Glycobiology vol. 10 no. 6 pp. 623–628, 2000

Transcriptional regulation of human β-galactoside α2,6-sialyltransferase (hST6Gal I) gene during differentiation of the HL-60 cell line

Akiyoshi Taniguchi[1], Yuko Hasegawa[2], Koji Higai and Kojiro Matsumoto

Department of Clinical Chemistry, School of Pharmaceutical Sciences, Toho University, 2–2–1, Miyama, Funabashi, Chiba 274–8510, Japan

Received on November 1, 1999; revised on December 26, 1999; accepted on January 3, 1999

We have previously shown that the expression of β-galactoside α2,6-sialyltransferase (hST6Gal I) mRNA decreases during HL-60 differentiation induced with dimethyl sulfoxide (DMSO) and that transcriptional regulation depends on the P3 promoter that exists 5′-upstream of exon Y (A.Taniguchi *et al.*, *FEBS Lett.*, 441, 191–194, 1998). The regulation of hST6Gal I may be important for the expression of sialyl-Le^x in HL-60 cells. In the present report, we studied the transcriptional regulation of hST6Gal I gene during DMSO-induced differentiation of HL-60 cells. To elucidate the molecular basis of hST6Gal I gene expression, the genomic region containing the P3 promoter of hST6Gal I was isolated and functionally characterized. Using a luciferase assay, we identified a functional DNA portion that confers an enhancer, located at nucleotide number (nt) –317 to –174 within the P3 promoter of hST6Gal I genomic DNA. This element contains two sequences similar to Sp1 (GC-box) and one sequence similar to Oct-1 recognition motifs (octamer sequence). Site-directed mutagenesis of Sp1 and Oct-1 sites showed that two Sp1 motifs and one Oct-1 motif are essential for transcriptional activity in HL-60 cells. Enhancer activity is suppressed during HL-60 cell differentiation induced with DMSO. These results suggest that GC-box and octamer sequence may play a critical role in the transcriptional regulation of the hST6Gal I gene during HL-60 cell differentiation.

Key words: sialyltransferase/gene expression/transcriptional regulation/sialyl-Le^x/HL-60

Introduction

Sialic acids on the reducing ends of sugar chains are key determinants for cell-to-cell interaction and cell differentiation (Tsuji, 1996; Kelm and Schauer, 1997; Varki, 1997). The expression of sialic acids differs in among various tissues; even within the same tissue, remarkable changes have been observed during cell differentiation (Taatjes and Roth, 1988; O'Hanlon *et al.*, 1989; Paulson *et al.*, 1989; Wang *et al.*, 1990a,b; O'Hanlon and Lau, 1992). Differentiation-dependent regulation of α2,3- and α2,6-linked sialic acids on sugar chains is associated with mRNA regulation of α2,3- and α2,6-sialyltransferase genes (Wen *et al.*, 1992; Vertino-Bell *et al.*, 1994; Taniguchi and Matsumoto, 1998, 1999; Taniguchi *et al.*, 1998). Moreover, β-galactoside α2,6-sialyltransferase (hST6Gal I) mRNA levels are altered by treatment with glucocorticoids, cytokines, secondary bile acids, and phorbol myristate acetate (PMA) (Wang *et al.*, 1990b; Hanasaki *et al.*, 1994; Li *et al.*, 1998). These results suggest that the expression levels of cell surface α2,3- and α2,6-sialic acids are mainly regulated at the mRNA level.

Among sialyltransferases, hST6Gal I is the most thoroughly studied. Multiple hST6Gal I mRNA forms, differing only in the 5′-untranslated region, have been described (Aasheim *et al.*, 1993; Wang *et al.*, 1993; Lo and Lau, 1996a,b). A short mRNA form (Form 1) has been isolated from liver. A large transcript (Form 3), containing two 5′-untranslated exons (exon Y + Z), has been isolated from several human cell types. A distinct transcript (Form 2) containing exon X, but not exon Y + Z, has been isolated from human B cell lymphoblastoma cell lines. Transcription of the three mRNA forms is initiated by promoter regions P1, P2, and P3.

The leukemia cell line HL-60 consists predominantly of promyelocytes which can be induced to differentiate into myelocytes, metamyelocytes, and granulocytes by the addition of dimethyl sulfoxide (DMSO) to the growth medium. Cell surface glycoprotein and glycolipid profiles are specifically altered during differentiation (Gahmberg *et al.*, 1979; Fukuda *et al.*, 1981; Nojiri *et al.*, 1984; Nakamura *et al.*, 1992). Therefore, this cell line is utilized as a useful model for studying the regulation of carbohydrate expression. Previous studies have demonstrated that DMSO-induced differentiation of HL-60 cells resulted in an increase in cell surface expression of sialyl-Le^x. This model of differentiation has been used to investigate glycosyltransferase activities (Skacel *et al.*, 1991; Clarke and Watkins, 1996). These results suggest that the increased expression of sialyl-Le^x is related to α2,6-sialyltransferase activity. Therefore, the regulation of hST6Gal I may be important for the expression of sialyl-Le^x in HL-60 cells.

