

Bis is Induced by Oxidative Stress *via* Activation of HSF1

Hyung Jae Yoo^{1,*}, Chang-Nim Im^{1,2,3,*}, Dong-Ye Youn¹, Hye Hyeon Yun^{1,2}, and Jeong-Hwa Lee^{1,2,3}

¹Department of Biochemistry, ²Cancer Research Institute, ³Cancer Evolution Research Center, College of Medicine, The Catholic University of Korea, Seoul 137-701, Korea

The Bis protein is known to be involved in a variety of cellular processes including apoptosis, migration, autophagy as well as protein quality control. Bis expression is induced in response to a number of types of stress, such as heat shock or a proteasome inhibitor via the activation of heat shock factor (HSF)1. We report herein that Bis expression is increased at the transcriptional level in HK-2 kidney tubular cells and A172 glioma cells by exposure to oxidative stress such as H₂O₂ treatment and oxygen-glucose deprivation, respectively. The pretreatment of HK-2 cells with N-acetyl cysteine, suppressed Bis induction. Furthermore, HSF1 silencing attenuated Bis expression that was induced by H₂O₂, accompanied by increase in reactive oxygen species (ROS) accumulation. Using a series of deletion constructs of the bis gene promoter, two putative heat shock elements located in the proximal region of the bis gene promoter were found to be essential for the constitutive expression as well as the inducible expression of Bis. Taken together, our results indicate that oxidative stress induces Bis expression at the transcriptional levels *via* activation of HSF1, which might confer an expansion of antioxidant capacity against pro-oxidant milieu. However, the possible role of the other cis-element in the induction of Bis remains to be determined.

Key Words: Bis, HSF1, Oxidative stress, ROS

INTRODUCTION

Bis (Bcl-2 interacting cell death suppressor), also known as BAG3 and CAIR-1, was originally identified as a Bcl-2-binding protein that enhances the antiapoptotic activity of Bcl-2 and as a HSP70-binding protein which inhibits Hsc70/Hsp70 chaperone activity [1-3]. Subsequent studies have provided a growing body of evidence to indicate that Bis exerts diverse functions through specific interactions with various partners. While the pro-survival activity of Bis is assigned to its binding to Bcl-2 or Hsp70 through the BAG domain [1,4], cellular motility and invasion activity appear to be modulated by Bis via interactions with a variety of proteins including FAK, MMP-2, PDZGEF and CCT [5-8]. In addition, recent studies strongly suggest that, as the result of complex formation with HspB8 or HspB6, Bis also participates in promoting autophagy and the subsequent degradation of misfolded and aggregated proteins [9-11]. Bis is also reported to be involved in the differ-

entiation process of promyelocytes, glia cells as well as cardiomyoblasts, although the molecular mechanism for this is unclear [12-14].

Considering these diverse functions of Bis, the maintenance of Bis expression in an appropriate level is prerequisite for appropriate responses various changes that occur in cells. Bis is normally expressed in most tissues at different levels with the highest levels in cardiac and skeletal muscles [1]. However, Bis expression is constitutively up-regulated in human tumor tissues from various origins compared to corresponding normal tissues [15]. On the other hand, Bis expression is efficiently induced by a variety of stressors, including heat shock, heavy metal, ultrasound, UV, proteasome inhibitors, small thiol compounds, lipid oxidation products and HIV infection [16-21]. Among those, several stimuli such as heat shock, proteasome inhibitors and pyrrolidine dithiocarbamate (PDTC) are known to induce Bis expression via activation of the heat shock factor (HSF)1 and subsequent interaction with the heat shock element (HSE) in the Bis gene promoter [20-23]. Activation of the bis gene promoter was also implicated in the upregulation of Bis expression through stimulation of the transcription factor Egr-1 and c-jun, which were directed by the fibroblast growth factor (FGF-2) and serum enrichment, respectively [24,25]. Moreover, in some cell types of glial ori-

Received May 26, 2014, Revised July 24, 2014,
Accepted July 26, 2014

Corresponding to: Jeong-Hwa Lee, Department of Biochemistry, College of Medicine, The Catholic University of Korea, 222 Banpo-daero, Seocho-gu, Seoul 137-701, Korea. (Tel) 82-2-2258-7293, (Fax) 82-2-596-4435, (E-mail) leejh@catholic.ac.kr

*These authors contributed equally to this work.



This is an Open Access article distributed under the terms of the Creative Commons Attribution Non-Commercial License (<http://creativecommons.org/licenses/by-nc/3.0>) which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

ABBREVIATIONS: Bis, Bcl-2 interacting cell death suppressor; HSF, heat shock factor; HSE, heat shock element; ROS, reactive oxygen species; PDTC, pyrrolidine dithiocarbamate; OGD, oxygen-glucose deprivation; SOD, superoxide dismutase; siRNA, small interfering RNA; DCF-DA, 2',7'-dichlorofluorescein diacetate; NAC, N-acetyl-L-cysteine.

gin, Bis protein enhances the transcriptional activity of its own promoter, probably through the BAG domain [26]. Thus, Bis expression is mainly regulated by transcriptional levels in response to diverse types of cellular stress, involving distinct transcription factors and cis-elements.

