Sp1 elements in *SULT2B1b* promoter and 5′-untranslated region of mRNA: Sp1/Sp2 induction and augmentation by histone deacetylase inhibition

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Abstract The steroid/sterol sulfotransferase gene (*SULT2B1*) encodes for two isozymes of which one (SULT2B1b) sulfonates cholesterol and is selectively expressed in skin. The human *SULT2B1* gene contains neither a TATAAA nor a CCAAT motif upstream of the coding region for SULT2B1b; however, this area is GC-rich. Of five Sp1 elements identified two had regulatory activity utilizing immortalized human keratinocytes: one element is located above the ostensible transcription initiation site, whereas the other is located within the 5′-untranslated region of the SULT2B1b mRNA. Sp1 and Sp2 transcription factors identified by supershift analyses induced reporter gene activity, an effect markedly augmented by histone deacetylase inhibition.

Keywords: Sulfonation; SULT2B1 promoter; Cis-element; Trans-factor; Chromatin structure

1. Introduction

Enzymes that catalyze the sulfoconjugation of hormones and neurotransmitters comprise a superfamily of cytosolic sulfotransferases (SULT), of which the SULT2 family, consisting of two subfamilies (SULT2A1 and SULT2B1), is engaged in the sulfoconjugation of steroids/sterols [19]. The gene for human *SULT2B1*, as a result of an alternative exon 1 and differential splicing, encodes for two mRNAs, i.e., SULT2B1a and SULT2B1b [10]. The use of exon 1A produces SULT2B1a, whereas to produce SULT2B1b exon 1B plus a portion of exon 1A is required. SULT2B1b efficiently sulfonates cholesterol, while SULT2B1a avidly sulfonates pregnenolone [6]. Cholesterol sulfate, quantitatively the most significant sterol sulfate in human plasma, has emerged as a multifaceted molecule with noteworthy physiologic actions [26]. In the realm of keratinocyte development and barrier formation, cholesterol sulfate will activate isozymes of protein kinase C [5], inhibit cholesterol synthesis [29], induce the gene for transglutaminase I, an essential cross-linking enzyme involved in barrier formation [14], and regulate the gene for involucrin, a major cross-linked protein constituent of the insoluble cornified cell envelope [9]. Interestingly, cholesterol sulfate is a ligand for the retinoic acid-related orphan nuclear receptor α (RORα), whereby it significantly increases transcriptional activity [13]; furthermore, RORα is highly expressed in skin [25]. We previously reported on the selective expression of the SULT2B1b isoform in human skin as well as primary cultures of normal human epidermal keratinocytes undergoing calcium-induced differentiation [11], and in this communiqué present initial studies regarding transcriptional control of expression of the *SULT2B1* gene in human keratinocytes.

2. Materials and methods

2.1. Cell culture

Immortalized but highly differentiated human keratinocytes (HaCaT cells) {originally developed by Boukamp et al. [2] and a generous gift of Dr. Shyh-Ing Jang at the NIAMS, NIH, Bethesda, MD} were grown in Dulbecco's modified essential medium (DMEM) supplemented with antibiotics and 10% fetal bovine serum unless otherwise specified.

2.2. Isolation of human SULT2B1b 5′-flanking region and preparation of reporter gene constructs

DNA sequence of human *SULT2B1* gene obtained from the human genome project (GenBank Accession No. AC008403) was used to produce the 5′-flanking region of the SULT2B1b isoform by employing the Advantage Genomic PCR kit (BD Biosciences Clontech), human genomic DNA (BD Biosciences Clontech) as template and the appropriate sense and antisense primers flanked by *Kpn*1 and *Xho*1, respectively. PCR was performed as follows: denature at 95 °C for 30 s followed by 40 cycles of denaturation at 95 °C for 15 s then annealing/elongation at 68 °C for 3 min. The −3389 to −27 PCR product was gel purified (Qiagen) and sub-cloned into the pCR2.1 TA cloning vector (Invitrogen) prior to ligation with the pGL3 luciferase reporter gene expression vector (Promega) at *Kpn*1 and *Xho*1 restriction sites to produce the pGL3(−3389/−27) construct. Nota bene: the nucleotide (nt) numbering system employed throughout this manuscript complies with recommended practice [4]; thus, the nt immediately upstream of the translation initiation codon (ATG) has been denoted as −1.

