Life Sciences 87 (2010) 294–301

Contents lists available at ScienceDirect

Life Sciences

journal homepage: www.elsevier.com/locate/lifescie

Regulation of the human *CHOP* gene promoter by the stress response transcription factor ATF5 via the AARE1 site in human hepatoma HepG2 cells

Takashi Yamazaki, Asako Ohmi, Haruka Kurumaya, Kenji Kato, Takanori Abe, Hiroyuki Yamamoto, Noriko Nakanishi, Ryuichi Okuyama, Mariko Umemura, Toshikazu Kaise, Ryuya Watanabe, Yoshiko Okawa, Shigeru Takahashi *, Yuji Takahashi

The Laboratory of Environmental Molecular Physiology, School of Life Sciences, Tokyo University of Pharmacy and Life Sciences, Hachioji, Tokyo 192-0392, Japan

ARTICLE INFO

Article history:
Received 8 December 2009
Accepted 1 July 2010

Keywords:
ATF5
CHOP
GADD153
DDIT3
Arsenite
Apoptosis

ABSTRACT

Aims: Activating transcription factor (ATF) 5 is a member of the cAMP response element-binding protein (CREB)/ATF family of transcription factors. We have shown that ATF5 is a stress response transcription factor that responds to amino acid limitation, arsenite exposure, or cadmium exposure. In this study we investigated whether ATF5 is involved in the regulation of CCAAT/enhancer-binding protein (C/EBP) homologous protein (*CHOP*) gene expression.

Main methods: We used a transient transfection system to express ATF5 and analyzed the regulation of *CHOP* gene promoter in human hepatoma, HepG2 cells. We also studied the effect of ATF5 knockdown on arsenite-induced CHOP protein expression and arsenite-induced cell death of HepG2 cells.

Key findings: We showed that ATF5 activates the *CHOP* gene promoter in HepG2 cells. Both deletion analysis and point mutations of the promoter revealed that amino acid response element (AARE) 1 is responsible for ATF5-dependent promoter activation. Furthermore, the existence of either AARE1 or activating protein-1 (AP-1) site is sufficient for transcriptional activation of the *CHOP* gene promoter by arsenite exposure, although complete induction requires the existence of both elements. We also demonstrated that knockdown of ATF5 reduced arsenite-induced CHOP protein expression and arsenite-induced cell death of HepG2 cells.

Significance: These results suggested that the *CHOP* gene is a potential target for ATF5, and that ATF5 raises the arsenite-induced *CHOP* gene expression level via the AARE1 site in HepG2 cells.

Introduction

All cells regulate gene expression in response to external environmental stresses such as heat shock, oxygen tension, heavy metals, and starvation. The CCAAT/enhancer-binding protein (C/EBP) homologous protein (*CHOP*, also called GADD153 or DDIT3) gene encodes a transcription factor related to the C/EBP family. The *CHOP* gene is induced by a wide variety of stresses, including amino acid limitation and oxidative stresses, and its expression is regulated at both the transcriptional and post-transcriptional level (Bruhat et al. 2000; Jousse et al. 2001; Oyadomari and Mori 2004). A region of the *CHOP* promoter (between amino acids −313 to −295) constitutes the amino acid response element (AARE) 1, and amino acid limitation-induced *CHOP* gene expression requires both activating transcription factor 4 (ATF4) and ATF2 in HeLa cells (Averous et al. 2004). Guyton et al. demonstrated that human *CHOP* gene expression is up-regulated by arsenite exposure via the activating protein-1 (AP-1) site in HeLa cells (Fawcett et al. 1999; Guyton et al. 1996).

ATF5 (formerly designated ATFx) is a transcription factor in the cAMP response element-binding protein/activating transcription factor (CREB/ATF) family that was first identified as a protein that binds to C/EBPγ (Nishizawa and Nagata 1992). It contains a DNA-binding and dimerization domain (bZIP domain) and regulates cellular differentiation (Angelastro et al. 2003), the cell cycle (Pati et al. 1999), and apoptosis (Devireddy et al. 2001; Persengiev et al. 2002). Angelastro et al. (2003) demonstrated that ATF5 represses cAMP response element (CRE)-mediated expression of neural genes and represses neural differentiation. Cdc34 is the G2 check-point gene, and ATF5 is a target of Cdc34-dependent ubiquitin-mediated proteolysis (Pati et al. 1999) whose expression is affected by the cell cycle.

The amino acid sequence of ATF5 is closely related to the sequence of ATF4, another member of the CREB/ATF family. *ATF4* expression is affected by amino acid availability and regulates target genes in an amino acid-dependent manner (Averous et al. 2004). Barbosa-Tessmann et al. (2000) identified a *cis*-element in the asparagine synthetase (*AS*) gene promoter, which is a nutrient-sensing response unit (NSRU) that is

* Corresponding author. School of Life Sciences, Tokyo University of Pharmacy and Life Sciences, 1432-1 Horinouchi, Hachioji, Tokyo 192-0392, Japan. Tel.: +81 42 676 7018; fax: +81 426 76 6811.
E-mail address: shigeru@ls.toyaku.ac.jp (S. Takahashi).