In a previous study, we reported that Bis expression was increased by ischemia-reperfusion in reactive astrocytes in vivo [27,28]. An *in vitro* study using C6 glioma cells demonstrated that oxygen-glucose deprivation significantly enhanced Bis protein expression, and Bis knockdown resulted in an increase in ROS levels and cell death upon oxygen-glucose deprivation (OGD), concomitant with an impairment in the induction of superoxide dismutase (SOD) activity [29]. Even though some oxidants such as 4-hydroxynonenal (HNE), a diffusible lipid species, have been reported to induce Bis expression via activating HSF1 [20], the issue of whether oxidative stress directly activates the Bis gene promoter remains unclear. Therefore, the primary purpose of this study is to investigate whether ROS derived from oxidative stresses such as H₂O₂ or OGD directly induce Bis expression at transcriptional levels. We also intended to examine the involvement of HSF1 in the activation of Bis and subsequent effect on cellular anti-oxidant capacity, by SHF1 silencing. Finally, we tried to define the cis-element for HSF1-binding on Bis gene promoter to prove the specific interaction between HSF1 and Bis gene promoter upon oxidative stresses.

METHODS

Cell culture and treatment

HK-2 human kidney-2 cells and A172 human glioma cells were purchased from American Type Culture Collection (ATCC) and maintained in DMEM (Hyclone) contained with 10% heat-inactivated FBS, 100 units/ml penicillin and 100 mg/ml streptomycin at 37°C in 5% CO₂ atmosphere. HK-2 cells were exposed to 50 μM of H₂O₂ in glucose/serum free DMEM for 3 h and then further incubated in the normal medium supplemented with glucose and serum. To observe the protective effect of ROS scavenger on the induction of Bis, 1 mM of N-acetyl-L-cysteine (NAC, Sigma-Aldrich) was treated to HK-2 cells before exposure to H₂O₂. For OGD, A172 cells were washed twice with degassed DMEM without glucose and serum (WelGene), and exposed to an anaerobic chamber containing 85% (v/v) N₂, 10% (v/v) H₂, 5% (v/v) CO₂ (Thermo Forma) at 37°C for 5 h. And then A172 cells were recovered in the normal medium in the indicated times.

MTT assay

The cells were transfected with siRNA into 48-well plates and then maintained for 48 h at 37°C in an incubator in a 5% CO₂ atmosphere. Cells were exposed to H₂O₂ in glucose/serum free DMEM for 3 h and then further incubated for 6 h in the normal medium supplemented with glucose and serum. Thiazolyl blue (3-[4, 5-dimethylthiazol-2-yl]-2, 5-diphenyltetrazolium bromide reagent, Sigma) was added to each well, to a final concentration of 0.5 mg/ml. After incubation at 37°C in an incubator under a 5% CO₂ atmosphere, the solution was suctioned and 200 μl of acid isopropyl alcohol (Sigma) was added to each well. Absorption was then measured at a wavelength of 570 nm. After ex-

traction with background value, the relative viability was determined by ratio of values to those of control in terms of mean±standard errors.

Quantitative real time PCR analysis

Total RNA was isolated using AccuZol (Bioneer, Daejeon, Korea), and first strand of cDNA was synthesized by reverse transcribed to cDNA using a ReverTra Ace qPCR kit (Toyobo). A quantitative real-time PCR was performed to validate the expression level of Bis and HSF1 mRNA using SYBR[®] Premix Ex Taq[™] (TaKaRa Bio) on Applied Biosystems 7300 machine (Applied Biosystems). The specific primers for real time PCR include following: bis, 5'-AGCCCTCAG-CACTGCCCTGCAGAA-3' and 5'-GCAGCTCTTTGGTC-AAATACTCTTC-3'; HSF1, 5'-GGCCATGAAGCATGAGAA-TG-3' and 5'-GTTTCAGCATCAGGGGGATCT-3' and β-actin, 5'-AGTACTCCGTGTGGATCGGC' and 5'-GCTGATCCAC-ATCTGCTGGA-3'. The relative values for HSF1 and Bis mRNA was calculated after normalization to the Ct value from β-actin in the same sample using the ddCt method [30].

