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Transcriptional regulation of human cyclic GMP-AMP synthase gene

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ABSTRACT

Cyclic GMP-AMP synthase (cGAS, cGAMP synthase) plays crucial roles in autoimmune disease, anti-tumor response, anti-senescence and anti-inflammatory response. Many studies have focused on cGAS-mediated signaling pathway. However, transcriptional mechanisms of cGAS gene have remained largely unknown. Here, we cloned the cGAS promoter region and characterized the molecular mechanisms controlling the cGAS transcriptional activity. By a series of 5′ deletion and promoter constructions, we showed that the region (−414 to +76 relatives to the transcription start site) was sufficient for promoter activity. Mutation of Sp1 and CREB binding sites in this promoter region led to an apparent reduction of the cGAS promoter activity. Overexpression of Sp1 and CREB could obviously enhance promoter activity, whereas knocking-down of endogenous Sp1 and CREB markedly restrained the cGAS promoter activity. Sp1 and CREB binding to the cGAS promoter region in vivo was verified by Chromatin immunoprecipitation assay. These results pointed out that transcription factors Sp1 and CREB regulate the transcription of the cGAS gene.

1. Introduction

Virus infection is a huge threat to human health. The occurrence of some cancers is related to the infection of certain viruses. The innate immune system fights against invading virus [1]. During this process, various germline-encoded pattern recognition receptors (PRRs) play an important role. PRRs include Nod-like receptors (NLRs), Toll-like receptors (TLRs) and RIG-I-like receptors (RLRs), etc. [2]. PRRs recognize nucleic acids when pathogens invade the organism. Then chemokines and type-I interferons (IFNs) are released in numerous ways [3,4]. PRRs play critical roles in the innate immune system [5]. It also detects typical molecules of pathogens [6].

The innate immune system recognizes invading pathogens by special PRRs, which sensing pathogen-associated molecular patterns (PAMPs). While viruses attack organisms, double strand DNA was produced as PAMPs.PRRs-DNA sensors including DDX11, cGAS, etc. interacted with PAMPs [6–9]. Cyclic GMP-AMP synthase (cGAS, cGAMP synthase) is called C6orf150 (chromosome 6 open reading frame 150) and MB21D1 (MAB-21 domain-containing protein 1), which uses ATP and GTP as substrate and then forming cyclic GMP-AMP (cGAMP), being part of the nucleotidyltransferase family. cGAS binds to DNA which invades the cytoplasm and promotes the synthesis of cGAMP. cGAMP is known as a second messenger [10]. cGAS activates the endoplasmic reticulum protein STING and induces type-I IFNs production. On the one hand, the cGAS–STING pathway participates in immune defense against viruses. On the other hand, it acts as a congenital immune sensor for retroviral (such as HIV-1) infection [11–13]. Apart from antiviral activity, cGAS has proven to be activated by cellular stresses; for instance, senescence, DNA damage and genome instability [14,15]. Recently, Ge B et al. systematically illustrated the nuclear function of cGAS completely independent of DNA recognition, and provides a newly theoretical basis for the development of new anticancer drugs based on the intervention of cGAS into the nucleus [16].

What's more, many researchers paid close attention to cGAS. Numerous studies have focused on the regulatory pathway of cGAS. However, transcriptional mechanisms have remained largely undiscovered. Here, we tend to explore the core region of the cGAS promoter and illustrated that transcription factors Sp1 and CREB regulate the cGAS gene.

Abbreviations: cGAS, Cyclic GMP-AMP synthase; PRRs, pattern recognition receptors; TLRs, Toll-like receptors; NLRs, Nod-like receptors; RLRs, RIG-I-like receptors; IFNs, Interferons; PAMPs, pathogen-associated molecular patterns; C6orf150, chromosome 6 open reading frame 150; MB21D1, MAB-21 domain-containing protein 1; cGAMP, cyclic GMP-AMP; PCR, polymerase chain reaction; RIPA, Radio Immunoprecipitation Assay; PVDF, polyvinylidene difluoride; TBST, Tris-buffered saline-Tween; ChIP, Chromatin immunoprecipitation; TFBSs, transcription factors binding sites; CREB, cAMP response element-binding protein

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Table 1
Sequences of oligonucleotides used in site-directed mutagenesis.

Name	Sequence[a]
CREB- F	GTTGGCCAGGCTGGTCTTGAACTCCTGACCTCAGGTGATC
CREB- R	GGAGACCGAGGTGGGCGGATCACCTGAGGTCAGGAGTTCA
CREB-MUT F	GTTGGCCAGGCTGGTCTTGAACTCCTCTCAGGTGATC
CREB-MUT R	GGAGACCGAGGTGGGCGGATCACCTGAGAGGAGTTCA
USF1 F	CTCCTGACCTCAGGTGATCCGCCCACCTCGGTCTCCCAGATTGC
USF1 R	GGTGGGCGGATCACCTGAGGTCAGGAGTTCAAGACCAGCCTGGC
USF1-MUT F	CTCCTGACCTCAGATCCGCCCACCTCGGTCTCCCAGATTGC
USF1-MUT R	GGTGGGCGGATCTGAGGTCAGGAGTTCAAGACCAGCCTGGC
Sp1 F	CCTCAGGTGATCCGCCCACCTCGGTCTCCCAGATTGCTGGGAT
Sp1 R	GACCGAGGTGGGCGGATCACCTGAGGTCAGGAGTTCAAGACCAG
Sp1-MUT F	CCTCAGGTGATCCACCTCGGTCTCCCAGATTGCTGGGAT
Sp1-MUT R	GACCGAGGTGGATCACCTGAGGTCAGGAGTTCAAGACCAG
C-JUN F	TACAGGCGTGAGTCAGTGTGCCCAGGCAACACACACACACACAT
C-JUN R	TGCCTGGGCACACTGACTCACGCCTGTAATCCCAGCAATCTGGG
C-JUN–MUT F	TACAGGCGTGAGGTGTGCCCAGGCAACACACACACACACAT
C-JUN–MUT R	TGCCTGGGCACACCTCACGCCTGTAATCCCAGCAATCTGGG
RAP1 F	AGGTCTTGCTGGGTTGCCCAGGCTGGTCTCAAACTCCTGAGCTC
RAP1 R	CCAGCCTGGGCAACCCAGCAAGACCTGGTCTTTCTTATAATTAA
RAP1-MUT F	AGGTCTTGCTGGCCCAGGCTGGTCTCAAACTCCTGAGCTC
RAP1-MUT R	CCAGCCTGGGCCAGCAAGACCTGGTCTTTCTTATAATTAA

