Transcriptional Regulation of the N-Acetylgalactosaminyltransferase V Gene in Human Bile Duct Carcinoma Cells (HuCC-T1) Is Mediated by Ets-1*

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N-Acetylgalactosaminyltransferase V (GnT-V) catalyzes the transfer of N-acetylgalactosamine from UDP-N-acetylgalactosamine to α-6-β-mannoside to produce the β1–6 linked branching of N-glycan oligosaccharides, which controls the polyolactosamine content. The expression of N-acetylgalactosaminyltransferase V, which contains 17 exons and spans 155 kilobase pairs, is expressed in a tissue- and cell type-specific manner and is regulated at the level of transcription by multiple promoters (Saito, H., Gu, J., Nishikawa, A., Ihara, Y., Fujii, J., Kohgo, Y., and Taniguchi, N. (1995) Eur. J. Biochem. 233, 18–26). To elucidate the mechanism by which the GnT-V gene is expressed in a cell- and tissue-specific manner, cell-restricted expression was analyzed using the 5'-upstream regions of the human GnT-V gene spanning base pairs −2760 to +23 in a human bile duct carcinoma cell line, HuCC-T1. We characterized two cis-acting elements that are potentially important in HuCC-T1 cell-specific expression. The two elements each contain an Ets-1 binding site, 5'-GGA-3'. Specific binding of Ets-1 to the respective elements was demonstrated by competition analysis as well as by antibody supershift experiments. Cotransfection of an Ets-1 expression plasmid along with a GnT-V promoter-luciferase reporter plasmid revealed the participation of Ets-1 in the regulation of the GnT-V gene transcription. These data indicated that the transcriptional regulation of the GnT-V gene was mediated by transcription factor Ets-1.

Cell surface oligosaccharides linked to asparagine residues of membrane glycoproteins are thought to participate in a variety of specific biological interactions (1). The high branching of N-glycans seemed to be related to malignancy potential, in particular, increased β1–6 branching of N-glycans being directly linked to increased tumor metastasis (2–6). This structure is synthesized by UDP-N-acetylgalactosamine:α-6-β-mannoside β1–6-N-acetylgalactosaminyltransferase (GnT-V) (EC 2.4.1.155), as shown in Scheme 1. The expression of the GnT-V gene is induced by viral and oncogene transfection, transformation growth factor-β, and phorbol ester (7–10), suggesting that complicated mechanisms for the induction exist. We and another group succeeded in its purification and cloning of its cDNA (11, 12). Messenger RNA was detected as two bands for HepG2 and MCF7 cells, and LEC rat liver, but the level is not always consistent with its enzymatic activity (13–15). To determine the details of the gene regulation, analysis of the 5'-upstream regions of GnT-V was needed. Recently, we isolated the human GnT-V genomic DNA, which contains 17 exons and spans 155 kb. Some putative consensus sequences for the tissue-specific transcription factors have been found in two possible promoter regions of the 5'-upstream regions of the GnT-V gene (16).

The Ets transcription factor family shares a common DNA binding domain that interacts specifically with sequences containing a common core trinucleotide sequence, GGA (17). Ets binding sites have been identified in the regulatory regions of human T cell receptor-α (18) and -β (19) and interleukin-2 receptor (20), as well as other cellular and viral enhancers, and these Ets binding sites regulate their transcriptional activities (21–26). Ets-1 was first described as a cellular homolog of v-ets in replication-defective retrovirus E26 and is thought to be associated with tumorigenesis and embryogenesis (27), whereas c-ets-2 appears to be important in cartilage/bone development and is one of the major factors of Down’s syndrome (28). The expression of c-ets-1 is associated with the invasion of tumor cells in both in vitro and in vivo systems (29, 30). One possible mechanism by which Ets-related proteins promote invasion of tumor cells is that they enhance the transcription of matrix metalloproteinase genes (31–34). In contrast, Suzuki et al. (35) reported that overexpression of Ets-related protein in colon cancer cells reversed the transformed phenotype and tumorigenicity. These reports suggested that complicated systems of gene regulation may be mediated through Ets proteins.

