Expression of the *ets-1* Proto-Oncogene in Human Colorectal Carcinoma

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The proto-oncogene, *ets-1*, is a transcription factor known to control the expression of a number of genes involved in extracellular matrix remodeling and has been postulated to play a role in cell migration and tumor invasion. To elucidate the involvement of *ets-1* in human colorectal carcinomas, we examined 41 cases of colorectal adenoma and 122 cases of colorectal carcinoma by immunohistochemistry and compared the degree of Ets-1 expression with the depth of carcinoma invasion. In adenomas, 12 of 41 cases (29.3%) showed immunopositivity for Ets-1. 12 of 27 cases (44.4%) of adenoma with high grade dysplasia showed immunopositivity for Ets-1. However, there was no positive case in low or moderate dysplasia of adenoma. In contrast, 103 of 122 cases (84.4%) of colorectal adenocarcinoma showed immunoreactivity for Ets-1 in the carcinoma cells themselves. We investigated the relationship between pathological features in colorectal carcinoma and Ets-1 immunoreactivity of the tumor cells. Among the 122 cases of invasive carcinomas, Ets-1 immunoreactivity was significantly correlated with the depth grading of tumor invasion ($P < .0001$), the presence of lymph node metastasis ($P < .05$), lymphatic invasion ($P < .01$) and venous invasion ($P < .05$). However, Ets-1 expression did not correlate with histological differentiation. *In situ* hybridization also confirmed the presence of ets-1 mRNA in colorectal carcinomas. Expression of ets-1 mRNA was also detected in two of three human colorectal carcinoma tissues and in four of six different kinds of carcinoma cell lines by the reverse transcription polymerase chain reaction method. These findings suggest that the expression of Ets-1 is one of the important factors related to carcinogenesis and/or tumor invasion of colorectal carcinoma.

KEY WORDS: Colorectal carcinoma, *ets-1*, Immunohistochemistry, *In situ* hybridization.

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The prognosis of colorectal cancer patients is based on the depth of invasion and the presence of lymph node metastasis. Usually, these parameters can be determined by microscopic examination of tissue sections from the primary neoplasm and lymph nodes (1). However, only histopathological examination of primary colorectal carcinoma specimens cannot always elucidate the prognosis (1). Recently, the occurrence and progression of cancer is suggested to be related to a series of genetic events affecting the structure and/or the expression of a number of oncogenes, anti-oncogenes and growth factors. However, the mechanism of the invasion of colorectal carcinomas has not been fully elucidated.

ets-1 was originally characterized as a *v-ets* retroviral gene, 1 of the 2 oncogenes (*v-myb* and *v-ets*) of the avian leukemia retrovirus, E26 (2). The *ets* family of genes encodes transcription factors for mesodermal cell development during the embryonal period (3). Ets-1 plays a role in the regulation of physiological processes such as cell proliferation and differentiation (4). Ets-1 is also associated with invasive processes in the stromal tissues of human carcinomas (5). We have reported that Ets-1 is correlated with the progression of the carcinoma cells of the stomach (6), pancreas (7) and thyroid (8). However, the biological function of the *ets-1* proto-oncogene remains unknown. The processes of tumor invasion and metastasis are thought to depend on the increased proteolytic activity of the invading tumor cells (9). Matrix metalloproteinases and plasminogen activator have been proposed to participate in the metastatic cascade (9–11). Ets-1 protein interacts with the urokinase-type plasminogen ac-

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tivator (u-PA) gene enhancer and with the promoters of the stromelysin-1 and matrix metalloproteinase-1(MMP-1) genes (5, 12). Ets-1, therefore, is suggested to regulate increased tumor invasion by activating the expression of u-PA, stromelysin and collagenase. Based on these findings, we hypothesized that *ets-1* may be involved in the progression and/or the invasion of colorectal carcinomas.

The purpose of the present study was to evaluate the role of *ets-1* in the carcinogenesis and/or the invasion of human colorectal carcinoma.

