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Expression of *S100A8* is induced by interleukin-1α in TR146 epithelial cells through a mechanism involving CCAAT/enhancer binding protein β

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Abstract. Calprotectin in mucosal epidermal keratinocytes has an important role in fighting microbial infections. S100A8 belongs to the S100 protein family and is a subunit of calprotectin (heterodimer complex of S100A8/A9). Interleukin-1α (IL-1α) is one of the cytokines produced by oral keratinocytes. The primary aims of the present study were to investigate the effect of IL-1α on the expression of *S100A8* and its underlying molecular mechanism in oral epithelial cells. Determining the molecular mechanism of the induced expression of *S100A8* by IL-1α aims to improve current understanding of the roles of calprotectin during the infection of mucosal epithelial cells. The expression analysis indicated that IL-1α significantly induced the expression of *S100A8* in human TR146 epithelial cells at the mRNA and protein levels, respectively. The reporter assay demonstrated that the upregulatory effect of *S100A8* induced by IL-1α was dependent on the *S100A8* promoter specific region (-165/-111). The results of electrophoresis mobility shift assay and chromatin immunoprecipitation assay also demonstrated that the CCAAT/enhancer binding protein β (C/EBPβ) binding site (-113/-109) in the *S100A8* promoter region was involved into the upregulatory effect on the expression of *S100A8* induced by IL-1α. Taken together, these results suggested that the activation of the expression of *S100A8* induced by IL-1α in TR146 epithelial cells involves a mechanism by which the binding activity of C/EBPβ to the specific site (-113/-109) of the *S100A8* promoter is increased.

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Introduction

Mucosal epidermal keratinocytes release proinflammatory cytokines during the fast innate immune response to microbial infection (1-3). Interleukin-1α (IL-1α) is one of the cytokines produced by oral keratinocytes (3-8). The binding of IL-1α to its cell surface IL-1 receptor induces the activation of nuclear factor-κB, c-Jun N-terminal kinase and p38-mitogen activated protein kinase (MAPK) target gene transcription (9,10). Functionally, pro-IL-1α has a nuclear localization signal at its N-terminus and binds HS-1-associated protein X-1 to allow for translocation into the nucleus; it then binds transcription activators to directly affect target gene transcription (9,10). In response to IL-1α, the expression levels of antimicrobial proteins/peptides, including calprotectin (a heterodimer complex of S100A8/A9), defensin and adrenomedullin, are significantly upregulated in epidermal keratinocytes (1-3,5-7). Calprotectin has a variety of antimicrobial activities in keratinocytes and is important in mucosal innate immunity (1-8). In addition, tumor-suppressive roles of calprotectin in head and neck squamous cell carcinoma (HNSCC) have been reported (11,12). Mechanistically, calprotectin negatively regulates G_2/M cell cycle progression and growth in a protein phosphatase 2α-dependent manner in HNSCC (11,12).

The human *S100A8* promoter has been well characterized (13). Transcription factors that can regulate the expression of human *S100A8* include CAAT enhancer binding protein α (C/EBPα) (14), C/EBPβ (15) and hypoxia inducible factor-1 (16). However, several copies of Ets transcription factor, E-Box and C/EBPβ consensus sequences have been observed in the murine *S100A8* promoter region (17).

Although IL-1α has been reported to increase the expression of *S100A8* in the HaCaT human keratinocyte cell line (5,6), the mechanism underlying the effect of IL-1α on human *S100A8* in keratinocytes remains to be fully elucidated. Determining the molecular mechanism underlying the expression of *S100A8* induced by IL-1α may provide a better understanding of the roles of calprotectin during the infection of mucosal epithelial cells. Therefore, to the best of our knowledge, the results of the present study are the first to provide a conceivable mechanism underlying the effects of human *S100A8* induced by IL-1α in epidermal keratinocytes.

Materials and methods

Cell culture and IL-1α treatment. The human TR146 epithelial cancer cells (ATCC, Manassas, VA, USA) were maintained in Dulbecco's modified Eagle's medium (Thermo Fisher Scientific, Inc., Waltham, MA, USA) supplemented with 10% FBS (Gemini Bio-Products, Sacramento, CA, USA). The TR146 cells were incubated at 37˚C in an incubator with 5% CO_2. Recombinant IL-1α (Sino Biological, Inc., Beijing, China) was dissolved and cell treatment was then performed as described previously (1). Briefly, cells were seeded 10^5 per well into 24-well plates. Following overnight incubation at 37˚C, cells were washed with Dulbecco's phosphate-buffered saline (DPBS), different concentrations of IL-1α were added or bovine serum albumin (BSA; vehicle, 50 μg/ml BSA in DPBS).