[a] Primers for site-directed mutagenesis of the cGAS gene: the underlined bases were deleted after point mutations.

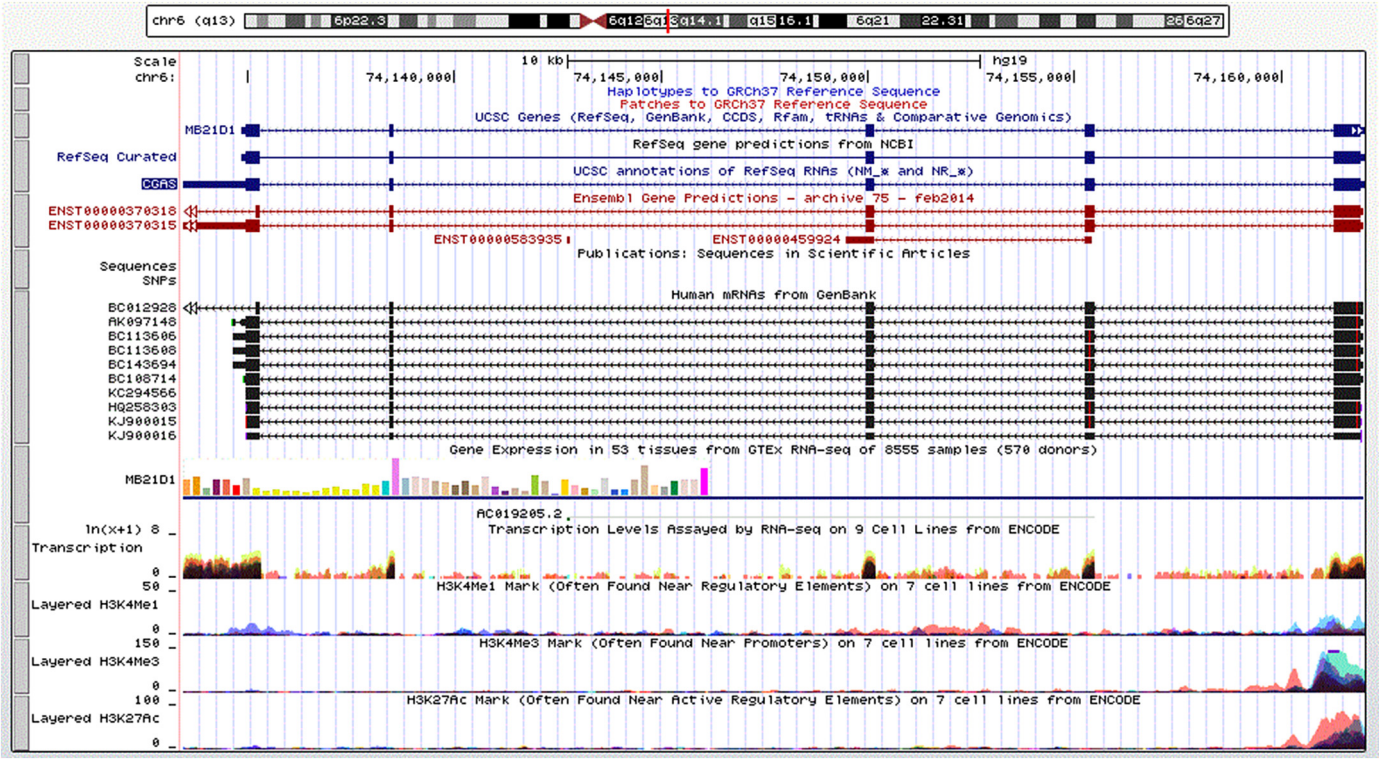

Fig. 1. DNase I Hypersensitivity sites in 5′ upstream of the cGAS gene by ENCODE. The cGAS gene (NM_138441, chr6: 74,134,856-74,162,043, 27,188 nt) was analyzed at UCSC Genome Browser on Human Feb. 2009 (GRCh37/hg19) Assembly. The transcription track shows transcription levels assayed by sequencing of polyadenylated RNA from a variety of cell types. The Layered H3K4Me3 track shows a histone mark associated with promoters. The DNase I Hypersensitivity tracks represent where chromatin is hypersensitive to the DNase enzyme. Regulatory regions tend to be DNase-sensitive, and promoters are particularly DNase-sensitive. At the functional level, DNase hypersensitivity suggests that a region is very likely to be regulated.

