

Progressive Suppression of Selenium Binding Protein 1 in Gastric Adenoma and Adenocarcinoma

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Background : Human selenium binding protein 1 (SELENBP1) is a protein that binds selenium as a cofactor. The decreased expression of SELENBP1 in several types of carcinomas and its association with a poor prognosis have previously been reported on. In this study, we evaluated the expression of SELENBP1 in low-grade and high-grade epithelial dysplasia/adenomas and adenocarcinomas. **Methods :** We analyzed 45 cases of low-grade epithelial dysplasia/adenomas, 42 cases of high-grade epithelial dysplasia/adenomas and 64 cases of adenocarcinomas and all of them were obtained from endoscopic mucosal resection or endoscopic submucosal dissection. We analyzed all of them for their SELENBP1 expression by immunohistochemistry. Eight triple-paired cases of gastric mucosa, adenoma and adenocarcinoma from the same patient were selected for RT-PCR analysis. **Results :** There was a progressive decrease in the expression of SELENBP1 from the low-grade dysplasia/adenomas (42/45, 93%) to the high-grade dysplasia/adenomas (29/42, 69%) and finally to the adenocarcinomas (24/64, 37%), ($p < 0.001$). The progressive decrease in the SELENBP1 expression was also evident in the eight paired cases that were analyzed by RT-PCR. **Conclusions :** Our findings demonstrate that the SELENBP1 expression is suppressed in gastric epithelial dysplasia/adenomas and adenocarcinomas. The suppression of SELENBP1 was significantly more frequent and severer in the adenocarcinomas than that in the low-grade dysplasia/adenomas, and this implies that the suppression of SELENBP1 is a late event in gastric carcinogenesis.

Key Words : Selenium binding protein 1, Human; Stomach; Adenoma; Adenocarcinoma

Human selenium binding protein 1 (SELENBP1), which binds covalently to selenium is a member of selenium-containing proteins. The *SELENBP1* gene is located on chromosome 1q21-22 and it encodes a protein of 472 amino acids.^{1,2} SELENBP1 has been implicated to play a role in the detoxification processes,³ cell-growth regulation,⁴ intra-Golgi protein transport,⁵ and lipid metabolism.⁶ However, the detailed functions of this protein are not known. SELENBP1 is expressed in the epithelium of the stomach, colorectum, liver, lung, breast, prostate, thyroid gland, and kidney.^{7,8} The decreased expression of SELENBP1 has been reported in several carcinomas, including lung adenocarcinoma,⁹ colorectal adenocarcinoma,^{7,10} prostate adenocarcinoma,⁸ stomach adenocarcinoma,¹¹ ovarian epithelial carcinoma,¹² and thyroid papillary carcinoma.¹³ The association between the decreased expression of this protein and a poor prognosis has been demonstrated for lung adenocarcinomas⁹ and colorectal adenocarcinomas.^{7,10} Additionally, it has been report-

ed that the decreased expression of this protein is a late event in colorectal carcinogenesis.⁷ Loss of heterozygosity and methylation of promoter CpG islands do not account for the suppression of this protein in lung adenocarcinomas and colorectal carcinomas.^{7,9,10}

Gastric carcinoma is one of the leading causes of death worldwide, despite of a marked decline of its incidence in the West.¹⁴ Especially in Korea, gastric carcinoma is the second leading cause of death.¹⁵ It is widely accepted that gastric carcinogenesis is a multistep process that progresses from chronic gastritis, atrophy, intestinal metaplasia to dysplasia/adenoma, and it finally leads to gastric carcinoma, especially for the intestinal type of gastric carcinoma.¹⁶ There are many molecular alterations involved in the gastric adenoma-carcinoma sequence, including *APC* mutation, *p53* mutation and loss of heterozygosity, the reduced expression of *p27*, and the increased expression of cyclin E.¹⁷ The sequential changes of the SELENBP1 expression in gastric dys-

plasia/adenomas to early stage carcinomas can provide insights into the carcinogenic pathways and this can aid in the detection of molecular targets for making a proper diagnosis or selecting a specific treatment. In this study, we evaluated the changes of the SELENBP1 expression in low-grade and high-grade epithelial dysplasia/adenomas and adenocarcinomas by immunohistochemistry, with a focus on the multistep carcinogenesis of intestinal-type adenocarcinoma, on the formalin-fixed paraffin-embedded specimens obtained from endoscopic mucosal resection or endoscopic submucosal dissection. We also evaluated the changes of the SELENBP1 mRNA expression by RT-PCR on the snap-frozen tissues obtained from total or subtotal gastrectomy.