Reverse transcription-quantitative polymerase chain reaction analysis (RT-qPCR) analysis. Total RNA was extracted using TRIzol reagent (Thermo Fisher Scientific, Inc.). Reverse transcription was performed with a FastQuant RT kit and gDNase (Tiangen Biotech Co., Ltd., Beijing, P. R. China). RT-qPCR with SYBR Green I was then conducted. Primers for the qPCR amplification of human *S100A8* [primer pair S100A8-1 forward (F)/S100A8-1reverse (R)] (1), and β-actin (*ACTB*; primer pair ACTB-1F/ACTB-1R) (18) were used (Table I). Each reaction was performed in a 20 μl volume containing 1XSYBR qPCR MasterMix (Fermentas; Thermo Scientific, Inc.), 50 nM of each primer and 1 μl cDNA. The cycling conditions were: 2 min at 95˚C, followed by 40 cycles at 95˚C for 5 sec and 60˚C for 30 sec. The mRNA expression levels of human *S100A8* were standardized to the mRNA expression of β-actin. The RT-qPCR results were quantified using the $2^{-\Delta\Delta Cq}$ method (19).

Plasmid construction. A PCR fragment (primer pair PA8-1/PA8-2) was amplified from human blood genomic DNA (Promega Corporation, Madison, WI, USA) using Herculase® II Fusion DNA Polymerase (Agilent Technologies, Inc., Santa Clara, CA, USA), which was then ligated into pGL3-basic (Promega Corporation) via the *Nhe*I and *Bgl*II restriction sites, to generate the pGL3(-3096/+246) construct. The cycling conditions were as follows: 2 min at 94˚C, followed by 36 cycles at 94˚C for 20 sec, 63˚C for 30 sec and 72˚C for 90 sec, followed by a final extension at 72˚C for 3 min. A 7,906-bp fragment (PA8-3/pGL3-S) was amplified from pGL3 (-3096/+246) and this fragment was then self-ligated to generate the pGL3(-3096/-1) construct. To generate *S100A8* promoter 5'deletion mutants, fragments of 6,697 bp (DPA8-1/pGL3-AS), 5,739 bp (DPA8-2/pGL3-AS), 5,488 bp (DPA8-3/pGL3-AS), 5,229 bp (DPA8-4/pGL3-AS), 5,053 bp (DPA8-5/pGL3-AS) and 4,882 bp (DPA8-6/pGL3-AS) were amplified from pGL3 (-3096/-1), respectively (Table I). The products were purified and self-ligated to generate pGL3(-1887/-1), pGL3(-929/-1), pGL3(-678/-1), pGL3(-419/-1), pGL3(-243/-1) and pGL3 (-72/-1), respectively. Similarly, fragments of 5,067 bp (DDA8-1/pGL3-AS), 5,042 bp (DDA8-2/pGL3-AS), 5,003 bp (DDA8-3/pGL3-AS), 4,975 bp (DDA8-4/pGL3-AS) and 4,921 bp (DDA8-5/pGL3-AS) were amplified from the pGL3 (-419/-1) construct (Table I), and these the fragments were purified and self-ligated to generate pGL3(-257/-1), pGL3(-232/-1), pGL3(-193/-1), pGL3(-165/-1) and pGL3(-111/-1), respectively. The cycling conditions for amplification of self-ligated fragments were: 2 min at 94˚C, followed by 36 cycles at 94˚C for 20 sec, 63˚C for 30 sec and 72˚C for 4 min, followed by a final extension at 72˚C for 5 min. Primer pairs (M3-1/M3-2) and pGL3(-257/-1) were used for amplification to generate the pGL3(-257/-1)-M3 constructs using the QuikChange XL site-directed mutagenesis kit (Stratagene; Agilent Technologies, Inc.; Table I). All plasmid constructs were confirmed by automated sequencing (Sangon Biotech Co., Ltd., Shanghai, China).

RNA interference. The C/EBPβ small interfering (si)-RNA sequence (5'-UUGGCCACUUCCAUGGGUCUAAAGG-3'), as described previously (20), was synthesized by Sangon Biotech Co., Ltd. C/EBPβ was silenced by transfecting the cells with 25 nM C/EBPβ siRNA using HiperFect transfection reagent (Qiagen, Inc., Valencia, CA, USA), following which the cells were collected for protein expression analysis. Non-specific siRNA (Sangon Biotech Co., Ltd.) was used as the negative control.

Transfection and dual luciferase assay. The TR146 cells were grown to 60-80% confluency, following which the cells were transfected with a firefly luciferase construct and a *Renilla* luciferase construct, pRL-TK (20:1 ratio), using lipofectamine 3000™ (Thermo Fisher Scientific, Inc.). The luciferase activities were measured after 40 h (Promega Corporation). The luciferase activity was normalized to *Renilla* luciferase activity.