In this study, we focused on verifying the possible mechanisms related to transcriptional regulation of the GnT-V gene in human bile duct carcinoma HuCC-T1 cells. GnT-V, a glycosyltransferase related to tumor metastasis, has many Ets binding sites in its 5'-upstream regions. There is a possibility that Ets regulates the metastatic potential of tumor cells by enhancing or suppressing the GnT-V gene. In the present study, we investigated the role of Ets-1 in GnT-V gene transcription by gel mobility assay, mutation analysis, and cotransfection assay, and we identified specific cis-regulatory elements. These results show that the corresponding Ets-1 transcription factor was found to be potentially involved in the expression of the HuCC-T1-restricted GnT-V transcripts.

MATERIALS AND METHODS

Cell Culture—The human bile duct carcinoma HuCC-T1 cells were obtained from the Japanese Cancer Research Resources Bank. They were cultured in RPMI 1640 medium (Nakarai Tesque, Japan) supplemented with 10% fetal calf serum (Life Technologies, Inc.), 50 mg/ml streptomycin sulfate, and 50 units/ml penicillin.

Plasmid Construction—A fragment encoding the full-length se-
sequence of Ets-1 was obtained by polymerase chain reaction and then cloned into the pSVK3 vector (Pharmacia Biotech Inc.). The nucleotide sequences were confirmed by general sequence analysis. The plasmid was digested with Xhol/SacI, and then the Ets-1 cDNA was subcloned into the Xhol/SacI site of the pET19b vector (Novagen), in which proteins were expressed under the control of a T7 promoter in *Escherichia coli* BL21(DE3). 5′-Upstream regions of the GnT-V gene were inserted in front of the promoterless luciferase gene in the Xhol site (Klenow-filled) of the plasmid vector, Life Technologies, Inc.). The constructs were termed pGV-2760/-440 (SalI/XhoI, Klenow-filled), pGV-600/-23 (XhoI/PvuII, Klenow-filled), and pGV+23/+1123 containing 2.2 and 0.6 kb of the upstream regions of exon 1, and 1.0 kb of intron 1, respectively. A series of 5′-unidirectional deletions of pGV-2760/-440 and pGV+600/-232 was generated by the nested deletion method as described previously (36). The deleted nucleotide sites were confirmed by general sequence analysis. The plasmids, pGV-23/+23 and pGV-123/+23, of the upstream of exon 1, 200 and 100 bp in length, respectively, were amplified by polymerase chain reaction using a pair of oligonucleotide primers (sense, 5′-ggagctgTCTTACCATATA-GAAC-3′; antisense, 5′-ggagctgGCTATAGTACGTC-3′; and antisense, 5′-ggagctgGCCTTACTGTTTTC-3′). The polymerase chain reaction products were subcloned into the pT7 Blue-T vector (Novagen), sequenced, and then their sequences were compared with that of the 2.76-kb fragment. Each fragment was digested with the combination of MluI and XhoI, followed by subcloning into a pGV enhancer vector (*MluI/XhoI*) site, which only contains a SV40 enhancer element.

**Gel Electrophoresis DNA Binding Assay**—The DNA fragment containing the putative Ets binding sequences of the upstream regulatory regions of the GnT-V of Ets-1 consensus binding sequence was synthesized as follows: E266, 5′-GGAGGATATGATGATGTA-3′; E565, 5′-CTTGTAAAGAGAAGGAGGCACT-3′; E278, 5′-GGGCGGAGGAACT-TACGTT-3′; and EB, 5′-CGGCAACCGAGGATAGGTC-3′. The mutant sequence was also synthesized as mE266, 5′-GGGGATATGATGATGTA-3′; and mE565, 5′-GGGCGGAGGAACTTACGTT-3′. Complementary oligonucleotides were annealed and used as probes or competitors. The probes were labeled at their 5′-extended ends with [γ-32P]dATP (Amersham Corp.) and T4 polynucleotide kinase (Takara, Osaka, Japan). For the gel mobility shift assay, DNA (10,000 cpm, 32P-labeled) and nuclear extracts or proteins that had been prepared by *in vitro* transcription/translation were mixed, in a total volume of 20 ml, with a buffer comprising 65 mM KCl, 25 mM Tris-HCl (pH 7.9), 6 mM MgCl2, 0.25 mM EDTA, and 10% glycerol. Two μg of poly(dI-dC) (Sigma) was also added to each reaction mixture. For competition analysis, proteins were incubated in the presence of excess unlabeled oligonucleotides. For the supershift assay, anti-Ets-1 IgG (Cambridge Research Biochemicals), which does not cross-react with the Ets-2 protein, was added to the reaction mixture, followed by incubation for 1 h at room temperature. Samples were loaded on to 6% nondenaturing polyacrylamide gels (acylamide: bisacrylamide, 29:1), 0.5 × TBE (1 × TBE = 89 mM Tris, 89 mM boric acid, 2 mM EDTA), and then electrophoresis was carried out at 4 °C at 150 V for 1 h. After the electrophoresis, the gels were dried with a gel dryer and then exposed to x-ray films (Kodak, Tokyo, Japan).