MATERIALS AND METHODS

Case selection and histologic review

We reviewed three consecutive series of 45 low-grade epithelial dysplasia/adenomas that were obtained between November 2006 and May 2007, 42 high-grade epithelial dysplasia/adenomas that were obtained between December 2006 and March 2008, and 64 adenocarcinomas that were obtained between September 2006 and March 2008, and all the specimens were the products of endoscopic mucosal resection or endoscopic submucosal dissection. These specimens were obtained from the archives of the Department of Pathology, Yonsei University, Seoul, Korea. Authorization for the use of these tissues for research purposes was obtained from the Institutional Review Board of Yonsei University of College of Medicine. In this study, we adopted the WHO system which is a two-tiered system of low-grade and high-grade epithelial dysplasia/adenoma, for the classification of the neoplastic precursor lesions. We used the definition of carcinoma as invasion into the lamina propria or beyond, as recommended by the WHO.¹⁷ For the selection of adenocarcinomas, in order to avoid confusion between high-grade dysplasia, carcinoma in situ and intramucosal adenocarcinoma, we only included those tumors that had invaded the muscularis mucosa or submucosa. The cases containing concomitant low-grade and high-grade dysplasia/adenoma or high-grade dysplasia/adenoma and adenocarcinoma were grouped according to the worst grade. All the specimens were routinely fixed in 10% buffered formalin, serially sectioned and embedded in paraffin, cut at 3 μ m thickness and then stained with hematoxylin-eosin. Each lesion was independently reviewed by two pathologists (Hy Kim and Ho Kim). The gross type, size and state of the non-neoplas-

tic mucosa were reevaluated. For the validation of the SELENBP1 expression at the RNA level, eight triple-paired cases of non-neoplastic mucosa, dysplasia/adenoma and adenocarcinoma from the same patient were selected and evaluated by RT-PCR analysis. All the eight cases were snap-frozen samples that were obtained immediately at the time of surgery. Of the eight cases, five cases (case 2, 3, 6, 7, and 8) had separate low-grade epithelial dysplasia/adenoma and the other three cases (case 1, 4, and 5) had low-grade dysplasia/adenoma that bordered adenocarcinoma. The carcinomatous components of case 6 and case 8 were the signet ring cell type and the mucinous type, respectively. Each specimen was microdissected on a cryostat and then fractionated to enrich the tumor cell population, which was ultimately comprised of greater than 70% tumor cells.

Immunohistochemistry

The representative 4 μ m-thick sections were deparaffinized and rehydrated through a graded series of xylene and alcohol solution. The endogenous peroxidase was blocked with 3% aqueous hydrogen peroxide. The slides were pretreated in a microwave oven for antigen retrieval. The sections were incubated with anti-SELENBP1 antibody (mouse antihuman monoclonal antibody, M061-3, clone 4D4, MBL, Nagoya, Japan) at a 1:500 dilution for 1 h at room temperature and then overnight at 4°C. The antibodies were detected using the avidin-biotin complex method with diaminobenzidine as a chromogen. The slides were counterstained with hematoxylin. In the cases containing concomitant low-grade and/or high-grade dysplasia/adenoma and/or adenocarcinoma, the expression of SELENBP1 was evaluated only in the worst lesion. A semi-quantitative scale from 0 to 100% was used to grade the percentage of SELENBP1-stained tumor cells. For Table 2, 3, the cases with more than 10% immunoreactive tumor cells were categorized as positive and the cases with 10% or less than 10% immunoreactive tumor cells were categorized as negative.

RT-PCR

Total RNA was extracted from 100-200 mg of the microdissected frozen tissues with using a RNeasy Mini kit (Qiagen, Valencia, CA, USA), according to the manufacturer's instructions. First-strand cDNA was synthesized from 1 μ g of the total RNA with using random hexamer primers (Qiagen) and M-MLV Reverse Transcriptase (Invitrogen, San Diego, CA, USA) according to the manufacturer's instructions. Twenty nanograms

of cDNA from each sample was used in each reaction. The reaction was performed with the primers for SELENBP1 and for β -actin in a duplex reaction. The sequences of the forward and reverse primers for SELENBP1 and β -actin were as follows: forward primer for SELENBP1: 5'-CCCAGTCTCATCTCCTCTCG-3', reverse primer for SELENBP1: 5'-GTCTCTCCCA-TGTCCTTC-3', forward primer for β -actin: 5'-TGCTATCCCTGTACGCCTCT-3', reverse primer for β -actin: 5'-GTA-CCTGCGCTCAGGAGGAG-3'. The SELENBP1 primers were designed to contain two sites of the exon-exon junction in the PCR product, and β -actin primers were designed to contain an exon-exon junction. β -actin was used as an internal control. After the RT-PCR, 5 μ L aliquots of the products were subjected to 2% agarose gel electrophoresis and then staining was done with ethidium bromide.