In myeloid cells, as in other cell types, the expression of most genes is regulated at the transcriptional level by a class of proteins called transcription factors. Several transcription factors that are involved in myeloid cells-specific gene regulation have been characterized, such as MZF-1 (Morris *et al.*, 1995; Robertson *et al.*, 1998), Sp1 (Rao *et al.*, 1998; Hauses *et*

[1]To whom correspondence should be addressed

[2]Present address: Department of Obstetrics and Gynecology, School of Medicine, Keio University, 35 Shinanomachi, Shinjuku, Tokyo, 160–8582, Japan

al., 1998), and Oct-1 (Kim *et al.*, 1995; Lopez-Rodriguez *et al.*, 1997; Corbi and Lopez-Rodriguez, 1997; Tenen *et al.*, 1997). The Sp1 protein is ubiquitously expressed in proliferating cells and contributes to the activation of many growth-promoting genes (Deng *et al.*, 1986; Kadonaga *et al.*, 1987; Swick *et al.*, 1989; Dynan and Tjian, 1983; Melton *et al.*, 1984).

We have previously shown that DMSO-, all trans-retinoic acid (ATRA)– and phorbol myristate acetate (PMA)–induced differentiation elicits negative regulation of hST6Gal I at the mRNA level in the HL-60 cell line and that transcriptional regulation of hST6Gal I depends on the P3 promoter that exists 5′-upstream of exon Y (Taniguchi *et al.*, 1998). We report here transcriptional regulation of the hST6Gal I gene during HL-60 cell differentiation. To elucidate the molecular basis of hST6Gal I gene expression, the genomic region containing the P3 promoter of hST6Gal I was isolated and functionally characterized. The present results suggest that the Sp1 binding site (GC-box) and Oct-1 binding sites (octamer sequence) of the P3 promoter may play a critical role in transcriptional regulation of the hST6Gal I gene during HL-60 cell differentiation.

The nucleotide sequence data reported in this paper will appear in the DDBJ, EMBL, and GenBank nucleotide sequence databases with the accession number AB030576.

Results

A differentiation responsive element exists within the hST6Gal I P3 promoter

We have previously shown that the expression of hST6Gal I decreases during HL-60 differentiation and that transcriptional regulation of hST6Gal I depends on the P3 promoter that exists 5′-upstream of exon Y (Taniguchi *et al.*, 1998). In order to study the transcriptional regulation of hST6Gal I gene during HL-60 differentiation, we cloned and identified the 5′-flanking region of the hST6Gal I P3 promoter from a human genome library. The sequence of the 5′-flanking region of the hST6Gal I P3 promoter is shown in Figure 1. The transcription start site of 5′-RACE is marked with an arrow. The 5′-flanking region lacks canonical TATA or CCAAT boxes, but contains several putative transcriptional factor binding sites such as MZF-1, AML-1a, AP-1, Sp1, and Oct-1.

In order to identify the element that responds to differentiation, we made luciferase constructs carrying the 5′-deleted hST6Gal I P3 promoter (Figure 2). These luciferase plasmids were then transfected into untreated HL-60 cells and cells treated with DMSO. In undifferentiated HL-60 cells, extension of the 5′-deletion of the P3 promoter to nt –174 (pGL-174-P3) reduced luciferase expression to approximately 10% of that of the promoter with deletion to nt –317 (pGL-317-P3) (Figure 2). This result suggests that the nt –317 to –174 region acts as an enhancer in undifferentiated HL-60 cells. We found that approximately 80% of the luciferase expression was suppressed by the addition of DMSO to pGL-876-P3, pGL-676-P3, pGL-460-P3, and pGL-317-P3 transfected HL-60 cells, whereas DMSO had little effect on luciferase expression of the other plasmids, pGL-174-P3 and pGL-84-P3 (Figure 2). The results suggested that a differentiation responsive element exists within nt –317 to –174 in P3 promoter.

```
-876 CAGCTTGGTT AACCTCTTTC CTTCCCCAGC AGCCCCACCG TCGTCCGGGT
                              MZF1
-826 GAAAAGAAAG GAACCTCGCT CCCTTCACCA TCCAGGCTGA GGCTTCAGTC

-776 TTTCAGCCTG CAGCTTGGGG CTTGGGGTGG ACGTCTCTGG GTGCAGGGGT
                             AML1a
-726 TCGAGGACGC TCCTCTCCTT CTGTATTTGC CATTTGAGAG CGCCCAGTAG

-676 GAGTCATGCC TCAATGTTAG GAGGTTAATG CCAAACGCAG TGAAAGATTT

-626 TGTTCCTTCC TATTCTAGAT AAGAGCAACA CGAGTCTTCG GAGAGGTTAA

-576 GCGACTTCCT CAGGGTCTCG CAGTCAGCCG GTAGCAGAGA CCGGGTGTAC
                            AP-1
-526 AGCACCCGCA TGTTAGGACC AAAAGCCGGA CACTGCTGGA TTGACCCCTC

-476 GCGGCCAAGC GGGGAAGAGG CGTTTAGGGG TCCTTGCGCA GCCGCGTGGC
               MZF1
-426 CCCTCCCTCG GCCCTCGATC CACCCTCCTT GAGGGCAGGC AGGATTCTAG

-376 CATCGCCCAG TGCCTTCATC AGGAGTCTTT AACCCCAAGC CCGCTCGCTA
                                      AML1a
-326 GCGACCTCCA GGCCGGTTTG GGGCGCGGTG TGGCCGGTGG CGAGAGGTGG

-276 AGTGGGGGCG GAGACCGACC CAGTCTTCGC AACTCTATTT GCATACGGAG
       Sp1                                      Oct-1
-226 GCGACTCGCT TCCTTCCCGC CCCTCCGCCG CGCTCTTCTT CCTTCCTTCT
                  Sp1
-176 CCAGTCCCTT CCACTGTGCG TCTTCTGTCC CCCGTTCTTC CCCAGCGGAC
                                   MZF1        MZF1
-126 CCCTCTTTCG AGACTCCCTA GTGGGGTTCC CAGCTCCCGG GCGATCCTGC
                             AML1a
-76  CCTTGCCGAG CGCGTTTTCT GGAGTCACCT GGGGGAGGGG AGTCCTGGCA
                                              MZF1
-26  GGGCCGGGCT GGGGAACCAT GCGACTGCCC GGCGTTAACA AAGGG
           MZF1                  +1
```

