Regulation of the GnT-V Promoter by Transcription Factor Ets-1 in Various Cancer Cell Lines*

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The glycosylation of cell surface glycoproteins is thought to play a critical role in a variety of specific biological interactions (1). Numerous studies have concluded that alterations of cell surface glycoprotein oligosaccharides cause significant changes in the adhesive or migratory behavior of a cell (2). The high degree of branching of N-glycans, in particular, the β1–6 branching type, appears to be related to a potential for the development of malignancy (3). UDP-GlcNAc, α-mannoside β-1,6-N-acetylglucosaminyltransferase V (GnT-V),1 represents an enzyme that catalyzes such branching. To understand the molecular basis of this oligosaccharide structure in terms of tumor metastasis, we and other groups have purified GnT-V from rat kidney (4) and from a human lung cancer cell line QG (5) and cloned the gene. The expression of GnT-V is enhanced by malignant transformation through the ras oncogene (6), by cell proliferation (7), and by hepatocarcinogenesis (8). However, GnT-V is also highly expressed in normal tissues, suggesting that the synthesis of oligosaccharides by GnT-V might be different in normal cells versus cancer cells (9). The most important issue, however, is how GnT-V modifies the metastatic potential of tumor cells.

When the GnT-V gene was transfected into a lung epithelioid cell, the transfectant showed an increased tumorigenicity, as evidenced by an assay involving the subcutaneous injection of the cells into nude mice (10). Interestingly, this cell showed an altered transformed cell morphology, as is often observed for oncogenically transformed cells. In addition, the overexpression of GnT-V resulted in a decrease in serum growth requirements of the contact-inhibited parental cells, but the migration rates of controls increased by 3- to 10-fold, and they adhered less well to fibronectin or collagen type IV. In contrast, when the overexpression of other glycosyltransferases, such as GnT-III, inhibited the action of GnT-V through substrate availability, lung metastasis of melanoma cells was dramatically suppressed (11). These data suggest that GnT-V is able to affect the phenotypes of cells directly, that a high level of GnT-V expression in tumor cells with a greater potential for malignancy may not be the result of malignant transformation by other factors, and that GnT-V itself may well be the key biological factor. For these reasons, it is important to elucidate the mechanism by which GnT-V is expressed.

We recently isolated human GnT-V genomic DNA clones and showed that the gene consists of 17 exons and spans 155 kilobases (12). The sequence analysis of the 5’ flanking region of GnT-V revealed that some putative consensus sequences for tissue-specific transcription factors, such as AP-1 and ets, are present in the promoter regions (Fig. 1). The AP-1 site is known to form complexes with the proto-oncogenes c-Jun and c-Fos in response to a wide variety of growth factors (13). The Ets family is a novel class of trans-acting phosphoproteins that play important roles in the control of growth and development (14). The family is defined by a highly conserved Ets domain (15), which encodes a winged helix-turn-helix DNA-binding motif (16), ets binding sites, which contain a common core trinucleotide sequence. GGA, have been identified in the regulatory regions of human T-cell receptor α (17) and β (18) and the interleukin 2β receptor (19), as well as other cellular and viral enhancers.

Whereas the stromal expression of c-Ets-1 is correlated with tumor metastasis (20), the overexpression of Ets-1 in colon cancer cells has been shown to result in suppression of tumorigenesis (21). These findings suggest that Ets-1 is a multifunctional transcription factor. To demonstrate an involve-

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1 The abbreviations used are: GnT-V, UDP-GlcNAc-α-mannoside β-1,6-N-acetylglucosaminyltransferase V; GFP, green fluorescence protein; AP-1 activator protein 1; TBS-T, Tris-buffered saline containing 0.05% Tween-20; EMSA, electrophoretic mobility shift assay.
Regulation of the GnT-V Promoter by Ets-1