Statistical analysis

The statistical analysis was performed using Pearson's χ^2 test for Table 1, 2, and by one-way ANOVA for Fig. 2. The statistical calculations were performed with the SPSS version 13.0 for Windows software (SPSS Inc, Chicago, IL, USA) and a p-value <0.05 was considered statistically significant.

Table 1. Clinicopathologic characteristics of the low-grade and the high-grade epithelial dysplasia/adenomas and the adenocarcinomas

	Epithelial dysplasia, low-grade (n=45)	Epithelial dysplasia, high-grade (n=42)	Adenocarcinoma (n=64)
Age (years, mean \pm SD)	63 \pm 8.6	65 \pm 10	63 \pm 10
Sex			
Male	31	25	40
Female	13	17	24
Tumor size (cm, mean \pm SD)	0.8 \pm 0.4	0.7 \pm 0.5	1.3 \pm 0.6
Location ^a			
Cardia/fundus/body	21 (47%)	13 (31%)	13 (20%)
Antrum/pylorus	24 (53%)	29 (69%)	51 (80%)
Gross type ^b			
Protruded (I)/elevated (IIa)	22 (49%)	5 (12%)	7 (11%)
Flat (IIb)/depressed (IIc)/ulcerated (III)	23 (51%)	37 (88%)	57 (89%)
Non-neoplastic mucosa			
Normal histology	1	1	2
Helicobacter gastritis	5	3	9
Intestinal metaplasia	29	27	35
Atrophy	10	11	18

^a, Low-grade dysplasia vs high-grade dysplasia+adenocarcinoma, p=0.007; ^b, Low-grade dysplasia vs high-grade dysplasia+adenocarcinoma, p<0.001.

RESULTS

The clinicopathologic characteristics of the gastric adenomas and the adenocarcinomas

The study group was composed of 45 cases of low-grade dysplasia/adenomas, 42 cases of high-grade dysplasia/adenomas and 64 cases of adenocarcinomas. There were no significant differences between these groups for the age distribution or the male-female ratio. Although the difference was not statistically significant, the adenocarcinomas were larger than the low-grade and the high-grade dysplasia/adenomas (1.3 \pm 0.6 cm vs 0.8 \pm 0.4 cm and 0.7 \pm 0.5 cm, respectively). The low-grade dysplasia/adenomas were located in the cardia/body/fundus significantly more often than were the high-grade dysplasia/adenomas and adenocarcinomas (p=0.007), and the low-grade dysplasia/adenomas were significantly more commonly associated with protruded (I) or elevated lesions (IIa), when compared with the high-grade dysplasia/adenomas and adenocarcinomas (p<0.001). The state of the non-neoplastic mucosae was not different among the three groups (Table 1).

The expression of SELENBP1 in the non-neoplastic mucosae

SELENBP1 was found to be expressed in the gastric mucosa, including the foveolar glands, the antral proper glands and the fundic/body proper glands (Fig. 1A, B). It was also expressed in the gastric mucosa with intestinal metaplasia and/or atrophy. The other mesenchymal cells, including smooth muscle cells, endothelial cells, inflammatory cells and fibrocytes/fibroblasts, were completely negative for SELENBP1.

Table 2. Progressive suppression of SELENBP1 expression in the low-grade and the high-grade epithelial dysplasia/adenomas and the adenocarcinomas

	SELENBP1 expression ^a	
	Negative (\leq 10%)	Positive (>10%)
Epithelial dysplasia, low-grade (n=45)	3 (7%)	42 (93%)
Epithelial dysplasia, high-grade (n=42)	13 (31%)	29 (69%)
Adenocarcinoma (n=64)	40 (63%)	24 (37%)

^a, p<0.001 by Pearson's χ^2 test.

Low-grade vs high-grade dysplasia, p=0.005 by Fisher exact test.

High-grade dysplasia vs adenocarcinoma, p=0.001 by Pearson's χ^2 test.