Electrophoretic mobility shift assays (EMSA). The EMSA was conducted as previously described (21). Briefly, 5'-biotin-labeled single-strand probes were synthesized by Sangon Biotech Co., Ltd. Double-stranded oligonucleotide probes were prepared by diluting equimolar quantities of complementary oligonucleotides in 1X STE buffer (100 mM NaCl, 50 mM Tris-HCl and 1 mM EDTA, pH 8.0), incubated at 95˚C for 3 min, and then slowly cooled to room temperature. Nuclear extracts from the TR146 cells were extracted using a nuclear extraction kit (Biyuntian, Shanghai, China). The EMSA reaction mixtures were incubated on ice for 30 min with or without unlabeled competitor, prior to adding end-labeled oligonucleotides for 20 min on ice. For the competitive assays, a 200-fold molar excess of cold oligonucleotides was added to the binding reaction prior to the addition of the hot-labeled oligonucleotides. The binding reactions were analyzed by transferring the reactants to positively charged nylon membranes (cat. no. 11209299001, Roche Diagnostics, Indianapolis, IN, USA). For the supershift assay, the nuclear extracts containing 5 μg protein were incubated with 500 ng C/EBPα (D-5, cat. no. sc-365318; Santa Cruz Biotechnology, Inc., Dallas, TX, USA) or C/EBPβ antibodies (cat. no. 23431-1-AP; Wuhan Sanying Biotechnology, Wuhan, China) on ice for 30 min prior to adding end-labeled oligonucleotides for 20 min on ice. The samples were electrophoresed on a 5% non-denaturing polyacrylamide gel in 0.5X Tris-borate-EDTA buffer. Detection was conducted using Lightshift electrophoretic mobility shift reagent (Pierce; Thermo Fisher Scientific, Inc.).

Table I. Oligonucleotides used in the present study.

Primer name	Oligonucleotide sequence (5'-3')[a]
RT-qPCR analysis	
S100A8-1F	GGGCATCATGTTGACCGAGC
S100A8-1R	GTAACTCAGCTACTCTTTGTGGCTT
ACTB-1F	GACGACATGGAGAAAATCTG
ACTB-1R	ATGATCTGGGTCATCTTCTC
Plasmid construction	
PA8-1	CTAGCTAGCAGGGACTGAGCCCTTTCCTGTAAACATG
PA8-2	GAAGATCTGTCCAGCCTAGGAGACAATGTGCC
PA8-3	GCAGGGCTGAGAGGCAGCTCC
pGL3-S	AGATCTGCGATCTAAGTAAGCTTGGCATTC
DPA8-1	CCCGGACATGGGAAAAGCTCAG
DPA8-2	GGTGGGGAGAGGATTTGTTCCTCC
DPA8-3	CTCCATCTCCCAGGGCATGGTC
DPA8-4	TGCGGTCTTTGGACCCTTTGAAAC
DPA8-5	AAGCAAGTGGATGCCAGCAGC
DPA8-6	TCTGATGGCCTGAAGCTGTGGG
pGL3-AS	GGCTAGCACGCGTAAGAGCTCGGTAC
DDA8-1	CCAGCAGCCCAGAAAAAGAGCC
DDA8-2	CTACCTGCTTTTTCCTTCTGGGCAC
DDA8-3	TGCCTTCCTCTTTCCGCTTCTCC
DDA8-4	TCCCCACCCAAAATTTTCATTCTGC
DDA8-5	CAACTCTGGCAGGGAGAAGCTGTC
EMSA assays	
EP1s	TCCCCACCCAAAATTTTCATTCTGC
EP1as	GCAGAATGAAAATTTTGGGTGGGGA
EP2s	CTGCACAGTGATTGCCACATTCACC
EP2as	GGTGAATGTGGCAATCACTGTGCAG
EP3s	CATTCACCTGGTTGAGAAACCAGAGAC
EP3as	GTCTCTGGTTTCTCAACCAGGTGAATG
ChIP assay	
Ch-1	TGCCTTCCTCTTTCCGCTTC
Ch-2	CAGCTGCCCACAGCTTCAG
Ch-3	GTACATGATGTGGGAAGGAG
Ch-4	ACCTAGTGATGTGGACATTAC
Mutagenesis of C/EBPβ binding sites[a]	
M3-1	GCCACATTCACCTGGTTGA**GCCTT**CAGAGACTGTAGCAACTC
M3-2	GAGTTGCTACAGTCTCTG**AAGGCT**CAACCAGGTGAATGTGGC

[a]Mutated nucleotides are shown in bold. RT-qPCR, reverse transcription-quantitative polymerase chain reaction; F, forward; R, reverse; ACTB, β-actin; EMSA, electrophoretic mobility shift assays; ChIP, chromatin immunoprecipitation; C/EBPβ, CCAAT/enhancer binding protein β.