Table I

<table>
<thead>
<tr>
<th>Oligonucleotides used in cDNA cloning</th>
<th>Sequence of the oligonucleotide</th>
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<tr>
<td>ND-ets-1-N</td>
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<tr>
<td>ND-ets-1-C</td>
<td>5′-TGGACGGGCAGCAAGGGGCTAG-3′</td>
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<tr>
<td>ets-2-N</td>
<td>5′-CAATAAGCTATTTAACACTACCA-3′</td>
</tr>
<tr>
<td>ets-2-C</td>
<td>5′-GTACAGTGTTGCCCTTCCGACGC-3′</td>
</tr>
<tr>
<td>ND-ets-2-N</td>
<td>5′-ATGGCTTGAGAAGAGGATTC-3′</td>
</tr>
<tr>
<td>ND-ets-2C</td>
<td>5′-TTCTCCATGTCAGGACGGCTCTAG-3′</td>
</tr>
<tr>
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<td>erg-1-C</td>
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<td>fli-1-C</td>
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</tr>
<tr>
<td>jun-C</td>
<td>5′-GTCGCAAGCGGGGGGGCGGCTCGAAG-3′</td>
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</table>

Regulation of the Ets family in GnT-V gene regulation, we showed that the GnT-V gene is regulated by Ets-1 protein in a human bile duct carcinoma cell line using a gel mobility assay (22). Buckhaults et al. also reported that the regulation of the GnT-V gene is mediated by the src oncogene in the BHK (baby hamster kidney) fibroblast cell line via the involvement of ets transcription factor (23). These results suggest that the GnT-V gene is regulated by ets transcription factors in a cell-specific manner. Ets is also known to cooperate with AP-1 in the transcriptional regulation of genes such as interleukin 2 (19), collagenase (24), and human tumor necrosis factor (23). These results suggest that regulation of the GnT-V gene is mediated by ets transcription factors in a cell-specific manner. Ets is also known to cooperate with AP-1 in the transcriptional regulation of genes such as interleukin 2 (19), collagenase (24), and human tumor necrosis factor (25). To provide more broad evidence concerning the role of ets proteins in enhancing GnT-V gene expression, a more general relationship between GnT-V and ets expression in cancer cells would be desirable.

This study was undertaken to investigate the issue of whether the expression of GnT-V is correlated with that of ets family mRNAs in 16 human and murine cancer cells and to better understand the effects of overexpression of ets-1 and ets-2 and dominant negative ets-1 and ets-2 on GnT-V expression as well as coordination of AP-1 and GnT-V genes.

Materials and Methods

Cell Lines—Human cancer cell lines MKN45 and Kato-III (gastric cancer), A549, Lu99B and Lu65A (lung cancer), PacalI (pancreatic cancer), MB231 (mammary cancer), Hu6, HepG2 (hepatoblastoma), Huh7 (hepatocellular carcinoma), A172 (glioblastoma), mouse melanoma cell line B16-F1, and rat hepatoma cell line AH66tc were obtained from the Japanese Cancer Research Resource (Tokyo, Japan), and Hep3B (hepatocellular carcinoma) and Colo201 and Colo205 (colon cancer) were obtained from American Type Culture Collection (Manassas, VA). These cell lines were cultured under standard conditions (RPMI 1640 medium or Dulbecco’s modified Eagle’s medium (Nikkon Kagaku, Kyoto, Japan) containing 10% fetal calf serum and antibiotics. The antibiotics to Ets-1 and Ets-2 were purchased from Santa Cruz Biotechnology.

Plasmids—Human c-ets-1 cDNA was cloned into the pSVK3 vector, as described previously (22). To express the human ets-1 cDNA, its mRNA was amplified by the polymerase chain reaction using oligonucleotide primers (Table I). The polymerase chain reaction products were cloned into the T vector (Novagen, Madison, WI) and confirmed with DNA sequencing (Applied Biosystem, Chiba, Japan). Antisense c-jun was prepared from a clone in which the Fast fragment (0.6 kilobase) had been cloned in a reverse manner into the EcoRI site of plasmid pCAGGS. Various DNA sequence analyses were performed with DNAstar version 3.6 (Hitachi).

