J. Biochem. 2010;148(2):171–178 doi:10.1093/jb/mvq047

JB THE JOURNAL OF BIOCHEMISTRY

Identification and characterization of the promoter of human *ATF5* gene

Received April 11, 2010; accepted April 21, 2010; published online April 27, 2010

Yuanyan Wei, Yuqing Ge, Fengbiao Zhou, Hong Chen, Chunhong Cui, Dan Liu, Zhiyuan Yang, Guoqiang Wu, Jianxin Gu and Jianhai Jiang*

Key Laboratory of Glycoconjuates Research, Ministry of Public Health & Gene Research Center, Shanghai Medical College of Fudan University, Shanghai, People's Republic of China, 200032

*Prof. Jianhai Jiang, Shanghai Medical College of Fudan University, Shanghai, People's Republic of China 200032, Tel.: +86 21 54237660, Fax: +86 21 54237660, E-mail: jianhaijiang@fudan.edu.cn

ATF5, a member of ATF/CREB family of b-ZIP transcription factors, is highly expressed in a wide variety of neoplasms and regulates cell differentiation, cell survival and apoptosis. However, the mechanism of human ATF5 transcriptional regulation has not been clarified. Here, we identified the transcription start site of the ATF5 gene, cloned its 5′-flanking region and identified the region −105 to +3 relative to the transcription start site as that having promoter activity. This region contained potential binding sites for several transcription factors, including EBF1, Sp1 and E2F1. EBF1 transcription factor binds to the ATF5 promoter and regulates the ATF5 transcription in an EBF-binding site independent manner. Thus, our studies not only provided molecular basis of ATF5 transcriptional regulation, but also identified ATF5 as a target gene of EBF1 transcription factor.

Keywords: ATF5/EBF1/promoter/transcription factors/transcriptional regulation.

Abbreviations: CHIP, Chromatin immunoprecipitation; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; PCR, polymerase chain reaction; RACE, Rapid amplification of cDNA ends; RT, reverse transcription; shRNA, short hairpin RNA.

ATF5 transcription factor, a member of ATF/CREB family of b-ZIP transcription factors, plays a critical role in regulating CRE-dependent genes (*1*), and interacts with a variety of proteins including Cyclin D3 (*2*), GABAβ receptor (*3*), HTLV-1 viral protein Tax (*4*), E2 ubiquitin-conjugating enzyme Cdc34 (*5*), PRL-1 and DISC1 (*6*, *7*).

ATF5 has been reported to regulate cell differentiation, proliferation and survival (*8*–*17*). ATF5 expression in neural progenitor cells appeared to be necessary for maintaining the proliferation and counteracted the effect of extracellualr differentiation factors, such as NGF, NT3 and CNTF (*12*, *18*, *19*). In addition, accumulated evidences indicated that ATF5-controlled transcription pathways were integrated with the transduction cascades that control programmed cell death (*8*, *9*, *20*–*22*). ATF5 mRNA expression was inhibited after induction of apoptosis by growth factor deprivation in multiple cell lines and primary cells and its overexpression suppressed apoptosis resulting from cytokine deprivation in an IL-3-dependent cell line (*22*). Consistent with this, we have previously reported that down-regulation of proteasome-mediated degradation of ATF5 mediated by Cdc34 contributed to cisplatin-induced cell apoptosis (*20*, *23*). Moreover, ATF5 was over-expressed in human glioblastoma and human and rat glioma cell lines (*24*). Interference with ATF5 expression in glioma cells led to their death both *in vitro* and *in vivo* (*24*), indicating that ATF5 might be an attractive target for therapeutic intervention in glioblastoma. Consistent with this, ATF5 is highly expressed in a wide variety of neoplasms, including breast ductal and lobular carcinomas, renal cell carcinomas, lung caner, lymphomas and seminomas (*9*). The widespread ATF5 expression in carcinogenesis may indicate that it is part of 'the molecular signature' in many neoplasms. Although the progresses in ATF5 interacting partners, function and its relation to some diseases have been made in recent years, the precise molecular mechanisms of ATF5 transcriptional regulation remain unknown.

To increase the knowledge about ATF5 transcriptional regulation, we carried out luciferase reporter assays with ATF5 promoter construct pGL3(−1953/+47) and a series of its 5′-deleted constructs. We identified the region −105/+3 relative to the transcription start site as that having promoter activity. This region was found to contain potential binding sites for several transcription factors, including EBF1, Sp1 and E2F. EBF1 transcription factor binds to ATF5 promoter and regulates ATF5 transcription. These data identified *ATF5* as a target gene of EBF1.

Materials and Methods

Materials

Restriction enzymes, bovine calf serum, Dulbecco's modified Eagle's medium (DMEM), Trizol regent and Lipofectamine reagent were purchased from Invitrogen. The anti-EBF1, anti-ATF5 and anti-GAPDH antibodies were purchased from Santa Cruz Biotechnology and Sigma.

Cell culture and cell transfections

Human cervical carcinoma cell line HeLa, human lung caner cell lines 95C and 95D, human glioma cell line U251, human breast cancer cell lines MCF-7 and T47D cells were grown at 37°C in DMEM supplemented with 10% fetal calf serum in a humidified 95% air, 5% CO2 incubator. Human kidney cell line HEK293 cells were grown at 37°C in MEM supplemented with 10% fetal calf serum in a humidified 95% air, 5% CO2 incubator. Cell transfection was performed with Lipofectamine 2000 (Invitrogen) according to the manufacturer's instructions.