MATERIALS AND METHODS

Cases and Tissues

We studied 41 colorectal adenomas and 122 primary human colorectal adenocarcinomas. All specimens of adenoma had been resected by endoscopy and all specimens of adenocarcinoma were obtained from patients operated on at Nagasaki University Hospital between 1996 and 1999. Each tumor was assigned a histological type according to the World Health Organization classification (13) and a depth grading of infiltration according to the TNM staging system by the American Joint Commission on Cancer (14). Fifteen specimens of normal colon mucosal tissue, that were taken from patients without colorectal cancer, were evaluated as the normal control.

The desmoplastic stromal reaction was graded according to the extent of the stromal area involved. It was defined as "slight" (when the fibrous stromal area was less than 25% of the whole tumor), "moderate" (between 25 and 50%), and "extensive" (when it exceeded 75% of the whole tumor) based on the overall pattern (15). The examination was performed on routine slides to identify lymphatic, venous and perineural invasion. In addition to hematoxylin and eosin staining, we also used Elastica van Gieson stain in all cases. Each parameter was defined as "present" when invasion was identified with certainty, but defined as "absent" when either not observed at all or not observed with certainty (16, 17). Lymph node metastasis was defined as "present" only when histologically proven. Diagnosis was established by two independent pathologists (TN, MI), and cases of questionable diagnosis were omitted from the study.

Immunohistochemistry

Formalin-fixed and paraffin-embedded tissues were cut into 4 μm sections, deparaffinized in xylene and rehydrated in phosphate-buffered saline. Deparaffinized sections were preincubated with normal bovine serum to prevent nonspecific binding, and then incubated overnight at 4°C with an optimal dilution (0.1 μg/mL) of a primary polyclonal antibody against human Ets-1 (C-20, raised against the C-terminal domain of the Ets-1 protein; Santa Cruz Biotechnology, Inc., Santa Cruz, CA) (18). The slides were sequentially incubated with an alkaline phosphatase-conjugated horse antirabbit immunoglobulin antibody, and the reaction products were resolved using a mixture of 5-bromo-4-chloro-3-indolyl phosphate and nitroblue tetrazolium chloride (BCIP/NBT; BRL, Gaithersberg, MD). Preabsorption of the primary antibody with excess recombinant ets-1 peptide (Santa Cruz Biotechnology, Inc.) was used as a negative control. Adrenal gland tissue (3) served as the internal positive control for Ets-1 immunostaining. Analysis of the immunohistochemical staining was performed by two investigators (TN, MI). Ets-1 expression was classified into three categories, depending on the percentage of cells stained: −, 0 to 10% positive cells; +, 10 to 50% positive tumor cells; and ++, more than 50% positive tumor cells.

In Situ Hybridization

In situ hybridization for the detection of human ets-1 mRNA was performed using an oligonucleotide probe complementary to a fragment of human ets-1 mRNA (19). The sequence of the oligonucleotide probe is 5'-GCCCAGCTTCATCACAGAGTCCTA TCAGAC-3', and it does not cross-hybridize with other mRNA sequences. The probe was labeled with 5' tailed digoxigenin (DIG, Greiner Japan, Inc., Tokyo, Japan) and purified by high performance liquid chromatography. Four cases of human colorectal adenocarcinoma were studied by *in situ* hybridization. In all of these cases, we were able to obtain relatively fresh (within 6 months) paraffin embedded sections. The presence of cytoplasmic RNA was confirmed through the use of a methyl green pyronine staining solution (Muto Pure Chemicals, Tokyo, Japan). Prehybridization was carried out as described previously (20). The sections were treated with 0.2 N HCl for 20 minutes and digested with 100 μg/mL of proteinase K (Sigma, St. Louis, MO) for 10 minutes at 37°C. After postfixation in 4% paraformaldehyde, each section was covered with 20 μL of a denatured hybridization mixture containing 4% dextran sulfate, 125 μg/mL sonicated salmon sperm DNA, 9% deionized formamide, 2.5 μg/mL yeast tRNA, 5 × Denhardt's medium, 1 mM EDTA (ethylenediamine-tetraacetic acid, pH 7.4), 0.6 M NaCl, 10 mM Tris-HCl, and 1 μg/mL digoxigenin-labeled Ets-1 oligonucleotide probe, and placed in a moist chamber, where it was incubated at 37°C for 15 hours. After washing, *in situ* detection was accomplished with a DIG detection kit (GIBCO/BRL). Briefly, the slides were incubated with 100 μL of blocking solution for 15 min