Fig. 1. Nucleotide sequence of 5′-flanking sequence adjacent to exon Y. Potential regulatory elements are underlined and indicated below the sequence. The transcription start site is marked with an arrow. Nucleotides are numbered with the transcription-initiation site designated as +1. The nucleotide sequence of the P3 promoter has been submitted to the GenBankTM/EMBL Data Bank with accession number AB030576.

Nt –317 to –174 region acts as a differentiation responsive element

To determine whether the nt –317 to –174 region functions as a differentiation responsive element, two different reporter plasmids, in which the luciferase-gene is transcribed under control of a heterologous (SV40)-promoter, were prepared (Figure 3). The nt –317 to –174 fragment was ligated in front of the SV40 promoter in both orientations. The luciferase activities of pGL3PRO-317-174 and pGL3PRO-174-317 were 4- to 5-fold higher than the activity of pGLPRO in undifferentiated HL-60 cells (Figure 3). The results demonstrated that DMSO negatively regulated luciferase expression in pGL3Pro-317-174 and pGL3Pro-174-317 transfected HL-60 cells (Figure 3). These results indicate that the nt –317 to –174 region acts as a differentiation responsive element in HL-60 cells.

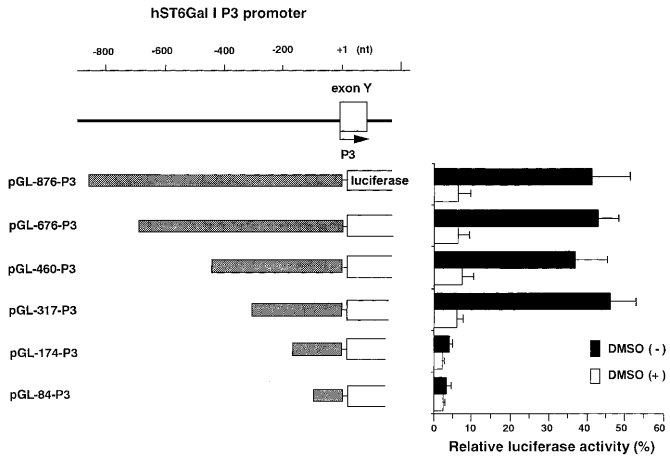

Fig. 2. Deletion analysis of hST6Gal I P3 promoter. The structure of the hST6Gal I gene is shown above the luciferase constructs. The relative luciferase activity of each of these constructs is indicated on the right. Each firefly luciferase construct was cotransfected into HL-60 cells with a *Renilla* luciferase expression vector (pRL-SV40) as the internal control. Following transfection luciferase activity was measured after a 24 h incubation with (open bars) or without (solid bars) DMSO. Relative luciferase activity was normalized to the luciferase activity of the pGL3-Control, which contains the SV40 promoter-enhancer sequences upstream of the luciferase gene. Data is expressed as the mean ± standard deviation (n = 3).

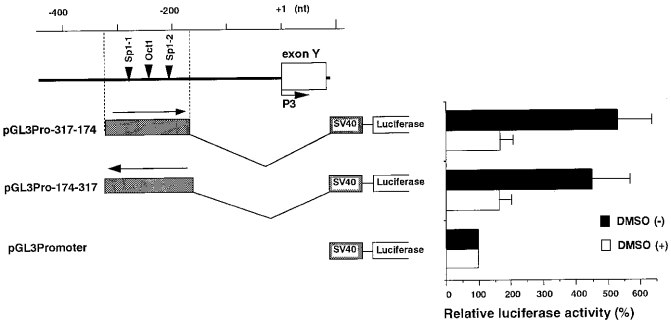

Fig. 3. Differentiation responsive element of hST6Gal I P3 promoter. The orientation of the nt –317 to –174 fragment is indicated by → or ←. Each firefly luciferase construct was cotransfected into HL-60 cells with the *Renilla* luciferase expression vector (pRL-SV40) as the internal control. Following transfection luciferase activity was measured after a 24 h incubation with (open bars) or without (solid bars) DMSO. Relative luciferase activity was normalized to the luciferase activity of the pGL3-Control. Data is expressed as the mean ± standard deviation (n = 3).

