A GATA binding site is involved in the regulation of 15-lipoxygenase-1 expression in human colorectal carcinoma cell line, Caco-2

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Received 22 October 1999; received in revised form 11 January 2000

Edited by Shozo Yamamoto

Abstract The data presented implicate a GATA binding site in the transcriptional regulation of 15-lipoxygenase-1 (15-LO-1) gene expression in human colorectal carcinoma Caco-2 cells. High expression of GATA-6 mRNA and protein was observed, while GATA-4 mRNA was expressed at a very low level in Caco-2 cells. The expression of GATA-6 was down-regulated, while 15-LO-1 expression was dramatically up-regulated after treatment with sodium butyrate (NaBT). A study using an electrophoretic mobility shift assay indicated that a GATA binding site of the 15-LO-1 promoter region binds to GATA proteins present in both undifferentiated and, to a lesser extent, NaBT-treated (differentiated) Caco-2 cells. Moreover, that DNA binding shift band was disrupted after the addition of GATA-6 antibody in a supershift assay in the absence of NaBT, suggesting that GATA-6 is bound to the GATA binding site of the 15-LO-1 promoter in undifferentiated cells. In contrast, the addition of GATA-6 antibody did not affect the DNA binding ability in NaBT-induced differentiated cells. On the other hand, mutation of the GATA site of the 15-LO-1 promoter decreased the transactivation of the 15-LO-1 promoter as measured by luciferase activity in both FBS and NaBT cultured cells, indicating an unknown GATA binding protein to up-regulate 15-LO-1 expression. These implicate the GATA site at −240 of the proximal region of the 15-LO-1 promoter in the basic transcription of 15-LO-1 gene expression in Caco-2 cells, with GATA-6 acting to repress 15-LO-1 expression.

Key words: 15-Lipoxygenase-1; GATA; Differentiation; Transcription factor

1. Introduction

The GATA family of transcription factors contain one or two DNA binding zinc finger domains of the distinctive form C-X-N-C-(X_{17})-C-N-X-C and recognize the consensus DNA sequence (A/T)GATA(A/G), which is an essential *cis* element in the promoters or enhancers of a variety of genes [1]. GATA-1/2/3 are expressed in the hematopoietic cell lineage and play critical roles in the development of hematopoietic stem cells [2]. In contrast, the more recently identified GATA-4/5/6 genes represent a subfamily of factors that are expressed in cardiovascular tissues [3], gut [3–5], urogenital system [6] and smooth muscle cells [7]. A possible role for GATA-4/5/6 in regulating gut-specific gene expression is proposed based on the expression of GATA-4/5/6 in the intestinal tissues. The presence of GATA binding sites has been reported in the α- and β-subunit promoter encoding the H^+/K^+-ATPase in stomach [8,9], rat histidine decarboxylase gene in gastric endocrine cells [10] and the intestinal fatty acid binding protein gene in the intestinal derived cells [11]. Furthermore, recent evidence suggests that GATA-4/5/6 are essential for development and differentiation in gut [11–16]. Nevertheless, the precise role of GATA-4/5/6 remains unclear in intestinal derived cells and tissues.

Lipoxygenases are dioxygenases that incorporate one molecule of oxygen at a specific position of unsaturated fatty acids such as arachidonic acid [17]. Among the members of the lipoxygenase family, reticulocyte-type 15-lipoxygenase (15-LO-1) [18] is unique in its ability to oxidize complex substrates such as biomembranes [19]. 15-LO-1 is also expressed during distinct stages of reticulocyte development [20], in macrophages of atherosclerotic lesions [21], in human tracheal epithelial cells during retinoid-induced mucocilial differentiation [22] and in the eye lens in conjunction with organelle degradation [23]. These reports suggest the possible involvement of differentiation-related transcription factors in the regulation of the 15-LO-1 gene. We observed the induction of 15-LO-1 in the human colorectal carcinoma cell line, Caco-2 after treatment with NaBT, concomitant with cell differentiation [24], and subsequently in human colorectal carcinoma tissues [25]. Only a few reports describe the transcriptional regulation of the 15-LO-1 gene. The 15-LO-1 gene is regulated by cytokines, IL-4 and IL-13 in monocytes [26,27] and in airway cells [28,29]. A specific Stat6 response element located at 952 base pairs upstream of the translational start codon is responsible for the regulation of 15-LO-1 by IL-4 [30]. However, the regulation of 15-LO-1 by cytokines appears to be different from that by NaBT during cell differentiation. Unlike cytokines, butyrate is a short-chain fatty acid, a product of fermentation of luminal carbohydrates, and is found in millimolar concentration in the lumen of the intestinal epithelium [31]. Moreover, NaBT is often used as a model for cellular differentiation in intestinal derived cell lines [32].