Protein analysis

Cells were lysed with RIPA buffer (150 mM NaCl, 1% NP-40, 0.5% sodium deoxycholate, 0.1% sodiumdodecyl sulphate, 50 mM Tris-HCl pH 8.0) with protease inhibitor (Roche Diagnostics) on ice for 30 min. Equal amounts of protein were separated on 10% sodiumdodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to polyvinylidene difluoride membranes (Millipore). The membranes were incubated for 1 h with 5% dry skim milk in TBST (20 mM Tris, 137 mM NaCl, 0.1% Tween 20) buffer and then incubated with antibodies against Bis [1], HSF1 (Cell Signaling), or beta-actin (Sigma-Aldrich). After incubation with horseradish peroxidase-conjugated anti-mouse or anti-rabbit IgG (1 : 5,000; Santa Cruz Biotechnology), the immunoreactive bands were visualized by an enhanced chemiluminescence substrate (Thermo Fisher Scientific). Quantification for the intensities of each band was carried out on Multi Gauge 2.2 software (Fuji Photo Film Co.).

Small interfering RNA (siRNA)

Suppression of Bis or HSF1 expression was performed by transfection of specific siRNA targeted for Bis (5'-AAGGUUCAGACCAUCUUGGAA-3') or HSF1 (5'-AAGGUUCAGACCAUCUUGGAA-3') with G-fectin (Genolution Pharmaceuticals) according to manufacturer's instruction. AccuTarget[™] Control siRNA (Bioneer, 5'-CCUACGCCACC-AAUUUCGU-3) was used for negative control.

Luciferase assay

The 5'-flanking region of human bis genomic DNA with various length was amplified by PCR from HEK 293 genomic DNA using different distal primers and proximal primers, ranging -1080 and +289 (+1 represents the transcription initiation site) and subcloned into the reporter plasmid pGL4 (Promega). After transfection into HK-2 cells with Fugene 6 (Roche Applied Science), the luciferase activity was determined using the Dual-Luciferase reporter assay system (Promega). After normalization with renilla ac-

tivity, the luciferase activities are presented as fold change relative to the normalized firefly luciferase activity in cells transfected with pBis-1(-1080/+289) without oxidative stress, which was taken as 1.0.

ROS determination

ROS accumulation in HK-2 cells following H₂O₂ treatment was determined using the oxidation sensitive fluorescent probe 2',7'-dichlorofluorescein diacetate (DCF-DA, Molecular Probes). This assay is based on the principle that the nonpolar, nonionic H₂-DCFDA crosses cell membranes and is enzymatically hydrolyzed into nonfluorescent H₂-DCF by intracellular esterases. In the presence of ROS, H₂-DCF is rapidly oxidized to become highly fluorescent DCF. At the indicated time points, cells were washed three times with phosphate buffered saline, incubated for 10 min with 10 μ M of DCF-DA, and then washed twice with PBS. Quantification of ROS levels were performed by flow cytometry analysis (BD FACSCalibur; BD Bioscience) with excitation at 488 nm and emission at 525 nm. Mean fluorescence intensities were obtained by histogram statistics using the BD FACSDiva software (BD Bioscience).

Statistics

Student's *t*-tests were employed in order to compare the

differences between two different groups. *p* value of <0.05 was considered statistically significant.

RESULTS

Bis expression is up-regulated by oxidative stress at transcriptional level

We previously reported that Bis expression was induced by oxygen-glucose deprivation in C6 glioma cells, as evidenced by a western blot assay [29]. To examine whether oxidative stress-induced Bis expression was restricted only to glioma-derived cells, we determined Bis expression levels after a H₂O₂ treatment in HK-2 cells, human kidney proximal cells. As shown in Fig. 1A, treatment of the HK-2 cells with 50 μ M of H₂O₂ for 3 h resulted in an increase in Bis expression at protein level by about 3.4-fold, which was further increased after additional incubation in normal media in a time dependent manner, showing an increase of about 4.6-fold after 6 h. Quantitative real time PCR assays revealed that Bis transcripts levels were also increased by exposure to H₂O₂ by about 3.3-fold, and then further increased to 4.8-fold after incubation for 6 h in normal media, with a similar pattern of protein levels (Fig. 1B). The increase in Bis expression in recovery or reperfusion period was verified in A172 glioma cells which were exposed to