**Transfection and Luciferase Assay**—HuCC-T1 cells were plated at 1.7 × 105 cells/60-mm dish for 1 day before transfection. They were transfected with a lipofectamine reagent (Life Technologies, Inc.) with 2–5 μg of various expression vectors containing 2 μg of reporter plasmids, various expression vectors, and pCH110 (Novagen), a β-galactosidase expression vector being used to measure the transfection efficiency. Briefly, 2–5 μl of diluted DNA and 5 μl of lipofectamine reagent were mixed with 200 μl of serum-free RPMI 1640 to form a DNA-liposome complex. After incubation for 30 min, 800 μl of serum-free medium was overlaid on the prerinsed cells. The cells were incubated with the complex for 6 h, followed by the addition of 1 ml of 20% fetal calf serum medium and incubation for a further 18 h. After the complex had been removed by washing, the cells were cultured in RPMI 1640 containing 10% fetal calf serum for 24 h. Luciferase activity in cell lysates was determined with the use of a PicoGene™ Luminescence Kit (Toyo Ink Co.). The β-galactosidase activity in cell lysates was determined as reported previously (37) and used as an internal control for variations in transfection efficiency. The results represent the averages of three to five independent experiments.

**RESULTS**

**Identification of a Functional Regulatory Element within the 5′-Untranslated Regions of the Human GnT-V Gene in HuCC-T1 Cells**—We demonstrated previously that the 5′-upstream regions of both exon 1 and intron 1 exhibited promoter activity when they were transiently expressed in COS-1 cells (16). HuCC-T1 cells were also found to transcribe two types of message on 5′-rapid amplification of cDNA end (RACE) analysis (16). To elucidate the transcriptional mechanism for the human GnT-V gene in more detail, in particular to identify functional elements necessary for this cell line-restricted expression, GnT-V activity was measured (64.5 pmol/mg/h), and two types of transcripts of GnT-V were confirmed as well (previously (16)). To examine the 2.76-kb SalI/PvuII upstream regions of exon 1 and 1.0 kb of intron 1 (Fig. 1A), which were isolated from a genomic clone of HuCC-T1 cells (16), we inserted the 2.76-kb SalI/PvuII fragment and 1.0 kb of intron 1 into the reporter plasmid pGV enhancer upstream of the luciferase gene to generate constructs pGV-2760/+23 and pGV+23/+1123 (Fig. 1A). We only detected luciferase activity for pGV+23/+1123 (Fig. 1B), detecting no luciferase activity for pGV-2760/+23 (data not shown). To rule out the possibility that the genomic insert in the pGV-2760/+23 construct included negative element(s), two additional pGV constructs containing smaller genomic fragments were analyzed (pGV-2760/-440 and pGV-600/-23). Although both constructs expressed luciferase activity, the pGV-2760/-440 one showed lower promoter activity compared with the SV40 promoter vector as a control. However, the corresponding deleted construct, pGV-1460/-440, showed strong promoter activity (313-fold) compared with that of the control plasmid (Fig. 2A). Among these constructs, pGV-600/-23 proved to be the most active because it showed 182-fold luciferase activity, pGV-2760/-440 and pGV+23/+1123 showing 124-fold and 169-fold luciferase activity, respectively, compared with the promoterless vector. These results strongly suggest that these two fragments act as promoters and that a negative element is present in the −2760/-1460 pGV construct.