We focused on transcription factors GATA-4/5/6 for regulation of 15-LO-1 expression in the intestinal derived cells. The genomic DNA of 15-LO-1 was isolated as a clone and a part of the sequence of the 5′ flanking region was determined [33,34]. The GATA binding site exists at −240 of the 5′

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Abbreviations: LO, lipoxygenase; EMSA, electrophoretic mobility shift assay; FBS, fetal bovine serum; NaBT, sodium butyrate; EGFR, epidermal growth factor receptor

PII: S0014-5793(00)01155-8

flanking region in 15-LO-1 gene. Thus, based on the possible involvement of GATA in cell differentiation and the regulation of 15-LO-1 expression during NaBT-induced differentiation, we evaluated the expression of GATA family transcription factors, especially GATA-4/5/6. Furthermore, nuclear extracts from Caco-2 cells were used to study their binding properties at the GATA binding site. In this study, we report the presence of a binding protein for the GATA binding site in the promoter region of the 15-LO-1 gene and present evidence implicating the GATA binding site in the transcription of 15-LO-1 in Caco-2 cells.

2. Materials and methods

2.1. Cell culture

The human colon carcinoma cell line, Caco-2, was obtained from the American Type Culture Collection (ATCC). The cells were grown in Eagle's minimal essential medium (EMEM)/15% fetal bovine serum (FBS) supplemented with 1 mM sodium pyruvate (GIBCO/BRL) and gentamicin (1 mg/100 ml) (GIBCO/BRL). FBS was from Summit and NaBT was obtained from Sigma. For treating cells with NaBT, cells were cultured in the appropriate media containing serum until near confluency. The medium was removed and replaced with FBS medium containing NaBT.

2.2. Northern blot analysis

15-LO-1 probe was purchased from Oxford and the G3PDH cDNA that was used as the internal standard was purchased from Clontech. The cDNA probes of GATA-4 and GATA-6 were generated by RT-PCR from the human heart RNA (Clontech) as the template. The primers and cDNA are as follows: G4S, AACGGAAGCCCAAGAACCTGA and G4A, TGCAGTGTGCTCGTGCTGAA for generation of GATA-4 cDNA which has 613 bp as the predicted size (GeneBank Accession Number D78260), G6S, AGGCCATTTGGTACACATCTCT and G6A, TAATGTAAACCAACCTGCCTGT for generation of GATA-6 cDNA that has 641 bp as the predicted size (GeneBank Accession Number U66075). Both cDNAs were extracted by using QIAquick gel extraction kit (QIAGEN) after agarose gel electrophoresis.

Extraction of total RNA from the cells, electrophoresis and hybridization were preformed as previous described [24].

2.3. Immunoprecipitation and Western blotting

Nuclear extracts from Caco-2 cells were prepared as previously described [35] and stored at −80°C. Protein concentration was determined by the bicinchronic acid method using BCA protein assay reagent (Pierce). Nuclear extract containing 0.3 mg protein was precleared with 5 μl of normal rabbit serum, 20 μl of protein G-agarose and 100 μl of protein A-Sepharose beads. The mixture was tumbled at 4°C for 1 h, and centrifuged at $10\,000\times g$ for 2 min, and the supernatant transferred to a new tube. Anti-GATA-4 or anti-GATA-6, 2 μg of each, was added to the supernatant and the samples were tumbled for 2 h at 4°C. For some experiments, 1 μg of blocking peptide for anti-GATA-6 or anti-epidermal growth factor receptor (EGFR; both from Santa Cruz Biotechnology) was also included. Then, 20 μl of protein G-agarose (Santa Cruz Biotechnology) and 100 μl of protein A-Sepharose (Sigma) beads were added and samples were tumbled for an additional 2 h. After centrifugation at $10\,000\times g$ for 2 min, the pellet was washed three times with 1 ml of 20 mM HEPES, pH 7.5, 150 mM NaCl, 0.1% Triton X-100, 10% glycerol. 2× protein sample buffer was added to the final pellet, the sample was boiled for 8 min and centrifuged at $10\,000\times g$ for 10 min. The supernatant was immediately loaded, run on SDS–PAGE (8% acrylamide gel) and electrophoretically transferred to a nitrocellulose membrane in 25 mM Tris, 192 mM glycine, 20% methanol with 0.1% SDS using Hoefer semi-dry electrophoresis equipment. Membranes were blocked in Tris-buffered saline containing 0.1% Tween 20 (TBST) with 5% non-fat milk at 4°C overnight. The blots were then incubated with anti-GATA-4 or anti-GATA-6 (1:500) in TBST plus 3% non-fat milk for 3 h at room temperature. The blots were washed five times in TBST and then incubated with horseradish peroxidase-conjugated anti-goat IgG (1:5000) in TBST containing 3% non-fat milk at room temperature for 1 h. The blots were again washed five times in TBST, and visualized using the Amersham ECL system.