at room temperature and incubated with the streptavidin-alkaline phosphatase complex. ets-1 mRNA expression was evaluated by comparing alkaline phosphatase staining using BCIP/NBT with the results obtained from the positive and negative controls. Each slide was studied in duplicate and negative controls were made using the digoxigenin-labeled sense oligoprobe (5'-GTCTGATAGGACTCTG TGATGAAGCTGGGC-3'). RNase treatment was carried out before hybridization as another negative control. Slides of a human adrenal gland served as a positive control (3).

Cell Culture

Six human colorectal carcinoma cell lines LoVo (poorly differentiated adenocarcinoma), DLD-1 (adenocarcinoma), WiDr (adenocarcinoma), Colo 201 (adenocarcinoma), Colo 320 (adenocarcinoma), and Sw 837 (adenocarcinoma) (21–26), were incubated at 37°C in a humidified atmosphere of 5% CO_2 and 95% air. Cultures of LoVo and Colo 201 were maintained in RPMI medium 1640 (GIBCO/BRL, Grand Island, NY) supplemented with 10% fetal calf serum (21, 22). DLD-1 was maintained in DMEM/F-12 (GIBCO/BRL) supplemented with 10% fetal calf serum (23). WiDr was maintained in Dulbecco's modified Eagle's medium (DMEM, GIBCO/BRL) supplemented with 10% fetal calf serum (GIBCO/BRL) (24). Colo 320 was maintained in RPMI 1640 medium (GIBCO/BRL) supplemented with 10% fetal calf serum (25) and Sw 837 was maintained in modified L-15 medium with 10% fetal calf serum (26). Plastic culture dishes were purchased from Becton Dickinson (Oxnard, CA). All of these cells were provided by the Health Science Research Resources Bank (Osaka, Japan).

Reverse Transcription-Polymerase Chain Reaction (RT-PCR)

Total RNA was prepared from three human colorectal carcinoma tissues and the carcinoma cell lines Lovo, DLD-1, WiDr, Colo 201, Colo 320 and Sw 837 (21–26) using the acid guanidine phenol method (27).

Cellular RNA (1 μg) was incubated at 37° C for 1 hour in 50 μL of reverse transcriptase buffer containing 20 units of RNAsin (Promega Corp., Madison, WI), 100 pmol of random hexamer primers (Boehringer Mannheim, Mannheim, Germany), and 400 units of Moloney murine leukemic virus reverse transcriptase (GIBCO/BRL). Reverse transcription was terminated by heating at 95° C for 10 minutes, and 20% of the resultant cDNA was removed for PCR. PCR samples were incubated with 50 pmol of each primer and 2.5 units of *Taq* DNA polymerase. The human *ets-1* PCR primers were 5'-GGGTGACGACTTCTTGTTTG-3' (sense) and 5'-GTTAATGGAGTCAACCCAGC-3' (antisense). The human §-actin PCR primers were 5'-TCCTCCCTG GAGAAGACTA-3' (sense) and 5'-AGTACTTGCG CTCAGGAGGA-3' (antisense). The *ets-1* and β-actin primers were predicted to amplify 274 and 313 bp DNA fragments, respectively. Both primer pairs were chosen to span introns of their respective human genes. Samples were subjected to 28 cycles of PCR amplification using a thermocycler. Each cycle included denaturation at 94° C for 1 minute, annealing at 57° C for 1 minute, and primer extension at 72° C for 1.5 minutes. An aliquot of each amplification mixture was subjected to electrophoresis on a 1.5% agarose gel, and DNA was visualized by ethidium bromide staining.

Statistical Analysis

The Stat View II program (Abacus Concepts, Inc., Berkeley, CA) was used for statistical analyses. Analyses comparing the intensities of Ets-1 expression were performed by the Mann-Whitney and Spearman's tests.