Northern Blot Analysis—Total cellular RNAs were extracted from cancer cell lines according to the method reported by Chomczynski and Sacchi (28). Total RNA (20 μg) was electrophoresed on a 1% agarose gel containing 2.2 μm formaldehyde. After blotting onto a Zeta probe (Bio-Rad) nylon membrane, the filter was hybridized with 32P-labeled human c-ets-1 (22) and ets-2 cDNAs (29) or GnT-V cDNA (30), other ets family cDNAs (31) at 42 °C in a hybridization buffer. The membrane filter was washed with 2× standard saline-citrate, pH 7.4, and 0.1% SDS twice for 10 min each at 55 °C and then washed with 2× standard saline citrate, pH 7.4, and 0.1% SDS for 30 min. The filter was then exposed to x-ray films (Kodak, Kyoto, Japan) with an intensifier screen at 80 °C for 1 day.

Transient Expression of ets-1 and ets-2—Various cancer cells were plated at 1.7 × 105 cells/well of a 60-mm dish 1 day before transfection. 10 μg each of ets-1 and the ets-2 expression vectors were introduced into the cells using a LipofectAMINE reagent (Takara, Shiga, Japan). To determine the efficiency of transfection, pEGFP-N1 (CLONTECH), which expresses the bacterial green fluorescence protein, was used as a control plasmid. Briefly, 10 μg of pEGFP-N1 and 20 μl of LipofectAMINE reagent were mixed with serum-free medium to form a DNA-liposome complex. After incubation for 20 min, 1 ml of media was overlayed on the preincubated cells. The cells were incubated with the complex for 4 h, and the complex was removed by washing with ice-cold phosphate-buffered saline. After incubation for 48 h, the cells were used for further analysis.

Western Blotting—Approximately 1 × 105 cells were washed with ice-cold phosphate-buffered saline, followed by lysis with Nonidet P-40 buffer (10 mM HEPES (pH 7.8) containing 10 mM KCl, 2 mM MgCl2, 0.25 mM EDTA, and 1% Triton X-100) containing 5 μl of 2-mercaptoethanol and subjected to 10% SDS-polyacrylamide gel electrophoresis. The separated proteins were electroblotted onto a nitrocellulose membrane (Schleicher & Schuell). Nonspecific sites on the blot were blocked by incubating the membrane in TBS-T supplemented with 5% skim milk. The filters were then probed with a primary rabbit antibody to Ets-1 (N-276; Santa Cruz Biotechnology) and Ets-2 (C-20) for 2 h at room temperature. The blots were washed with TBS-T for 30 min and then incubated with anti-rabbit horseradish peroxidase-conjugated secondary antibody for 30 min at room temperature. After washing for 30 min, the membranes were developped using ECL (Amersham Pharmacia Biotech) according to the manufacturer’s protocol.

Electrophoretic Mobility Shift Assay (EMSA)—Nuclei were isolated from cultured cells as described above. Extracts were cleared by centrifugation at 14,000 × g for 15 min at 4 °C. Aliquots of the resulting supernatants were frozen at −70 °C. The protein concentration of the nuclear extract was measured using a BCA kit (Pierce) with bovine serum albumin as the standard. These nuclear proteins were used for the electrophoretic mobility shift assay. The DNA fragment containing the putative ets binding sequences of the upstream regulation regions of GnT-V was synthesized as described in Table II. Complementary oligonucleotides were annealed and used as probes or competitors. The probes were end-labeled with γ-[32P]ATP (Amersham Pharmacia Biotech) using T4 polynucleotide kinase (Takara, Osaka, Japan). For the gel mobility shift assay, DNA (10,000 cpm; labeled with 32P) and 5 μg of nuclear proteins were preincubated for 10 min at room temperature with 400 ng of poly(dI-dC) (Sigma) in 20 μl of binding buffer (25 mM Tris-HCl, pH 7.9, 65 mM KCl, 6 mM MgCl2, 0.25 mM EDTA, and 10% glycerol). For the competition assay, the unlabeled competitor oligonucleotide was added before the addition of labeled oligonucleotide. The mixture was incubated for 20 min at 37 °C and then incubated with anti-rabbit horseradish peroxidase-conjugated secondary antibody for 30 min at room temperature. After washing for 30 min, the membranes were exposed to x-ray films (Kodak, Tokyo, Japan) with an intensifier screen at 80 °C for 1 day.
Consensus ets-1 binding