2.4. Electrophoretic mobility shift assays (EMSA)

Three different oligonucleotide probes were used in this study. The commercially available oligonucleotide of wild-type, N-GATA, CGCCGCAGAGATAAGGCACTGCC (Geneka Biotechnology) including GATA binding site was used. Two oligonucleotide probes were synthesized based on the sequence of the promoter region of 15-LO-1 including GATA site: WT-15LO-GATA, TCCCGTCAAGATAGTGGTTTCCA (nucleotides −249 to −227) and Mu-15LO-GATA, TCCCGTCAAT**AT*ATG*G**TGGTTTCCA in which GATA site is mutated. Each complementary oligonucleotide was annealed at a concentration of 250 mM in 250 mM Tris (pH 7.8) at 95°C for 15 min and then cooled slowly to room temperature. Probes were labeled using [γ-^{32}P]ATP (Amersham) and T4 polynucleotide kinase (Pharmacia). The GATA-1 gel shift kit was obtained from Geneka Biotechnology and the procedure for the reaction between the probe and nuclear extracts was followed according to the manufacturer's instruction. Supershift assays were also performed by preincubating with the following anti-GATA antibodies, GATA-1 (c-20), GATA-2 (c-20), GATA-3 (c-18), GATA-4 (c-20), GATA-5 (Y-19) and GATA-6 (c-20) (all antibodies were purchased from Santa Cruz). Bound and free probes were resolved by non-denaturing polyacrylamide gel electrophoresis. The entire contents of each reaction were loaded onto 5% polyacrylamide (38:2) gel, pre-cooled to 4°C in 1×Tris–glycine electrophoresis buffer. Gels were dried under vacuum on Whatman 3 mm paper (Whatman) and exposed to X-ray film.

2.5. Transient transfection and luciferase assay

15-LO-1 promoter/reporter plasmids were constructed in pGL2 (Promega) as previously described [34]. The numbering of nucleotides in the 5′ flanking region of 15-LO-1 was defined by defining the translational start codon as +1. The location of the transcriptional initiation site is at −27 [33,34]. In this study, -628/-23pGL2, Mu-628/-23pGL2 and pGL-Basic (Promega) plasmids were used (Fig. 4A). Mu-628/-23pGL2 plasmid was generated by the technique of site-directed mutagenesis and has an alternative sequence in the GATA site. For transfection, cells were plated in 6-well plates at a density of 1×10^5 cells/well and incubated in FBS medium for 24 h. After washing the cells with PBS, 1 μg of luciferase reporter and 10 ng of pRL-null internal control construct (Promega) were transfected using 5 μl of Lipofectamine (Life Technologies, Inc.). The medium was replaced after 6 h with or without 5 mM NaBT and the cells were incubated for 48 h.

The cells were harvested using 500 μl of passive lysis buffer (Promega). Firefly luciferase and renilla (sea pansy) luciferase activities were measured sequentially using a dual-luciferase reporter assay system (Promega) and a model TD-20/20 Luminometer (Turner Design). After measuring the firefly luciferase signal (LA_F) and the renilla luciferase signal (LA_R), the relative luciferase activity (RLA) was calculated as $RLA = LA_F/LA_R$.

2.6. Site-directed mutagenesis

Mutations were generated by QuikChange site-directed mutagenesis kit (Stratagene) for Mu-628/-23pGL2. Primer S-GA, TCTCTCCTCCCGTCAA**TTG**AGTGGTTTCCACTCCCT and its reverse complement were used for *Pfu* polymerase amplification under conditions specified by the manufacturer's instruction. The transformed, white colonies were picked and plasmid DNA was extracted by SNAP Miniprep kit (Invitrogen). The product was sequenced to verify the mutations.