RESULTS

Of the 122 primary human colorectal adenocarcinomas there were 17 submucosal infiltrative carcinomas (T1), 12 carcinomas invading proprial muscle layers (T2), 82 carcinomas reaching the subserosa (T3), and 11 carcinomas through serosa or invading contiguous organs (T4). Histologically, of the 122 primary human colorectal adenocarcinomas, there were 41 well differentiated adenocarcinomas, 66 moderately differentiated adenocarcinomas, 8 poorly differentiated adenocarcinomas, 7 mucinous adenocarcinomas. The 27 specimens of adenoma with high grade dysplasia (Tis) and the 14 specimens of adenomas with low or moderate grade dysplasia had been resected by endoscopy.

The results of immunohistochemical staining are summarized in Tables 1 and 2. In Table 1, Ets-1 antigen was expressed heterogeneously in adenomas and carcinomas. Strong staining of Ets-1 was observed in 84.4% of the carcinomas (103 of 122) and there was significant differences between total adenocarcinoma and adenoma/normal mucosa, respectively ($P < .01$). In adenomas, 12 of 41 cases (29.3%) showed immunopositivity for Ets-1. Twenteen of 27 cases (44.4%) with high grade dysplasia of adenoma (Tis) showed immunopositivity for Ets-1. However, there was no positive case in 14 cases with low or moderate dysplasia of adenoma. There was significant difference in Ets-1 expressions with between high grade dysplasia and low/moderate dysplasia of adenomas ($P < .01$). And all 15 cases of normal mucosa were negative for Ets-1.

TABLE 1. Ets-1 Expression in Colorectal Tumors

	n	Ets-1 Expression, *n* (%)			Note
		−	+	++	
Invasive carcinoma (T1–4)	122	19 (15.6)	30 (24.6)	73 (59.8)	[a]
Adenoma	41	29 (70.7)	4 (9.8)	8 (19.5)	
High-grade dysplasia (Tis)	27	15 (55.6)	4 (14.8)[c]	8 (29.6)[c]	[b]
Low- or moderate-grade dysplasia	14	14 (100.0)	0 (0.0)	0 (0.0)	
Normal mucosa	15	15 (100.0)	0 (0.0)	0 (0.0)	

[a] Significant differences between total adenocarcinoma and adenoma/normal mucosa, respectively ($P < 0.01$).
[b] Significant differences between high-grade dysplasia and low/moderate dysplasia of adenoma, respectively. ($P < 0.01$).
[c] Ets-1 was expressed in only high-grade dysplastic area of adenoma.

Ets-1 immunoreactivity of tumor cells in relation to pathological features in invasive carcinomas is shown in Table 2. Every type of colorectal carcinomas stained strongly for Ets-1. Ets-1 expression was found in 75.6% (31/41) of well-differentiated adenocarcinomas, 87.9% (58/66) of moderately differentiated adenocarcinomas, 100.0% (8/8) of poorly differentiated adenocarcinomas and 85.7% (6/7) of mucinous carcinomas. There were no statistical correlations in between Ets-1 immunoreactivity and the differentiation of colorectal carcinomas.

Ets-1 immunoreactivity was compared with the depth grading of tumor invasion. Ets-1 expression was found in 41.2% (7/17) of T1 tumor, in 83.3% (10/12) of T2 tumor, in 92.7% (76/82) of T3 tumor, and in 90.9% (10/11) of T4 tumor, respectively. A significant correlation was found between Ets-1 immunoreactivity and the depth grading of tumor invasion ($P < .0001$).