2. Materials and methods

2.1. Cell culture

Human embryonic kidney 293 T (HEK 293 T) cells, HPV-18 infected human cervical cell lines: HeLa, HPV-16 infected cervical cancer cell lines: Siha, were obtained from the American Type Culture Collection (ATCC) (http://www.atcc.org). Complete growth medium was utilized by adding 10% fetal bovine serum to Dulbecco's modified Eagle medium (DMEM). Cells were incubated at 37 °C in 5% carbon dioxide.

2.2. 5′ rapid amplification of cDNA ends (5′ RACE)

RNA extraction was conducted by using TRIzol (life) and phenol-chloroform. Hela cells' RNA was invoked as template. SMARTer® RACE (Takara) was performed according to the manufacturer's instructions.

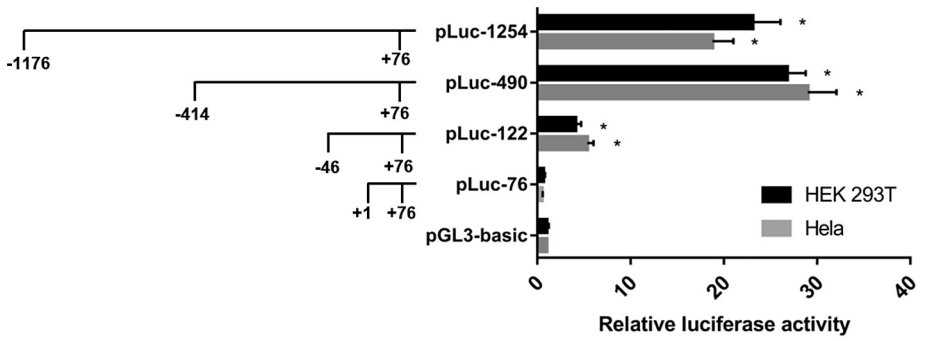

Fig. 2. Deletion analysis of the cGAS promoter in Hela and HEK 293 T cells. Diagram of the cGAS promoter reporter gene constructs were shown on the left. The relative location was marked. Promoter reporter plasmids and pGL3-Basic plasmids were transfected together with Renilla into Hela and HEK 293 T cells. Every bar represented the mean ± SD of four independent experiments ($n = 4$, $^*p < .05$ vs. pGL3-Basic).

We designed two antisense primers to synthesis the first-strand cDNA. Primer sequences were shown below: GATTACGCCAAGCTTCTTGGGT GCTAGCAGGCCAGCTAC, GATTACGCCAAGCTTGTAGCTGGCCTGCTA GCACCCAAGAAGG. PCR products were sequenced after electrophoresed in 1% agarose gel.

2.3. Polymerase chain reaction (PCR)

Using Hela cells' whole genomic DNA as template, we cloned cGAS gene promoter fragment. Oligo 7 software was used to design primers. *Kpn I* and *Bgl II* (NEB, USA) restriction sites (bold) were added. The forward primer was 5′- GG**GGTACC**CAGTGGCTCATGCCTACAAT-3′ and the reverse primer was 5'-GA**AGATCT**CTGTTGGAAACCAAGCACT ACT-3′. PCR was conducted by using EXtaq (Takara, Japan) as polymerase.

Then, promoter-less reporter gene vector pGL3-Basic and PCR fragment were digested and ligated. After sequencing correctly, the plasmid was designated as pLuc-1254. Similarly, we amplified the truncated sequences. The truncated plasmids were constructed. Then, these plasmids were described as pLuc-490(−414~ + 76) and pLuc-122(−46~ + 76), pLuc-76(+1~ + 76).

2.4. Plasmids and siRNAs

pcDNA and CREB plasmids were preserved by our team. Dr. Guntram Suske gave us the Sp1 and PN3 plasmids. Genepharma Company (Shanghai, China) synthesized siRNAs and the negative control (si-Control). Sequences can be seen as follows: si-Sp1: 5′- CCUCA CAGCCACACAACUUTT-3′; si-CREB: 5′- GCCACAGATTGCCACATTA −3′; si-Control: 5′-UUCUCCGAACGUGUCACGUTT-3′.

2.5. Site-directed mutagenesis

Point mutation was realized by QuickChange Site-directed Mutagenesis kit (TAKARA, Japan). Primers can be seen in Table 1.

2.6. Transient transfections and luciferase activity

With the help of Lipofectamine™2000 (Invitrogen, USA), transient transfection assay was implemented. Hela or HEK 293 T cells were planked in a 48-well culture plate. After 16 h, these cells grew to 70–80%. To check the promoter activity, 400 ng recombinant plasmids and 4 ng renilla (as conrtol) were transfected into the cells. Expression plasmids (200 ng) or siRNA (50 nM) were used to explore the relationship between Sp1, CREB and cGAS promoter. After 1 day, cells were harvested. FB12 luminometer (Berthold, Germany) and the Dual Reporter assay system (Promega, USA) were applied to measure the luciferase activity. Every experiment was done at least 3 times.

2.7. RNA purification and quantitative PCR

Total RNA was extracted. Then, cDNA was synthesized by reverse transcription. Next, Roche LightCycler 96 real-time PCR system was used to amplify the gene together with SYBR Green I dye (Vazyme Biotech Co.,Ltd). It is convenient to design the primers according to the Primer Primier 6 and Oligo 7 software. Sequences were presented in Table 1. The reference gene was GAPDH. Melting curves were checked to ensure the specificity of amplification.