RNA ligase-mediated rapid amplification of the 5′ cDNA end

RNA ligase-mediated rapid amplification of cDNA ends by polymerase chain reaction (PCR) was performed with total RNA extracted from HEK293 cells and was used to determine single or multiple transcription start points. Rapid amplification of 5′ cDNA ends (5′-RACE) was performed using the FirstChoice RLM-RACE kit (Ambion) according to the manufacturer's instructions. The PCR conditions were as recommended, with 10 pmol of gene-specific primer (GSP) (5′-GGCGAAGACAGGCACCAAGG CGAAA-3′), 1.25 U of SuperTaq polymerase (Ambion), 10 pmol of 5′-RACE primer (5′-GCTGATGGCGATGAATGAACACTG -3′) (Ambion), 1 × SuperTaq PCR buffer (Ambion), 100 μM deoxynucleoside triphosphates, 1 ng of first-strand cDNA reaction mixture per microlitre, and H_2O to a volume of 50 μl. The PCR product was subjected to electrophoresis in a 2% agarose gel, stained with ethidium bromide and visualized under ultraviolet (UV) lamp. A DNA fragment comprising 223 bp was obtained, cloned into pGEM-T Easy vector and subsequently sequenced.

Plasmids

Expression constructs for pcDNA3.0, pcDNA3.0-E1AF, pGL3Basic and pRL-SV40 have been described previously (*20*, *25*, *26*). Expression construct for E2F1 was a generous gift from Prof. Cress Doug. PEVR2-Sp1 vector was kindly provided by Dr. Guntram Suske (Marburg, Germany). Expression construct for EBF1 was obtained from Dr. Jim Hagman and Dr. Barbara L. Kee. A 2500-bp fragment (containing nucleotides −1953 to +487 of ATF5 promoter) was prepared by PCR amplification of human genomic DNA using a sense primer containing a KpnI restriction site and an anti-sense primer containing an XhoI restriction site. Primers were synthesized on the basis of the reported genomic sequence for human ATF5, sense 5′-tatggtaccgggtgcagtcaagtttt gaactcatataat-3′ and anti-sense 5′-aaactcgagagagactgtgatccgag gacaggga-3′. Following digestion with restriction enzymes, the fragment was directionally cloned into the pGL3-Basic vector (Promega) to generate a 'full-length' ATF5 reporter construct, and the correct insertion was confirmed by sequencing. Reporter genes containing sequentially truncated fragments of the ATF5 promoter were prepared in a similar manner. PGL3(−105/+27) site-directed mutagenesis constructs were derived from pGL3(−105/+27) by PCR amplification using TakaRa MutanBEST mutagenesis kit. The LacZ shRNA and EBF1 shRNA plasmids were constructed as previously described (*27*). The following sequences were targeted to silence LacZ and EBF1 by shRNA expression: TCGCTGATTTGTGTA GTCG (LacZ) and GAACAGCCTGCAAGCGATA (EBF1).

Dual luciferase assay and reverse transcription PCR

Dual luciferase assay and reverse transcription (RT) PCR were performed as described previously (*20*, *25*). Primers used for PCR were as follows: ATF5 sense 5′-AGTTGCGGGACTGGGAGGTG-3′ and ATF5 anti-sense 5′-GTAGCA CAGAGGGCCAAAA-3′. The PCR products for ATF5 were 184 bp. GAPDH mRNA expression served as a loading control.

Chromatin immunoprecipitation assay

The association of EBF1 with ATF5 chromatin DNA in U251 cells was confirmed using a chromatin immunoprecipitation (ChIP) assay kit (Upstate Biotechnology) with anti-EBF1 antibody as described by the manufacturer. Normal anti-rabbit IgG was used as a negative control. The region of −200 to +3 of ATF5 promoter was amplified by conventional PCR.

Results

Mapping of the transcription start site of the human ATF5 gene

To determine the position of the transcription start site of the human ATF5 gene, 5′-RACE analysis was performed using total RNA from HEK293 cells and two sets of oligonucleotide primers, 5′-RACE Primer and GSP (gene specific primer) (Fig. 1A). As shown in Fig. 1B, the primer PCR using 5′-RACE Primer and GSP produced a 200-bp product. The product was extracted from the agarose gel and was cloned into pGEM-T Easy vector for sequencing. The results

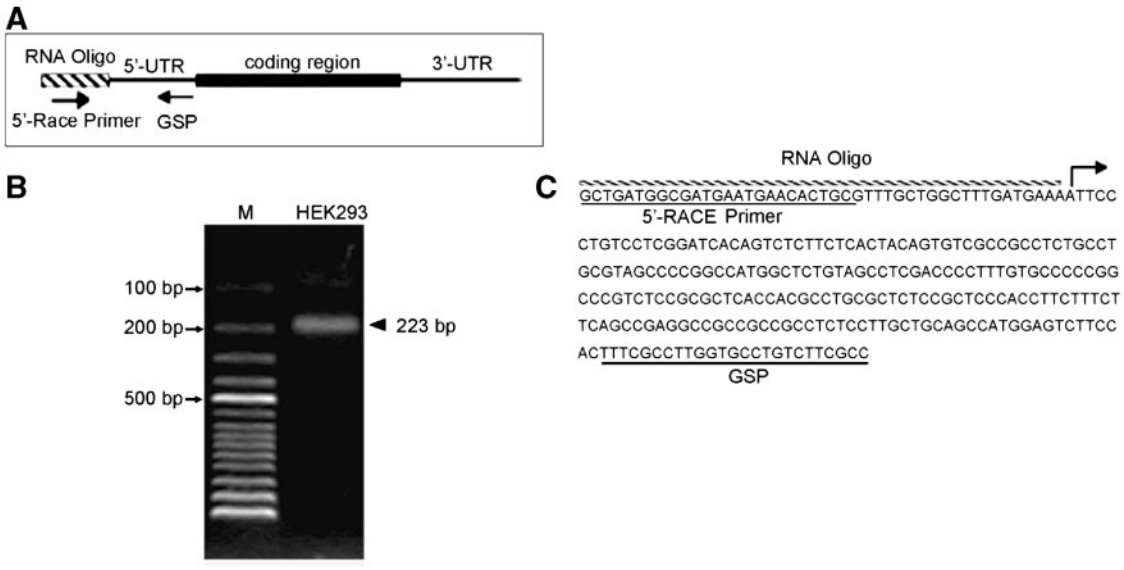

Fig. 1 RLM-RACE analysis of the transcription start site of the human *ATF5* gene. (A) Schematic representation of the primers used in RLM-RACE analysis. *UTR* indicated the untranslated region. The 5′-Race Primer and GSP (ATF5 gene specific primer) were indicated. (B) Ethidium bromide staining of the PCR products on an agarose gel. M indicated the 100-bp DNA ladder which was used as a molecular size marker. (C) Nucleotide sequence of the PCR product. The 5′-Race Primer and the complementary sequence for GPS used in the PCR were underlined. The sequence of the GeneRacer RNA Oligo linked to the ATF5 cDNA was overlined. The arrow indicated the transcription start site of ATF5.

showed that RNA Oligo was linked to an adenine residue at nucleotide position 223 upstream from the translation initiation codon (Fig. 1C). Prolonging the extension time in the PCRs did not amplify any larger PCR products (data not shown). Therefore, the transcription start site of the ATF5 gene in HEK293 cells was located at position 223 upstream from the translation initiation codon.