2

2

2

and Colo201 cell lines were subjected to an EMSA analysis. The expression of ets-1, GnT-V, ets-2, erg, and fli-1 mRNAs was significantly lower in MKN45 cells and Kato-III cells. In contrast, their expression was relatively high in HepG2, Huh7, and Colo205 cells, suggesting that Ets-2 could play a significant role in the regulation of these genes. Whereas A549, Lu65A, Lu99B, MKN45, PacaII, MB231, Huh6, Hep3B, and AH66tc cells showed lower levels of both ets-1 and GnT-V mRNAs, the expression of ets-2 was quite high.

RESULTS

Table I

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<th>Name</th>
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<td>Consensus ets-1 binding</td>
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<td>-741 to -718</td>
</tr>
<tr>
<td>mE728</td>
<td>5'-ATGGGCGGAGAGCTTCTAGTTTAT-3'</td>
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<td>EJ565</td>
<td>5'-CGTGTAAAGATGAGCCACACCTCCCTCATTCGTTCTCGTTTGTAT-3'</td>
<td>-578 to -522</td>
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<td>mJ565</td>
<td>5'-CTGTTAAGATGAGCCACACCTCCCTCATTCGTTCTCGTTTGTAT-3'</td>
<td>-578 to -522</td>
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Relationships between GnT-V and ets-1 mRNA Expression in Various Cancer Cell Lines—According to the diagram of the GnT-V promoter region (Fig. 1), the results of our previous study (22), Ets-1 might be expected to have a high potential for influencing GnT-V expression. To examine the relationships between ets-1 and Gn-T-V expression, Northern blot analysis was performed on various cancer cell lines using ets-1, GnT-V, ets-2, erg, and Ets-1 cDNAs as probes (Fig. 2). Kato-III, HepG2, Huh7, Colo201, Colo205, and B16-F1 cells showed higher levels of ets-1 expression than the other cells. The expression of GnT-V mRNA showed a pattern similar to that of ets-1 expression. In contrast, A549, Lu65A, Lu99B, MKN45, PacaII, MB231, Huh6, Hep3B, and AH66tc cells showed lower levels of both ets-1 and GnT-V mRNA expression. The expression levels of GnT-V and ets-1 mRNAs, as quantitated by densitometry, were plotted (Fig. 2). A positive correlation was found between these mRNAs expressed in cancer cell lines (r = 0.97; p < 0.0001), suggesting that the control of GnT-V gene expression by Ets-1 is widely distributed in a variety of cell lines. The expression of ets-2 was positively correlated with GnT-V expression in some cells such as HepG2, Huh7, Colo205, and Kato-III cells. However, in the case of A172, Colo201, B16-F1, and MB231 cells, no correlations were observed between ets-2 and GnT-V expression. Another protein of the ets family, erg, showed a low level of expression in various cell lines and did not correlate with GnT-V expression. The expression of fli-1, which is known to be 98% homologous to erg (32), showed a pattern that was very similar to erg (data not shown). Whereas the cDNA sequence homology of ets-1 among human, mouse, and rat is in excess of 90%, cDNA of the ets-2 coding region between human and mouse was less than 70% homologous. In the B16-F1 cell line, low levels of ets-2 could be due to its low sequence homology. A high level of expression of ets-2, as well as GnT-V and ets-1, was observed in Kato-III, HepG2, Huh7, and Colo205 cells, suggesting that ets-2 could also regulate gene expression of GnT-V in these cells. Whereas MKN45 cells expressed low levels of GnT-V and ets-1, the expression of ets-2 was quite high.

To investigate the expression of ets-1 and ets-2 at the protein level, Western blot analysis was performed using A549, B16-F1, HepG2, Huh7, Kato-III, and MKN45 cells (Fig. 3). A high level of expression of ets-1 and ets-2 was observed in HepG2, Huh7, and Kato-III cells. In contrast, their expression was quite low in A549 cells, but a high expression of ets-2 was observed in MKN45 cells. These results were consistent with the mRNA expression levels.