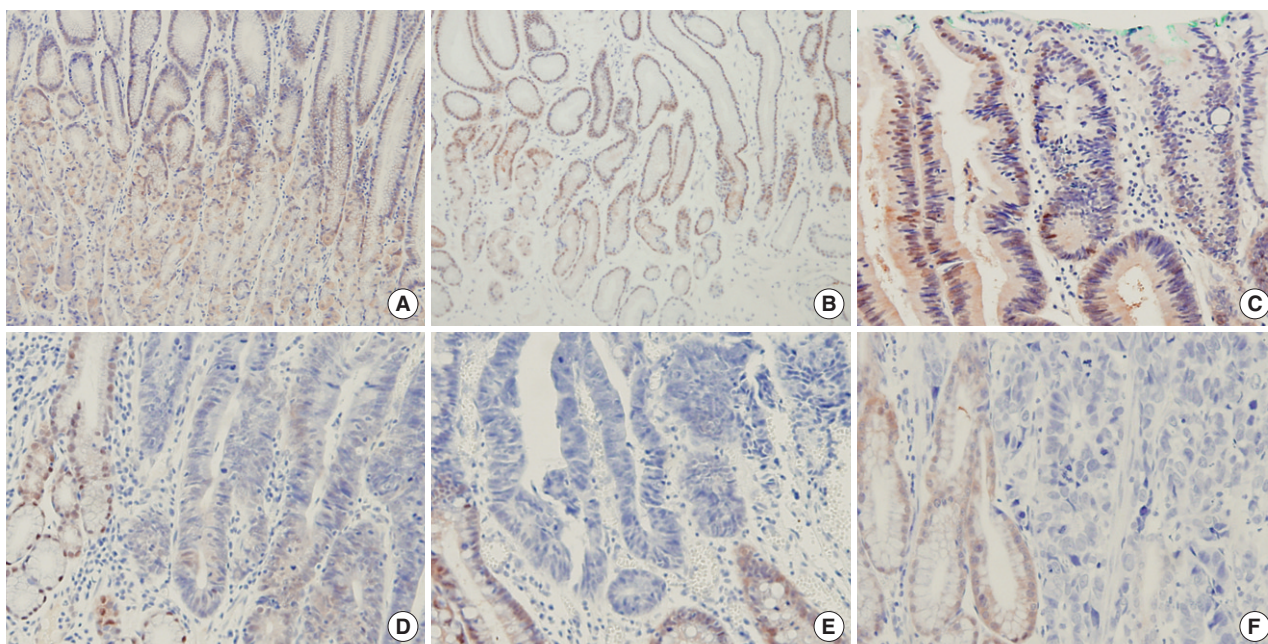


Fig. 1. Immunohistochemical staining for SELENBP1 shows a SELENBP1 expression in a non-neoplastic gastric mucosa of the fundus/body (A) and the antrum (B). The expression of SELENBP1 is slightly decreased in a low-grade epithelial dysplasia/adenoma (C), further decreased in a high-grade dysplasia/adenoma (D) and markedly decreased in adenocarcinomas (E, F).

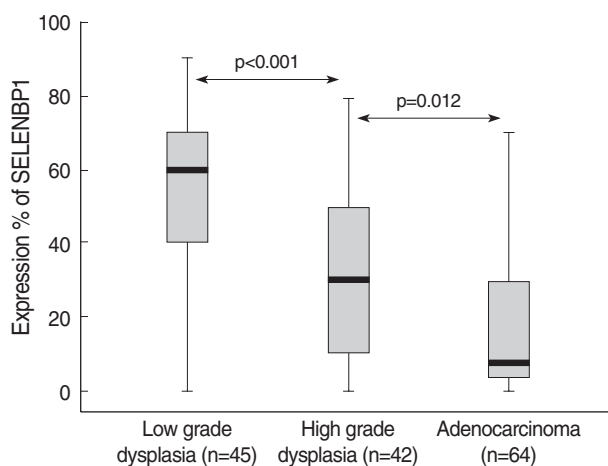


Fig. 2. Progressive suppression of SELENBP1 in the low-grade and the high-grade epithelial dysplasia/adenomas and the adenocarcinomas is statistically significant. The box plot shows median (thick solid line in the box), interquartile range from the first quartile to the third (box), maximum (upper thin solid line) and minimum (lower thin solid line).

