, p21 and p53 were performed using tissue microarray analysis of 175 surgically resected SIACs. **Results:** Of 175 SIACs, loss of S100A14 expression was observed in 128 cases (73.1%). Loss of S100A14 expression was associated with lymph node metastasis ($p = 0.009$) and advanced disease stage ($p = 0.013$), and was more frequently observed in distal than duodenal tumors ($p = 0.043$). The majority of SIACs lost p21 expression (93.7%), and significant loss of p21 expression was observed in cancers with high pT stages (pT₃ and pT₄; $p = 0.011$), lymph node metastasis ($p = 0.029$) and advanced cancer stage defined by the American Joint Committee on Cancer ($p = 0.005$). Overexpression of p53 was

found in 23.4% of cases. Positive expression of p53 was associated with distally located SIACs (jejunum or ileum; $p = 0.006$). There was no association between the expression of S100A14 and p21 or p53. **Conclusion:** Loss of S100A14 in SIAC is common and is associated with higher metastatic potential and advanced clinical stage.

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Introduction

Small intestinal adenocarcinoma (SIAC) is an exceedingly rare tumor even though the small intestine occupies about 90% of the absorptive surface area of the human gastrointestinal tract [1]. It comprises <5% of whole gastrointestinal tract tumors [2, 3]. Despite recent advances in the techniques used to approach the small intestine [4], the early detection of SIAC is quite challenging [5]. Due to its rarity, the molecular carcinogenesis of SIAC is still poorly understood [5–7].

S100A14 is one of the least-known subtypes of the 20 S100 family members and was only recently identified on

chromosome 1q21 [8, 9]. *S100A14* mRNA has been reported to be expressed in several normal human tissues, including the colon, and the functional role of *S100A14* in malignant tumors is organ specific. Loss of *S100A14* expression has been reported in tumors of the kidney, esophagus and colorectum, while overexpression of *S100A14* has been reported in several cancers, including cancers of the ovary, urinary bladder, breast and uterus [8, 10–13]. A recent study has reported that the low expression of *S100A14* is associated with a high metastatic potential and poor prognostic outcome in patients with colorectal cancer [14]. *S100A14* induces cell cycle arrest or apoptosis in oral and esophageal squamous cell carcinomas [10, 15, 16], and regulates the cell cycle in a p53- or receptor for advanced glycation end-products (RAGE)-dependent manner. However, there are no published studies on *S100A14* expression in SIAC. To identify the role of *S100A14* in SIAC, we investigated the expression of *S100A14*, p21 and p53 in these tumors using immunohistochemistry.

Materials and Methods

Patients and Specimens

One hundred and ninety-seven surgically resected SIACs were collected from 22 institutions in Korea. Representative hematoxylin and eosin slides, paraffin tissue blocks and the appropriate approvals from the institutional review board of each institution that participated in the Korean Small Intestinal Cancer Study Group were obtained. Primary carcinomas arising from the duodenum, jejunum and ileum were included in this study. Malignant tumors from other gastrointestinal tract organs, such as the stomach, ampulla of Vater, pancreas or appendix with extension to the small intestine, were excluded. Data were collected by reviewing the medical records of the patients, including each patient's sex, age, other accompanying tumors, results of the most recent follow-up examination and survival status. The pathological characteristics that were evaluated included growth patterns, tumor location, tumor size, histological subtype, differentiation status, depth of invasion, lymph node metastases and the presence of lymphatic invasion.

Tissue Microarray

In order to construct the tissue microarrays of the 197 cases, tissue cores that were 2 mm in diameter were obtained from morphologically representative areas of the formalin-fixed paraffin-embedded blocks after being reviewed by two experienced gastrointestinal tract pathologists (G.K. and S.-M.H.). Areas where tumor cells occupied >75% of cells with major histological differentiation and without accompanying tumor necrosis were selected. The tissue array blocks consisted of 2 or 3 cores from each tumor tissue, 1 core from a corresponding metastatic lymph node and 1 core from a matched normal tissue.