To determine whether the expression of GnT-V is dependent on Ets-1 protein, nuclear extracts from the MKN45, HepG2, and Colo201 cell lines were subjected to an EMSA analysis. EMSA was performed using the 24-base pair GnT-V promoter-derived oligonucleotide E728 (741/718) (22), which has been shown to have moderate binding affinity among three ets binding sites, which are located in the 5′-untranslated regions of the GnT-V gene. As shown in Fig. 4, when the radiolabeled Et-B, E728 oligonucleotides were incubated with nuclear extracts prepared from MKN45, HepG2, and Colo201, retarded protein-DNA complexes were detected (lanes 1 and 2). The intensities of the complexes of HepG2 and Colo201 were higher than those of MKN45. Specificity of binding was identified with a mutant oligonucleotide (mE728; Fig. 4, lane 3). To confirm ets antibody-specific binding, the supershift assay was performed by means of the addition of anti-Ets-1 and anti-Ets-2. Whereas both antibodies were shown to shift in the HepG2 cells, a supershifted band by the Ets-2 antibody was clearer than that of the Ets-1 antibody in MKN45 cells. The supershifted band by Ets-1 and Ets-2 was a competitive pattern in Colo201 cells (Fig. 4, lanes 4 and 5). This is because Colo201 cells are not adhering cells like HepG2 and MKN45 cells, and the amounts of nuclear proteins in 5 µg of proteins applied on EMSA might be relatively small as compared with the others. As a result, its supershift might appear as a competitive pattern. Collectively, these results strongly reflect the importance of Ets-1 and Ets-2 in GnT-V expression in each cell.

AP-1 Does Not Cooperate with Ets-1 in GnT-V Expression—To investigate the cooperative trans-activation of c-Jun on the GnT-V promoter through the c-Jun binding element (AP-1) and the ets binding element, we first performed a Northern blot analysis in various cancer cell lines. The expression of c-jun mRNA was observed in nearly all cells (Fig. 5) and was not correlated with GnT-V expression. To directly investigate the cooperation of AP-1 and ets binding elements in GnT-V expression, an EMSA was performed using the 58-base pair GnT-V promoter-derived oligonucleotide EJ565 (757/732), which contains the ets and AP-1 sites (Table II). When the radiolabeled EJ565 oligonucleotide was incubated with unlabeled competitors prepared from HepG2, protein-DNA complexes were retarded (Fig. 6, lane 1). The radiolabeled mJ565, which contained a mutant AP-1 site and was designed to detect the specificity of the AP-1 site, was incubated with nuclear extracts. The level of a retarded protein-DNA complex pattern was the same as when EJ565 was used (Fig. 6, lane 2). Specific binding was confirmed using competition analysis and the supershift assay. Levels of retarded protein-DNA complexes were decreased during an incubation with unlabeled EJ565 (Fig. 6, lane 3), and the bands were supershifted during incubation with anti-Ets-1 in the case of both EJ565 and mJ565 (Fig. 6, lanes 4 and 5). These results indicate that the DNA-protein complex with E565 nucleotide is dependent on ets binding, but not on AP-1.
To further confirm that AP-1 and the Ets-1 site do not cooperate, antisense c-jun was transfected to A549 and PacaII cells. Although these cells expressed low levels of both ets-1 and GnT-V (Fig. 2), the transfection of a vector alone (mock transfectant) to these cells brought a slight enhancement of GnT-V mRNA expression (Fig. 7). This result was reproduced in three separate experiments. When antisense c-jun was transfected into cells, GnT-V mRNA expression was unchanged (data not shown), suggesting that c-Jun could not be linked to GnT-V expression.