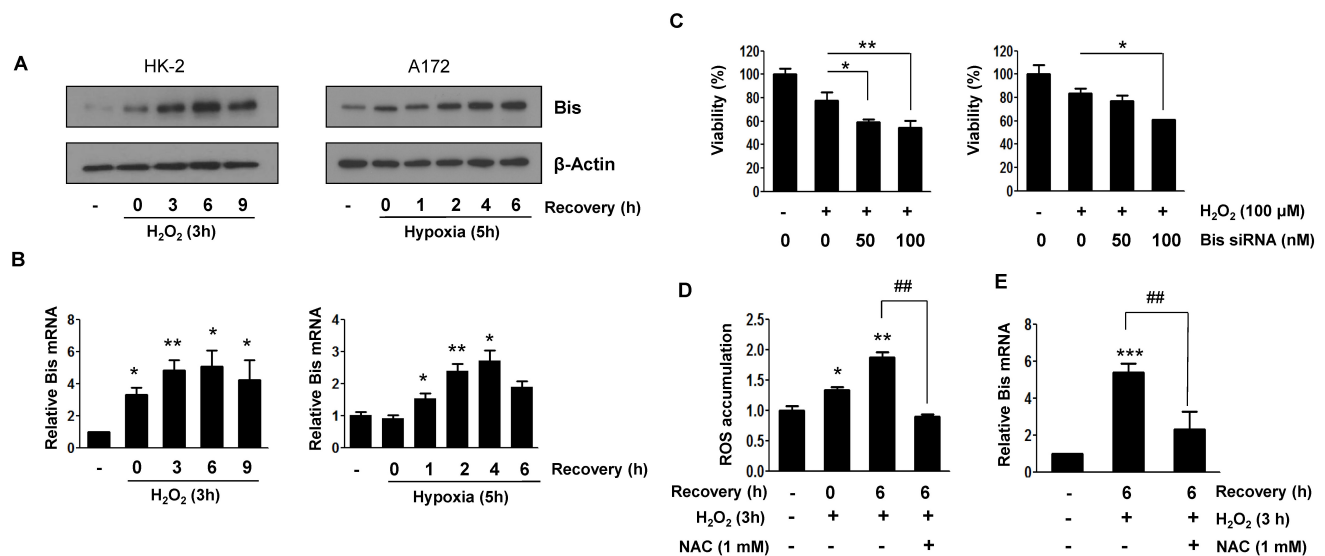


Fig. 1. Induction of Bis expression by oxidative stress in HK-2 and A172 cells. (A) Western blot assay for Bis expression in HK-2 (left column) and A172 (right column) cells after exposure to H₂O₂ and OGD, respectively, and then incubated in the normal medium for indicated times. Beta-actin expression was used as a loading control. (B) The relative levels of Bis mRNA from HK-2 (left column) or A172 (right column) cells at the indicated conditions were determined by quantitative real time RT-PCR analysis after normalizing with beta-actin mRNA level in the same sample. The mean values from three independent experiments are shown with SD. The value from the control cells before exposure to oxidative stress is designated as 1.0. **p*<0.05 and ***p*<0.01 vs. the value from control cells. (C) HK-2 (left column) or A172 (right column) cells were transfected with control or Bis specific siRNA with indicated doses for 48 h and then treated with 100 μ M of H₂O₂ for 3 h followed by additional 6 h in normal medium. The relative cell viability was determined using MTT assay as described in METHODS section. Values from triplicate experiments are provided as mean \pm SE. **p*<0.05 and ***p*<0.01 vs. the value from control cells treated only with H₂O₂. (D) ROS accumulation was determined in HK-2 cells after treatment of H₂O₂ by measuring DCF-DA fluorescence intensity using flow cytometric analysis. 1 mM of NAC was pretreated before exposure to H₂O₂. Fold changes in the mean from three experiments are provided as mean \pm SE. **p*<0.05 and ***p*<0.01 vs. the value from control cells, ###*p*<0.01 vs. the value in the absence of NAC. (E) Effect of NAC on the induction of Bis mRNA was determined by pretreatment of NAC prior to H₂O₂ treatment and then Bis mRNA level was determined as in (B). The mean values from four independent experiments are present with SD. ****p*<0.001 vs. the value from control cells, ###*p*<0.01 vs. the value in the absence of NAC.

OGD and subsequently allowed to recover in normal media supplemented with 5% O₂ (Fig. 1A and B). It thus appears that the signals for Bis induction are persistently activated even after oxidative stress source is eliminated. To define the physiological significance of Bis induction by oxidative stress, we evaluated the susceptibility to cell death induced by H₂O₂ following Bis silencing. Bis depletion resulted in the significant decrease of the relative viability both in HK-2 and A172 cells : 84% and 78% were decreased to 61% and 55% by Bis depletion with 100 nM of Bis siRNA in HK-2 and A172 cells, respectively (Fig. 1C). Thus, the induction of Bis upon oxidative stress might exhibit pro-survival activity to protect cells from entering apoptosis program. As determined by DCF-DA staining and subsequent FACS analysis, cellular ROS levels were higher in the recovery period than in H₂O₂ exposure period (Fig. 1D). Furthermore, the pretreatment with NAC, an antioxidant, significantly repressed ROS levels as well as Bis mRNA levels (Fig. 1D and E), indicating that ROS accumulation directly stimulates Bis expression at the transcriptional level.