Immunohistochemistry and Interpretation

Serial 4- μ m sections were applied to 3-aminopropyltriethoxysilane-coated slides (Sigma, St. Louis, Mo., USA). Deparaffinization and rehydration were performed using xylene and alcohol. Endogenous peroxidase was blocked using a 3% solution of aqueous hydrogen peroxide. The slides were pretreated with citrate buffer (pH 6.0) in a microwave oven for antigen retrieval. The sections were incubated at room temperature for 60 min with mouse monoclonal anti-*S100A14* (1:200; Santa Cruz Biotechnology, Santa Cruz, Calif., USA), mouse monoclonal anti-p21 (4D10, 1:100; Novocastra, Newcastle, UK) and mouse monoclonal anti-p53 (DO-7, 1:100; Novocastra). The sections were reacted with peroxidase-conjugated streptavidin for 30 min. Diaminobenzidine was used as the chromogen, and the sections were counterstained with hematoxylin. Immunohistochemical staining was successfully performed on 175 of 197 SIACs on the tissue microarrays. Because several tissue cores were lost during sectioning or the staining process, they were not successfully immunolabeled. Both the percentage of positive tumor cells and the intensity of the positive staining were graded.

An intensity scale was categorized from 0 to 3 as follows; 0, corresponding to no labeling of neoplastic epithelial cells; 1, corresponding to weak labeling of neoplastic epithelium which could best seen at 10 $\times$ objective; 2, corresponding to unequivocal labeling of epithelial cells, and 3, corresponding to intense labeling, which was previously described elsewhere [17]. Loss of *S100A14* expression was defined as positive immunostaining in <30% of the tumor cells, as previously described [14]. Sections were considered positive for p21 and p53 when $\geq 10\%$ of the tumor cell nuclei were stained [18].

Statistical Analysis

In order to compare any associations between the immunohistochemical markers and clinicopathological parameters, the χ^2 and Fisher exact tests were performed. Overall patient survival was defined as the time from surgical resection of the SIAC to death of the patient or last follow-up examination. The survival rate was calculated using the Kaplan-Meier method. Comparisons of the survival rates and associations with the various clinicopathological factors were performed using the log-rank test. Statistical analyses were performed using IBM SPSS 19.0 software (SPSS, Chicago, Ill., USA). A p value <0.05 was considered statistically significant.

Results

Clinical and Pathological Characteristics

The study group consisted of 175 cases (111 men and 64 females) with a mean age of 58.1 years (range: 23–86 years). Fifteen cases were in stage 0–I, 70 were in stage II and 90 were in stage III (table 1). There were 103 duodenal carcinomas and 72 jejunal/ileal tumors. The case numbers in each clinical stage were 0–I, 10 (9.7%); II, 40 (38.8%), and III, 53 (51.5%) in duodenal carcinomas, and 0–I, 5 (6.9%); II, 30 (41.7%), and III, 37 (51.4%) in jejunal/ileal tumors. However, there was no significant difference in clinical stages between the two groups (p = 0.79).

Association between the Loss of S100A14 Expression and Clinicopathological Factors in Primary SIAC