Effect of substitution the Sp1 and Oct-1 motifs upon transcriptional activity

The nt –317 to –174 region contains three sequences similar to the two Sp1 recognition elements (Sp1-1 and Sp1-2) (Briggs *et al.*, 1986; Kriwacki *et al.*, 1992) and one sequence similar to the Oct-1 recognition element (Verrijzer *et al.*, 1992). To examine the contribution of each Sp1 and Oct-1 recognition element to the transcriptional activity of the P3 promoter, we mutated the Sp1 and Oct-1 recognition elements by introducing base substitutions. The mutated pGL-317-P3 plasmids were generated from pGL-317-P3 and transfected into HL-60 cells, and then luciferase activity was measured.

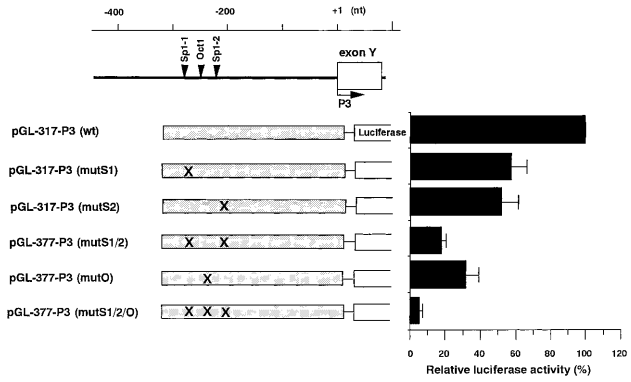

Fig. 4. Effect of mutations in the Sp1 and Oct-1 sites on hST6Gal I P3 promoter activity. The sites of mutation are denoted by (X). Each firefly luciferase construct was cotransfected into HL-60 cells with the Renilla luciferase expression vector (pRL-SV40) as the internal control. Following transfection luciferase activity was measured after a 24 h. Relative luciferase activity was normalized to the luciferase activity of the pGL-317-P3. Data is expressed as the mean + standard deviation (n = 3).

A mutation of the Sp1-1 (mutS1) and Sp1-2 (mutS2) sites reduced luciferase activity about 60% (Figure 4). Double mutants at of Sp1-1 and Sp1-2 (mutS1/2) sites reduced expression to ~20% of that of the pGL-317-P3 (wt). A mutation of the Oct-1 (mutO) site resulted in about 70% reduction in promoter activity. A triple mutation of the two Sp1 sites and one Oct-1 site (mutS1/2/O) resulted in about 90% reduction in promoter activity. These results suggest that Sp1-1, Sp1-2, and Oct-1 sites are necessary for full enhancer activity.

Discussion

In this study, we studied the transcriptional regulation of hST6Gal I gene during HL-60 cell differentiation induced by DMSO. Our results suggest that Sp1 and Oct-1 sites may play a critical role in the transcriptional regulation of the hST6Gal I gene during HL-60 cell differentiation. Several transcription factors, such as Sp1 (Rao *et al.*, 1998; Hauses *et al.*, 1998) and Oct-1 (Kim *et al.*, 1995; Lopez-Rodriguez *et al.*, 1997; Corbi and Lopez-Rodriguez, 1997), which are involved in myeloid cells specific gene regulation have been characterized, however, the functional roles of Sp1 and Oct-1 remain unknown.

There are three known Sp1-related proteins, Sp2, Sp3, and Sp4 (Hagen *et al.*, 1992; Hagen *et al.*, 1994), but because Sp2 binds to GT rather than GC box (Verrijzer and van der Vliet, 1993), Sp4 expression is restricted to the nervous system (Hagen *et al.*, 1992), and the level of Sp3 expression is considerably lower than Sp1 abundance in HL-60 cells (Rao *et al.*, 1998), only Sp1 is likely to contribute to GC box binding in HL-60 cell extracts. The Sp1 protein is ubiquitously expressed in proliferating cells and contributes to the activation of many growth-promoting genes (Deng *et al.*, 1986; Kadonaga *et al.*, 1987; Swick *et al.*, 1989; Dynan and Tjian, 1983; Melton *et al.*, 1984). However, little is known about the regulation and mechanisms of Sp1-mediated transcriptional regulation during HL-60 differentiation. Rao *et al.* (1998) observed the truncation of Sp1 by myeloblastin in undifferentiated HL-60 cells. This result suggested that Sp1 truncation is important for GC-

box mediate gene regulation during myeloid cell differentiation.