Chromatin immunoprecipitation (ChIP) assays. ChIP assays were performed using the Magna ChIP™ A/G kit (EMD Millipore, Billerica, MA, USA) according to the manufacturer's protocol. Antibodies against C/EBPβ (cat. no. 23431-1-AP; Wuhan Sanying Biotechnology) were combined with protein A/G magnetic beads and were then incubated for 4 h at 4˚C and rotated. Normal mouse IgG (Santa Cruz Biotechnology, Inc.) was used as the negative control. Elution of the protein/DNA complexes and reverse cross-links of the protein/DNA complexes to free DNA were then performed. For amplification of the *S100A8* promoter (-193/-45), the Ch-1/Ch-2 primer pairs (Table I) were used. Serving as the control for the absence of C/EBPβ binding sites, the Ch-3/Ch-4 primer pairs (Table I) were used to amplify the upstream fragment of human *S100A8* (-2861/-2723). The thermocycling conditions were as follows: 2 min at 94˚C, followed by 32 cycles at 94˚C for 20 sec, 59˚C for 30 sec, and 72˚C for 30 sec, and then a final extension at 72˚C for 3 min. The PCR products were detected by 1.5% agarose gel electrophoresis.

Western blot analysis. The cells were washed with Dulbecco's phosphate-buffered saline (Gibco; Thermo Fisher Scientific, Inc.) and were then extracted using mammalian cell lysate buffer (Biyuntian). The cell extracts were centrifuged at 12,000 x g for 5 min at 4˚C and the supernatants were collected. The protein concentrations were determined using a bicinchoninic acid protein concentration detection kit (Biyuntian). The cell extracts (20 μg protein) were separated by 12% DS-PAGE, transferred onto 0.22-μM nitrocellulose membranes, and incubated overnight at 4˚C with rabbit anti-myeloid-related protein-8 (an alias of S100A8; cat. no. ab196689, Abcam, Cambridge, MA, USA) or mouse anti-β-actin (cat. no. TA-09, OriGene Technologies, Inc., Beijing, China) at 1:2,000 dilution. The membranes were washed and then incubated 2 h at room temperature with horseradish peroxidase-conjugated goat anti-rabbit antibodies or goat anti-mouse antibodies (cat. nos. EM35111-01 and EM35110-01, EMAR Biotechnology, Beijing, China) at 1:3,000 dilution. The immunoreactions were visualized using Clarity™ Western ECL substrate (Bio-Rad Laboratories, Inc., Hercules, CA, USA) and exposed to Amersham Hyperfilm ECL film (Amersham; GE Healthcare Bio-Sciences, Pittsburgh, PA, USA). The protein bands were evaluated using Quantity One software (version 4.6.5, Bio-Rad Laboratories, Inc.).

Bioinformatics analysis and statistical analysis. TRANSFAC 7 (http://gene-regulation.com/pub/databases.html) online prediction software was used to analyze transcription factor binding for *S100A8* promoter region. Between three and six independent experiments were conducted in the present study. Statistical analysis was performed with SPSS version 19.0 (IBM Corp., Armonk, NY, USA). Comparisons between two groups were performed with Student's t-test, and multiple comparisons were conducted with one-way analysis of variance followed by Bonferroni's post hoc test. P<0.05 was considered to indicate a statistically significant difference.

Results

IL-1α activates the expression of S100A8 in TR146 epithelial cells. The effect of IL-1α on the expression of *S100A8* in human TR146 epithelial cells was investigated by RT-qPCR and western blot analyses. The results revealed that treatment with various concentrations (5-100 ng/ml) of IL-1αsignificantly upregulated the expression levels of *S100A8* at the mRNA level (Fig. 1A). The maximal induction effects were observed with 10-50 ng/ml IL-1α. The results also indicated that the inductive effects on the expression of *S100A8* induced by various concentrations (5-100 ng/ml) of IL-1α were detected at the protein level (Fig. 1B). Taken together, these results suggested that IL-1α significantly induced the expression of *S100A8* in human TR146 epithelial cells through a mechanism associated with transcriptional regulation.