S100A14 expression was observed on the cellular membrane of normal small intestinal mucosal epithelial cells (fig. 1a). Surface epithelial cells expressed S100A14 most strongly. S100A14 expression decreased in the crypts and disappeared in Brunner's glands. Of 175 SIACs, loss of S100A14 expression was observed in the majority of cases (128, 73.1%; fig. 1b, c). Loss of S100A14 expression was demonstrated in 4 of 8 accompanying adenoma cases (50%; data not shown). Loss of S100A14 expression was more commonly observed in ulceroinfiltrative (103 of 133, 77.4%) or polypoid tumors (21 of 31, 67.7%) than flat tumors (4 of 11, 34.6%; $p = 0.012$; fig. 1d). Loss of S100A14 expression was more frequently observed in distal (jejunum or ileum) tumors (59 of 72, 81.9%) than proximal (duodenum) tumors (69 of 103, 67%; $p = 0.043$). Cases with lymph node metastasis (74 of 90, 82.2%) demonstrated increased loss of S100A14 expression compared with cases without lymph node metastasis (54 of 85, 63.5%; $p = 0.009$). The proportion of cases with loss of S100A14 expression significantly increased with increasing American Joint Committee on Cancer (AJCC) cancer staging (stage 0–I, 8 of 15, 53.3%; stage II, 46 of 70, 65.7%; stage III, 74 of 90, 82.2%; $p = 0.013$; table 1). There was no association between loss of S100A14 expression and the other clinicopathological factors.

Expression of p21 and p53

Because some previous studies have reported that the functional role of S100A14 in cell cycle arrest is regulated by p21 and p53 [10, 15], we used immunohistochemical analysis to study p21 and p53. Both p21 and p53 are located in the nuclei of cancer cells (fig. 2). The associations between the protein expression levels of p21 and p53 and the examined clinicopathological factors are summarized in table 2. Of 175 SIAC cases, 164 cases demonstrated loss of p21 expression (93.7%). We found that SIACs that demonstrated the loss of p21 expression were more likely to invade into lymphovascular spaces ($p = 0.031$). Significant loss of p21 expression was more frequently observed in high pT cancers (pT₃–pT₄, 152 of 159 cases, 95.6%) than in low pT tumors (pT_{is}–pT₂, 12 of 16 cases, 75%; $p = 0.011$), in carcinomas with lymph node metastasis (pN₁ + pN₂, 88 of 90, 97.8%) than those without lymph node metastasis (pN₀, 76 of 85, 89.4%; $p = 0.029$) and advanced-stage cancers (stage 0–I, 11 of 15, 73.3%; stage II, 65 of 70, 92.2%; stage III, 88 of 90, 97.8%; $p = 0.005$). Aberrant p53 protein expression was ob-

Table 1. Correlation between S100A14 expression and clinicopathological characteristics of SIACs

Clinicopathological information	n	S100A14 expression, n (%)		p value
		loss	intact	
Sex				0.642
Male	111	83 (74.8)	28 (25.2)	
Female	64	45 (70.3)	19 (29.7)	
Age				0.482
<60 years	91	64 (70.3)	27 (29.7)	
≥60 years	84	64 (76.2)	20 (23.8)	
Type of growth				0.012^a
Polypoid	31	21 (67.7)	10 (32.3)	
Flat	11	4 (36.4)	7 (63.6)	
Ulceroinfiltrative	133	103 (77.4)	30 (22.6)	
Location				0.043
Duodenum	103	69 (67)	34 (33)	
Jejunum + ileum	72	59 (81.9)	13 (18.1)	
Histological subtype				0.491 ^a
Adenocarcinoma	159	114 (71.7)	45 (28.3)	
Mucinous carcinoma	9	8 (88.9)	1 (11.1)	
Signet ring cell carcinoma	4	3 (75)	1 (25)	
Undifferentiated carcinoma	3	3 (100)	0 (0)	
Differentiation				0.362
Well/moderate	135	96 (71.1)	39 (28.9)	
Poor/undifferentiated	40	32 (80)	8 (20)	
Lymphovascular invasion				0.412
Absent	86	60 (69.8)	26 (30.2)	
Present	89	68 (76.4)	21 (23.6)	
T classification				0.192
pT _{is} –pT ₂	16	9 (56.3)	7 (43.8)	
pT ₃ –pT ₄	159	119 (74.8)	40 (25.2)	
N classification				0.009
pN ₀	85	54 (63.5)	31 (36.5)	
pN ₁ + pN ₂	90	74 (82.2)	16 (17.8)	
AJCC staging				0.013
0–I	15	8 (53.3)	7 (46.7)	
II	70	46 (65.7)	24 (34.3)	
III	90	74 (82.2)	16 (17.8)	
Total	175	128 (73.1)	47 (26.9)	

Significant differences are highlighted in bold.