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Abbreviations: SULT, Sulfotransferase; nt, nucleotide; TSS, transcription start site; UTR, untranslated region; RT, reverse transcription; EMSA, electrophoresis mobility shift assay; HDAC, histone deacetylase; TSA, trichostatin A

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Various segments of SULT2B1b 5′-flanking region, i.e., −3189/−27, −951/−27, −401/−27, −331/−27, −212/−27, −179/−27, −148/−27 and −125/−27 were obtained by performing nested-PCR using the pGL3(−3389/−27) construct as template, appropriate sense primers and the common antisense primer (−55/−27) designed to be located well downstream of the 5′-end of the 5′-untranslated region (UTR) of SULT2B1b mRNA. Nested PCR was performed with pfu-Ultra DNA polymerase (Stratagene) as follows: denature at 95 °C for 1 min followed by 30 cycles of denaturation at 95 °C for 30 s, annealing at 62 °C for 30 s, and elongation at 72 °C for appropriate durations (1 min/kb of the expected length of each PCR product). PCR products were subcloned into the firefly luciferase expression vector at *Kpn*1 and *Xho*1 restriction sites. The additional constructs pGL3(−2940/−27), pGL3(−2286/−27), pGL3(−1740/−27) and pGL3(−548/−27) were generated by double digestions of the pGL3(−3389/−27) construct at, respectively, *Kpn*1/*Spe*1, *Kpn*1/*Sma*1, *Kpn*1/*Aat*II, and *Kpn*1/*Mlu*1 restriction sites followed by blunting and ligation.

2.3. Reverse transcription (RT) PCR analysis

Total RNA was extracted from HaCaT cells using Absolutely RNA RT-PCR Miniprep kit (Stratagene). RT was performed using the ThermoScript RT-PCR system (Invitrogen). Briefly, the first cDNA strand was synthesized at 50 °C for 60 min using 1 μg of total RNA as template and random hexamer primers. PCR was performed using 2 μl cDNA reaction mix as template and gene-specific primer pairs using platinum *Taq* DNA polymerase (Invitrogen) under the conditions of denaturing at 94 °C for 2 min, followed by 30 cycles of denaturizing at 94 °C for 30 s, annealing at 58 °C for 30 s and extension at 72 °C for 30 s.

2.4. Transient transfection and reporter gene assay

HaCaT cells were seeded in 6-well plates at 2.5×10^5 cells/well 1 day prior to transfections that were performed with a selective pGL3/SULT2B1b upstream construct with or without *Renilla* luciferase plasmid (Promega) as indicated in figure legends. Transfections were carried out using a 3-fold excess of SuperFect (Qiagen). Cells were harvested 48 h after transfection and both firefly luciferase and *Renilla* luciferase activities were measured (Promega). All experiments were carried out in triplicate and repeated twice. In some experiments, 0.3 μM trichostatin A (TSA; Sigma) was added to the incubation medium 12 h prior to harvesting.

2.5. Site-directed mutagenesis

Transcription factor binding elements were altered using Quick Change Site-Directed Mutagenesis (Stratagene). Briefly, PCR was performed using 50 ng of the pGL3(−548/−27) construct and 10 pmol of sense and antisense primers (Table 1) under the conditions of denaturation at 95 °C for 30 s, followed by 18 cycles of denaturation at 95 °C for 30 s, annealing at a temperature determined by a formula supplied with instructions, and extension at 68 °C for 10 min. Reaction mixtures were treated with Dpn1. PCR products were amplified with XL10-Gold competent cells (Stratagene) and sequenced.