**Deletion Analysis of the 5′-Untranslated Regions of the Human GnT-V Gene**—To characterize further potential regulatory domains including Ets binding sites within the 5′-untranslated region of exon 1, we prepared a series of 5′-deleted constructs of the GnT-V 5′-untranslated region using plasmids pGV-2760/-440 and pGV-642/+23 and then transfected the deleted constructs into HuCC-T1 cells for luciferase activity determination (Fig. 1A). HuCC-T1 cells express endogenous GnT-V activity, which suggests that the cells contain the nuclear factors required for GnT-V promoter activity. The results are shown in Fig. 2, A and B, the pGV-1460/-440 construct exhibited a 2.5-fold increase in luciferase activity compared with pGV-2760/-440, indicating that the proximal region is more active than the full-length of the distal region. Further deletion, to nucleotide −710 bp, removes a potential Ets-1 binding site (position −728) and results in a 3.4-fold decrease in luciferase activity. Compared with construct pGV-642/+23, pGV-362/+23 exhibited more promoter activity, showing that this region could function as a core promoter of the GnT-V gene. The deletion in this region, extending to nucleotide −243 bp, removes another Ets-1 binding site (position −266), resulting in a 13.2-fold reduction in luciferase activity. Therefore, the most proximal region (−362/−243) is about 3.9-fold more active than the distal region (−1460/−710), showing the extremely high luciferase activity in the upstream region of the GnT-V gene. These results strongly suggest that the proximal region (−362/−243) and the distal region (−1460/−710) act as positive regulatory elements that up-regulate the promoter activity of the GnT-V
gene in HuCC-T1 cells. However, further deletion of approximately 100-bp (pGV2112/123) results in an 8.3-fold increase in activity in HuCC-T1 cells, suggesting the presence of negative regulatory elements in the 2243/2112 region which may down-regulate the transcription of GnT-V in HuCC-T1 cells like the 22760/21460 element.

Analysis of Binding of the Ets-1 Protein to the Putative Sequence of the Regulatory Regions of the GnT-V Gene—The deletion analysis described above indicated that the 21460/2710 and 2362/243 regions of this promoter act as cis-acting elements for HuCC-T1-restricted transcription. We have shown that several putative binding sites for LBP-1 (positions 21334, 21038, 2935, and 2932), AP-2 (positions 21264 and 2227), nuclear factor–interleukin-6 (positions 21385, 21005, 2965, and 2255), c-myb (position 2287), and Ets-1 (positions 2728 and 2266) are included in these two elements (16). We chose to focus our analysis on three putative binding sites for transcription factor Ets-1, which is a product of proto-oncogenes related to the malignant transformation and metastasis of tumors (31–34). To determine whether or not the Ets-1 protein can bind to these putative binding sites of the GnT-V gene regulatory regions, gel mobility shift experiments were performed using 23-bp GnT-V promoter-derived oligonucleotides E266, E565, and E728, corresponding to the three putative Ets binding sites in the 5'-untranslated regions of the GnT-V gene (Table I). At first, we confirmed that the in vitro transcribed/
The unlabeled E266 and E728 oligonucleotides were found to compete with proteins binding to the labeled E266 and E728 probes, indicating the specific and high affinity binding of the Ets-1 protein to the Ets binding site of the GnT-V regulatory regions. The protein-DNA complex could be efficiently cross-competed with an excess of either E266 or E728, whereas oligonucleotides mE266 and mE728 containing a mutated Ets binding sequence did not compete (data not shown). These findings suggest that the in vitro transcribed/translated truncated Ets-1 protein recognizing the Ets consensus sequence can bind to the GnT-V promoter sequences, 5'-GGAGTGAGGATGATGTAGGGAAG-3' and 5'-ATGGGGCAGAGGAACTTACGT-TAT-3', at positions -266 and -728.

To determine whether or not GnT-V promoter-derived Ets binding sites are associated with nuclear factors in the cells expressing Ets-1, we performed a gel mobility shift assay using the same GnT-V promoter-derived Ets binding sequence incubated with nuclear extracts prepared from MOLT4 cells, which highly expressed the Ets-1 protein. As shown in Fig. 3C, when the radiolabeled E266 and E728 oligonucleotides were incubated with nuclear extracts prepared from MOLT4 cells, retarded protein-DNA complexes were detected, and specific binding was identified on competition analysis (Fig. 3C, lanes 3 and 7). Binding of Ets was confirmed by an Ets-specific antisera. The addition of anti-Ets-1 IgG resulted in competition with the protein-DNA complex (Fig. 3B, lanes 4 and 8), whereas the mE266 and mE728 oligonucleotides containing a mutated Ets binding sequence did not compete (Fig. 3D, lanes 4 and 7). These results revealed the binding of an Ets-related factor to the 23-bp Ets binding site of the HuCC-T1-restricted promoter region of the GnT-V gene.