^a p value determined using the Fisher exact test.

served in 41 of 175 SIAC cases (23.4%). Abnormal p53 expression was more commonly noted in distally located SIACs (25 of 72 cases, 34.7%; $p = 0.006$; table 2). There was no correlation between the expression of S100A10 and p21 or p53 (table 3).

Fig. 1. Representative images of S100A14 labeling of normal small intestinal mucosa and SIAC. **a** S100A14 labeling on the cell membrane of normal small intestinal epithelial cells. **b, c** Moderately and poorly differentiated SIAC cells expressing S100A14. **d** SIAC demonstrating the loss of S100A14.

Color version available online

Fig. 2. Representative images of nuclear p21 and p53 immunolabeling of SIAC.

Color version available online

Correlation between S100A14 Expression and Clinical Outcomes

The median survival time of the patients that demonstrated loss of S100A14 expression was 30 months, while patients with intact S100A14 expression showed a median survival time of 47.6 months; however, there was no significant survival difference between patients with loss of S100A14 expression and those with intact S100A14 expression ($p = 0.619$; fig. 3).

Discussion

The S100 family consists of calcium-modulated EF-hand-type proteins with intra- and extracellular functions. The functions of some of the S100 subfamily mem-

bers, such as the role of S100A4 in metastasis, are well characterized [19]. However, because S100A14 was just recently characterized using a small cell lung carcinoma cell line, its function in human tumors is largely unknown [8, 20]. We previously reported that distal SIACs demonstrate more pT₄ tumors, and that patients with distal SIACs demonstrate a significantly shorter median survival than patients with proximal (duodenal) cancers [21]. In the present study, we found that the loss of S100A14 expression is associated with distally located SIACs, and this result indirectly implies a possible correlation between loss of S100A14 expression and a poor prognostic outcome.

Regarding cancers of the gastrointestinal tract, only a recent study that used human colorectal cancer tissues has ever reported the clinical significance of S100A14 ex-

Table 2. Correlation between p21 and p53 expression and the clinicopathological characteristics of SIACs

Clinicopathological information	n	p21, n (%)		p value	p53, n (%)		p value
		negative	positive		negative	positive	
Location				0.529 ^a			0.006
Duodenum	103	95 (92.2)	8 (7.8)		87 (84.5)	16 (15.5)	
Jejunum + ileum	72	69 (95.8)	3 (4.2)		47 (65.3)	25 (34.7)	
Lymphovascular invasion				0.031^a			0.097
Absent	86	77 (89.5)	9 (10.5)		71 (82.6)	15 (17.4)	
Present	89	87 (97.8)	2 (2.2)		63 (70.8)	26 (29.2)	
T classification				0.011^a			0.766 ^a
pT _{is} -pT ₂	16	12 (75.0)	4 (25.0)		13 (81.3)	3 (18.8)	
pT ₃ -pT ₄	159	152 (95.6)	7 (4.4)		121 (76.1)	38 (23.9)	
N classification				0.029^a			0.614
pN ₀	85	76 (89.4)	9 (10.6)		67 (78.8)	18 (21.1)	
pN ₁ -pN ₂	90	88 (97.8)	2 (2.2)		67 (74.4)	23 (25.6)	
AJCC staging				0.005^a			0.641 ^a
0-I	15	11 (73.3)	4 (26.7)		13 (86.7)	2 (13.3)	
II	70	65 (92.9)	5 (7.1)		54 (77.7)	16 (22.9)	
III	90	88 (97.8)	2 (2.2)		67 (74.4)	23 (25.6)	
Total	175	164 (93.7)	11 (6.3)		134 (76.6)	41 (23.4)	

Significant differences are highlighted in bold. ^a p value determined using the Fisher exact test.