Promoter region of -165/-111 is responsible for the upregulation of S100A8 by IL-1α treatment. To localize the promoter region that is responsible for the upregulation of *S100A8* induced by IL-1α treatment, a series of promoter fragments of *S100A8* were cloned into a luciferase reporter gene vector, pGL3-basic, to generate several deletion mutants. Following

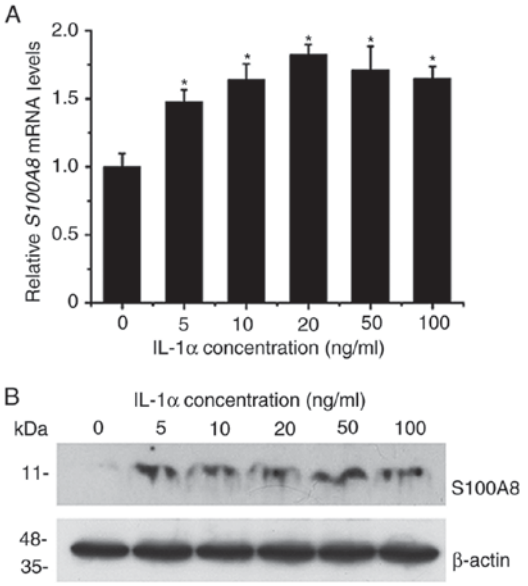

Figure 1. Induction of the expression of *S100A8* by IL-1α in TR146 epithelial cells. (A) RT-qPCR analysis of the mRNA expression of *S100A8*; β-actin was used as the internal control. (B) Western blot analysis was conducted to analyze the protein expression of S100A8. TR146 cells were treated with vehicle (50 μg/ml bovine serum albumin in Dulbecco's phosphate-buffered saline) or various concentrations of IL-1α in fresh medium and incubated for 24 h, following which the cells were collected for RT-qPCR or western blot analyses. Bars represent the mean ± standard error of the mean from three to six determinations. *P<0.05 vs. control. IL, interleukin; RT-qPCR, reverse transcription-quantitative polymerase chain reaction.

the transfection of these constructs into TR146 cells, the cells were treated with IL-1α, and were then collected for luciferase activity assays. The results revealed that IL-1α treatment significantly enhanced promoter activity following transfection with the pGL3 (-1887/-1), pGL3 (-929/-1), pGL3 (-678/-1), pGL3 (-419/-1) and pGL3 (-243/-1) constructs, but not with the pGL3 (-72/-1) construct (Fig. 2A). These results indicated that the promoter region potentially responsible for the induced gene expression of *S100A8* by IL-1α may be located in the-243/-72 promoter region. In order to further locate the associated promoter region for the induced gene expression of *S100A8* by IL-1α, a series of deletion mutants were also constructed. The luciferase assays showed that IL-1α treatment significantly induced promoter activity for the pGL3 (-257/-1), pGL3 (-232/-1), pGL3 (-193/-1) and pGL3 (-165/-1) reporter gene constructs, but not for the pGL3 (-111/-1) reporter gene construct (Fig. 2B). Taken together, these results suggested that the -165/-111 promoter region of *S100A8* may be responsible for the inductive effects of IL-1α.

IL-1α treatment induces the binding of C/EBPβ to a specific site in the promoter region. As IL-1α can affect gene transcription via the transcription factor C/EBPβ (22,23), the present study analyzed the -165/-111 promoter region of human *S100A8* using online prediction software for transcription factor binding, TRANSFAC 7 (http://gene-regulation.com/pub/databases.html) (24). The results revealed that there are three potential transcription factor C/EBPβ binding sites in this region. Subsequently, three pairs of EMSA primers (-166/-142 for E1; -145/-121 for E2 and -128/-102 for E3) were designed to detect whether IL-1α treatment affects the combination of