**Trans-activation of the Human GnT-V Promoter**—To confirm the importance of Ets-1 in the regulation of GnT-V transcription in living cells, Ets-1 cDNA was subcloned into the pSVK3 vector, in which the SV40 promoter drives the expression of c-Ets-1 cDNA in mammalian cells. To determine whether or not the GnT-V gene can be activated by exclusive Ets stimulation, construct pGV–1460–440 was cotransfected into HuCC-T1 cells along with an Ets-1 expression plasmid, SVETS-1. As shown in Fig. 4A, after HuCC-T1 cells had been transiently cotransfected with SVETS-1 and pGV–1460–440, the luciferase activity increased more than 2.1-fold in the presence of 1 μg of the Ets-1 expression vector, whereas promoter activity decreased in the presence of 2 μg of the Ets-1 expression vector. However, we could observe a dose-dependent increase in the promoter activity when SVETS-1 and pGV–362/ +23 were cotransfected into HuCC-T1 cells (Fig. 4B). Although the cause of this differential effect is unclear, this trans-activation result suggests that Ets-1 may activate the GnT-V promoters that contain its binding sites. To investigate the regulation function in more detail, we used antisense Ets-1 mRNA to block the formation of Ets-1, which would interfere with the promoter activity of the human GnT-V gene in HuCC-T1 cells. This construct (SVETS-SA) was transiently cotransfected with pGV–1460–440 and pGV–600/+23 into HuCC-T1 cells. How-

### Table I

**Oligonucleotides used in gel mobility shift assay**

| Name     | Sequence | Position |
|----------|----------|----------|
| EB       | 5'-GGGCAACGGGAGGACGTTGAC-3' | Consensus Ets-1 site |
| E266     | 5'-GGGTGTAGGATGATGGGAA-3' | 278 to -255 |
| E565     | 5'-CTTGTTAAGGATTGGTAGGAC-3' | 578 to -554 |
| E728     | 5'-ATGGGGGAGGACAGTTCTGATT-3' | 741 to -718 |
| mE266    | 5'-GGGTGTAGGATGATGGGAA-3' | Mutant of E265 |
| mE728    | 5'-ATGGGGGAGGACAGTTCTGATT-3' | Mutant of E728 |

### Fig. 2

**Transcriptional activity of the GnT-V promoter and its deletion constructs linked to the luciferase reporter gene upon transfection into HuCC-T1 cells.** The relative luciferase activity of each deletion mutant derived from pGV–2764–440 (panel A) or pGV–600/+23 (panel B) is expressed as a percentage of the activity of the pGV enhancer vector in HuCC-T1 cells, means ± S.D. for three to five independent experiments being presented.

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translated truncated Ets-1 protein and nuclear extract of MOLT4 cells indeed bind to the Ets-1 consensus sequence (Fig. 3A). When radiolabeled E266, E565, and E728 were incubated with the in vitro transcribed/translated Ets-1 protein, as described under “Materials and Methods,” protein-DNA complexes were retarded in the cases of both E266 and E728, but not that of the E565 oligonucleotide (Fig. 3B, lanes 2, 7, and 12). Competition analysis was performed using excess unlabeled E266, E565, and E728 oligonucleotides, as shown in Fig. 3B. The unlabeled E266 and E728 oligonucleotides were found to compete with proteins binding to the labeled E266 and E728 probes, indicating the specific and high affinity binding of the Ets-1 protein to the Ets binding site of the GnT-V regulatory regions. The protein-DNA complex could be efficiently cross-competed with an excess of either E266 or E728, whereas oligonucleotides mE266 and mE728 containing a mutated Ets binding sequence did not compete (data not shown). These findings suggest that the in vitro transcribed/translated truncated Ets-1 protein recognizing the Ets consensus sequence can bind to the GnT-V promoter sequences, 5'-GGAGTGAGGATGATGTAGGGAAG-3' and 5'-ATGGGGCAGAGGAACTTACGT-TAT-3', at positions -266 and -728.