3. Results

3.1. GATA-4 and GATA-6 are expressed in Caco-2 cells

The expressions of GATA-4/5/6 were evaluated in Caco-2 cells. Based on the cDNA sequence indicated in GeneBank, three sets of primers corresponding to GATA-4/5/6 were synthesized and RT-PCR was performed. GATA-4 and GATA-6 were expressed, while GATA-5 was undetectable (data not shown). We next measured by Northern analysis the temporal expression of GATA-4 and 6, as well as 15-LO-1 (Fig. 1A) in

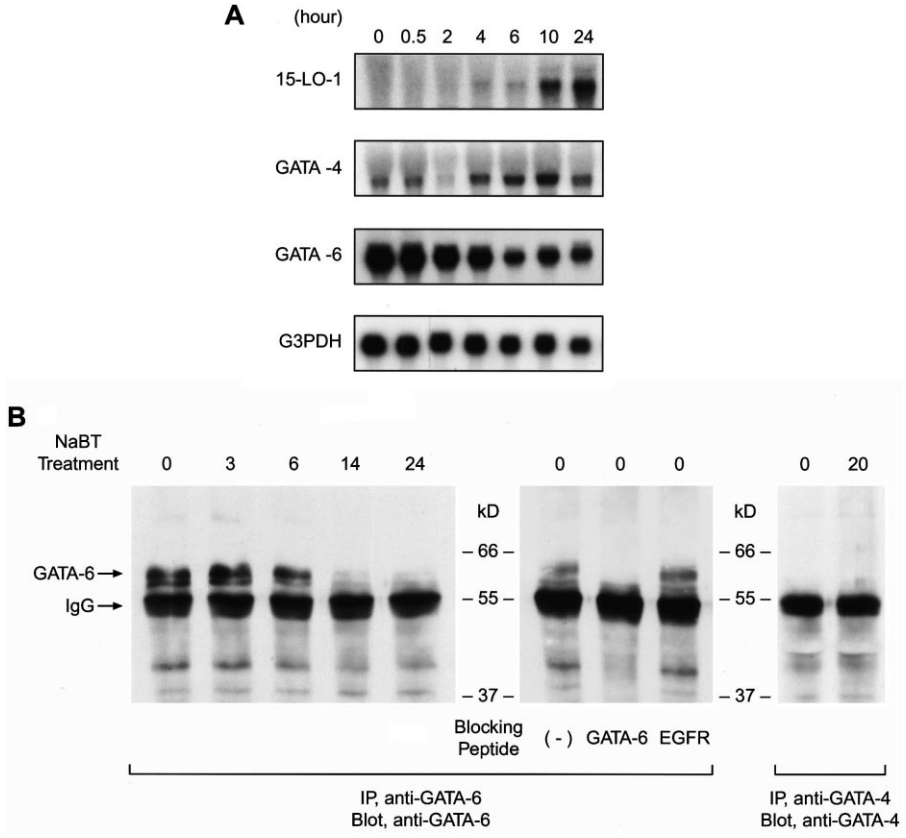

Fig. 1. Expression of GATA-4, 6 and 15-LO-1 in Caco-2 cells. A: Temporal expression after treatment with 5 mM NaBT by Northern blot analysis using human specific probes of total RNA (20 μg per sample). Time points are shown at 0, 0.5, 2, 4, 6, 10, 24 h after treatment with NaBT, respectively. Lane was evaluated by equivalent density of hybridization to the glyceralaldehyde-3-phosphate dehydrogenase (G3PDH) cDNA. Exposure times for the blots are 72 h, 120 h, 24 h, and 15 h for 15-LO-1, GATA-4, GATA-6, and G3PDH, respectively. B: Immunoprecipitation and Western analysis. GATA-4 or GATA-6 was immunoprecipitated from 0.3 mg of nuclear protein extract of Caco-2 cells untreated or treated with 5 mM NaBT for indicated times (20 h for GATA-4, and 3, 6, 14 and 24 h for GATA-6) and the amount of protein expression was analyzed by Western analysis. To demonstrate the specificity of the band representing GATA-6, 1 μg of blocking peptide for anti-GATA-6 or anti-EGFR, as a control, was included in the immunoprecipitation of GATA-6 from some samples.

order to determine the involvement of GATA proteins in NaBT-induced expression of 15-LO-1. A faint expression of GATA-4 was observed before and after treatment with NaBT. The expression was increased by NaBT treatment, although still weak, and the maximum level was observed 10 h after treatment. In contrast, a strong expression of GATA-6 was detected before treatment with NaBT and gradually decreased after NaBT treatment.