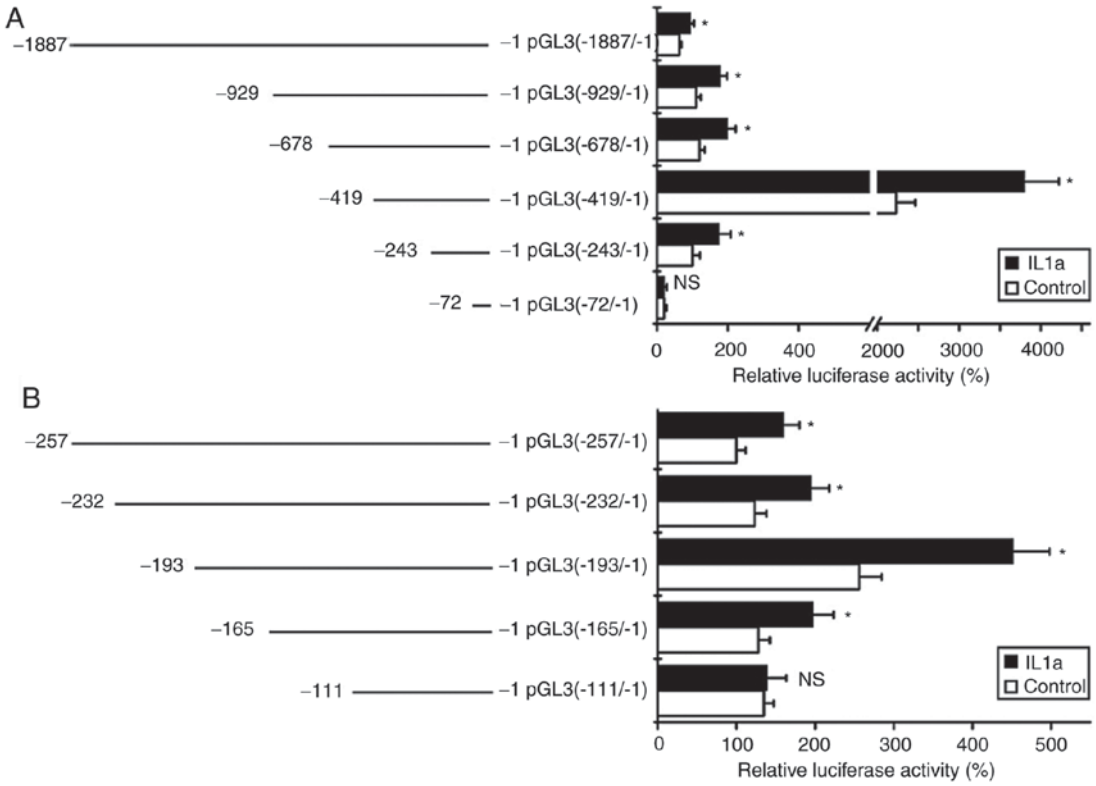

Figure 2. Localization of the promoter region responsible for the upregulation of *S100A8* induced by IL-1α in TR146 cells. (A) Deletion analysis for the promoter -1887/-1 region. (B) Deletion analysis for the promoter -257/-1 region. Constructs were transfected into TR146 cells for 10 h, followed by the addition of 20 ng/ml IL-1α. After 12 h, the cells were collected for the luciferase activity assay. Bars represent the mean ± standard error of the mean from three to six determinations. *P<0.05 vs. control. NS, not significant; IL, interleukin.

transcription factors to these primers (Fig. 3A). No significant differences were observed when comparing C/EBPβ binding and alterations in IL-1α treatment with E1 primers (Fig. 3B). In addition, the EMSA results revealed no significant difference in C/EBPβ binding following IL-1α treatment with the E2 primer (Fig. 3C). Notably, the results demonstrated that the binding activity between the C/EBPβ binding site (-113/-109) in E3 primer pairs and the transcription factor C/EBPβ were significantly enhanced following IL-1α treatment (Fig. 3D). In addition, the ChIP assay verified the binding activity between C/EBPβ and the-193/-45 promoter region *in vivo* (Fig. 3E). These results suggested that the C/EBPβ binding site (-113/-109) of the *S100A8* gene promoter may be associated with the upregulatory effect on the expression of *S100A8* induced by IL-1α.

C/EBPβ is critical in the process of S100A8 activation induced by IL-1α. Finally, to further elucidate the role of the transcription factor C/EBPβ binding site (-113/-109) in the *S100A8* gene promoter, the pGL3 (-257/-1) reporter gene vector was used as a template to generate the mutated C/EBPβ binding site construct pGL3 (-257/-1)-M3 (mutation at the -113/-109 site) (Fig. 4A). Following transfection with these constructs, the cells were collected subsequent to IL-1α treatment, and the effect of IL-1α treatment on *S100A8* promoter activity was analyzed. The results demonstrated that the inductive effect of IL-1α treatment was attenuated following pGL3(-257/-1)-M3 transfection, whereas the inductive effect remained following of pGL3(-257/-1) transfection (Fig. 4B). By contrast, silencing C/EBPβ significantly decreased *S100A8* promoter activity following pGL3(-419/-1) construct transfection (Fig. 4C and D). Taken together, these results suggested that activation of the expression of *S100A8* induced by IL-1α in TR146 epithelial cells may involve a mechanism associated with the increased binding activity of C/EBPβ to a specific site (-113/-109) of the *S100A8* promoter.

Discussion

Due to the important roles of human *S100A8* in infectious diseases and tumors (1-8,11,12), a number of studies have investigated the mechanism underlying the transcriptional regulation of human *S100A8* (15-17,25-27). The upregulation of *S100A8* by fibroblast growth factor-2 and IL-1β, and its downregulation by transforming growth factor-β in murine fibroblasts has been previously observed (25). Through activation of the protein kinase A signaling pathway and subsequent stimulation of C/EBPβ binding to the *S100A8* promoter, prostaglandin E2 has been reported to upregulate the expression of human *S100A8* (15). Mechanistically, the process of the induced expression of *S100A8* by glucocorticoids was positively regulated by protein kinase A and negatively regulated by protein kinase C (26). Glucocorticoids increase the transcription and mRNA half-life of human *S100A8*; the upregulation process requires new protein synthesis, IL-10, products of the cyclooxygenase-2 pathway, and both the extracellular signal-regulated kinase (ERK)-1/2 and p38 MAPK signaling pathways (26). Furthermore, the expression of human *S100A8* is induced by polyinosinic:polycytidylic acid, a