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**Trans-activation of the Human GnT-V Promoter**—To confirm the importance of Ets-1 in the regulation of GnT-V transcription in living cells, Ets-1 cDNA was subcloned into the pSVK3 vector, in which the SV40 promoter drives the expression of c-Ets-1 cDNA in mammalian cells. To determine whether or not the GnT-V gene can be activated by exclusive Ets stimulation, construct pGV–1460–440 was cotransfected into HuCC-T1 cells along with an Ets-1 expression plasmid, SVETS-1. As shown in Fig. 4A, after HuCC-T1 cells had been transiently cotransfected with SVETS-1 and pGV–1460–440, the luciferase activity increased more than 2.1-fold in the presence of 1 μg of the Ets-1 expression vector, whereas promoter activity decreased in the presence of 2 μg of the Ets-1 expression vector. However, we could observe a dose-dependent increase in the promoter activity when SVETS-1 and pGV–362/ +23 were cotransfected into HuCC-T1 cells (Fig. 4B). Although the cause of this differential effect is unclear, this trans-activation result suggests that Ets1 may activate the GnT-V promoters that contain its binding sites. To investigate the regulation function in more detail, we used antisense Ets-1 mRNA to block the formation of Ets-1, which would interfere with the promoter activity of the human GnT-V gene in HuCC-T1 cells. This construct (SVETS-SA) was transiently cotransfected with pGV–1460–440 and pGV–600/+23 into HuCC-T1 cells. How-
ever, as shown in Fig. 5A, pGV–1460/-440 caused a 60% decrease in luciferase activity when cotransfected with 1 μg of antisense expression plasmid (SVETSA), and a 100% reduction in luciferase activity was observed on cotransfection with 10 μg of anti-Ets-1 expression plasmid, whereas no change was observed in the promoter activity of pGV–600/+23 when it was cotransfected with the antisense expression vector (Fig. 5B). These findings may suggest that the transcription factor Ets-1 protein might positively regulate GnT-V gene transcription mediating complicated transcription mechanisms.

**DISCUSSION**

In most human epithelial tissues, the β1–6 branching of both N-linked and O-linked oligosaccharides controls the polylac-
tosamine content, which may be followed by sialic acid addition as a terminal sugar residue (38, 39). The increased β1–6 branching of oligosaccharides on the cell surface may influence the structure and function of specific glycoproteins required for the metastatic dissemination of tumor cells and malignant transformation (2–6). For example, increased β1–6 branching was observed in human breast carcinomas (5), human uroepithelial cell lines (3), polyoma and Rous sarcoma virus-transfected baby hamster kidney cells (8, 40), and H-ras- and v-fps-transfected rat2 fibroblast cell lines (41). Consistent with these observations, GnT-V, the enzyme which initiates the branch of oligosaccharides on the cell surface, although at a level lower than that seen in the carcinomas (30). Using in vitro transcribed/translated Ets-1 protein, gel mobility shift and supershift assays showed that the Ets protein binds to the –286 and –728 sites but not to the 565 site, consistent with the observation that two cis-acting elements are involved in Ets binding sites. Transient cotransfection analysis showed that the sense-Ets-1 expression plasmid increased the luciferase activity of the GnT-V gene in HuCC-T1 cells, suggesting that these two Ets binding sites may act as positive elements in the transcription regulation of the GnT-V gene in HuCC-T1 cells. However, the antisense Ets-1 expression plasmid decreased the luciferase activity of the GnT-V gene in HuCC-T1 cells, but we did not identify these down-regulation factor(s).

Recent studies have shown that the expression of GnT-V in epithelial cells promotes features of transformation, including reduced substratum adhesion, release of cell growth from contact inhibition, and promotion of metastasis in the latter stages of tumor progression (6). However, the transcriptional regulation of the GnT-V gene remains unresolved. We have demonstrated that the human GnT-V gene uses a multiple promoter system and that its expression may be regulated in a tissue- and cell type-specific manner (16).