TABLE 2. Ets-1 Expression in Colorectal Tumor (122 Cases)

	n	Ets-1 Expression, *n* (%)		
		−	+	++
Total Adenocarcinoma	122	19 (15.6)	30 (24.6)	73 (59.8)
Histological Differentiation				
Well	41	10 (24.4)	8 (19.5)	23 (56.1)
Moderate	66	8 (12.1)	18 (27.3)	40 (60.6)
Poor	8	0 (0.0)	3 (37.5)	5 (62.5)
Mucinous carcinoma	7	1 (14.3)	1 (14.3)	5 (71.4)
Invasive grade[a]				
T1	17	10 (58.8)	4 (23.5)	3 (17.6)
T2	12	2 (16.7)	5 (41.7)	5 (41.7)
T3	82	6 (7.3)	19 (23.2)	57 (69.5)
T4	11	1 (9.1)	2 (18.2)	8 (72.7)
Desmoplastic stromal reaction[b]				
Slight	27	7 (25.9)	8 (29.6)	12 (44.4)
Moderate	65	9 (13.8)	16 (24.6)	40 (61.5)
Extensive	30	3 (10.0)	6 (20.0)	21 (70.0)
Lymph node metastasis[c]				
Absent	72	18 (25.0)	14 (19.4)	40 (55.6)
Present	50	1 (2.0)	16 (32.0)	33 (66.0)
Lymphatic invasion[d]				
Absent	26	9 (34.6)	8 (30.8)	9 (34.6)
Present	96	10 (10.4)	22 (22.9)	64 (66.7)
Venous invasion[e]				
Absent	67	14 (20.9)	19 (28.4)	34 (50.8)
Present	55	5 (9.1)	11 (20.0)	39 (70.9)
Perineural invasion				
Absent	97	16 (16.5)	25 (25.8)	56 (57.7)
Present	25	3 (12.0)	5 (20.0)	17 (68.0)

[a] $P < 0.0001$, Spearman's test.
[b] $P < 0.05$, Spearman's test.
[c] $P < 0.05$, Mann-Whitney *U* test.
[d] $P < 0.01$, Mann-Whitney *U* test.
[e] $P < 0.05$, Mann-Whitney *U* test.

The relationship between Ets-1 immunoreactivity of the tumor cells and the desmoplastic stromal reaction were investigated. There were significant associations between Ets-1 immunoreactivity and the stromal reactions ($P < .05$).

The incidence of lymph node metastasis, lymphatic invasion, venous invasion, and perineural invasion was 36.1%, 78.7%, 45.1% and 20.5%, respectively. Ets-1 immunoreactivity was significantly correlated with the presence of lymph node metastasis ($P < .05$), lymphatic invasion ($P < .01$) and venous invasion ($P < .05$). However, there was no correlation between Ets-1 expressions and the perineural invasion.

Also, the invasive front and/or the peripheral parts of the primary tumor were intensely stained compared with the superficial and central parts of the tumor in almost all cases of invasive carcinomas.

Figure 1A and B show a representative example of strong immunohistochemical Ets-1 staining in a carcinoma invading the subserosa. Ets-1 protein was detected in both the cytoplasm and the nucleus of almost all carcinomas (Fig. 1A). In a few cases, Ets-1 protein was predominantly located in the nucleus (Fig. 1B). Figure 1C and D show the adenoma with high grade dysplasia. Ets-1 was expressed only in the area with high grade dysplasia, but not in area with moderate or low grade dysplasia (Fig. 1D).

As with the antigenic intensity shown by immunostaining, ets-1 mRNA expression demonstrated by *in situ* hybridization was positive in both the cytoplasm and the nucleus of colorectal adenocarcinomas (Fig. 1E). And there are correlative expression of Ets-1 in between *in situ* hybridization and immunohistochemistry (Fig. 1F). No specific hybridization was observed with the sense labeled probe. RNase treatment of the sections hybridized with the Ets-1 oligonucleotide probe yielded no positive signals.

The results from RT-PCR of ets-1 mRNA in human colorectal cancer tissues and carcinoma cell lines are shown in Figure 2. Two of three human