Silencing HSF1 suppressed Bis expression and ROS accumulation upon oxidative stress

Several types of stress such as heat shock and proteasome inhibitor have been reported to stimulate Bis expression through activation of HSF1 and subsequent its interaction

with HSEs in the promoter of bis gene [22,23]. To investigate if oxidative stress-induced Bis induction is also mediated by the activation of HSF1, we investigated the effect of HSF1 siRNA on Bis induction upon a H₂O₂ treatment. As shown in Fig. 2A, the transfection of HSF1 siRNA effectively suppressed HSF1 protein expression. Silencing HSF1 had no effect on the basal expression of Bis at the transcriptional level as well as the protein level. However, H₂O₂-induced Bis expression was decreased in HSF1-silenced cells. The increase in Bis expression by H₂O₂ in HSF1-silenced cells was 2.3-fold and 3.5-fold at protein and the mRNA levels, respectively, which correspond to 63% and 48% of the control cells. To define the physiological significance of Bis induction mediated by ROS and HSF1, we measured ROS levels after a H₂O₂ treatment in control or HSF1 siRNA-treated HK-2 cells. As shown in Fig. 2C, the transfection of HSF1 siRNA had no effect on basal ROS levels. Treatment with H₂O₂ resulted in an increase in ROS accumulation by about 2.7-fold, which was further increased the 3.4-fold by HSF1 silencing. Thus, the HSF1 deficiency appeared to be associated with anti-oxidant defense, probably via the regulation of Bis expression.

HSF1 is involved in Bis expression upon oxidative stress

It has been reported that three putative HSEs are present in the promoter of the Bis gene [22,31]. To define which region of the Bis gene promoter is responsible for oxidative stress-mediated Bis induction, we prepared a series of deletion constructs of the Bis gene promoter for use in a reporter assay (Fig. 3A). pBis-1, a luciferase plasmid containing all the three putative HSEs, showed similar time course profiles for the induction of luciferase activity with an increase in the endogenous Bis transcripts, showing a peak activity of about a 5-fold increase at 6 h after recovering from the H₂O₂ treatment. Thus, oxidative stress induced Bis expression can primarily be attributed to the activation of promoter activity, resulting in an increase in the transcripts level. The basal luciferase activity of pBis-2, which contain two HSEs (H1 and H2), was 1.5-fold higher compared to pBis-1. In addition, the transcriptional induction of pBis-2 by oxidative stress was also greater than pBis-1, by about 7-fold and 5-fold, respectively. Further deletion of HSE2 (pBis-3) or HSE1(pBis-4) resulted in a marked reduction in basal activity of 9 and 15% of pBis-1, while partially restored oxidative stress-responsive ability by about 2-fold leading to 16 and 33% of pBis-1, respectively. Finally, as observed in endogenous Bis induction, HSF1 silencing clearly suppressed the transcriptional activation of pBis-2 upon H₂O₂ exposure, from 2.6-fold to 0.8-fold (Fig. 3D). Taken together, these results suggest that both HSE1 and HSE2 are required for basal expression as well as oxidative stress-inducible expression of Bis.

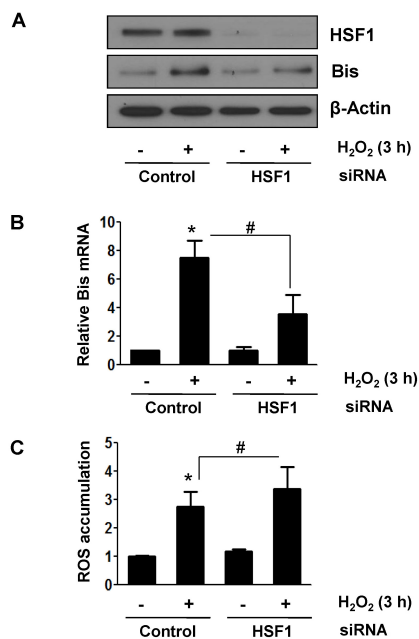


Fig. 2. HSF1 suppressed induction of Bis mRNA upon oxidative stress. HK-2 cells were transfected with control or HSF1-specific siRNA and incubated with H₂O₂ for 3 h followed by additional 6 h in normal medium. HSF1 and Bis expressions were evaluated by Western assay (A) and by real-time RT-PCR (B) as in Fig. 1. (C) Effect of downregulation of HSF1 on ROS accumulation upon H₂O₂ treatment was examined by DCF-DA staining and FACS analysis. Data are presented with fold changes in the mean intensities from three independent experiments with SE. *p<0.05 vs. the value from control cells, #p<0.05 vs. the value from the control siRNA-treated cells.