Expression of the H2B histone mRNA is high in undifferentiated HL-60 cells and suppresses during differentiation of the HL-60 cells (Kim *et al.*, 1995), which is similar to the case of hST6Gal I gene. Kim *et al.* (1995) also demonstrated that the level of Oct-1 decreased during HL-60 cell differentiation. This result suggested that the transcriptional repression of hST6Gal I gene during HL-60 cell differentiation may be mediated by reduced level of Oct-1.

Sialyl-Le^x is highly expressed on the surface of mature granulocytes and is believed to be a major ligand recognized by E- and P-selectin (Philips *et al.*, 1990; Walz *et al.*, 1990; Tiemeyer *et al.*, 1991). Previous studies demonstrated that DMSO-induced differentiation of HL-60 cells resulted in an increase in cell surface expression of sialyl-Le^x, and have been performed on α2,3-, α2,6-sialyltransferase and α1,3-fucosyltransferase activities (Skacel *et al.*, 1991). These results suggest that the reduced expression of sialyl-Le^x in undifferentiated HL-60 cells is related to the presence of the strong α2,6-sialyltransferase, which uses the precursor at the expense of the α1,3-fucosyltransferase and competes the synthesis of Le^x and sialyl-Le^x. We have previously shown that differentiation elicits negative regulation of hST6Gal I at the mRNA level in the HL-60 cell line and that transcriptional regulation of hST6Gal I depends on the P3 promoter that exists 5′-upstream of exon Y (Taniguchi *et al.*, 1998). Therefore, transcriptional regulation of hST6Gal I P3 promoter during the differentiation of HL-60 cells may play an important role in the expression of sialyl-Le^x.

Materials and methods

Cell culture

The myeloid cell line HL-60 cell line was grown in RPMI 1640 (Nissui, Japan) at 37°C in a humidified atmosphere with 5% CO_2. The medium was supplemented with 100 U of penicillin, 100 mg of streptomycin, and 10% heat-inactivated fetal bovine serum (JRH Biosciences, USA).

Cloning of the 5′-flanking region of the hST6Gal I P3 promoter

Cloning and isolation of 5′-flanking region of P3 promoter was performed using GenomeWalker kit (Clonetech, USA) according to the manufacturer's instructions. EcoRV digested human genomic DNA was ligated with a double stranded oligonucleotide containing an anchor sequence, which functioned as a primer binding site for subsequent PCR amplification. Primary PCR was performed with the provided adapter primer (AP1) and a gene specific primer, GSP1(5′-CTATTATCCATGGGAGGGAAGGTTTAT-3′) for exon Y of hST6Gal I. Secondary PCR using the provided adapter primer (AP2) and gene specific primer (GSP2, 5′-CCAAGGCCCATTTTTCTCAGGATGATC-3′) was required. The PCR amplicon was ligated into pCR2.1-TOPO (Invitrogen, USA), and sequenced using GeneRapid DNA sequencing system (Amersham Pharmacia Biotech, UK).

PCR Amplification of the 5′-cDNA end (5′-RACE)

Amplification of the 5′ end of hST6Gal I cDNA was performed according to the manufacturer's instructions (5′-full RACE core set, Takara, Japan) (Maruyama *et al.*, 1995; Taniguchi and Matsumoto, 1998). First-strand cDNA was synthesized from 3 mg of total RNA using the gene specific primer, 5′-pTCTGAGACTGGCCGA-3′. After digestion of template mRNA with RNase H at 30°C for 1 h, cDNA was precipitated with ethanol and ligated using T4 RNA ligase at 16°C for 16 h. A 1 in 10 dilution of the ligation mixture was used as the template for the first PCR amplification using 5′-TTCTTTTCCTTCCACACACAGATG-3′ as the sense primer and 5′-AAAGGGAGTTACTATGATTCCTTT-3′ as the anti-sense primer. Twenty-five cycles of denaturation at 94°C for 30 s, annealing at 56°C for 30 s, and extension at 72°C for 1 min were performed. The resulting PCR products were diluted 100-fold with sterile water, and amplified under the same conditions using 5′-TGCAAACAGAAGAAAGACCAGGAC-3′ as the sense primer and 5′-CCATGGGGTCTGATTCCCAGTCTG-3′ as the anti-sense primer. The final PCR products were subcloned into the pCR2.1 vector using the Original TA cloning kit (Invitrogen, CA), and then sequenced.

Construction of plasmids for luciferase assay

The oligonucleotides primers designed and used were: pGL-876-P3; 5′-AGCTCGAGCAGCTTGGTTAACCTCTTT-3′, pGL-pGL-676-P3; 5′-ATCTCGAGTCATGCCTCAATGTTAGGA-3′, pGL-460-P3; 5′-ATCTCGAGGCGTTTAGGGGTCCTT-3′, pGL-317-P3; 5′-ACCTCGAGGCCGGTTTGGGGCGCGGTG-3′, pGL-174-P3; 5′-ATCTCGAGTCCCTTCCACTGTGCGTCT-3′, pGL-84-P3; 5′-ATCTCGAGATCCTGCCCTTGCCGA-3′ and 5′-GCAAGCTTAACGCCGGGCAGTCGC-3′, and (pGLPRO-317-174 and pGLPRO-174-317; 5′-ACTCTGAGGCCGGTTTGGGGCGCGGTG-3′, and 5′-CTCTCGAGCTGGAGAAGGAAGGAAGAAGA-3′), respectively. The underlined nucleotides indicate restriction sites that were incorporated into the primers. Twenty-five cycles of PCR amplification consisting of denaturation at 98°C for 20 sec, and annealing and extension at 68°C for 1 min was carried out in a Parkin Elmer/Cetus thermal cycler. A single band was obtained by agarose gel electrophoretic analysis. PCR products were digested using the XhoI and HindIII restriction enzymes and cloned into the XhoI and HindIII sites of the pGL3-Basic Vector or into the XhoI site of the pGLPRO vector (Promega, USA). The identity of the amplification products was verified by sequence analysis.