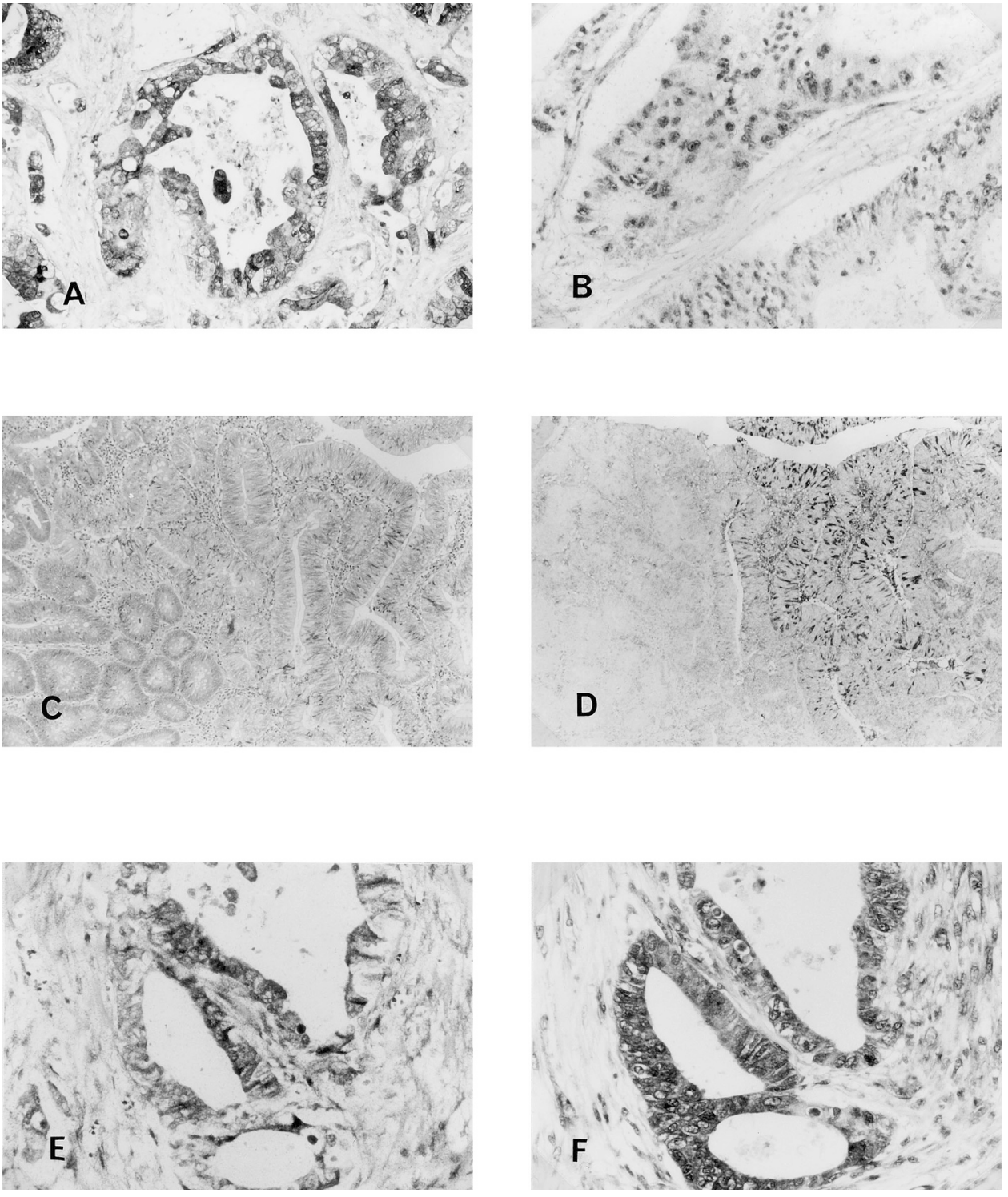

FIGURE 1. Immunohistochemical studies of Ets-1 reveals positive staining in cytoplasm and nucleus in human colorectal carcinoma cells (**A**). Ets-1 protein is located predominantly in the nucleus (**B**). Hematoxylin–eosin staining of polypoid neoplasia in the human colon (**C**). Left area of this figure shows adenoma with moderate grade dysplasia and right area shows adenoma with high grade dysplasia. Immunohistochemical studies of Ets-1 reveal positivity only for the area of adenoma with high grade dysplasia, but not for the area of adenoma with moderate dysplasia (**D**). Using the *in situ* hybridization method, ets-1 mRNA is expressed in cytoplasm of carcinoma cells (**E**) and showing the immunohistochemical staining in same area of *in situ* hybridization for comparison (**F**). Immunoalkaline phosphatase staining; magnification: **A**, 100×; **B**, 200×; **D**, 60×; **E**, 200×; **F**, 200×. And hematoxylin-eosin staining; magnification: **C**, 60×.