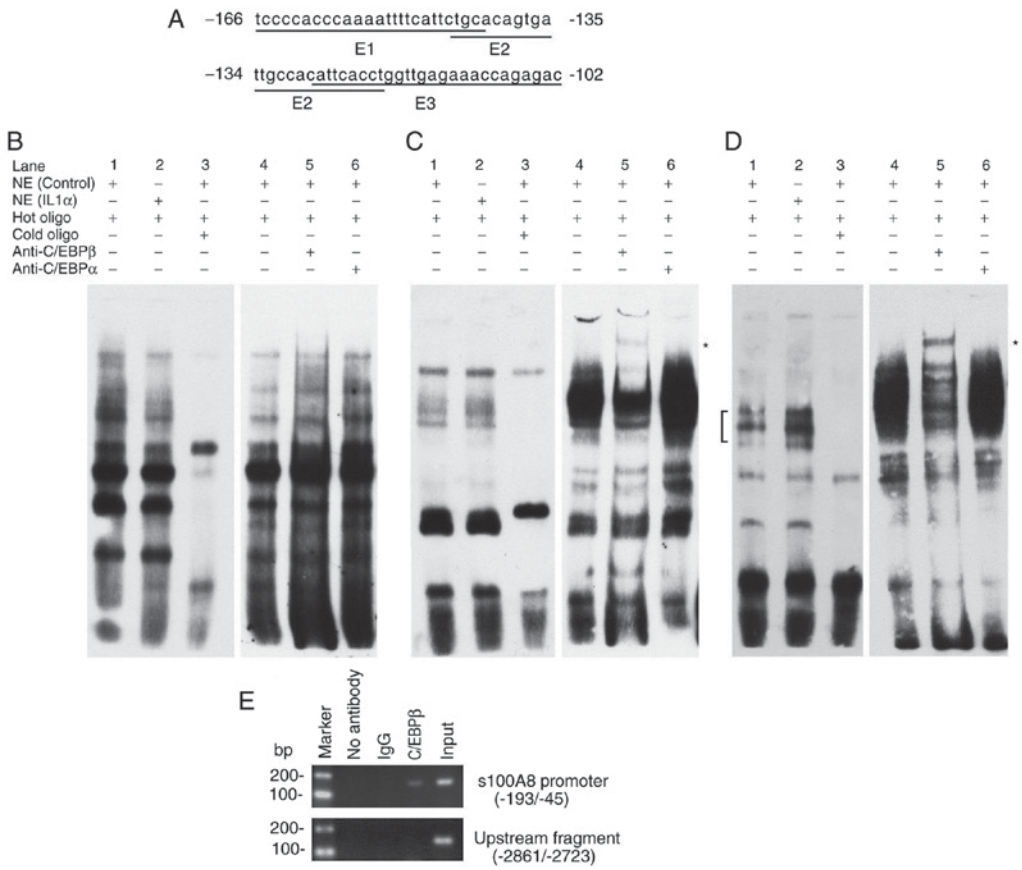

Figure 3. Induction of C/EBPβ binding to the specific site in the *S100A8* promoter region by IL-1α. (A) Oligonucleotide sequences used for EMSA analysis. Bold letters indicate the potential binding sites for transcription factor C/EBPβ. (B) Promoter region (-166/-142) sequence used for EMSA analysis. (C) Promoter region (-145/-121) sequence used for EMSA analysis. (D) Promoter region (-128/-102) sequence used for EMSA analysis. (E) C/EBPβ binding to the *S100A8* promoter *in vivo*. Chromatin immunoprecipitation assays were performed using DNA from TR146 cells and specific antibodies. Compared with the competitive assay (left-hand panels of B-D), a longer exposure time was performed for the supershift assay (right-hand panels of B-D). Lanes: 1, no IL-1α; 2, IL-1α; 3, 200-fold cold oligo; 4, no antibody (negative control); 5, C/EBPβ antibody; 6, C/EBPα antibody. Square brackets indicate the position of the DNA/protein complex involving C/EBPβ. *Positions of supershift bands. At least three experiments were performed. NE, nuclear extracts; Input, sheared DNA prior to immunoprecipitation was used for amplification; C/EBPβ, CCAAT/enhancer binding protein β; EMSA, electrophoretic mobility shift assays.