0024-3205/$ – see front matter © 2010 Elsevier Inc. All rights reserved.
doi:10.1016/j.lfs.2010.07.006

responsible for amino acid-dependent transcriptional regulation. Transcription factors, ATF4, ATF2, and C/EBPβ, were shown to regulate transcription of the *AS* gene in response to amino acid limitation. Recently, Al-Sarraj et al. (2005) demonstrated that ATF5 activated *AS* reporter gene transcription when the promoter contained a NSRU. We observed that amino acid limitation also resulted in a marked increase in *ATF5* mRNA in HeLaS3 cells (Watatani et al. 2007). These results prompted us to investigate whether ATF5 also activates the *CHOP* gene promoter via the AARE1 site. The purpose of this study was to evaluate the effect of ATF5 on cellular stress-induced *CHOP* gene expression. Our results suggest that the *CHOP* gene is a potential target for ATF5 and that ATF5 raises the arsenite-induced *CHOP* gene expression level via the AARE1 site.

Materials and methods

Chemicals

Dulbecco's modified Eagle's medium (DMEM) powder was purchased from Nissui Pharmaceutical (Tokyo, Japan). L-glutamine and sodium arsenite ($NaAsO_2$) were purchased from Wako Pure Chemical Industries (Osaka, Japan). $NaAsO_2$ was dissolved in H_2O and $NaAsO_2$ treatment of cells was conducted in serum-free medium.

Cell culture and DNA transfection

Human cervical carcinoma cells (HeLaS3), human hepatoma cells (HepG2), human glioblastoma cells (T98G), and human osteosarcoma cells (U2OS) were obtained from the RIKEN Cell Bank (Tsukuba, Japan). Human glioma cells, U251MG, were obtained from the Health Science Research Resources Bank (Osaka, Japan). Human glioma cells, U87MG, were purchased from the American Type Culture Collection (Manassas, VA, USA). These cells were maintained in DMEM supplemented with 10% fetal bovine serum and 2 mM glutamine. The cells were kept at 37 °C in CO_2 (5%)/air (95%) in a humidified atmosphere. DNA transfection was performed using the FuGENE6 transfection reagent (Roche, Indianapolis, IN, USA) according to the manufacturer's instructions.

Construction of plasmids

Luciferase reporter plasmids containing the human *CHOP* promoter regions (−954 to +91), (−649 to +91), or (−249 to +91), were designated phCHOP-LUC(−954), phCHOP-LUC(−649), and phCHOP-LUC(−249) in this study. These plasmids were kindly provided by Dr. P. Fafournoux (U.R. 238 de Nutrition Cellulaire et Moléculaire, INRA de Theix, Saint Genès Champanelle, France). The phCHOP-LUC(−777) luciferase reporter plasmid was constructed as follows. The human *CHOP* promoter region (−777 to −650) was amplified by PCR using the *CHOP* promoter region (−954 to +91) as a template and a *Mlu*I-ended primer, CHOP-AARE2wt-F, 5′-CGCACGCGTGTTTCACCATGTTGGTCAGGC-3′, and a *Xho*I-ended primer, CHOP-AARE2-R, 5′-TCGACATCTCGAGTCTTCTTAAAGAGGTCT-3′. This fragment was digested with *Mlu*I/*Xho*I and ligated into *Mlu*I/*Xho*I-digested phCHOP-LUC(−954) to produce phCHOP-LUC (−777). Further deletion mutants were constructed as follows. The human CHOP promoter region (−546 to +91), (−446 to +91), (−336 to +91), or (−116 to +91), were amplified by PCR using the CHOP promoter region (−777 to +91) as a template and a *Nhe*I-ended primer, CHOP-Dmt3-F, 5′-CGCGCTAGCCCCTTATAGTGGGGGTAAAACG-3′, CHOP-Dmt4-F, 5′-CGCGCTAGCACCAAGGCTGATAGCCGTTGG-3′, CHOP-Dmt5-F, 5′-CGCGCTAGCGCGCGTGCAGACACCGGTTGC-3′, or CHOP-Dmt6-F, 5′-CGCGCTAGCGGACGGGGCGGGGCCAATGCC-3′, and a *Hin*dIII-ended primer, CHOP-0-Hind-R, 5′-CAGTACCGGAATGCCAAGCTTTCTCCTC-3′. These fragments were digested with *Nhe*I/*Hin*dIII and ligated into *Nhe*I/*Hin*dIII-digested pGL3-Basic to produce phCHOP-LUC(−546), phCHOP-LUC (−446), phCHOP-LUC(−336), or phCHOP-LUC(−116).