The expression of SELENBP1 in the low-grade and the high-grade dysplasia/adenomas, and the adenocarcinoma

The expression of SELENBP1 progressively decreased from the low-grade and the high-grade dysplasia/adenomas to the adenocarcinomas. The expression of SELENBP1 was markedly suppressed in the adenocarcinomas as compared with the non-

Table 3. Comparison of the SELENBP1 expression according to the histologic type, the differentiation, and the invasion depth in the 64 adenocarcinomas

	SELENBP1 expression	
	Negative ($\leq 10\%$)	Positive ($> 10\%$)
Histologic type		
Tubular adenocarcinoma ^a (n=61)		
Well differentiated	26	18
Moderately differentiated	9	5
Poorly differentiated	2	1
Signet ring cell carcinoma (n=3)	3	0
Invasion depth ^b		
Muscularis mucosa (n=25)	13	12
Submucosa (n=39)	23	16

^a, $p > 0.05$ by Pearson's χ^2 test; ^b, $p > 0.05$ by Pearson's χ^2 test.

neoplastic mucosae (Fig. 1C-F). SELENBP1 was expressed in the surrounding mucosa in all the cases. In contrast, 3 of the 45 cases of low-grade dysplasia/adenomas, 13 of the 42 cases of high-grade dysplasia/adenomas and 40 of the 64 cases of adenocarcinomas showed a marked suppression of SELENBP1. The high-grade dysplasia/adenomas revealed a statistically significant loss of the SELENBP1 expression when compared to the low-grade dysplasia/adenomas ($p = 0.005$). The adenocarcinomas revealed a significant loss of the SELENBP1 expression rather than the high-grade dysplasia/adenoma ($p < 0.001$), (Table 2).

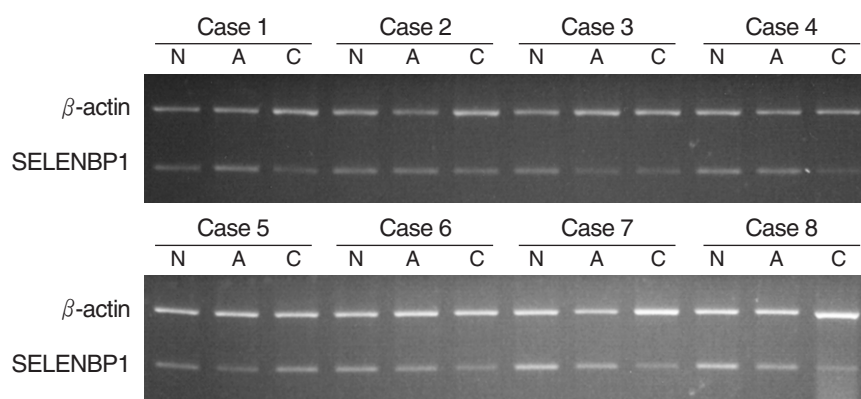


Fig. 3. RT-PCR results of SELENBP1 in the paired gastric dysplasia/adenomas and adenocarcinomas shows a normal SELENBP1 expression in adenomas and a decreased expression in adenocarcinomas (case 1, 2, 4, 7, and 8), a concomitant decreased SELENBP1 expression in both adenomas and adenocarcinomas (case 3 and 6), and a normal SELENBP1 expression in both adenoma and carcinoma (case 5).

The percentage of the SELENBP1 expression among the three groups was also significantly different (Fig. 2). Twenty (44%) of the 45 cases of low-grade dysplasia/adenomas, 8 (19%) of the 42 cases of high-grade dysplasia/adenomas and 4 (6%) of the 64 cases of adenocarcinomas showed a focal, but intensive expression of SELENBP1. The SELENBP1 expression in the adenocarcinomas did not vary according to the differentiation and the invasion depth (Table 3).

The SELENBP1 RNA expression in eight paired cases of gastric adenomas and adenocarcinomas

The reduced expression of SELENBP1 was validated by RT-PCR for the eight paired cases. Of the eight paired cases, five cases (case 1, 2, 4, 7, and 8) showed a normal SELENBP1 expression in the adenomas and a severely suppressed expression in the adenocarcinomas. Two cases (cases 6 and 3) showed a concomitant SELENBP1 suppression in both the adenomas and the adenocarcinomas. The remaining case (case 5) exhibited a normal SELENBP1 expression in both the adenoma and the carcinoma. Overall, two dysplasia/adenomas (25%) and seven carcinomas (87.5%) showed a decreased SELENBP1 expression (Fig. 3). These RT-PCR findings support the immunohistochemical findings, and suggest that the suppression of SELENBP1 occurs at the transcriptional level.