The Effects of Ets-1 Transfection on GnT-V Expression—To determine whether GnT-V mRNA expression is controlled by the overexpression of ets-1, two types of Ets-1-expressing vectors were transiently transfected into A549, MKN45, and PacaII cells, which were shown to express low levels of both ets-1 and GnT-V (Fig. 2). The transfection efficiency was about 15–20% of the total cell numbers, as judged by fluorescence microscopy (data not shown). Although intrinsic GnT-V expression was quite low, as shown in Fig. 2, the transfection of a vector alone (mock transfectant) slightly enhanced the expression of GnT-V mRNA (Fig. 7). This enhancement was very reproducible, but the details of its mechanism are presently unknown. When ets-1 was transfected into A549 and PacaII cells that expressed low levels of Ets-1, the expression of GnT-V was increased in comparison with control or mock-transfected cells (Fig. 7). In the case of MKN45, however, the level of GnT-V expression was not increased by ets-1 transfection, suggesting that Ets-1 is not sufficient for GnT-V gene expression in MKN45 cells.

The Effects of Dominant Negative ets-1 Transfection on GnT-V Expression—To demonstrate the enhancement of GnT-V by Ets-1 proteins by a different approach, the action of Ets-1 was inhibited by the transfection of dominant negative ets-1 in Kato-III and B16-F1 cells, which showed high levels of intrinsic ets-1 and GnT-V expression. It has been reported that the dominant negative mutant containing the DNA-binding domain of ets-1 (N70) specifically inhibits both ras stimulation...
and the constitutive α-domain activity of Ets-1 (26). When the dominant negative mutant of ets-1 was transfected into Kato-III and B16-F1 cells, the expression of GnT-V was decreased in comparison with a mock transfectant, suggesting that Ets-1 regulates GnT-V expression in these two cell lines (Fig. 8).

The Effects of ets-2 and Dominant Negative ets-2 Transfection on GnT-V Expression—To determine whether GnT-V is regulated by ets-2, we performed transfections with an expression vector for ets-2 and a dominant negative ets-2 construct (pCAGGS) into A549, MKN45, and PacaII cells, which express a very low level of ets-1 and GnT-V mRNA. Total RNA (30 μg) was extracted from each cell line and analyzed by Northern blot (Fig. 7). GnT-V expression levels were described according to their relative intensity compared with the mock transfectant of each cell.
mRNA expression is controlled by Ets-2, ets-2- or dominant negative ets-2-expressing vector was transiently transfected into A549, MKN45, PacaII, Kato-III, and B16-F1 cells, respectively. The transfection efficiency was about 15–20% of the total cell numbers, as judged by cotransfection of a GFP-producing vector, followed by fluorescence microscopy (data not shown). When ets-2 was transfected into A549 and PacaII cells that expressed low levels of ets-2, the expression of GnT-V was not changed in comparison with control or mock-transfected cells (Fig. 9). In contrast, whereas the level of GnT-V expression in MKN45 cells was not increased by ets-2 transfection, transfection of a dominant negative ets-2 suppressed GnT-V mRNA at the 40% levels of GnT-V expression in mock transfectedants. This suggests that Ets-2 regulates gene expression of GnT-V in MKN45 cells. When the dominant negative mutant of ets-2 was transfected into Kato-III and B16-F1 cells, the expression of GnT-V was not changed, suggesting that ets-1 is a key factor for the control of GnT-V expression in these cells (Fig. 9). Transfection of ets-2 into Kato-III cells induced increases in the expression of urokinase plasminogen activator mRNA (data not shown).

**FIG. 8.** GnT-V expression by transfection of the dominant negative ets-1 mutant. A dominant negative mutant of ets-1 was transfected into Kato-III and B16-F1 cancer cells, which showed high levels of expression of both ets-1 and GnT-V. Total RNAs (30 μg) were extracted from these cells, electrophoresed on a 1% agarose gel containing formaldehyde, and analyzed by Northern blot. Lane 1 contains the transfectant of the control plasmid pEGFP-N1; lanes 2 and 4 contain the mock transfectant (pCAGGS), and lanes 3 and 5 contain the transfectant of the dominant negative mutant of ets-1. The mRNA of the dominant negative ets-1 is indicated by an arrowhead. GnT-V expression levels are according to their relative intensity compared with mock transfectant of each cell. Experimental details are described under “Materials and Methods.”