DISCUSSION

The findings of the present study demonstrate that Bis expression is increased at the transcriptional level in response to oxidative stress such as H₂O₂ and OGD. Various types of stress, such as heat shock or proteasome inhibitors have been reported to increase Bis expression through HSF1 activation and subsequent interaction with HSEs that are located in the bis gene promoter [22,23]. Our result

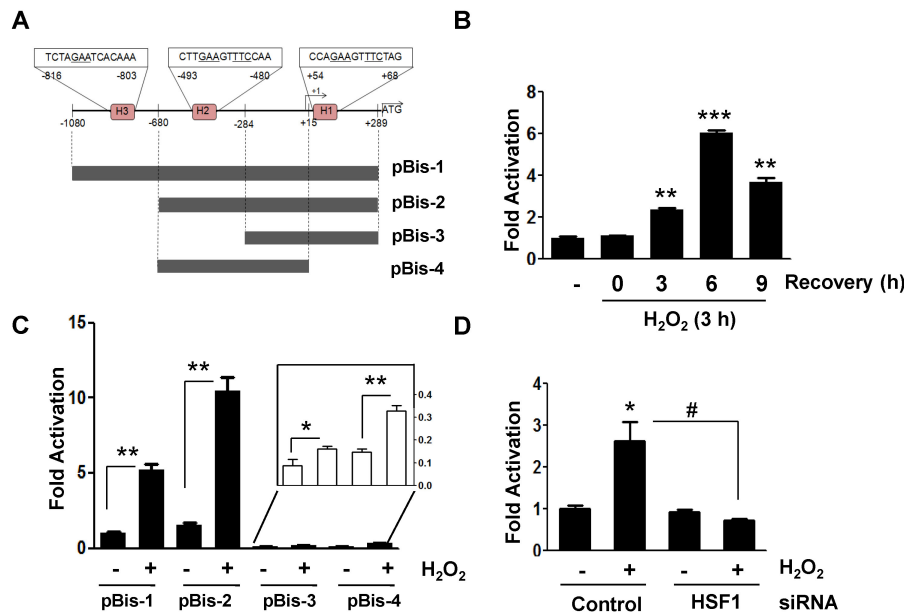


Fig. 3. Identification of oxidative stress-responsive region in the Bis promoter (A) Schematic diagram of the deletion mutants for proximal region of Bis promoter, which were cloned into pGL3 basic vector. The relative position to the transcription site (+1) and the locations of putative HSE (H) are shown. (B) Transcriptional activation of pBis-1 was determined at the indicated time points following treatment of H₂O₂ after normalization with renilla activity. Luciferase activity before exposure to H₂O₂ is designated at 1.0. The mean value from triplicate experiments are present with SD. **p<0.01 and ***p<0.001 vs. the value from control cells. (C) Fold activation of various deletion mutants of Bis promoter in response to H₂O₂ treatment was shown. The luciferase activity of pBis-1 before exposure to H₂O₂ is designated at 1.0. The values for pBis-3 and pBis-4 were provided as a magnified graph (inlet). The mean values from triplicate experiments are present with SD. *p<0.05 and **p<0.01 vs. the value from H₂O₂-untreated cells in each construct. (D) Effect of HSF1 knockdown on the expression of pBis-2 was shown. Expression of HSF1 was suppressed by transfection of HSF1 specific siRNA for 24 h and then HK-2 cells were transfected with pBis-2 construct as described in Materials and Methods section. Fold change in luciferase activity compared with that of control HK-2 cells are presented as mean value with SD. *p<0.05 vs. the value from H₂O₂-untreated control cells, #p<0.05 vs. the value from the control siRNA-treated cells.

also show that the silencing of HSF1 significantly suppressed the induction of endogenous Bis mRNA as well as luciferase activity derived pBis-2 which include two HSEs upon H₂O₂ treatment, suggesting that HSF1 is involved in the activation of the bis gene promoter upon exposure to oxidative stress (Figs. 1 and 3). It should be noted that the H₂O₂-mediated increase of endogenous Bis mRNA was inhibited in 48% of control cells upon HSF1 siRNA while the induction of luciferase activity derived from pBis-2 was completely inhibited by HSF1 siRNA. As a possible explanation for this discrepancy, in addition to HSEs, another cis-element located outside the sequences that was used in the reporter assay, appears to participate in the activation of Bis gene transcription in response to oxidative stress. It is noteworthy that, the effect of regulatory elements in the coding region, if present, on the promoter activity should be excluded in the reporter assay. Furthermore, the Bis protein is reported to auto-regulate its own promoter in glia cells in a positive feedback manner [26]. Thus, it is likely that the activity of the Bis gene promoter in cells represents the net effects contributed from the interactions of individual cis-elements and the corresponding trans-acting factors, such as HSF1, Bis or other unknown factors.

We, and other groups, have reported that Bis expression is increased by oxidative stress or pro-oxidants, both *in vivo* and *in vitro* [20,27,28]. The physiological significance of Bis induction upon PDTC or HNE was verified by the knock-down of HSF1 [20,21], showing that HSF1-mediated Bis ex-

pression confers cellular protection from pro-apoptotic and inflammatory stress. In the present study, we observed that the knockdown of HSF1 increased ROS accumulation upon H₂O₂ treatment (Fig. 2C), suggesting that HSF1 is involved in cellular homeostasis for oxidative status via the modulation of Bis expression. In glioma cells, we also observed that the knockdown of Bis increases susceptibility to cell death upon OGD [29]. In addition, we recently demonstrated that diabetic nephropathy was aggravated in bis heterozygote (bis^{+/-}) mice by increased ROS accumulation and apoptosis in kidney tubular and mesangial cells, which was accompanied by an impaired induction in SOD activity [32]. Thus, a HSF1 deficiency or inactivation may result in a significant impairment in the antioxidant system, through the modulation of Bis expression and subsequent SOD. However, the survival of neuronal cells of the hippocampus following neonatal hypoxia-reperfusion was higher in bis-deleted (bis^{-/-}) mice than in the wild type (bis^{+/+}) mice, possibly because of the modulation of galectin 3 levels [28]. Taken together, these results suggest that the modulation of Bis expression resulted in diverse effects related to cell survival depending on the cellular contexts.