2.8. Western blotting

Washing cells with cold 1 x PBS (phosphate buffer saline). Then, protease inhibitor cocktail, phosphatase inhibitor cocktail and PMSF (Kangchen bio-tech, China) were mixed with Radio Immunoprecipitation Assay (RIPA) lysis buffer. Next, we harvested and transferred the protein into tubes. Soon afterwards, loading 20 μg boiled protein and 5× SDS into 10% SDS-PAGE gel. After electrophoresis and electrophoretic transfer, proteins were transferred to polyvinylidene difluoride (PVDF) membranes. Immersing the PVDF membranes in 5% milk for an hour at 37 °C to block non-specific binding sites. Subsequently, protein blots were incubated overnight together with primary antibodies at 4 °C. GAPDH (Santa Cruz, USA), Sp1, CREB and cGAS (Abcam, UK) antibodies were selected. The next day, membranes were treated with HRP-conjugated secondary antibodies (GeneCopoeia Inc.) at 37 °C for an hour and washed 3 times every 5 min with TBS-T. Lastly, reactive protein was visualized by enhanced chemiluminescence (Millipore, USA).

2.9. Chromatin immunoprecipitation (ChIP) assay

According to the manufacturer's instructions, we performed ChIP™ (Millipore, USA) assay. 1×10^7 cells were needed. Siha cells were fixed with 1% formaldehyde for ten minutes. After fixation, every dish was filled with 2 mL cold PBS and 1 × Protease Inhibitor Cocktail II. Cells were collected by centrifugation at the temperature of 4 °C. Using nuclear lysis buffer to resuspend the pellets. Sonicator Ultrasonic Processor (Qsonica, LLC) works at the condition of 10 s pulses and 20 s rest recycling 9 times. The strength is 60% of maximum output. The antibodies of IgG (Millipore, USA), Sp1 and CREB (CST, USA) were applied. IP Wash, Elution, Crosslink Reversal were followed. Then, DNA cleanup and PCR were realized. ChIP primers can be seen in Supplementary Table S1.

2.10. Statistical analysis

Data was presented in the form of mean ± SD. All experiments were carried out in four copies independently. Paired two tailed student's-*t*-test was used to deal with the data. $P < .05$ indicates a statistical difference between the groups.