**DISCUSSION**

The present study demonstrated that GnT-V expression is regulated by Ets-1 in many different cancer cells. This evidence provides a possible new pathway of tumor metastasis via the up-regulation of GnT-V by Ets-1. As shown in Fig. 1, three ets binding sites and one AP-1 site are located in the 5’ flanking region of the GnT-V gene. It is already known that ets cooperates with AP-1 in the transcriptional regulation of genes such as interleukin 2, collagenase, and tumor necrosis factor. However, this was not observed in the regulation of the GnT-V gene by Ets-1 (Figs. 5 and 6). Expression of c-jun was not correlated with GnT-V levels, and antisense c-jun did not change the level of GnT-V expression.

Although other ets family proteins are able to bind to these ets binding sites, GnT-V expression was correlated most with ets-1 expression. Correlations between GnT-V and Ets-2 were not so high compared with Ets-1. Fig. 9 showed the noninvolvement of Ets-2 in GnT-V expression in Kato-III and B16-F1 cells. However, in MKN45 cells, Ets-2 protein is a key factor in gene regulation of GnT-V because a dominant negative of ets-2 suppressed GnT-V expression (Fig. 9), and overexpression of ets-1 showed no effects on GnT-V expression (Fig. 7). Thus, gene expression of GnT-V is regulated in a cell type-specific manner, although Ets-1 contributes to GnT-V gene regulation in a variety of cancer cell lines. Although an ets-2 dominant negative mutant contains a sequence that is similar to the DNA-binding domain of ets-1, it was not able to inhibit GnT-V expression, as did the ets-1 dominant negative mutant (Fig. 8). A similar phenomenon was reported in which the dominant negative mutant of ets-2 was unable to suppress ras stimulation and the constitutive a-domain activity as Ets-1 did (26). A similar correlation between the expression levels of ets-1 and GnT-V was observed in 12 human hepatoma tissues (data not shown), suggesting that Ets-1 regulates the expression of GnT-V in vivo.

The mechanism of tumor metastasis is complicated and is not fully understood at present. The ets family has been reported to be involved in tumor metastasis through angiogenesis and the enhancement of expression of metalloproteinase or collagenase (33, 34). In addition, it has been suggested that the increased expression of GnT-V is associated with metastasis and the invasion of tumor cells. Therefore, Ets-1 would be expected to promote tumor metastasis not only via angiogenesis and the expression of metalloproteinase, but also through enhancing the expression of GnT-V. Moreover, because changes of N-linked oligosaccharide structures in tumor cells are associated with the expression of tissue inhibitors of metalloproteinase I (35), the enhancement of GnT-V expression by ets-1 would be expected to participate in the regulation of metalloproteinase activity. These considerations are consistent with the suggestion that Ets-1 contributes to the malignancy potential of tumor cells via an increase in the expression of GnT-V, which, in turn, leads to alteration of N-linked oligosaccharides in tumor cells.

It has been reported that stromal fibroblasts adjacent to tumor cells may express Ets-1 in response to tumor invasion (20); thus, it is uncertain whether the high expression levels of ets-1 and GnT-V detected in human hepatoma tissues were derived from hepatoma cells. However, our analyses of a variety of cancer cells, including hepatoma cells, indicate that many of the cancer cells investigated in the present study expressed Ets-1.

It was recently reported that the use of antisense oligonucleotide for ets-1 is one of the strategies by which tumor growth is suppressed through the inhibition of angiogenesis (36). This
latter report also indicated that Ets-1 controls not only tumor angiogenesis but also normal vascular development. The issue of whether GnT-V plays a role in these vascular developments involving Ets-1 is not known and remains to be examined. We also indicated that the impairment of the action of GnT-V by transfection of GnT-III, the product of which prevents the addition of a β1–6 GlcNAc residue, dramatically suppresses lung metastasis of mouse melanoma cells that express high levels of GnT-V (11). This clearly suggests that a β1–6 branch of an N-glycan is involved in tumor metastasis. Therefore, it can be concluded that the pathophysiological significance of Ets-1 in malignancy potential such as tumor metastasis can be ascribed to the increased expression of GnT-V. The prevention of the action of GnT-V as well as Ets-1 would provide new insights into cancer therapy.

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REFERENCES
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