HSF1 has been known to orchestrate the transcriptional activation of heat shock proteins to protect cells from severe stresses by control of protein structures for proper folding for essential target proteins [33,34]. Bis was previously shown to be induced by heat shock, heavy metal as well as proteasome inhibitor, in addition to oxidative stress [16,

23,35]. Furthermore, Bis was shown to play an important role in protein quality control as a co-chaperone and as an activator for macroautophagy by aggresomal targeting of mis-folded proteins [2,9-11,36]. Thus, the ability of HSF1 in the activation of Bis implicates that, under various stresses including aggregation-prone stressor, HSF1-mediated regulation of Bis levels might determine the cellular fates by affecting the proper folding or solubility of specific proteins which are essential for cell viability.

In conclusion, Bis expression was found to be induced by oxidative stress at the transcriptional level. Although HSF1 is an important regulator of Bis induction in oxidative stress as in other types of stress, further studies will be required to define the role of other cis elements in the regulation of constitutive and inducible Bis expression.

ACKNOWLEDGEMENTS

This research was supported by Basic Science Research Programs through the National Research Foundation of Korea (NRF), funded by the Minister of Education, Science and Technology (2012R1A1A2007589 and 2012R1A5A2047939).

REFERENCES

- Lee JH, Takahashi T, Yasuhara N, Inazawa J, Kamada S, Tsujimoto Y. Bis, a Bcl-2-binding protein that synergizes with Bcl-2 in preventing cell death. *Oncogene*. 1999;18:6183-6190.
- Takayama S, Xie Z, Reed JC. An evolutionarily conserved family of Hsp70/Hsc70 molecular chaperone regulators. *J Biol Chem*. 1999;274:781-786.
- Doong H, Price J, Kim YS, Gasbarre C, Probst J, Liotta LA, Blanchette J, Rizzo K, Kohn E. CAIR-1/BAG-3 forms an EGF-regulated ternary complex with phospholipase C-gamma and Hsp70/Hsc70. *Oncogene*. 2000;19:4385-4395.
- Doong H, Rizzo K, Fang S, Kulpa V, Weissman AM, Kohn EC. CAIR-1/BAG-3 abrogates heat shock protein-70 chaperone complex-mediated protein degradation: accumulation of poly-ubiquitinated Hsp90 client proteins. *J Biol Chem*. 2003;278:28490-28500.
- Kassis JN, Guancial EA, Doong H, Virador V, Kohn EC. CAIR-1/BAG-3 modulates cell adhesion and migration by downregulating activity of focal adhesion proteins. *Exp Cell Res*. 2006;312:2962-2971.
- Suzuki M, Iwasaki M, Sugio A, Hishiya A, Tanaka R, Endo T, Takayama S, Saito T. BAG3 (BCL2-associated athanogene 3) interacts with MMP-2 to positively regulate invasion by ovarian carcinoma cells. *Cancer Lett*. 2011;303:65-71.
- Fontanella B, Birolo L, Infusini G, Cirulli C, Marzullo L, Pucci P, Turco MC, Tosco A. The co-chaperone BAG3 interacts with the cytosolic chaperonin CCT: new hints for actin folding. *Int J Biochem Cell Biol*. 2010;42:641-650.
- Iwasaki M, Tanaka R, Hishiya A, Homma S, Reed JC, Takayama S. BAG3 directly associates with guanine nucleotide exchange factor of Rap1, PDZGEF2, and regulates cell adhesion. *Biochem Biophys Res Commun*. 2010;400:413-418.
- Carra S, Seguin SJ, Lambert H, Landry J. HspB8 chaperone activity toward poly(Q)-containing proteins depends on its association with Bag3, a stimulator of macroautophagy. *J Biol Chem*. 2008;283:1437-1444.
- Gamerding M, Hajieva P, Kaya AM, Wolfrum U, Hartl FU, Behl C. Protein quality control during aging involves recruitment of the macroautophagy pathway by BAG3. *EMBO J*. 2009;28:889-901.