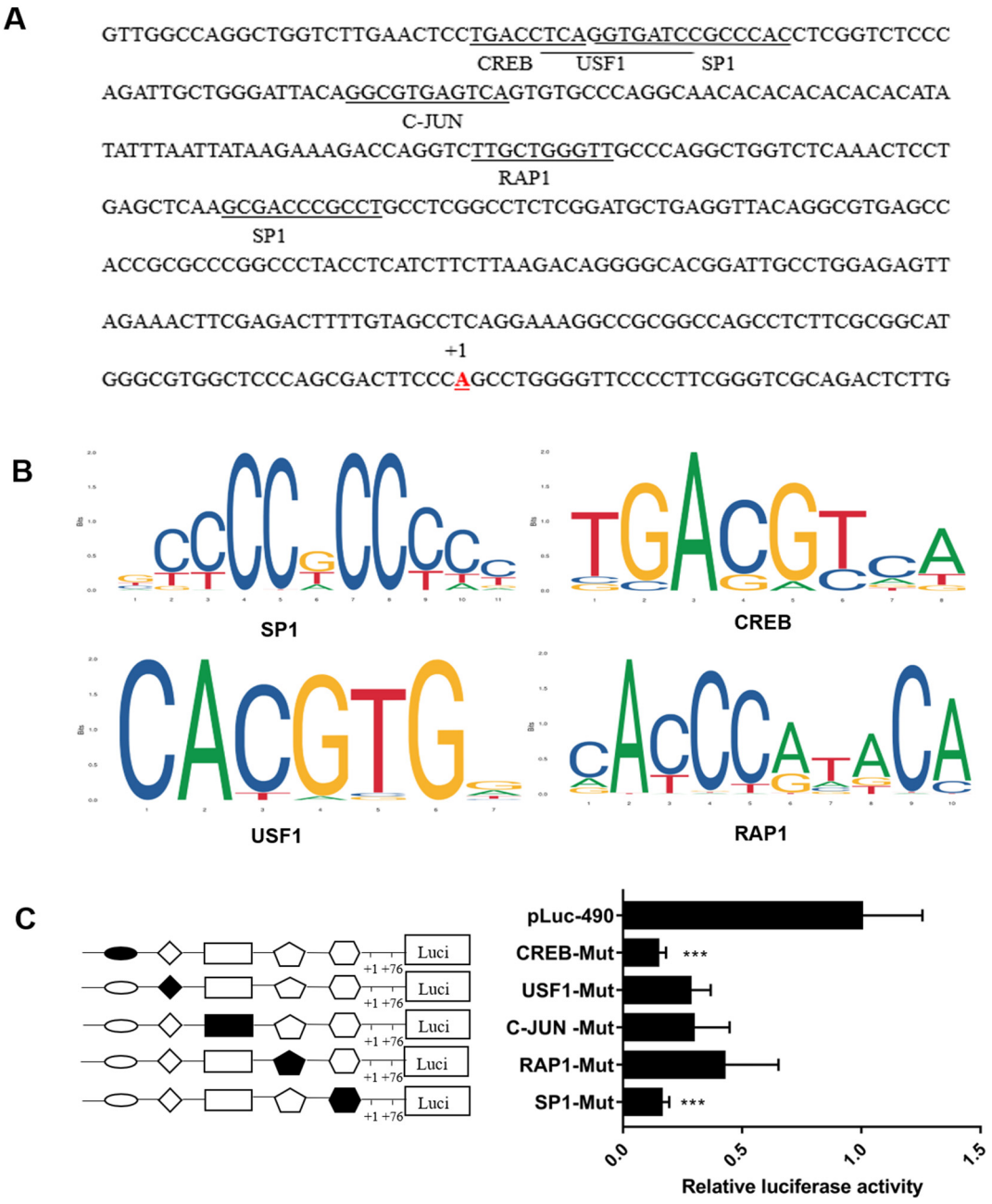

Fig. 3. Prediction and verification of transcription factors binding sites in cGAS core promoter region. (A)A sketch map showed the putative binding sites for transcription factors in the proximal promoter of the cGAS gene. TFSEARCH software was utilized to analyze the core region (from −414 to +76). The putative transcription factor binding sites were underlined. TSS was marked. (B) Conserved base sequence was listed and the size of base represents affinity between transcription factor and promoter. (C)Mutations of the cGAS minimal promoter in Hela cells. The binding sites of CREB, USF, C-JUN, RAP1, Sp1 were shown in different shapes on the left. The shape was blackened when the corresponding transcription factor binding sites were mutated. Meanwhile, the relative luciferase activities of pLuc-490 and mutational plasmids were presented on the right. The levels of firefly luciferase activities were calibrated with the Renilla luciferase activities. Each bar represented the mean ± SD of four independent experiments. ($n = 4$, * $p < .05$ vs. pLuc-490).

3. Results and discussion

3.1. Transcription start site (TSS) localization of human cGAS (hcGAS) promoter

5′ RACE was conducted to identify the TSS. On the 3rd exon of cGAS, two antisense primers were designed as nested PCR primers for RACE. Hela cells' RNA was used as a template. We obtained four products. PCR products were sent to sequencing. It is demonstrated that the TSS is located at 139 bp upstream of the initiation codon.

3.2. Bioinformatics analysis

From NCBI database, the full length of cGAS gene (chr6:73425133~73452320) was obtained and its promoter was predicted. Owing to Encyclopedia of DNA Element (ENCODE) at UCSC (http://genome.ucsc,edu/), cGAS gene promoter was analyzed (Fig.1). The 1000 bp upstream of the cGAS gene TSS shows several DNase I hypersensitive sites, increasing the possibility of DNA binding proteins. Hence, −1178 to +76 nt of cGAS gene was cloned.