Luciferase assay

Transient transfection of HL-60 cells was performed using Effectene Transfection Reagent (Qiagen, Germany). Cells were plated at a density of approximately $1–3 \times 10^5$ cells per 35 mm dish, and then transfected with 1 mg of pGL constructs and 0.1 mg of pRL-CMV (Promega, USA), containing the CMV promoter located downstream of the Renilla luciferase gene, as an internal control for variations in transfection efficiency. After 24 h, uninduced HL-60 cells or HL-60 cells induced to differentiate by treatment with 1.35% of DMSO, cells were harvested and cell lysates were prepared. Firefly and Renilla luciferase assays were performed using the Dual-Luciferase Reporter Assay System (Promega, USA).

Mutagenesis of the Sp1 and Oct-1 binding sites

Mutations with base substitutions were constructed for each Sp1 and Oct-1 motif using the GeneEditor *in vitro* site-directed mutagenesis system (Promega, USA) according to the manufacturer's protocol. The oligonucleotides used for site-directed mutagenesis were 5′-AGAGGTGGAGTGGGAACGGAGACGACC-3′ (for pGL-317-P3 mutS1, mutS1/2, and mutS1/2/O), 5′-ACTCGCTTCCTTCCCGAACCTCCGCCG-3′ (for pGL-377-P3mutS1/2, mutS2, and mutS1/2/O) and 5′-AGTCTTCGCAACTCTATAAGCATACGG-3′ (for pGL-377-P3mutO and mutS1/2/O). Mutation sites of these primers are underlined.

References

Aasheim,H.-C., Aas-Eng,D.A., Deggerdal,A., Blomhoff,H.K., Funderd,S. and Smeland,E.B. (1993) Cell-specific expression of human β-galactoside α 2,6-sialyltransferase transcripts differing in the 5′ untranslated region. *Eur. J. Biochem.*, **213**, 467–475.

Briggs,M.R., Kadonaga,J.T., Bell,S.P. and Tjian,R. (1986) Purification and biochemical characterization of the promoter-specific transcription factor, Sp1. *Science*, **234**, 47–52.

Clarke,J.L. and Watkins,W.M. (1996) α1,3-L-Fucosyltransferase expression in developing human myeloid cells. Antigenic, enzymatic and mRNA analyses. *J. Biol. Chem.*, **271**, 10317–10328.

Corbi,A.L. and Lopez-Rodriguez,C. (1997) CD11c integrin gene promoter activity during myeloid differentiation. *Leuk. Lymphoma*, **25**, 415–425.

Deng,T., Dawel,L., Jehn,C. and Johnson,L.F. (1986) Structure of the gene for mouse thymidylate synthase. Locations of introns and multiple transcriptional start sites. *J. Biol. Chem.*, **261**, 16000–16005.

Dynan,W.S. and Tjian,R. (1983) Isolation of transcription factors that discriminate between different promoters recognized by RNA polymerase II. *Cell*, **32**, 6669–6680.

Fukuda,M., Koeffler,H.P. and Minovada,J. (1981) Membrane differentiation in human myeloid cells: expression of unique profiles of cell surface glycoproteins in myeloid leukemic cell lines blocked at different stages of differentiation and maturation. *Proc. Natl. Acad. Sci. USA*, **78**, 6299–6303.

Gahmberg,C.G., Nilsson,K. and Anderson,L.C. (1979) Specific changes in the surface glycoprotein pattern of human promyelocytic leukemic cell line HL-60 during morphologic and functional differentiation. *Proc. Natl. Acad. Sci. USA*, **76**, 4087–4091.

Hagen,G., Muller,S., Beato,M. and Suske,G. (1992) Cloning by recognition site screening of two novel GT box binding proteins: a family of Sp1 related genes. *Nucleic Acids Res.*, **20**, 5519–5525.

Hagen,G., Muller,S., Beato,M. and Suske,G. (1994) Sp1-mediated transcriptional activation is repressed by Sp3. *EMBO J.*, **13**, 3843–3851.

Hanasaki,K., Varki,A., Stamenkovic,I. and Bevilacqua,M.P. (1994) Cytokine-induced β-galactoside α2,6-sialyltransferase in human endothelial cells mediates α2,6-sialylation of adhesion molecules and CD22 ligands. *J. Biol. Chem.*, **269**, 10637–10643.