colorectal carcinoma tissues and four carcinoma cell lines (Lovo, WiDr, Colo 201 and Sw 837) expressed ets-1 mRNA. However, one cancer tissue and two cell lines (DLD-1 and Colo 320) did not express ets-1 mRNA. β-actin mRNA, a control to demonstrate the equivalent amounts of tissue RNA, was used for cDNA synthesis and was detected in all samples.

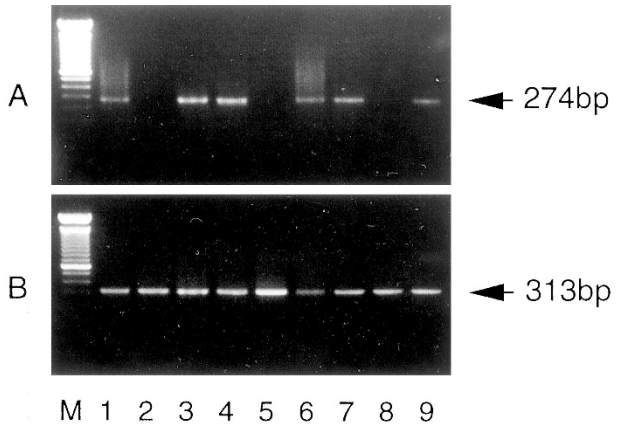

FIGURE 2. RT-PCR analysis of ets-1 mRNA expression in human colon carcinoma cells using the specific primer pairs predicted to amplify fragment size on the right (**A**, Ets-1, **B**, β-actin as internal control). Total RNA was prepared from three human colorectal carcinoma tissues (*Lanes 1–3) and six carcinoma cell lines (*Lane 4, Lovo; *Lane 5, DLD-1;* Lane 6, WiDr; *Lane 7, Colo 201;* Lane 8, Colo 320; *Lane 9, Sw 837). Size markers (*Lane M) consist of 100-bp DNA ladder markers (Takara, Tokyo, Japan).

DISCUSSION

There is the expression of the *ets* family of genes during embryonic life, particularly in the intestine, and this expression is dramatically reduced in later fetal age (3). In our study, Ets-1 was not expressed in the normal colon epithelium nor in colorectal adenomas with low or moderate dysplasia. However, 44.4% (12/27) of adenoma with high grade dysplasia (Tis) and 84.4% (103/122) of total carcinomas overexpressed Ets-1 protein (Tables 1 and 2). In submucosal carcinomas (T1) Ets-1 expression was weak (41.2% positive, 7/17), but was enhanced in carcinomas over submucosa (T2–4) (91.4% positive, 96/105) (Table2). And there was statistical correlation between Ets-1 expressions and invasive grade ($P < .0001$). Therefore, the degree of Ets-1 expression was correlated to the extent of colorectal carcinoma invasion. Multi-step carcinogenesis has been proposed for colorectal carcinoma, a theory that is generally accepted (28). These results suggest that *ets-1* has a role in the invasion of colorectal carcinoma, as one of the steps in carcinogenesis and in the development of invasive characteristics.

In adenoma with high grade dysplasia, Ets-1 expression was detected in 44.4% (12/27) of cases. Ets-1 was expressed only in the area with high grade dysplasia, but not in the area with moderate or low dysplasia. We reported previously that Ets-1 plays a role in tumor invasion (6–8). We suppose that the adenoma with high grade dysplasia has a higher degree of invasive activity than the adenoma with low or moderate grade dysplasia in the colorectum.

Poorly differentiated adenocarcinomas show invasive spreading histologically, and have a significantly poorer prognosis than well and moderately differentiated carcinomas (29). It has been reported that there was a significant correlation between the grade of histological differentiation and collagenolytic activity (30). However our results indicated no association between Ets-1 expression and tumor differentiation.