Sequence and functional analysis of the 5′-flanking region of the ATF5 gene

The nucleotide sequence of the 5′-flanking region of the ATF5 gene was determined to analyse its promoter function. No typical CAAT box sequence was found, but one TATA box located between positions −25 and −35 bp upstream from the transcription start site. The TRANSFAC search program predicted a number of potential transcription factor-binding sites near or upstream of the transcription initiation site, including Ets1, Ets2, E1AF, AP1, Sp1, E2F1 and EBF1 (Fig. 2).

To investigate the activity of the ATF5 promoter, a fragment extending from −1953 to +472 was amplified by PCR from human genomic DNA and cloned into pGL3-Basic, creating the luciferase reporter plasmid pGL3(−1953/+472). Upon transient transfection into several cell lines, pGL3(−1953/+472) exhibited a significant luciferase activity as compared with that of the pGL3-Basic (data not shown). Among the various cell types we have examined, the ATF5 gene promoter was activated mostly in HEK293 cells and 95D cells among the cell lines examined (Fig. 3A). Consistent with this, ATF5 was highly expressed in 95D cells and HEK293 cells (Fig. 3B).

Therefore, HEK293 cells were used to identify the promoter region of the ATF5 gene. To identify regulatory elements contributing to ATF5 transcriptional regulation, 'nine' additional reporter plasmids containing the promoter in variable lengths were constructed and transfected into HEK293 cells (Fig. 3C, left), and the promoter activities were determined. The results showed that pGL3(−105/+27), containing the sequence downstream to nucleotide position −105, retained relatively strong promoter activity, whereas the promoter activity was significantly reduced for pGL3(+3/+27) (Fig. 3C, right). These results highlighted that ATF5 core promoter was contained within the −105/+3 fragment and indicated the presence of positively regulatory elements between nucleotide positions −105 and +3 relative to transcription start site.

Activation of the human ATF5 gene by EBF1 transcription factor

To determine the transcription factors which contributed in the transcription regulation of ATF5, pGL3(−1953/+472) was transiently co-transfected into HEK293 cells with a variety of transcription factors or the empty control. The highest activation of *ATF5* promoter was obtained by EBF1 (Fig. 4A). To further determine the effect of EBF1 on the *ATF5* transcription, pGL3(−1953/+472) was transiently

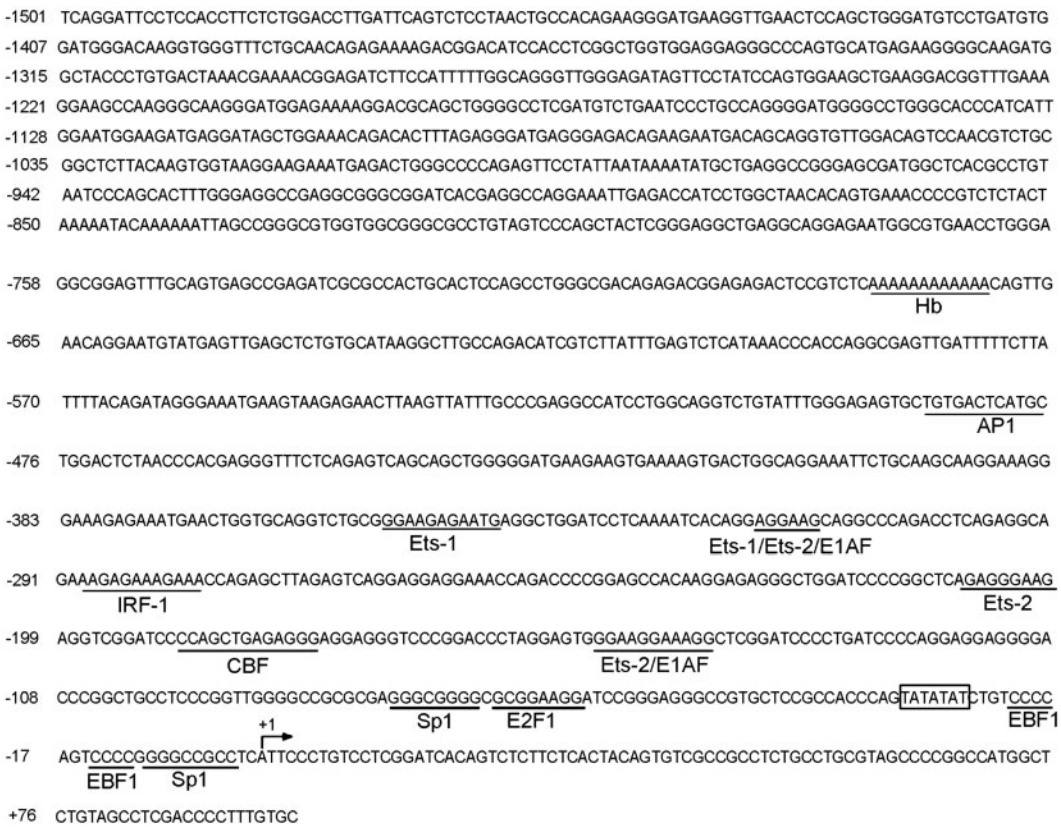

Fig. 2 Human ATF5 upstream genomic sequence and putative transcription factor binding sites. Presented above was the sequence of 1501 bp genomic region immediately upstream of the human ATF5 transcription start site and 100 bp sequence downstream of the human ATF5 transcription start site. Potential transcription factor-binding sites identified by searching TRANSFAC transcription factor database were underlined. Numbers at the left referred to the transcription start site, which was indicated with an arrow and taken as +1.