In this study, we cloned and sequenced a 2.76-kb fragment of the human 5′-untranslated regions of the GnT-V gene from the HuCC-T1 cell line. HuCC-T1 cells showed two transcription products of the GnT-V gene with low GnT-V activity, which were tumorigenic in nude mice (42). The luciferase activity obtained with the pGV–2760/–728, pGV–600/–23, and pGV–23/+1123 constructs reflected the promoter activity as well as the strong SV40 promoter activity of the control plasmid. This was not surprising since GnT-V transcripts were not abundantly expressed in HuCC-T1 cells (data not shown). Deletion mutant analysis showed that the 5′-untranslated regions of the GnT-V gene contained two cis-elements as positive regulation regions, both the distal region (–1460/–710) and proximal region (–362/–243) appearing to enhance the transcriptional activity of the GnT-V gene in HuCC-T1 cells. However, the upstream regions of the GnT-V gene (–2760/–1460 and –243/–112) contain negative elements that down-regulate the luciferase activity of the GnT-V gene in HuCC-T1 cells, but we did not identify these down-regulation factor(s). In fact, it is very interesting to note that these genomic inserts contain most of the putative regulatory elements that had been observed previously (16). We focused our analysis on the three binding sites for transcription factor Ets-1 located at positions –286, –565, and –728, which had been identified on computer analysis, because it has been reported that the Ets-1 protein interacts with the transcription of the matrix metalloproteinase gene and is associated with the invasion and tumorigenesis of tumor cells (31–34). However, to date no information regarding GnT-V gene regulation by Ets-1 is available. As demonstrated by the mutational analysis in Fig. 2, Ets-1 is an important factor regulating expression of the GnT-V promoter in HuCC-T1 cells. Indeed, destruction of this site results in 3.4-fold and 13.2-fold decreases in the activities of the two cis-acting elements. In addition, as shown in Fig. 4, Ets-1 could trans-activate the transcription of the GnT-V gene in HuCC-T1 cells.

It has been shown that Ets-1 expression is high in both T and B cell systems (17). However, it is expressed not only in the endothelial layer of developing vessels but also in the fibrocytes of tumor stroma, although at a level lower than that seen in the carcinomas (30). Using in vitro transcribed/translated Ets-1 protein, gel mobility shift and supershift assays showed that the Ets protein binds to the –286 and –728 sites but not to the 565 site, consistent with the observation that two cis-acting elements are involved in Ets binding sites. Transient cotransfection analysis showed that the sense-Ets-1 expression plasmid increased the luciferase activity of these two positive regulation regions of the human GnT-V gene in HuCC-T1 cells, suggesting that these two Ets binding sites may act as positive elements in the transcription regulation of the GnT-V gene in HuCC-T1 cells. However, the antisense Ets-1 expression plasmid...
mid decreased the luciferase activity of the distal region (~1460/–710) when it was transiently transfected with pGVT–1460/–440, which was consistent with the observations for the sense plasmid of Ets-1. A decrease in the luciferase activity of the proximal region (~362/–243) was not observed when it was transiently transfected with pGVT–362/–23, consistent with the strong affinity of the E266 site observed on competition analysis (Fig. 3).

Ets-1 is a nuclear phosphoprotein that binds to purine-rich DNA sequences and functions as a transcription factor (43, 44). The presence of Ets binding sequences in the promoters of c-myb and cdc-2, which are involved in the control of normal cell growth, and their deregulation in neoplasia have been reported, indicating that Ets family proteins are associated with transformation properties (45). In situ analysis of mouse embryos revealed much greater GnT-V expression on embryonic day 9.5, when rapid growth and organogenesis occur (6), and a significant level of Ets-1 was detected on day 9, when extensive organogenesis is occurring, and its expression is widespread throughout all organs (46). Furthermore, GnT-V expression is more restricted in the neuroepithelium of the brain, consistent with Ets-1 mRNA detected in the nervous tissue (6, 46). The key point is that Ets-1 trans-activates the expression of an extracellular matrix metalloproteinase (32–34), membrane-type metalloproteinases, stromelysin-1, collagenase, and stromelysin-3 being involved in the β1–6 branching of oligosaccharides (47). In the present study, transient cotransfection analysis showed that the Ets-1 protein binds to the Ets binding sites of two cis-acting elements and trans-activates the promoter activity of the human GnT-V gene in HuCC-T1 cells. Taken together, these findings lead to the hypothesis that transcription factor Ets-1 interacts with the expression of the GnT-V gene during malignant metastasis and thereby alters the extracellular matrix organization and increases the availability of growth factors, contributing to malignant metastasis. This suggests that the β1–6 branching of matrix proteins may reduce integrin-substratum binding or alter the extracellular matrix organization.