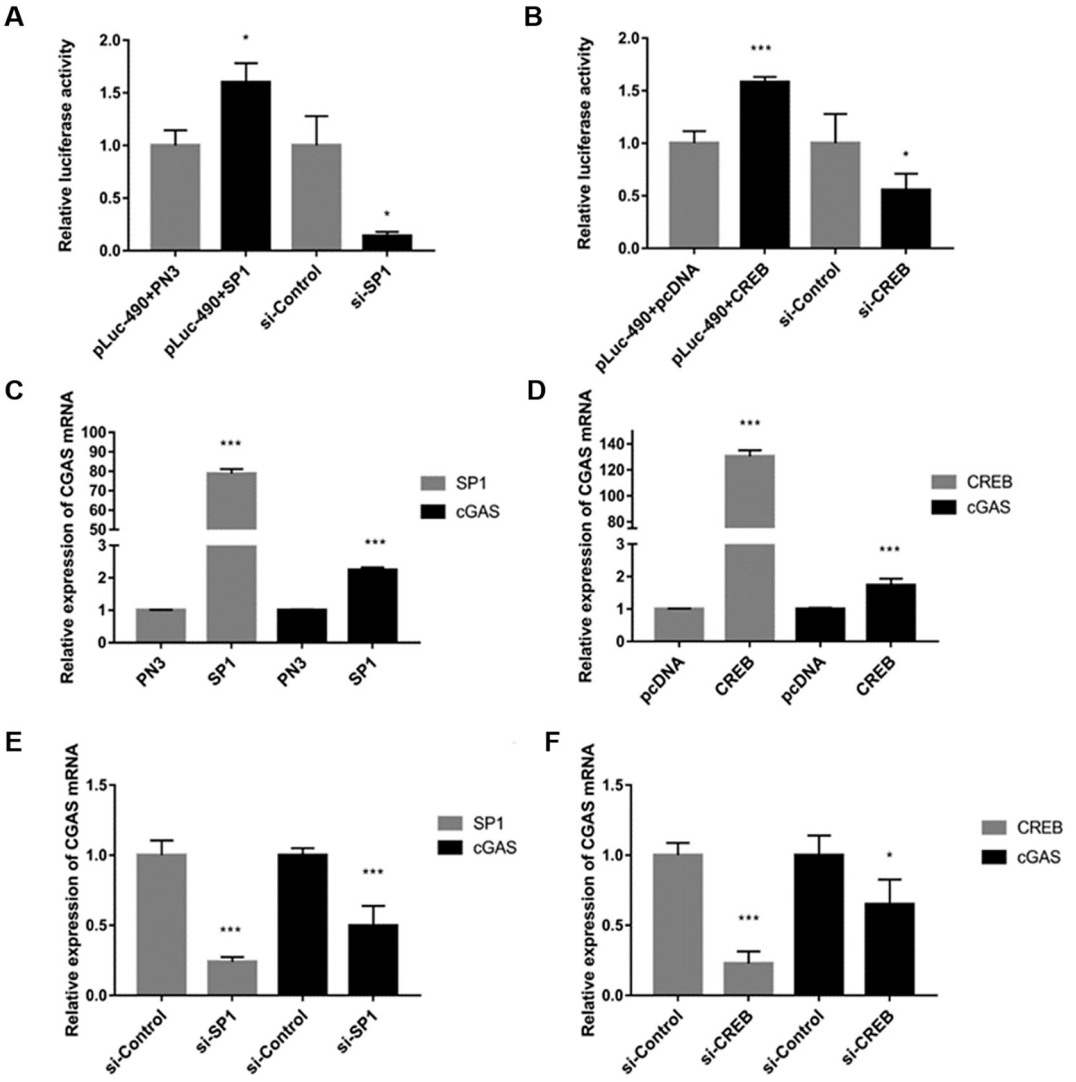

Fig. 4. Effects of transcription factors Sp1 and CREB on the promoter activity and mRNA level of the cGAS gene. (A, B) Overexpression and knockdown of Sp1 and CREB to measure the luciferase reporter gene activity in HEK 293 T cells. (C–F) The mRNA level of cGAS gene was significantly increased or reduced in Siha cells when Sp1 or CREB was overexpressed or knocked down. Each bar represented the mean ± SD of three independent experiments (*$P < .05$, ** $P < .01$, *** $P < .001$).

3.3. Cloning and analysis of human cGAS promoter

To corroborate the kernel of cGAS promoter, a series of luciferase reporter deletion plasmids were constructed. Newly generated plasmids were called pLuc-1254 (−1178 to +76 nt), pLuc-490 (−414 to +76 nt), pLuc-122 (−46 to +76 nt) and pLuc-76 (+1 to +76 nt). These recombinant plasmids and Renilla were co-transfected into Hela and HEK 293 T cells, then luciferase assays were performed. As is shown in Fig. 2, relative luciferase activity of pLuc-1254 is about 20 times higher than pGL3-Basic in two cell lines (HEK 293 T and Hela). Luciferase assays revealed pLuc-490 was enough for elementary promoter activity of the cGAS. Overall, we conclude that the region of cGAS gene promoter (−414 to +76 nt) can maintain the basal promoter activity.

3.4. Site-directed mutagenesis

Online software TFSEARCH ver.1.3, AliBaba2.1, PROMO (Fig. 3A) were used to forecast putative binding sites. JASPAR database was employed to predict the key nucleotides in transcription factors binding sites (TFBSs). Sp1, CREB, USF1, RAP1 were exhibited in Fig. 3B. In order to evaluate the vital binding sites, site-direct mutagenesis on the pLuc-490 was made. We transfected these mutational plasmids together with renilla into Hela cells. After 24 h, we gauged luciferase activities. There was minor difference between pLuc-490 and recombinant plasmids, which mutated USF1, RAP1 or C-JUN binding sites (Fig. 3C). Contrarily, mutations of Sp1 and CREB binding sites could reduce cGAS promoter activity to 16% and 15%. The difference is most obvious. To conclude, CREB and Sp1 regulate the fundamental transcription of the cGAS gene.

3.5. Sp1, CREB positively regulate the expression of cGAS

To determine the pivotal regulation of the Sp1 and CREB, the overexpression plasmids of Sp1 or CREB and pLuc-490 were co-transfected into HEK 293 T cells. The luciferase activity of pLuc-490 was increased almost 1.6 folds compared to the empty vector group, when Sp1 was overexpressed (Fig. 4A). At the same time, the overexpression of CREB can lead to 1.6 times increase of promoter activities (Fig. 4B). Furthermore, the plasmid pLuc-490 and siRNA-Sp1 (si-Sp1) or siRNA-CREB (si-CREB) or negative control (si-Control) were co-transfected. When knocking down Sp1, the luciferase activities were lowered to