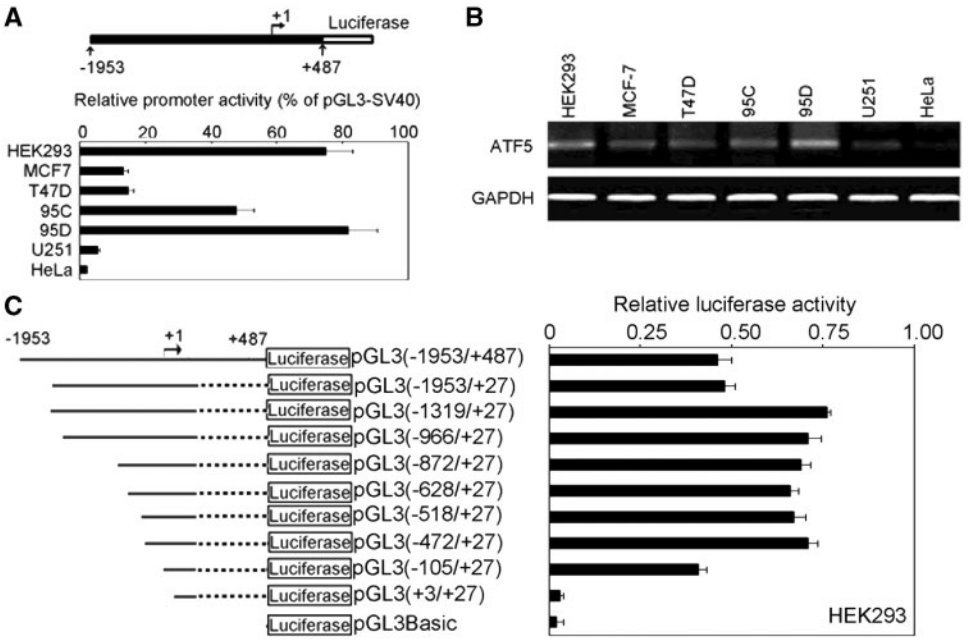

Fig. 3 Analysis of the promoter activity of human ATFS gene. (A) PGL3(−1953/+472) was transiently transfected into the indicated cells. Luciferase activity was normalized to the Renilla luciferase activity of a co-transfected internal control plasmid pRL-SV40 and was given as a percentage of the SV40 promoter activity in the indicated cells. Three experiments were conducted, and the data were shown as the mean values with standard errors. (B) RT-PCR analysis of ATF5 mRNA expression level in various cell lines HEK293, MCF7, T47D, 95C, 95D, HeLa and U251. GAPDH mRNA expression served as a loading control. (C) 5′-flanking regions of the ATF5 gene varying in length were fused to the luciferase reporter gene. The arrow indicated the transcription start site (left). HEK293 cells were transiently transfected with the indicated ATF5 promoter reporter construct, and the luciferase activity was determined 48 h after transfection. Transfection efficiency was adjusted by co-transfection with pRL-SV40 and parallel transfection with pGL3-Basic was used as negative control. Three experiments were conducted, and the data were shown as the mean values with standard errors (right).

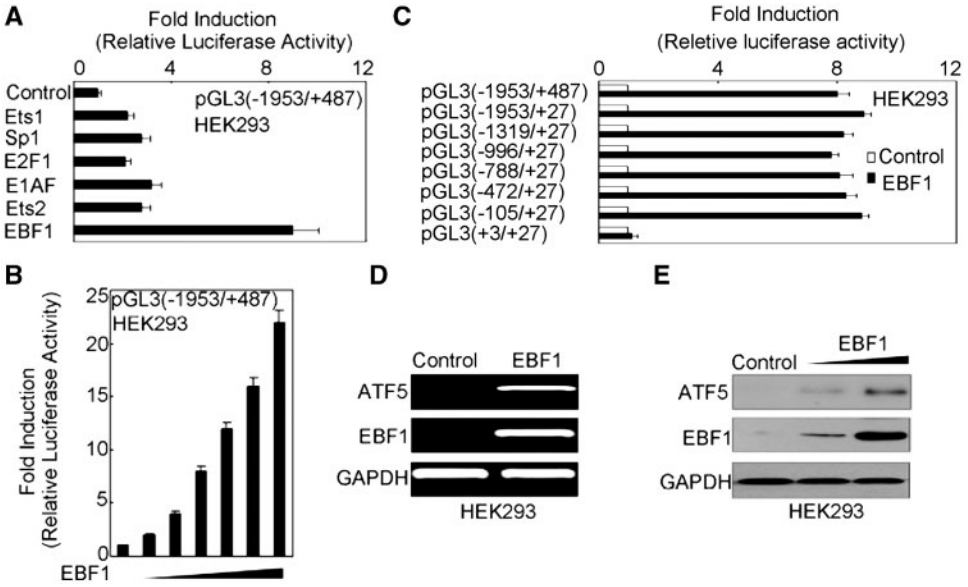

Fig. 4 The effect of EBF1 transcription factor on ATF5 promoter activity. (A) PGL3(−1953/+472) was transiently transfected into HEK293 cells with the indicated expression vector and the luciferase activity was determined 48 h after transfection. Results shown were the means ± SD of at least three independent experiments, expressed relative to activity in control cells transfected with empty expression vectors. (B) PGL3(−1953/+472) was transiently transfected into HEK293 cells with an increasing amounts of EBF1 expression vectors and the luciferase activity was determined 48 h after transfection as described above. (C) HEK293 cells were transiently co-transfected with pGL3(−1953/+472) construct or the truncated ATF5 promoter constructs shown above, and with or without EBF1 expression vector. The luciferase activity was determined 48 h after transfection as described above. (D) RT-PCR assay was performed to investigate the mRNA expression of *ATF5* gene from HEK293 cells transiently transfected with control or EBF1 expression vector using primers specific to the ATF5 and EBF1. Level of GAPDH mRNA expression served as a loading control. (E) Western blot assay was performed to investigate the protein expression of *ATF5* from HEK293 cells transiently transfected with control or EBF1 expression vector. Level of GAPDH protein expression served as a loading control.