2.6. Electrophoresis mobility shift assay (EMSA)

EMSA was carried out as follows: double stranded oligonucleotides (Table 1) were end-labeled with ^{32}P using [γ-^{32}P] ATP (3000 Ci/mmol, Perkin–Elmer Life Sciences) and T4 polynucleotide kinase [22] and column-purified with a NucTrap probe purification column (Stratagene). EMSA was performed using labeled oligonucleotides ($\sim 130 \times 10^3$ cpm), HaCaT cell nuclear extract ($\sim$5 μg) from Active Motive and a commercially available kit (Promega). Samples were subjected to electrophoresis using 6% (w/v) polyacrylamide gels in 0.5× Tris/borate/EDTA buffer (0.178 M Tris/borate and 4 mM EDTA) at 4 °C for 2 h. For supershift analyses, nuclear extracts were preincubated with $\sim$2 μg of antibody to human Sp1, Sp2, Sp3 or Sp4 (Santa Cruz Biotechnology) for 2 h at 4 °C prior to incubation with the probes and then subjected to electrophoresis using 3.5% polyacrylamide gels. Following electrophoresis, gels were dried and analyzed by autoradiography.

2.7. DNA sequence analysis

DNA sequence was analyzed for potential transcription factor binding using the TESS (Transcription Element Search System) web tool (http://www.cbil.upenn.edu/cgi-bin/tess/tess?RQ=WELCOME).

Table 1
Primers used for constructing, substitution mutants, RT-PCR and oligonucleotides for EMSA

Name	Sequence	Position
Primers for site-directed mutagenesis		
Sp1.2/M2(S)	5′-CACTGCTCCTCtttGCCCTCAGAGCAGGGTGGCTCC-3′	(−2327/−197)
Sp1.2/M2(A)	5′-GGAGCCACCCTGCTCTGAGGGCaaaGAGGAGCAGTG-3′	(−232/−197)
Sp1.5/M5(S)	5′-GAGAACCGGCTGGGTGCTGaaaCTCCCCTTGGGC-3′	(−154/−121)
Sp1.5/M5(A)	5′-GCCCAAGGGGAGtttCAGCACCCAGCCGGTTCTC-3′	(−154/−121)
Primers for RT-PCR		
RTP1(S)	5′-CTGCCCCTCCCCTTGGGCCGGGCACGGAGTAG-3′	(−138/−107)
RTP2(S)	5′-CTGGGTGCTGCCCCTCCCCTTGGGCCGGGCAC-3′	(−145/−114)
RTP3(S)	5′-AGAACCGGCTGGGTGCTGCCCCTCCCCTTGG-3′	(−153/−124)
RTP(S)	5′-AGCTGGGAGAACCGGCTGGGTGCTGCCCCTC-3′	(−160/−130)
RTP5(S)	5′-TAGCAGCTGGGAGAACCGGCTGGGTGCTG-3′	(−164/−136)
RTP6(S)	5′-TTGGAGGCGTGGATAGCAG-3′	(−177/−159)
RTP7(S)	5′-TGTTGGAGGCGTGGATAGCAGCTGGGAG-3′	(−179/−152)
RTP8(S)	5′-TGTTGGAGGCGTGGATAGC-3′	(−179/−161)
RTP9(S)	5′-GCCTCTCCCCGCTGTTGG-3′	(−191/−174)
RTP10(S)	5′-AGAGCAGGGTGGCTCCCTCTGGCCTCTC-3′	(−212/−185)
RTP11(A)	5′-AGATGATCTCGATCATCCAGGTCGTGCCTG-3′	(+212/+242)
Oligonucleotides for EMSA		
WT/Sp1.2	5′-ACTGCTCCTCCCCGCCCTCA-3′ 3′-IGACGAGGAGGGGCGGGAGT-5′	(−231/−212)
M2/Sp1.2	5′-ACTGCTCCTtttCGCCCTCA-3′ 3′-TGACGAGGAaaaGCGGGAGT-5′	(−231/−212)
WT/Sp1.5	5′-CGGCTGGGTGCTGCCCCTCCCCTTGGGCCG-3′ 3′-GCCGACCCACGACGGGGAGGGGAACCCGGC-5′	(−148/−119)
M5/Sp1.5	5′-CGGCTGGGTGCTGaaaCTCCCCTTGGGCCG-3′ 3′-GCCGACCCACGACtttGAGGGGAACCCGGC-5′	(−148/−119)

Underlined and lower case letters indicate mutated bases. S, sense; A, antisense; WT, wild type.