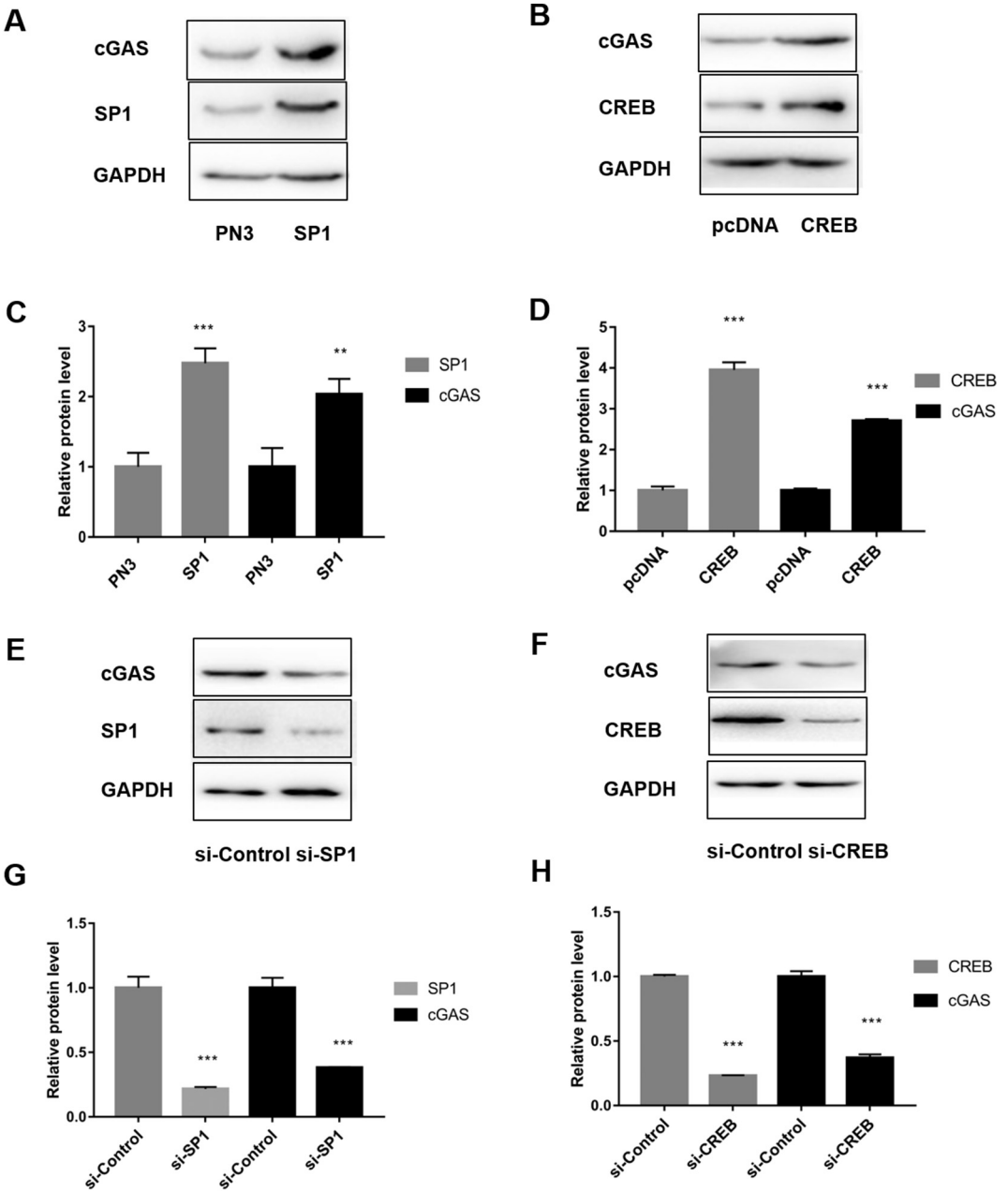

Fig. 5. Effects of Sp1 and CREB on protein levels of the cGAS gene in Siha cells. (A, B) Overexpression of Sp1 or CREB increased the protein levels of cGAS. (E, F) The protein levels of cGAS were significantly reduced when knocking down Sp1 and CREB. (C, D, G, H) Results of gray scanning was based on the homologous western blotting pictures (*P < .05, ** P < .01, *** P < .001).

14% of the activity (Fig. 4A). While knocking-down CREB, the luciferase activity was decreased by 45% (Fig. 4B).

To detect whether the mRNA and protein levels of cGAS were influenced in vivo, the overexpression and knockdown of Sp1 or CREB were realized in Siha cells. The results showed that the mRNA of cGAS were increased 2.2 and 1.7 times when Sp1(Fig. 4C) and CREB (Fig. 4D) were overexpressed. Knocking down Sp1(Fig. 4E) and CREB (Fig. 4F) caused a nearly 50% reduction of the mRNA.

At the same time, overexpression of Sp1(Fig. 5A,C) and CREB (Fig. 5B,D) enhanced the protein levels of cGAS by 2 and 2.5 folds. siRNA which targeted Sp1(Fig. 5E,G) and CREB (Fig. 5F,H) caused 70–80% and 60–70% decrease of protein levels.

Rely on these results, we concluded the expression of the endogenous cGAS gene was positively regulated by Sp1 and CREB in Siha cells.

3.6. Sp1 and CREB binding sites in the cGAS promoter

We performed ChIP assay in Siha cells to verify the binding of transcriptional factors and the cGAS promoter. Using Sp1 and CREB antibodies to immunoprecipitate the binding DNA fragments.

The kit provides normal rabbit IgG as negative control. Next, real-time PCR was used to amplify the Sp1 or CREB binding parts. It is suggested that anti-Sp1 and anti-CREB antibodies binding proteins were bound to the cGAS promoter region (Fig. 6). Moreover, semi-quantitative PCR was carried out to amplify the eluted DNA which contains Sp1 or CREB binding sites, respectively. Sp1 or CREB binding site was amplified successfully (Supplementary Fig. S1). However, primers of cGAS exon 3 failed to amplify a sequence. Moreover, normal rabbit IgG (negative control) could not precipitate proteins combined with this sequence. Our study revealed that transcription factor Sp1, CREB bind

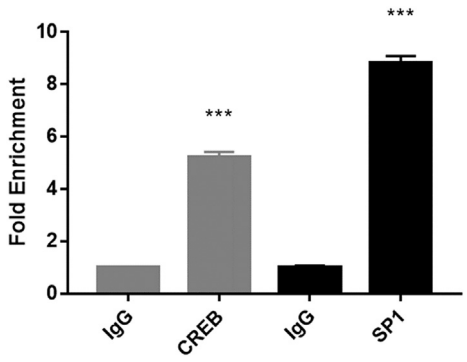

Fig. 6. ChIP assay of the cGAS proximal promoter was performed in Siha cells. The immunoprecipitated chromatin fragments were analyzed by quantitative PCR. Primer pairs were designed and spanned the putative Sp1 and CREB binding sites (the target locus). Data were expressed as fold enrichment. Each experiment was performed triplicated and $p < .05$ calculated with student's test (*$P < .05$, ** $P < .01$, *** $P < .001$).

to the cGAS promoter exclusively. Primer sequences were contained in Supplementary Table S1.

Ultimately, we use LinkedOmics (www.linkedomics.org/) to explore a correlative analysis of cGAS with Sp1 and CREB expressing levels in CESC (cervical squamous cell carcinoma and endocervical adenocarcinoma), which applied 304 patients' RNAseq results from TCGA (http://www.tcga.org/). As is shown in Fig.7, the expression of Sp1 or CREB was positively correlated with that of cGAS.