co-transfected into HEK293 cells with increasing amounts of EBF1 expression plasmids. Forced expression of EBF1 increased the activity of *ATF5* promoter in a dose-dependent manner in HEK293 cells (Fig. 4B). To identify the *cis*-element responsible for the effect of EBF1 on *ATF5* promoter, HEK293 cells were transiently co-transfected with pGL3 (−1953/+472) construct or the truncated ATF5 promoter constructs and with or without EBF1 expression vector. The luciferase assay showed that a deletion from −105 to +3 resulted in a loss of EBF1 activation (Fig. 4C), suggesting that the region between nucleotide −105 and +3 was responsible for the activation of *ATF5* transcription by EBF1. Furthermore, compared to control, the mRNA and protein expression levels of *ATF5* were dramatically increased in EBF1-transfected HEK293 cells (Fig. 4D and E). These data indicated that EBF1 might play an important role in the regulation of the human *ATF5* gene.

EBF1 transcription factor binds to ATF5 promoter

Sequence analysis revealed that the 109-bp fragment between nucleotide −105 and +3 contains several potential transcription factor binding sites, including one E2F-binding consensuses, one Sp1-binding consensuses and two EBF-binding consensuses (Fig. 2A). To further characterize the contribution of the various putative transcription factor binding motifs, mutations in each of the four sites were introduced into the pGL3(−105/+27) luciferase vector by site-directed mutagenesis and these mutant constructs were tested for their basal activity in HEK293 cells. Mutation in the E2F site or the EBF1 site 2 reduced the basal activity of the promoter by ∼4-fold, mutation in Sp1 site reduced the basal activity of the promoter by ∼30%, while the EBF1 site 1 mutant construct retained activity comparable to the wild-type pGL3(−105/+27) (Fig. 5A). These results indicate that the E2F site and EBF site 2 are important *cis*-elements for the transcriptional activation of the human *ATF5* gene. To determine whether these various putative transcription factor binding motifs were necessary for EBF1-induced ATF5 transcription, mutant constructs were co-transfected into HEK293 with control or EBF1 expression vector. Mutations in the E2F site, the Sp1 site or the EBF sites did not have obvious effect on EBF1-induced ATF5 transcription (Fig. 5B). To further identify the *cis*-element responsible for the effect of EBF1 on *ATF5* promoter, pGL3(−105/+27) luciferase construct or the truncated ATF5 promoter constructs were transiently transfected into HEK293 cells with control vector or EBF1 expression vector. The luciferase assay showed that a deletion from −73 to −52 resulted in a loss of EBF1 activation (Fig. 5C), suggesting that the region between nucleotide −73 and −52 was responsible for the activation of *ATF5* transcription by EBF1.

In the RT-PCR analysis of EBF1 expression in the various cell types we have examined revealed that EBF1 was highly expressed in U251 cells as compared to that in other cells, in which EBF1 was faintly expressed (Fig. 5D). Thus, we examined the binding of EBF1 transcription factor to ATF5 promoter in U251 cells. PCR primers that spanned the region between nucleotide −200 to +3 of *ATF5* promoter obviously detected ATF5 promoter DNA in CHIP samples using anti-EBF1 antibody (Fig. 5E). To further determine whether EBF1 regulates ATF5 transcription, U251 cells were co-transfected with pGL3(−1953/+487) luciferase vector and LacZ shRNA or EBF1 shRNA. Compared to LacZ shRNA, EBF1 shRNA effectively inhibited the EBF1 expression and reduced the activity of ATF5 promoter in U251 cells (Fig. 5F and G).

Discussion

Transcription factors represent an important class of genes that play key roles in controlling cellular proliferation, cell cycle modulation and attractive targets for cancer therapy. ATF5, a member of a large family of transcription factors originally identified more than a decade ago (*28*), contributes to stem cell differentiation and is involved in cell survival and apoptosis in response to a number of environment factors (*12–15*, *18*, *22*, *29*, *30*). Moreover, there is extensive cross-talk between the ATF5 expression and its biological function (*12–15*, *18*, *19*, *29*, *30*). Thus, it is important to elucidate the mechanisms by which ATF5 is transcriptionally regulated.

In the present study, we have functionally characterized for the first time the regulation of ATF5 transcription. We identified the transcription start site and cloned the promoter region of the ATF5 gene. Deletion analysis showed that the core promoter was contained within the '−105/+3' fragment relative to transcription start site. This region contained one canonical TATA box and several GC box and lacked a canonical CCAAT box. Mutation of the TATA box within pGL3(−105/+3) construct did not significantly alter the activity of ATF5 promoter (data not shown). Within the region between −105 and +3, there were several potential transcription factor binding sites including an E2F-binding sites, a Sp1-binding site and two EBF1-binding sites. Mutation of the E2F-binding site or EBF-binding site 2 obviously impaired the activity of ATF5 promoter, indicating that these two sites are important *cis*-elements for the transcriptional activation of the human *ATF5* gene. The transcription factors which bind to these two sites should be further explored.