Hauses,M., Tonjes,R.R. and Grez,M. (1998) The transcription factor Sp1 regulates the myeloid-specific expression of the human hematopoietic cell kinase (HCK) gene through binding to two adjacent GC boxes within the HCK promoter-proximal region. *J. Biol. Chem.*, **273**, 31844–31852.

Kadonaga,J., Carner,K.C., Masiarz,F.R. and Tjian,R. (1987) Isolation of cDNA encoding transcription factor Sp1 and functional analysis of the DNA binding domain. *Cell*, **51**, 1079–1090.

Kelm,S. and Schauer,R. (1997) Sialic acids in molecular and cellular interactions. *Int. Rev. Cytol.*, **175**,137–240.

Kim,K.Y., Kweon,K.R., Lee,M.S.,Kwak,S.T., Kim,K.E., Hwang,B.D. and Lim,K. (1995) Reduced level of octamer binding transcription factor (Oct-1) is correlated with H2B histone gene repression during differentiation of HL-60 cells by all-trans retinoic acid. *Biochem. Biophys. Res. Commun.*, **213**, 616–624.

Kriwacki,R.W., Schultz,S.C., Steitz,T.A. and Caradonna,J.P. (1992) Sequence-specific recognition of DNA by zinc-finger peptides derived from the transcription factor Sp1. *Proc. Natl. Acad. Sci. USA*, **89**, 9759–9763.

Li,M., Vemulapalli,R., Ullah,A., Izu,L., Duffey,M.E. and Lance,P. (1998) Downregulation of a human colonic sialyltransferase by a secondary bile acid and a phorbol ester. *Am. J. Physiol.*, **274**, G599–G606.

Lo,N-W. and Lau,J.T. (1996a) Transcription of the β-galactoside α2,6-sialyltransferase gene in B lymphocytes is directed by a separate and distinct promoter. *Glycobiology*, **6**, 271–279.

Lo,N-W. and Lau,J.T. (1996b) Novel heterogeneity exists in the 5′-untranslated region of the β-galactoside α2,6-sialyltransferase mRNAs in the human B-lymphoblastoid cell line, louckes. *Biochem. Biophys. Res. Commun.*, **228**, 380–385.

Lopez-Rodriguez,C., Zubiaur,M., Sancho,J., Concha,A. and Corbi,L.A. (1997) An octamer element functions as a regulatory element in the differentiation-responsive CD11c integrin gene promoter: OCT-2 inducibility during myelomonocytic differentiation. *J. Immunol.*, **158**, 5833–5840.

Maruyama,I.N., Reakow,T.L. and Maruyama H. I. (1995) cRACE: a simple method for identification of the 5′ end of mRNAs. *Nucleic Acids Res.*, **23**, 3796–3797.

Melton,D.W., Konecki,D.S., Brennand,J. and Caskey,C.T. (1984) Structure, expression and mutation of the hypoxanthine phosphoribosyltransferase gene. *Proc. Natl. Acad. Sci. USA*, **81**, 2147–2151.

Morris,J.F., Rauscher,F.J. III, Davis,B., Klemsz,M., Xu,D., Tenen,D. and Hromas,R. (1995) The myeloid zinc finger gene, MZF-**1**, regulates the CD34 promoter *in vitro*. *Blood*, **86**, 3640–3647.

Nakamura,M., Tsunoda,A., Sakoe,K., Gu,J., Nishikawa,A., Taniguchi,N. and Saito,M. (1992) Total metabolic flow of glycosphingolipid biosynthesis is regulated by UDP-GlcNAc:lactosylceramide β 1→3N-acetylglucosaminyltransferase and CMP-NeuAc:lactosylceramide α 2→3 sialyltransferase in human hematopoietic cell line HL-60 during differentiation. *J. Biol. Chem.*, **267**, 23507–23514.

Nojiri,H., Takaku,F., Tetsuka,T., Motoyoshi,K., Miura,Y. and Saito,M. (1984) Characteristic expression of glycosphingolipid profiles in the bipotential cell differentiation of human promyelocytic leukemia cell line HL-60. *Blood*, **64**, 534–541.

O'Hanlon,T.P., Lau,K.M., Wang,X.C. and Lau,J.T.Y. (1989) Tissue-specific expression of β-galactoside α2,6-sialyltransferase. Transcript heterogeneity predicts a divergent polypeptide. *J. Biol. Chem.*, **264**, 17389–17394.

O'Hanlon,T.P. and Lau,J.T.Y. (1992) Analysis of kidney mRNAs expressed from the rat β-galactoside α2,6-sialyltransferase gene. *Glycobiology*, **2**, 257–266.

Paulson,J.C., Weinstein,J. and Schauer,A. (1989) Tissue-specific expression of sialyltransferases. *J. Biol. Chem.*, **264**, 10931–10934.

Philips,M.L., Nudelman,E., Gaeta,F.C., Perez,M., Singhal,A.K., Hakomori,S. and Paulson,J.C. (1990) ELAM-1 mediates cell adhesion by recognition of a carbohydrate ligand, sialyl-Le^x. *Science*, **250**, 1130–1132.