DISCUSSION

In this study, we demonstrate that the expression of SELENBP1 is progressively suppressed in gastric adenomas and adenocarcinomas. The suppression of SELENBP1 has been reported in many cancers, including lung adenocarcinoma,⁹ colorectal adenocarcinoma,^{7,10} prostate adenocarcinoma,⁸ stomach adenocarci-

noma,¹¹ ovarian epithelial carcinoma,¹² and thyroid papillary carcinoma.¹³ The suppression of SELENBP1 in gastric adenocarcinoma suggests that SELENBP1 might be related to gastric carcinogenesis. One study has previously reported on the suppressed expression of SELENBP1 in gastric adenocarcinoma on 10 carcinomas specimens with two-dimensional electrophoresis.¹¹ However, there have not been any studies on the SELENBP1 expression in gastric adenomas. In this study, we demonstrated the suppression of SELENBP1 in gastric adenomas and adenocarcinomas by immunohistochemistry, and we compared the level of SELENBP1 expression between the groups. We found that the SELENBP1 expression was decreased in some gastric adenomas and we also found the progressive suppression of SELENBP1 in gastric carcinomas. These findings indicate that expression of SELENBP1 is severely suppressed in gastric cancer.

SELENBP1 is a protein that binds selenium as a cofactor. Selenium also becomes cotranslationally incorporated into a polypeptide chain as part of selenocysteine which is the 21st encoded amino acid.¹⁸ The molecular mechanism for the suppression of SELENBP1 in cancer cells is not presently clear. Promoter methylation and gene deletion are not the mechanisms in lung adenocarcinomas⁹ or colorectal adenocarcinomas.⁷ There have been many reports that selenium supplementation can decrease the risk of lung, colorectum and prostate cancers.¹⁹⁻²² The Nutritional Prevention of Cancer trial, a 13-year, double-blind, randomized, placebo-controlled clinical trial, was recently completed. The trial confirmed that selenium supplementation was associated with marked reductions of the risk for the total cancer incidence (relative risk [RR]=0.63) (for all sites except skin) and of the risk for carcinomas of the prostate (RR=0.51) and the colorectum (RR=0.46).²² The proposed mechanisms for the protective role of selenium against cancer include inhibiting carcinogen-induced covalent DNA adduct formation, impeding oxidative damage to DNA, lipids, and proteins, increasing apop-

tosis, inhibiting tumor cell growth, altering DNA, RNA, and protein synthesis, and increasing p53 expression.²³ Both our results and previous studies, which have demonstrated a decreased expression of SELENBP1 in several types of carcinomas, suggest that the decreased expression of SELENBP1 may play a role in the tumorigenesis of many types of carcinomas and that the decreased expression of SELENBP1 may abrogate the chemoprotective effects of selenium in tumorigenesis.

Although the percentage of the expression of SELENBP1 was slightly decreased in the low-grade dysplasia/adenomas, the expression of SELENBP1 was relatively preserved in the low-grade dysplasia/adenomas compared to that in the adenocarcinomas. This finding was correlated with the findings from a previous study that used a colorectal adenoma-carcinoma sequence model.⁷ This finding was also supported by the RT-PCR results. Among the eight paired cases, five showed a markedly decreased mRNA expression of SELENBP1 in the adenocarcinomas compared with that in the non-neoplastic mucosae and the dysplasia/adenomas. When the intensity of the RT-PCR product for the SELENBP1 was adjusted by that for the β -actin, the dysplasia/adenomas of cases 1, 2, and 4 showed a stronger expression of SELENBP1 than that of the paired non-neoplastic mucosae. This finding was also found on our immunohistochemical analysis. Although the percentage of the SELENBP1 expression was decreased in the low-grade dysplasia/adenomas compared with that of non-neoplastic mucosae, many low-grade dysplasia/adenomas showed a focal, but intensive expression of SELENBP1. In contrast, a generalized and severe suppression of SELENBP1 was noted in most carcinomas. Our immunohistochemistry and RT-PCR results implicate that the suppression of SELENBP1 is a late event in gastric carcinogenesis.

In conclusion, the SELENBP1 expression is markedly suppressed in gastric adenocarcinomas and is a late event in gastric carcinogenesis. Further study will be necessary to precisely determine the relationship between the SELENBP1 expression levels and the clinicopathologic and/or molecular factors of gastric carcinomas, including the histologic type according to Lauren, the specific tumor type such as signet ring cell type or mucinous type, the tumor stage, the prognosis, the presence of microsatellite instability and several other genetic characteristics.

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