Another interesting finding was that EBF1 regulates the transcription of ATF5. EBF1 belongs to a small family encoding closely related helix–loop–helix transcription factors, which have been implicated in early cell differentiation and in the tumourigenesis of diverse types of human cancer (*31–33*). The expression of ATF5 gene was regulated by EBF1, which was suggested by several evidences: (i) the ectopic expression of EBF1 obviously activated the activation of ATF5 promoter and the mRNA and protein expression level of ATF5; (ii) EBF1 down-regulation by RNA interference reduced the activation of ATF5 promoter; and (iii) ChIP assay revealed that EBF1 transcription factor binds to ATF5 promoter. Taking all these facts

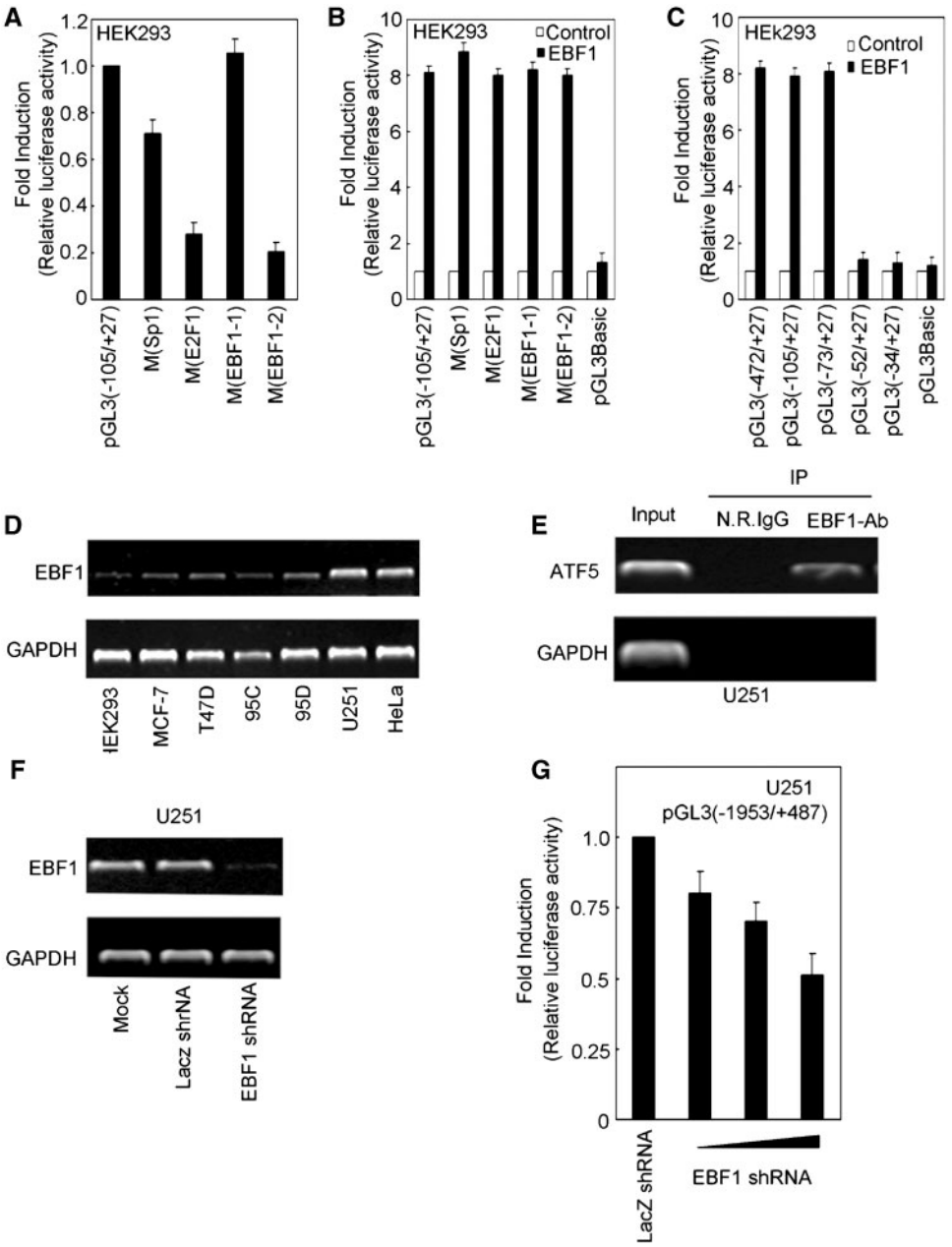

Fig. 5 EBF1 transcription factor binds to ATF5 promoter. (A) HEK293 cells were transiently transfected with pGL3(−105/+27) or mutated promoter construct. Firefly luciferase activities were normalized by Renilla luciferase activities. Each value is the mean ± SD of at least three independent experiments. (B) Control or EBF1 expression vector was transiently co-transfected into HEK293 cells with pGL3Basic, pGL3(−105/+27) or mutated promoter construct. Firefly luciferase activities were normalized by Renilla luciferase activities and standardized to the normalized activity from luciferase reporter construct with vector alone. Each value is the mean ± SD of at least three independent experiments. (C) Control or EBF1 expression vector was transiently co-transfected into HEK293 cells with pGL3(–472/+27) construct or the truncated ATF5 promoter constructs. Firefly luciferase activities were normalized by Renilla luciferase activities and standardized to the normalized activity from luciferase reporter construct with vector alone. Each value is the mean ± SD of at least three independent experiments. (D) RT-PCR analysis of EBF1 mRNA expression level in various cell lines HEK293, MCF7, T47D, 95C, 95D, HeLa and U251. GAPDH mRNA expression served as a loading control. (E) ChIP assay was performed in U251 cells using control control IgG or antibody against EBF1. PCR primers for the ATF5 and GAPDH promoters (negative control) were used to detect promoter fragments in immunoprecipitates. (F) RT-PCR analysis of EBF1 expression in U251 cells transfected with LacZ shRNA or EBF1 shRNA. GAPDH mRNA expression served as a loading control. (G) LacZ shRNA or EBF1 shRNA vector was transiently co-transfected into U251 cells with the indicated luciferase reporter constructs pGL3(−1953/+486). Firefly luciferase activities were normalized by Renilla luciferase activities and standardized to the normalized activity from luciferase reporter construct with LacZ shRNA. Each value is the mean ± SD of at least three independent experiments.

into account, we conclude that EBF1 regulates ATF5 transcription in an EBF1-binding site independent manner. EBF1 has been reported to regulate gene transcription via interaction with p300/CBP, ZNF423, ZNF521 and PAX5 (*34–37*). We presumed that EBF1 regulates ATF5 transcription via interaction other transcription factor, which should be further investigated.