Rao,J., Zhang,F., Donnelly,R.J., Spector,N.L. and Studzinski,G.P. (1998) Truncation of Sp1 transcription factor by myeloblastin in undifferentiated HL60 cells. *J. Cell Physiol.*, **175**, 121–128.

Robertson,K.A., Hill,D.P., Kelley,M.R., Tritt,R., Crum,B., Van Epps,S., Srour,E., Rice,S. and Hromas,R. (1998) The myeloid zinc finger gene (MZF-1) delays retinoic acid-induced apoptosis and differentiation in myeloid leukemia cells. *Leukemia*, **5**, 690–698.

Skacel,P.O., Edwards,A.J., Harrison,C.T. and Watkins,W.M. (1991) Enzymic control of the expression of the X determinant (CD15) in human myeloid cells during maturation: the regulatory role of 6-sialyltransferase. *Blood*, **78**, 1452–1460.

Swick,A.G., Blake,M.C., Kahn,J.W. and Azizkhan,J.C. (1989) Functional analysis of GC element binding and transcription in the hamster dihydrofolate reductase gene promoter. *Nucleic Acids Res.*, **17**, 9291–9304.

Taatjes,D.J. and Roth,J. (1988) Alteration in sialyltransferase and sialic acid expression accompanies cell differentiation in rat intestine. *Eur. J. Cell Biol.*, **46**, 289–298.

Taniguchi,A. and Matsumoto,K. (1998) Down-regulation of human sialyltransferase gene expression during *in vitro* human keratinocyte cell line differentiation. *Biochem. Biophys. Res. Commun.*, **243**, 177–183.

Taniguchi,A. and Matsumoto,K. (1999) Epithelial-cell specific transcriptional regulation of human Galβ1,3GalNAc/Galβ1,4GlcNAc α2,3-sialyltransferase (hST3Gal IV) gene. *Biochem. Biophys. Res. Commun.*, **257**, 516–522.

Taniguchi,A. Higai,K., Hasegawa,Y., Utsumi,K. and Matsumoto,K. (1998) Differentiation elicits negative regulation of human β-galactoside α2,6-sialyltransferase at the mRNA level in the HL-60 cell line. *FEBS Lett.*, **441**, 191–194.

Tiemeyer,M., Swiedler,S.J., Ishihara,M., Moreland,H., Schweingruber,H., Hirzter,P. and Brandley,B.K. (1991) Carbohydrate ligands for endothelial-leukocyte adhesion molecule 1. *Proc. Natl. Acad. Sci. USA*, **88**, 1138–1142.

Tenen,D.G., Hromas,R., Licht,J.D. and Zhang,D.E. (1997) Transcription factors, normal myeloid development and leukemia. *Blood*, **90**, 489–519.

Tsuji,S. (1996) Molecular cloning and functional analysis of sialyltransferase. *J. Biochem.*, **120**, 1–13.

Varki,A. (1997) Sialic acids as ligands in recognition phenomena. *FASEB J.*, **11**, 248–255.

Verrijzer,C.P., Alkema,M.J., van Weperen,W.W., Strating,M.J. and van der Vliet,P.C. (1992) The DNA binding specificity of the bipartite POU domain and its subdomains. *EMBO J.*, **11**, 4993–5003.

Verrijzer,C.P. and van der Vliet,P.C. (1993) POU domain transcription factors. *Biochim. Biophys. Acta*, **1173**, 1–21.

Vertino-Bell,A., Ren,J., Black,J.D. and Lau,J.T.Y. (1994) Developmental regulation of β-galactoside α2,6-sialyltransferase in small intestine epithelium. *Dev. Biol.*, **165**, 126–136.

Walz,G., Aruffo,A., Kolanus,W., Bevilacqua,M. and Seed,B. (1990) Recognition by ELAM-1 of the sialyl-Le^x determinant on myeloid and tumor cells. *Science*, **250**, 1132–1135.

Wang,X., O'Hanlon,T.P., Young,R.F. and Lau,J.T.Y. (1990a) Rat β-galactoside α2,6-sialyltransferase genomic organization: alternate promoters direct the synthesis of liver and kidney transcripts. *Glycobiology*, **1**, 25–31.

Wang,X., Smith,T.J. and Lau,J.T.Y. (1990b) Transcriptional regulation of the liver β-galactoside α2,6-sialyltransferase by glucocorticoids. *J. Biol. Chem.*, **265**, 17849–17853.

Wang,X., Vertino,A., Eddy,R.L., Byers,M.G., Jani-Sait,S.N., Shows,T.B. and Lau,J.T. (1993) Chromosome mapping and organization of the human β-galactoside α2,6-sialyltransferase gene. Differential and cell-type specific usage of upstream exon sequences in B-lymphoblastoid cells. *J. Biol. Chem.*, **268**, 4355–4361.

Wen,D.X., Svensson,E.C. and Paulson,J.C. (1992) Tissue-specific alternative splicing of the β-galactoside α2,6-sialyltransferase gene. *J. Biol. Chem.*, **267**, 2512–2518.