Next, we examined the protein expression of GATA-4/5/6 using nuclear extracts before and after treatment with NaBT. The expressions of GATA-4 (Fig. 1B) and GATA-5 (data not shown) were not detectable by Western analysis even with the concentration of the target protein by immunoprecipitation. In contrast, GATA-6 expression was clearly observed before NaBT treatment. NaBT caused a down-regulation of protein expression of GATA-6 (Fig. 1B), which is consistent with the temporal change in mRNA expression. GATA-6 expression was confirmed by the specific abrogation of the signal with a blocking peptide for anti-GATA-6, but not with that for anti-EGFR. Since 15-LO-1 is up-regulated by NaBT, the changes in the expression patterns of 15-LO-1 and GATA-6 appear to occur in opposition with each other.

3.2. Nuclear binding proteins recognize and bind to the GATA site in Caco-2 cells

Nucleotide sequences in approximately 1 kb of the 5′ flanking region of the promoter area of the 15-LO-1 gene have been analyzed, and a GATA site is located 240 bp upstream from the translational start codon [33,34]. Thus, we amplified a 979 bp fragment of the promoter region of 15-LO-1 gene from genomic DNA of Caco-2 cells and the sequence was determined (data not shown). The GATA site was located at the same position in Caco-2 cells as previously reported [33,34]. Then, we tested Caco-2 cells for the presence of a nuclear protein which was bound to DNA at the GATA site by EMSA (Fig. 2). Two different labeled DNA probes containing the GATA site were used and mixed with nuclear extracts. One is the 23-mer oligonucleotide which corresponds to the sequence of the promoter region of 15-LO-1 including 'GATA' (WT-15LO-GATA), and the other is the non-specific 23-mer oligonucleotide including 'GATA' (WT-N-GATA). Both oligonucleotides bound to the nuclear protein prepared from undifferentiated and NaBT-differentiated cells. The addition of a competitor (100-fold excess) of unlabeled wild-type oligo-probe completely blocked the binding activity at the

GATA site. In contrast, Mu-15-LO-GATA, which has mutations at the GATA site of WT-15LO-GATA, did not compete with WT-15-LO-GATA for its binding site, suggesting that there are nuclear binding proteins which recognize and bind to the GATA site in Caco-2 cells. Moreover, the intensity of each band in undifferentiated cells (FBS) was stronger than that in differentiated cells (NaBT). Possible explanations for these observations include: (1) butyrate treatment attenuates the binding ability at the GATA site, or (2) butyrate treatment decreases the amount of the DNA binding protein at the GATA site.

3.3. *Identification of GATA-6 as a GATA site binding protein in undifferentiated Caco-2 cells*

To identify the nuclear binding protein which bound to the GATA site, we performed the supershift assay using GATA antibodies. Incubation with 2 μg each of GATA-1, 2, 3, 4, 5 or 6 antibody did not result in a supershift band in either FBS- or NaBT-treated cells (data not shown). However, incubation with GATA-6 antibody remarkably reduced the intensity of the original band in undifferentiated cells. The mixtures of labeled probe and the nuclear protein were incubated with various concentrations of the GATA-6 antibody, and were examined by EMSA (Fig. 3). The original bands (arrow in Fig. 3) composed of the GATA site oligo-probe and binding proteins were disrupted by incubation with GATA-6 antibody in a concentration-dependent manner in FBS-treated cells. In contrast, the original band was not affected by incubation with 4 μg of GATA-6 antibody in NaBT-treated cells. These observations suggested that GATA-6 protein is bound to the GATA site in undifferentiated Caco-2 cells but not in NaBT-induced differentiated cells.