In summary, we functionally characterized for the first time the regulation of ATF5 transcription and identified the region −105/+3 relative to the

transcription start as that having promoter activity. In addition, EBF1 significantly activated the promoter of ATF5. These data identified ATF5 as a target gene of EBF1. Besides, the role of activation of ATF5 by EBF1 in tumour behaviour and cell differentiation should be next investigated.

Funding

This work was supported by Shanghai Rising-Star Program (08QA14013), Program for New Century Excellent Talents in University (NCET-08-0128), National Natural Scientific Foundation of China (30930025 and 30900248 and 30870542), a grant from the Development of Science and Technology of Shanghai (09ZR140340), Shanghai Educational Development Foundation (2007CG02) and Shanghai Leading Academic Discipline Project (B110).

Conflict of interest

None declared.

References

1. Hansen, M.B., Mitchelmore, C., Kjaerulff, K.M., Rasmussen, T.E., Pedersen, K.M., and Jensen, N.A. (2002) Mouse Atf5: molecular cloning of two novel mRNAs, genomic organization, and odorant sensory neuron localization. *Genomics* **80**, 344–350
2. Liu, W., Sun, M., Jiang, J., Shen, X., Sun, Q., Liu, W., Shen, H., and Gu, J. (2004) Cyclin D3 interacts with human activating transcription factor 5 and potentiates its transcription activity. *Biochem. Biophys. Res. Commun.* **321**, 954–960
3. White, J.H., McIllhinney, R.A., Wise, A., Ciruela, F., Chan, W.Y., Emson, P.C., Billinton, A., and Marshall, F.H. (2000) The GABAB receptor interacts directly with the related transcription factors CREB2 and ATFx. *Proc. Natl. Acad. Sci. USA* **97**, 13967–13972
4. Forgacs, E., Gupta, S.K., Kerry, J.A., and Semmes, O.J. (2005) The bZIP transcription factor ATFx binds human T-cell leukemia virus type 1 (HTLV-1) Tax and represses HTLV-1 long terminal repeat-mediated transcription. *J. Virol.* **79**, 6932–6939
5. Pati, D., Meistrich, M.L., and Plon, S.E. (1999) Human Cdc34 and Rad6B ubiquitin-conjugating enzymes target repressors of cyclic AMP-induced transcription for proteolysis. *Mol. Cell. Biol.* **19**, 5001–5013
6. Morris, J.A., Kandpal, G., Ma, L., and Austin, C.P. (2003) DISC1 (Disrupted-In-Schizophrenia 1) is a centrosome-associated protein that interacts with MAP1A, MIPT3, ATF4/5 and NUDEL: regulation and loss of interaction with mutation. *Hum. Mol. Genet.* **12**, 1591–1608
7. Peters, C.S., Liang, X., Li, S., Kannan, S., Peng, Y., Taub, R., and Diamond, R.H. (2001) ATF-7, a novel bZIP protein, interacts with the PRL-1 protein-tyrosine phosphatase. *J. Biol. Chem.* **276**, 13718–13726
8. Wang, H., Lin, G., and Zhang, Z. (2007) ATF5 promotes cell survival through transcriptional activation of Hsp27 in H9c2 cells. *Cell Biol. Int.* **31**, 1309–1315
9. Monaco, S.E., Angelastro, J.M., Szabolcs, M., and Greene, L.A. (2007) The transcription factor ATF5 is widely expressed in carcinomas, and interference with its function selectively kills neoplastic, but not nontransformed, breast cell lines. *International journal of cancer* **120**, 1883–1890
10. Watatani, Y., Kimura, N., Shimizu, Y.I., Akiyama, I., Tonaki, D., Hirose, H., Takahashi, S., and Takahashi, Y. (2007) Amino acid limitation induces expression of ATF5 mRNA at the post-transcriptional level. *Life Sci.* **80**, 879–885
11. Chow, L.S., Lam, C.W., Chan, S.Y., Tsao, S.W., To, K.F., Tong, S.F., Hung, W.K., Dammann, R., Huang, D.P., and Lo, K.W. (2005) Identification of RASSF1A modulated genes in nasopharyngeal carcinoma. *Oncogene* **25**, 310–316
12. Angelastro, J.M., Ignatova, T.N., Kukekov, V.G., Steindler, D.A., Stengren, G.B., Mendelsohn, C., and Greene, L.A. (2003) Regulated expression of ATF5 is required for the progression of neural progenitor cells to neurons. *J. Neurosci.* **23**, 4590–4600
13. Nishioka, T., Miyai, Y., Haga, H., Kawabata, K., Shirato, H., Homma, A., Shibata, K., and Yasuda, M. (2009) Novel function of transcription factor ATF5: blockade of p53-dependent apoptosis induced by ionizing irradiation. *Cell Struct. Funct.* **34**, 17–22
14. Li, G., Li, W., Angelastro, J.M., Greene, L.A., and Liu, D.X. (2009) Identification of a novel DNA binding site and a transcriptional target for activating transcription factor 5 in c6 glioma and mcf-7 breast cancer cells. *Mol. Cancer Res.* **7**, 933–943
15. Greene, L.A., Lee, H.Y., and Angelastro, J.M. (2009) The transcription factor ATF5: role in neurodevelopment and neural tumors. *J. Neurochem.* **108**, 11–22
16. Pascual, M., Gomez-Lechon, M.J., Castell, J.V., and Jover, R. (2008) ATF5 is a highly abundant liver-enriched transcription factor that cooperates with constitutive androstane receptor in the transactivation of CYP2B6: implications in hepatic stress responses. *Drug Metab. Disposit. Biol. Fate Chem.* **36**, 1063–1072
17. Gho, J.W., Ip, W.K., Chan, K.Y., Law, P.T., Lai, P.B., and Wong, N. (2008) Re-expression of transcription factor ATF5 in hepatocellular carcinoma induces G2-M arrest. *Cancer Res.* **68**, 6743–6751
18. Mason, J.L., Angelastro, J.M., Ignatova, T.N., Kukekov, V.G., Lin, G., Greene, L.A., and Goldman, J.E. (2005) ATF5 regulates the proliferation and differentiation of oligodendrocytes. *Mol. Cell. Neurosci.* **29**, 372–380
19. Angelastro, J.M., Mason, J.L., Ignatova, T.N., Kukekov, V.G., Stengren, G.B., Goldman, J.E., and Greene, L.A. (2005) Downregulation of activating transcription factor 5 is required for differentiation of neural progenitor cells into astrocytes. *J. Neurosci.* **25**, 3889–3899
20. Wei, Y., Jiang, J., Sun, M., Chen, X., Wang, H., and Gu, J. (2006) ATF5 increases cisplatin-induced apoptosis through up-regulation of cyclin D3 transcription in HeLa cells. *Biochem. Biophys. Res. Commun.* **339**, 591–596
21. Persengiev, S.P. and Green, M.R. (2003) The role of ATF/CREB family members in cell growth, survival and apoptosis. *Apoptosis* **8**, 225–228
22. Persengiev, S.P., Devireddy, L.R., and Green, M.R. (2002) Inhibition of apoptosis by ATFx: a novel role for a member of the ATF/CREB family of mammalian bZIP transcription factors. *Genes Dev.* **16**, 1806–1814
23. Wei, Y., Jiang, J., Liu, D., Zhou, J., Chen, X., Zhang, S., Zong, H., Yun, X., and Gu, J. (2008) Cdc34-mediated degradation of ATF5 is blocked by cisplatin. *J. Biol. Chem.* **283**, 18773–18781
24. Angelastro, J.M., Canoll, P.D., Kuo, J., Weicker, M., Costa, A., Bruce, J.N., and Greene, L.A. (2006) Selective destruction of glioblastoma cells by interference with the activity or expression of ATF5. *Oncogene* **25**, 907–916
25. Jiang, J., Chen, X., Shen, J., Wei, Y., Wu, T., Yang, Y., Wang, H., Zong, H., Yang, J., Zhang,