Table 3. Associations between the expression levels of S100A14, p21 and p53 in SIACs

Immunohistochemical markers	n	p21, n (%)		p value	p53, n (%)		p value
		negative	positive		negative	positive	
S100A14				0.277 ^a			0.837
Negative	128	122 (95.3)	6 (4.7)		97 (75.8)	31 (24.2)	
Positive	47	42 (89.4)	5 (10.6)		37 (78.7)	10 (21.3)	
Total	175	164	11		134	41	

^a The Fisher exact test was employed to determine significant differences.

pression. Low S100A14 expression is associated not only with a high metastatic potential but also with a poor prognostic outcome in patients with colorectal carcinomas [14]. Although we did not find a prognostic association with S100A14 expression in SIACs, our results show that the loss of S100A14 expression is correlated with the presence of lymph node metastasis and high AJCC staging in SIACs, which are concordant with the results of a previous study on colorectal cancers. The discrepancy of the correlation between lymph node metastasis and prognostic outcome could be explained by several possible reasons, such as lack of distant metastasis in our cases, no

association between loss of S100A14 expression and tumoral stages, and other unknown molecular interactions. Taken together, S100A14 may work as a potential tumor suppressor of SIACs.

The molecular pathogenesis of S100A14 and its association with tumor progression has been studied only in a limited number of malignant tumors. S100A14 regulates the expression of MMP1, MMP9, p21 and p53 in oral squamous cell carcinomas [15, 22]. For example, S100A14 represses the proliferation of oral squamous carcinoma cell lines with wild-type p53 by inducing G1 arrest in association with the upregulation of p21 [15]. In esophageal

Fig. 3. Kaplan-Meier survival analysis of SIACs according to S100A14 expression status. The median survival time of the patients who demonstrated loss of S100A14 expression was 30 months, while patients with intact S100A14 expression demonstrated a median survival time of 47.6 months. There was no statistically significant survival difference between cases that demonstrated a loss of S100A14 expression and cases with intact S100A14 expression ($p = 0.619$).

squamous cell carcinomas, S100A14 is involved in RAGE and p53 pathways [10, 16]. In particular, cancer cells with down-regulated S100A14 expression can escape apoptotic mechanisms that are regulated by p21 or p53. However, in the present study, we did not observe any association between S100A14 and p21 or p53 expression in SIACs. Even if S100A14 is downregulated in both esophageal carcinomas and SIACs, the role S100A14 plays in tumor progression or metastasis may be different. In addition, because the loss of p21 expression was correlated not only with higher pT and pN classifications but also AJCC staging, the expression of p21 could be a possible prognostic indicator of SIAC regardless of S100A14 expression. On the contrary, p53 expression did not demonstrate any clinical significance in SIAC. Although a single study on p21 expression in SIACs was previously reported, its clinical implications have not been revealed yet. The expression of p21 was found to be decreased in SIACs (46%) in comparison with small intestinal adenomas (50%) in the previous study [18]. In addition, the overexpression of p53 in SIACs has been reported to vary between 24 and 65%.

Little is known about the clinical significance of p53 overexpression in SIACs either [18, 23, 24].

The upregulation of S100A14 has been reported to occur predominantly in basal-like breast carcinoma, which is considered both a stem cell phenotype and a type of circulating breast cancer cell [11, 25]. This result implies the possibility that S100A14 expression could be associated with the cancer stem cell phenotype. This may suggest different levels of S100A14 expression depending on the organ. Further research on the role of S100A14 in cancer stem cells and SIAC is warranted.

In conclusion, loss of S100A14 expression was observed in a major subset of SIACs and associated with lymph node metastasis and advanced clinical stage. These findings suggest that S100A14 plays a role as a tumor suppressor in SIACs. To the best of our knowledge, this is the first report on the expression of S100A14 in SIACs.

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