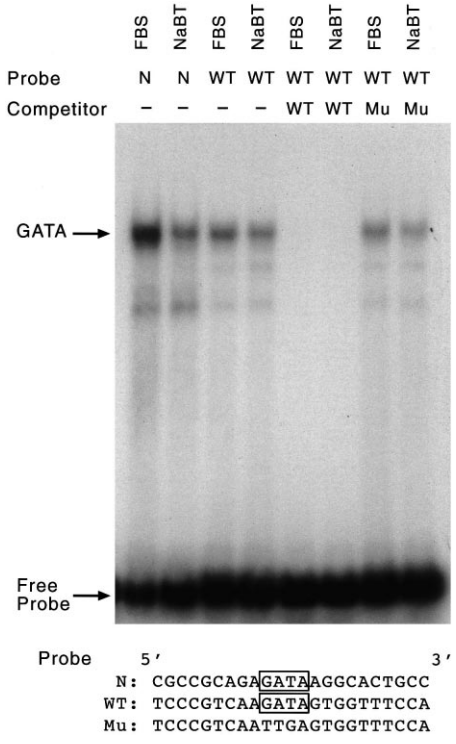

Fig. 2. Existence of a binding protein to the GATA site in Caco-2 cells. EMSA was carried out using nuclear extracts prepared from Caco-2 cells treated with NaBT for 20 h or FBS alone-treated cells. Labeled oligonucleotide containing the sequence of the GATA site required for the transactivation of the 15-LO-1 promoter from positions −249 to −227 from the translational initiation site was used as a WT probe. Another labeled probe (N) including 'GATA' sequence but without 15-LO-1 promoter sequence was also used. For the competitor study, unlabeled WT and Mu probes were used at 100-fold excess. The band of GATA protein and labeled probe complex is shown by an arrow. Unbound labeled probe is also indicated by an arrow as 'free probe'.

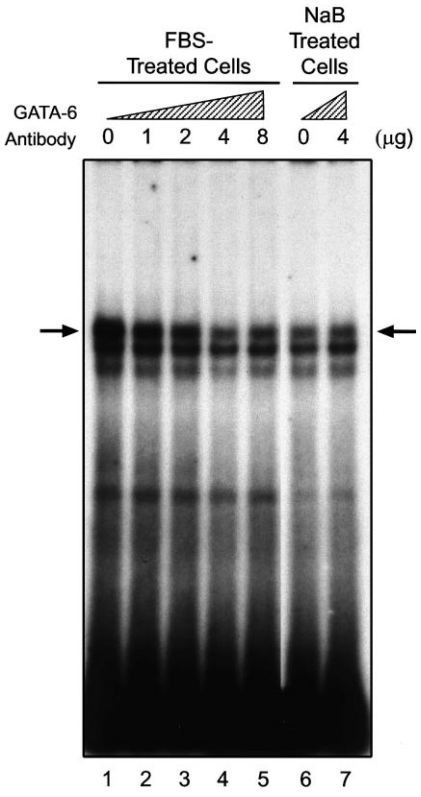

Fig. 3. GATA-6 bound to the GATA site in the 15-LO-1 promoter in FBS-treated cells, but not in NaBT-treated cells. A supershift study was carried out using GATA-6 antibody in FBS alone-treated cells and cells treated with 5 mM NaBT for 20 h. Labeled WT probe containing the GATA site was used for the probe template. Nuclear extracts (5 μg) and labeled probes were incubated with the varying concentrations of GATA-6 antibody; 0, 1, 2, 4, and 8 μg in FBS-treated cells, and 0 and 4 μg in NaBT-treated cells. The GATA-probe complex bands are indicated by an arrow.

3.4. *The GATA site of the 15-LO-1 promoter region is responsible for the basic transcription of 15-LO-1 in Caco-2 cells*

According to the above data, certain proteins, including GATA-6, bind to the GATA site in the 15-LO-1 promoter. The next question is whether the GATA site is critical for the transcription of 15-LO-1. To address that question, a reporter assay was performed using the promoter/reporter plasmid of 15-LO-1 gene shown in Fig. 4A. Since a single base mutation in a sequence of 'GATA' has a weak binding ability to GATA-6 protein [36], mutations of 'GATA' to 'TTGA' were constructed, and designated Mu-628/-23pGL2 as the mutant promoter/reporter plasmid. The mutations were confirmed by nucleotide sequence analysis (data not shown). Relative luciferase activity (RLA) was measured in the cells after treatment with or without NaBT as shown in Fig. 4B. In FBS alone condition, RLA of Mu-628/-23pGL2 was decreased to 10% of that observed with -628/-23pGL2 (0.50 ± 0.0021