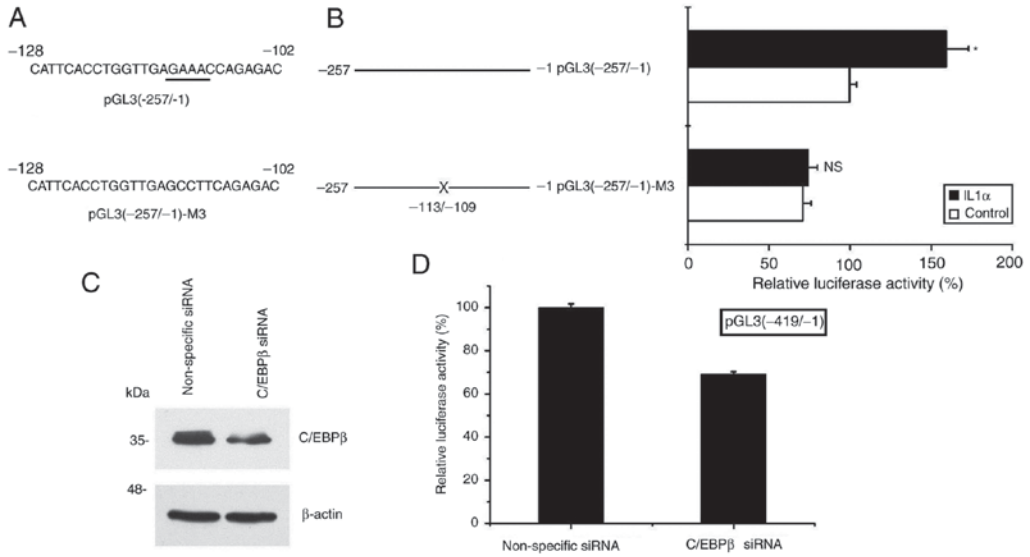

Figure 4. Role of C/EBPβ in the activation of *S100A8* expression induced by IL-1α. (A) Schematic illustration of the C/EBPβ binding site and its mutation in the *S100A8* promoter region, the C/EBPβ binding site (-113/-109) is underlined. The mutated oligos in the binding site are shown in bold. (B) Mutational analysis of the C/EBPβ binding site (-113/-109) in the *S100A8* promoter region and its effect on *S100A8* promoter activity. (C) siRNA silencing of C/EBPβ downregulated the expression of the protein. (D) siRNA silencing of C/EBPβ attenuated *S100A8* promoter activity. pGL3 (-419/-1) was used. Constructs were transfected into TR146 cells for 10 h, followed by the addition of 20 ng/ml IL-1α. After 12 h, the cells were collected for luciferase activity assays. Bars represent the mean ± standard error of the mean from three to six determinations. *$P<0.05$ vs. control. NS, not significant; C/EBPβ, CCAAT/enhancer binding protein β; IL-, interleukin; siRNA, small interfering RNA.

double strand RNA mimetic, and its induction is dependent on the p38, ERK MAPK and protein kinase R-dependent signaling pathways (27). Notably, the p38 MAPK signaling pathway is critical in the process of tumor necrosis factor-α- and IL-17A-induced expression of *S100A8* in human keratinocytes (28).

It has been demonstrated that IL-1α can affect gene transcription by increasing C/EBPβ-dependent transcriptional activity (22,23). A previous report also revealed that IL-1α promotes the expression of stromal-derived factor-1 in vascular smooth muscle cells by upregulating C/EBPβ in an inhibitor of NF-κB kinase β signaling-dependent manner (29). An IL-1α-induced increase in the binding of C/EBPβ to the 11β-hydroxysteroid dehydrogenase type 1 P2 promoter in human A549 epithelial cells has also been reported (30). In the present study, the results revealed that IL-1α treatment induced the expression of *S100A8* in TR146 epithelial cells, and the inductive effect occurred at the transcriptional level. In addition, the activated expression of *S100A8* induced by IL-1α in TR146 epithelial cells may involve a mechanism associated with increasing the binding activity of C/EBPβ to a specific site (-113/-109) of the *S100A8* promoter. Overall, the results of the present study are consistent with the findings reported in previous studies (22,23,29,30). Taken together, these similar findings support the hypothesis that IL-1α increases the binding activity of the transcription factor C/EBPβ to the promoter of specific genes and this may be a common regulatory mechanism that affects target gene expression.

In conclusion, the present study provided novel mechanistic insights into the transcriptional regulation of human *S100A8* in TR146 epithelial cells. However, the detailed molecular mechanism requires further clarification.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

MQ, YZ, KZ and XZ made substantial contributions to the conception and design of the study. YG and XZ analyzed the results. MQ, YG and XZ drafted the manuscript. All the authors read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors confirm that they have no competing interests.

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