This paper describes studies that address some of the transcriptional mechanisms regulating expression levels of cGAS and its promoter activity. Notably, using a series of 5'deletion and promoter constructs, that the region (−414 to +76 relatives to the transcription start site) is enough for the promoter activity of cGAS. Mutation of Sp1 and CREB binding sites in the promoter locations of cGAS culminates in the attenuation of the cGAS promoter activity.Concomitantly, overexpression of Sp1 and CREB can enhance promoter activity, whereas knocking-down of endogenous Sp1 and CREB markedly restrained the cGAS promoter activity.

The transcription factor SP family is involved in the transcriptional regulation of many tissue-specific, viral and inducible genes. The transcription factor Sp1 activates or inhibits transcription in response to physiological and pathological stimuli [17–19]. It has a high affinity for GC-rich motifs and regulates the expression of many genes involved in many processes, such as cell growth, apoptosis, differentiation and immune responses. Sp1 is highly regulated by post-translational modification (phosphorylated, Proteolytic enzymes, glycosylation and acetylation [20,21]), which also mediate cellular responses to DNA damage and chromatin remodeling.

CREB (cAMP response element-binding protein) is a cellular transcription factor [22]. It binds to specific DNA sequences called cAMP response elements (CRE), thereby the transcription of the genes was increased or decreased. CREB was first identified in 1987 as a CRE, involving in regulating the somatostatin gene [23]. CREB can repress the replication of HCMV. So it provides new strategies in the aspect of anti-HCMV [24]. In the course of studying Hodgkin lymphoma (HL), researchers found that CREB is a tumor suppressor gene which reduces the expression of several cell cycle-related genes. Therefore, it has influence on cell proliferation [25]. In our study, transcription factor CREB was involved in regulation of the human cGAS gene promoter. Collectively, we have reason to believe that CREB participates in antiviral and anti-tumor events.

Previously, Wang et al. [26] cloned porcine cyclic GMP–AMP synthase and explored it's function. In addition, Ma et al. [27] had published the paper to estimate the transcriptional regulation of mouse cGAS (mcGAS) several years ago. There are three ISREs and one STAT1 binding site that are potentially responsible for the induction of mouse cGAS by IFN-I. The homology of hcGAS and mcGAS is < 60%, so there are species differences in key regulatory sites. Interpreting the cGAS-STING pathway, including careful consideration of possible species-specific differences, will be helpful to further develop treatments for DNA-induced pathways.

In summary, this paper reports on the core region of cGAS proximal promoter (−414 to +76 nt). For the first time, we analyzed the human cGAS promoter and identified the transcription factors Sp1 and CREB which regulate human cGAS gene. cGAS is known as a cytosolic DNA sensor. It can activate the type-I interferon pathway. Understanding these processes may be helpful to reveal innate immune signaling events, autoinflammatory diseases and to provide potential therapeutic targets for drug intervention.

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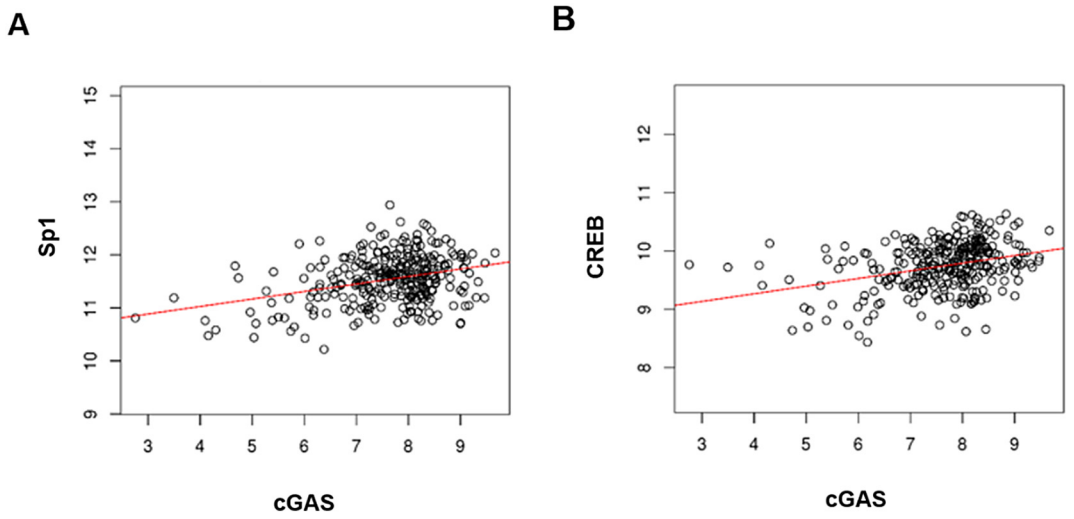

Fig. 7. A correlative analysis of cGAS with Sp1 and CREB expressing levels in CESC(cervical squamous cell carcinoma and endocervical adenocarcinoma).(A)The correlation between Sp1 and cGAS·(B)The correlation between CREB and cGAS.

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Declaration of Competing Interest

The authors declare no conflict of interest.

Acknowledgements

Conceptualization, G.Z. and H.X.; Data curation, H.C., X.P. and Y.Xu.; Formal analysis, H.C., X.P., Y.X., G.Z. and H.X.; Funding acquisition, H.X.; Project administration, H.X.; Supervision, H.X.; Writing-original draft, H.C.; Writing-review & editing, H.X. All authors read and approved the final manuscript.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cellsig.2019.109355.

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