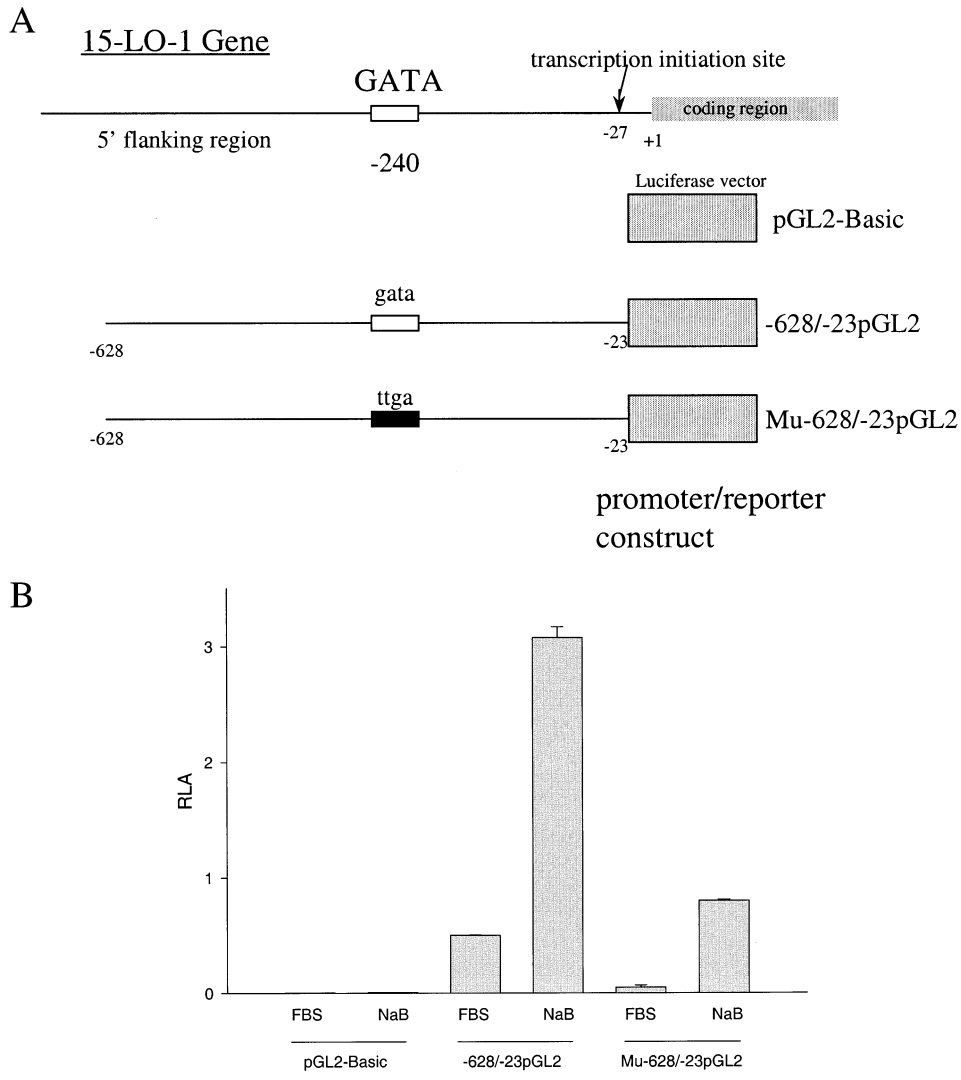

Fig. 4. Mutations of the GATA site in the promoter/reporter construct of 15-LO-1 attenuates luciferase activity. A: Promoter/reporter construct of 15-LO-1 gene. The fragment of 5′ flanking region from −628 to −23 of 15-LO-1 promoter was constructed in pGL2 basic luciferase reporter plasmid as -628/-23pGL2, which contains the GATA binding site at −240 position. The GATA binding site of -628/-23pGL2 was mutated by site-directed mutagenesis and the mutant promoter/reporter plasmid is designated as Mu-628/-23pGL2. B: Luciferase activity of 15-LO-1 promoter in Caco-2 cells by treatment with or without 5 mM NaBT. Caco-2 cells were transiently transfected with -628/-23pGL2 or Mu-628/-23pGL2 and pRL-null and then incubated for 48 h. Luciferase activity was measured and expressed as the RLA (relative luciferase activity): LA_F (firefly luciferase signal)/LA_R (renilla luciferase signal).