A mutation of the AARE1 in the CHOP promoter was introduced into phCHOP-LUC(−777) at positions −310 to −302 by replacement of the nucleotide sequence ATTGCATCA with CAGGCATCA by inverse PCR with the mutagenic primers: CHOP-AAREmt-F2, 5′-TGCCAAACATTGCATCATCCCCGCCCCCCTTTC-3′, and CHOP-AAREmt-R2, 5′-ACCGGTGTCTGCACGCGCACGCGCAGGGGACAC-3′. A mutation of the AARE2 in the CHOP promoter was introduced into phCHOP-LUC (−777) at positions −777 to −769 by replacement of the nucleotide sequence GTTTCACCA with CAGTCACCA by inverse PCR with the mutagenic primers: CHOP-AARE2-mt-F, 5′-AGAGACGGGCAGTCACCATGTTGGTCAGGCTGATCTC-3′, and CHOP-AARE2-mt-Rnew-neo, 5′-ACTAAAAATACAAAAATTAGCCGGGTGTGGTGATGCG-3′. A mutation of the AP-1 in the *CHOP* promoter was introduced into phCHOP-LUC (−777) at positions −244 to −238 by replacement of the nucleotide sequence TGACTCA with TGACTTG by inverse PCR with the mutagenic primers: CHOP-AP1-mt-F, 5′-GCGCATGACTTGCCCACCTCCTCCGTGAAG-3′, and CHOP-AP1-mt-R, 5′-GCGCGCGGAGGGGAGTGTAGCGG-3′. pTK(−81)-LUC was constructed as follows. pGL3-TK (Takahashi et al. 2002) was digested with *Bgl*II/*Eco*RI to truncate the TK promoter, it was then blunt-ended with T4 polymerase and self-ligated. pTK(−81)-AARE-LUC, pTK(−81)-AAREmt-LUC, pTK(−81)-AAREx3-LUC, pTK (−81)-AAREmtx3-LUC, pAARE-SV40-LUC, pSV40-AAREmt-LUC, pSV40-AAREx3-LUC, and pSV40-AAREmtx3-LUC, were constructed as follows. Oligonucleotides: C-AARE-F, 5′-TCGACAACATTGCATCATCCCCGCC-3′, and C-AARE-R, 5′-TCGAGGCGGGGATGATGCAATGTTG-3′, were annealed and inserted into *Xho*I-digested pTK (−81)-LUC or *Xho*I-digested pGL3-Promoter (Promega, Madison, WI, USA) to produce pTK(−81)-AARE-LUC or pAARE-SV40-LUC. Similarly, oligonucleotides: C-AAREmt-F, 5′-TCGACAACCAGGCATCATCCCCGCC-3′, and C-AAREmt-R, 5′-TCGAGGCGGGGATGATGCCTGGTTG-3′, were annealed and inserted into *Xho*I-digested pTK(−81)-LUC or *Xho*I-digested pGL3-Promoter to produce pTK(−81)-AAREmt-LUC or pSV40-AAREmt-LUC.

Finally, C-AARE-F and C-AARE-R were annealed and the resultant AARE fragment was ligated, then digested with *Sal*I/*Xho*I. The fragment containing three copies of AARE was extracted from 5% PAGE, and inserted into *Xho*I-digested pTK(−81)-LUC or *Xho*I-digested pGL3-Promoter to produce pTK(−81)-AAREx3-LUC or pSV40-AAREx3-LUC. Similarly, C-AAREmt-F and C-AAREmt-R were annealed and the resultant AAREmt fragment was ligated, then digested with *Sal*I/*Xho*I. The fragment containing three copies of AAREmt was extracted from 5% PAGE, and inserted into *Xho*I-digested pTK(−81)-LUC or *Xho*I-digested pGL3-Promoter to produce pTK (−81)-AAREmtx3-LUC or pSV40-AAREmtx3-LUC.

Luciferase assay

Cells were lysed in passive lysis buffer (Promega, Madison, WI, USA). The cell lysates were used to determine luciferase activity using the dual-luciferase reporter assay system (Promega) and Lumat LB 9501 (EG and G Berthold, Badwildbad, Germany). Luciferase activities were normalized with the sea pansy luciferase activity. All values are the means ± SE calculated from the results of at least three independent experiments.

Stealth RNAi transfection

Stealth RNAi (RNA interference) corresponding to human ATF5 (5′-UUCAGCUCGCGAUUCCGUGCCUCCA-3′ and 5′-UGGAGGCACGGAAUCGCGAGCUGAA-3′) was designed with BLOCK-iT RNAi Designer (Invitrogen, Carlsbad, CA, USA). Stealth RNAi Negative Control Duplex (Invitrogen) was used as a negative control. For transfection of stealth RNAi, we used a reverse transfection method as follows. To study the effect of ATF5 knockdown on the up-regulation of the CHOP promoter caused by ATF5 expression (Fig. 1), 12 pmol of stealth RNAi duplex was diluted in 100 μl of Opti-MEM I Medium (Invitrogen) without