S., Xie, J., Kong, X., Liu, W., and Gu, J. (2006) Beta1,4-galactosyltransferase V functions as a positive growth regulator in glioma. *J. Biol. Chem.* **281**, 9482–9489
26. Jiang, J., Wei, Y., Shen, J., Liu, D., Chen, X., Zhou, J., Zong, H., Yun, X., Kong, X., Zhang, S., Yang, Y., and Gu, J. (2007) Functional interaction of E1AF and Sp1 in glioma invasion. *Mol. Cell. Biol.* **27**, 8770–8782
27. Zhu, X., Jiang, J., Shen, H., Wang, H., Zong, H., Li, Z., Yang, Y., Niu, Z., Liu, W., Chen, X., Hu, Y., and Gu, J. (2005) Elevated beta1,4-galactosyltransferase I in highly metastatic human lung cancer cells. Identification of E1AF as important transcription activator. *J. Biol. Chem.* **280**, 12503–12516
28. Hai, T.W., Liu, F., Coukos, W.J., and Green, M.R. (1989) Transcription factor ATF cDNA clones: an extensive family of leucine zipper proteins able to selectively form DNA-binding heterodimers. *Genes Dev.* **3**, 2083–2090
29. Uekusa, H., Namimatsu, M., Hiwatashi, Y., Akimoto, T., Nishida, T., Takahashi, S., and Takahashi, Y. (2009) Cadmium interferes with the degradation of ATF5 via a post-ubiquitination step of the proteasome degradation pathway. *Biochem. Biophys. Res. Commun.* **380**, 673–678
30. Satake, H., Ito, K., Takahara, M., Furukawa, T., Takagi, M., Ogino, T., and Shinomura, T. (2009) Spatio-temporal expression of activating transcription factor 5 in the skeletal development of mouse limb. *Dev. Growth Different.* **51**, 669–676
31. Liberg, D., Sigvardsson, M., and Akerblad, P. (2002) The EBF/Olf/Collier family of transcription factors: regulators of differentiation in cells originating from all three embryonal germ layers. *Mol. Cell. Biol.* **22**, 8389–8397
32. Liao, D. (2009) Emerging roles of the EBF family of transcription factors in tumor suppression. *Mol. Cancer Res.* **7**, 1893–1901
33. Pongubala, J.M., Northrup, D.L., Lancki, D.W., Medina, K.L., Treiber, T., Bertolino, E., Thomas, M., Grosschedl, R., Allman, D., and Singh, H. (2008) Transcription factor EBF restricts alternative lineage options and promotes B cell fate commitment independently of Pax5. *Nat. Immunol.* **9**, 203–215
34. Hata, A., Seoane, J., Lagna, G., Montalvo, E., Hemmati-Brivanlou, A., and Massague, J. (2000) OAZ uses distinct DNA- and protein-binding zinc fingers in separate BMP-Smad and Olf signaling pathways. *Cell* **100**, 229–240
35. Bond, H.M., Mesuraca, M., Carbone, E., Bonelli, P., Agosti, V., Amodio, N., De Rosa, G., Di Nicola, M., Gianni, A.M., Moore, M.A., Hata, A., Grieco, M., Morrone, G., and Venuta, S. (2004) Early hematopoietic zinc finger protein (EHZF), the human homolog to mouse Evi3, is highly expressed in primitive human hematopoietic cells. *Blood* **103**, 2062–2070
36. Merluzzi, S., Moretti, M., Altamura, S., Zwollo, P., Sigvardsson, M., Vitale, G., and Pucillo, C. (2004) CD40 stimulation induces Pax5/BSAP and EBF activation through a APE/Ref-1-dependent redox mechanism. *J. Biol. Chem.* **279**, 1777–1786
37. Zhao, F., McCarrick-Walmsley, R., Akerblad, P., Sigvardsson, M., and Kadesch, T. (2003) Inhibition of p300/CBP by early B-cell factor. *Mol. Cell. Biol.* **23**, 3837–3846