(mean ± S.E.M.) to 0.048 ± 0.019). With NaBT treatment, RLA was observed with Mu-628/-23pGL2 (0.80 ± 0.095) only 26% of that observed with -628/-23pGL2 (3.08 ± 0.092). This observation indicated that the GATA site is important for the stimulation of 15-LO-1 promoter activity in both undifferentiated and NaBT-induced differentiated Caco-2 cells.

4. Discussion

The expression of 15-LO-1 is not constitutive in all tissues, and appears to occur during the process of differentiation [18,20,37–39]. We hypothesized that 15-LO-1 is regulated in colorectal cells by certain differentiation-related factors, and focused on the importance of GATA-4/5/6 expression as the transcription factors since these proteins appear to be associated with intestinal epithelial cell differentiation [11–16]. We confirmed the presence of a GATA binding site in the 15-LO-1 promoter of Caco-2 cells. By Northern analysis, the expression of GATA-4 and 6 was observed, but only GATA-6 expression was detectable by Western analysis in undifferentiated cells (Fig. 1A and B). Interestingly, the expression of GATA-6 was down-regulated in NaBT-differentiated cells, measured by both Northern and Western analyses. In contrast, expression of 15-LO-1 was up-regulated after treatment with NaBT. In addition to the study of expression, EMSA experiments indicated that the nuclear proteins extracted from Caco-2 cells bind to the GATA binding site present in the 15-LO-1 promoter region (Fig. 2). The anti-GATA-6 antibody we used competitively or allosterically disturbed the binding of GATA-6 protein to the GATA site in undifferentiated cells (Fig. 3). Therefore, GATA-6 is highly expressed and bound to the GATA site in undifferentiated cells (FBS alone), while GATA-6 protein is not detected in differentiated cells (NaBT). These results suggest that down-regulation of GATA-6 expression is likely to be linked to the regulation

of 15-LO-1 expression, possibly as a negative regulator (repressor) for 15-LO-1 gene.

On the other hand, the reporter assay using constructs with mutations of its GATA binding site at −240 in the 15-LO-1 promoter region (Fig. 4) supports the conclusion that the GATA site is responsible for the stimulation of 15-LO-1 promoter activity in both undifferentiated and NaBT-induced differentiated cells.

Based on these data, the following two hypotheses are proposed: (1) GATA-6 represses the expression of 15-LO-1 by binding the GATA site of the 15-LO-1 promoter. (2) There are uncharacterized proteins of the GATA family, or related proteins, which bind to the GATA site and activate the 15-LO-1 expression both in undifferentiated and NaBT-differentiated Caco-2 cells. Mano et al. reported that a down-regulation of GATA-6 is an essential feature for the phenotypic modulation of vascular smooth muscle cells [40]. GATA-6 appears to function primarily within the proliferating progenitor population in the process of intestinal cell differentiation in HT-29 cells [11]. In addition, a reduced GATA-6 level is reported in the progression of the cardiomyogenic differentiation program [41]. GATA proteins also repress PU.1 transactivation of critical myeloid target genes [42]. These reported findings suggest GATA transcription factors act as repressors for target genes. In our study, a disruption model (knockout model) of GATA-6 would be more informative to clarify the precise function of GATA-6 for the regulation of 15-LO-1 expression.

In contrast, not only GATA-6, but also GATA-4, 5 are implicated in adrenocortical tumorigenesis [43], in the myocardial gene expression [44], and in the regulation of cardiac morphogenesis [45]. We were not able to delete the GATA binding shift band using another antibody against GATA-1, 2, 3, 4, or 5 in the NaBT-treated condition (data not shown). However, we can not exclude the possibility that the antibodies used in our study did not recognize DNA-bound proteins very well. The second explanation for our results is that novel GATA site binding proteins such as FOG (friend of GATA)-1 [46] and FOG-2 [47] are bound to the GATA binding site of the 15-LO-1 promoter instead of the conventional GATA-1–6. Thus, transcriptional regulation of 15-LO-1 by the GATA family is still complex and not well understood. In future studies, post-translational modifications, such as phosphorylation [48] or acetylation [49,50], of GATA family must be considered in order to clarify the regulation of 15-LO-1 in intestinal derived cells.

Acknowledgements: We thank Mark Geller for his technical assistance in this study.

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