Tumor desmoplasia is a common feature in several malignant human tumors. It has been reported that a well-marked desmoplastic stromal reaction is associated with a poorer prognosis (31). Expression of MMP-1 that is regulated by Ets-1 has been observed in areas of extracellular matrix remodeling both in physiological and pathological situations (32). In our study, there were significant associations between Ets-1 immunoreactivity in carcinoma cells and the degree of desmoplastic stromal reaction ($P < .05$). We supposed that Ets-1 played some roles in stromal remodeling through the expression of MMP-1.

In invasive carcinomas, Ets-1 immunoreactivity was significantly correlated with the presence of lymph node metastasis ($P < .05$), lymphatic invasion ($P < .01$) and venous invasion ($P < .05$). Ordinarily, these results were understanded that the expression of Ets-1 induced the metastatic activity of carcinoma cells. However, in our study, the incidence of lymphatic invasion, venous invasion and lymph node metastasis increased with the depth of tumor invasion (data not shown). Therefore, we supposed that Ets-1 induced the deeper invasion of carcinoma cells, and that carcinoma cells infiltrated successively to lymph ducts and vein, and reached lymph nodes.

The product of *ets-1* is localized in the nucleus and binds to DNA (33). However, in other reports, Ets-1 protein was detected in the cytoplasm (34) or both in the cytoplasm and in the nucleus in human colorectal carcinoma cell lines (35). In this study, we showed immunohistochemically that Ets-1 protein was widely expressed in the cytoplasm of carcinoma cells as well as in the nucleus. We believe that Ets-1 protein is overproduced in the cytoplasm and bound to DNA in the nucleus of colorectal carcinoma cells. Recently, it was shown that Ets-1 protein regulates the gene expression of some cytokines and peptides, such as Fos and Jun (36), integrin (37), stromelysin (38), u-PA (5) and MMP-1 (12). The expression of these substances also has been observed in colorectal carcinoma cells (39–41), and they may play important roles in tumor invasion and progression.

It was necessary to determine whether Ets-1 is produced by mesenchymal cells or carcinoma cells. Ets-1 is thought to be involved not only in tumor invasion but in connective tissue remodeling (42). We observed increased Ets-1 expression during vascular smooth muscle cell migration and/or proliferation induced by serum stimulation *in vitro* and

by balloon injury *in vivo* (18). Ets-1 is known to be expressed in stromal fibroblasts around carcinoma cells (43). The expression of the *ets-1* gene also has been demonstrated in vascular sarcomas (44) and astrocytomas (45). In this study, however, we detected ets-1 mRNA in the colorectal carcinoma cells themselves, but not intensely in stromal fibroblasts, as shown by immunohistochemistry and *in situ* hybridization. RT-PCR of the ets-1 mRNA from the two human carcinoma tissues and the four carcinoma cell lines confirmed that it is produced in the carcinoma cells themselves, as we previously reported in carcinoma cells of the stomach (6), pancreas (7) and thyroid (8). These findings suggest that Ets-1 is expressed in carcinoma cells and we suppose that Ets-1 may play a critical role in the invasion of colorectal carcinoma cells.

The *ets-1* gene is localized to the q23 region of chromosome 11 and is translocated in certain leukemias and lymphomas (46). Recently, in colorectal carcinoma, loss of heterozygosity (LOH) of chromosome 11q23 has been reported (47), and it was suggested that the mutation of the *ets-1* gene was concerned with the genesis of colorectal carcinoma. Although we did not examine the LOH of chromosome 11q23 in this study, it has reported that wild type Ets-1 suppresses tumorigenicity of human colon cancer cells, but variant Ets-1 did not suppress it (48). Therefore, we suggest that the overexpressed Ets-1 protein in colorectal carcinoma is a variant form of the wild type and that this variant type does not have the normal function of wild type Ets-1 as a p53 proto-oncogene product. Further studies are necessary for confirmation of this hypothesis.

In conclusion, our results suggest that *ets-1* plays a role or roles in the carcinogenesis and/or the progression of colorectal adenocarcinoma. The overexpression of the *ets-1* proto-oncogene product may be one of the steps in the multi-step carcinogenesis of human colorectal carcinomas.

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