Although it was well known that Ets family members trans-activate natural target promoters in association with other transcription factors (48–52), the Ets family member PU1 is also able to interact directly with basal transcription interactions, the latter forming a complex with basal transcription factors (48–52), the Ets family member PU1 is also able to interact directly with basal transcription factors (48–52). The presence of Ets binding sequences in the promoters of Ets-1 is a nuclear phosphoprotein that binds to purine-rich DNA sequences and functions as a transcription factor (43, 44). Although it was well known that Ets family members trans-activate natural target promoters in association with other transcription factors (48–52), the Ets family member PU1 is able to interact directly with basal transcription factors (48–52). The presence of Ets binding sequences in the promoters of Ets-1 is a nuclear phosphoprotein that binds to purine-rich DNA sequences and functions as a transcription factor (43, 44). Although it was well known that Ets family members trans-activate natural target promoters in association with other transcription factors (48–52), the Ets family member PU1 is able to interact directly with basal transcription factors (48–52). The presence of Ets binding sequences in the promoters of Ets-1 is a nuclear phosphoprotein that binds to purine-rich DNA sequences and functions as a transcription factor (43, 44). Although it was well known that Ets family members trans-activate natural target promoters in association with other transcription factors (48–52), the Ets family member PU1 is able to interact directly with basal transcription factors (48–52). The presence of Ets binding sequences in the promoters of Ets-1 is a nuclear phosphoprotein that binds to purine-rich DNA sequences and functions as a transcription factor (43, 44). Although it was well known that Ets family members trans-activate natural target promoters in association with other transcription factors (48–52), the Ets family member PU1 is able to interact directly with basal transcription factors (48–52). The presence of Ets binding sequences in the promoters of Ets-1 is a nuclear phosphoprotein that binds to purine-rich DNA sequences and functions as a transcription factor (43, 44).
Transcriptional Regulation of the N-Acetylglucosaminyltransferase V Gene in Human Bile Duct Carcinoma Cells (HuCC-T1) Is Mediated by Ets-1

Rujun Kang, Hiroyuki Saito, Yoshito Ihara, Eiji Miyoshi, Nobuto Koyama, Yin Sheng and Naoyuki Taniguchi

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Additions and Corrections

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Transcriptional regulation of the N-acetylglucosaminyltransferase V gene in human bile duct carcinoma cells (HuCC-T1) is mediated by Ets-1.

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Page 26707, right-hand column, lines 44 and 55, Page 26708, Fig. 1A, and Page 26709, Fig. 2B: The name of plasmid pGV should be pGV-600+/+23.

Page 26709, Table I: Line 5 should be “...Mutant of E266.”

Page 26710, Fig. 3, lines 5 and 6 of the legend: Insert “(lanes 1–5)” after “transcribed/translated Ets-1 protein” and “(lanes 6–9)” after “nuclear extract of MOLT4 cells.” The last line of that legend should refer to “(lanes 4 and 7).”

Page 26709, right-hand column, last line, Page 26710, left-hand column, last line, Fig. 4, line 4 of the legend, and Page 26711, Fig. 5, line 3 of the legend: The name of the plasmid should be pGV-362+/+23.

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Secretion of biologically active recombinant fibrinogen by yeast.

Samar N. Roy, Bohdan Kudryk, and Colvin M. Redman

The following correction has been supplied by Drs. Colvin M. Redman and Bohdan Kudryk:

In this article, we reported that recombinant fibrinogen was expressed and secreted by transformed Saccharomyces cerevisiae. Despite resolute attempts, we have been unable to reproduce the results reported in that publication, and therefore we wish to retract the article.

We suggest that subscribers photocopy these corrections and insert the photocopies at the appropriate places where the article to be corrected originally appeared. Authors are urged to introduce these corrections into any reprints they distribute. Secondary (abstract) services are urged to carry notice of these corrections as prominently as they carried the original abstracts.