- Crippa V, Sau D, Rusmini P, Boncoraglio A, Onesto E, Bolzoni E, Galbiati M, Fontana E, Marino M, Carra S, Bendotti C, De Biasi S, Poletti A. The small heat shock protein B8 (HspB8) promotes autophagic removal of misfolded proteins involved in amyotrophic lateral sclerosis (ALS). *Hum Mol Genet*. 2010;19:3440-3456.
- Seo YJ, Jeon MH, Lee JH, Lee YJ, Youn HJ, Ko JH, Lee JH. Bis induces growth inhibition and differentiation of HL-60 cells via up-regulation of p27. *Exp Mol Med*. 2005;37:624-630.
- Yoon JS, Lee MY, Lee JS, Park CS, Youn HJ, Lee JH. Bis is involved in glial differentiation of p19 cells induced by retinoic acid. *Korean J Physiol Pharmacol*. 2009;13:251-256.
- De Marco M, Turco MC, Rosati A. BAG3 protein is induced during cardiomyoblast differentiation and modulates myogenin expression. *Cell Cycle*. 2011;10:850-852.
- Zhu H, Liu P, Li J. BAG3: a new therapeutic target of human cancers? *Histol Histopathol*. 2012;27:257-261.
- Pagliuca MG, Lerosé R, Cigliano S, Leone A. Regulation by heavy metals and temperature of the human BAG-3 gene, a modulator of Hsp70 activity. *FEBS Lett*. 2003;541:11-15.
- Chen L, Wu W, Dentchev T, Zeng Y, Wang J, Tsui I, Tobias JW, Bennett J, Baldwin D, Dunaief JL. Light damage induced changes in mouse retinal gene expression. *Exp Eye Res*. 2004;79:239-247.
- Tabuchi Y, Ando H, Takasaki I, Feril LB Jr, Zhao QL, Ogawa R, Kudo N, Tachibana K, Kondo T. Identification of genes responsive to low intensity pulsed ultrasound in a human leukemia cell line Molt-4. *Cancer Lett*. 2007;246:149-156.
- Wang HQ, Liu HM, Zhang HY, Guan Y, Du ZX. Transcriptional upregulation of BAG3 upon proteasome inhibition. *Biochem Biophys Res Commun*. 2008;365:381-385.
- Jacobs AT, Marnett LJ. HSF1-mediated BAG3 expression attenuates apoptosis in 4-hydroxynonenal-treated colon cancer cells via stabilization of anti-apoptotic Bcl-2 proteins. *J Biol Chem*. 2009;284:9176-9183.
- Song S, Kole S, Precht P, Pazin MJ, Bernier M. Activation of heat shock factor 1 plays a role in pyrrolidine dithiocarbamate-mediated expression of the co-chaperone BAG3. *Int J Biochem Cell Biol*. 2010;42:1856-1863.
- Franceschelli S, Rosati A, Lerosé R, De Nicola S, Turco MC, Pascale M. Bag3 gene expression is regulated by heat shock factor 1. *J Cell Physiol*. 2008;215:575-577.
- Du ZX, Zhang HY, Meng X, Gao YY, Zou RL, Liu BQ, Guan Y, Wang HQ. Proteasome inhibitor MG132 induces BAG3 expression through activation of heat shock factor 1. *J Cell Physiol*. 2009;218:631-637.
- Gentilella A, Passiatore G, Deshmane S, Turco MC, Khalili K. Activation of BAG3 by Egr-1 in response to FGF-2 in neuroblastoma cells. *Oncogene*. 2008;27:5011-5018.
- Li C, Li S, Kong DH, Meng X, Zong ZH, Liu BQ, Guan Y, Du ZX, Wang HQ. BAG3 is upregulated by c-Jun and stabilizes JunD. *Biochim Biophys Acta*. 2013;1833:3346-3354.
- Gentilella A, Khalili K. Autoregulation of co-chaperone BAG3 gene transcription. *J Cell Biochem*. 2009;108:1117-1124.
- Lee MY, Kim SY, Shin SL, Choi YS, Lee JH, Tsujimoto Y, Lee JH. Reactive astrocytes express bis, a bcl-2-binding protein, after transient forebrain ischemia. *Exp Neurol*. 2002;175:338-346.
- Cho KO, Lee KE, Youn DY, Jeong KH, Kim JY, Yoon HH, Lee JH, Kim SY. Decreased vulnerability of hippocampal neurons after neonatal hypoxia-ischemia in bis-deficient mice. *Glia*. 2012;60:1915-1929.
- Jung SE, Kim YK, Youn DY, Lim MH, Ko JH, Ahn YS, Lee JH. Down-modulation of Bis sensitizes cell death in C6 glioma cells induced by oxygen-glucose deprivation. *Brain Res*. 2010;1349:1-10.
- Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. *Methods*. 2001;25:402-408.
- Wu C. Heat shock transcription factors: structure and regulation. *Annu Rev Cell Dev Biol*. 1995;11:441-469.
- Lim JH, Youn DY, Yoo HJ, Yoon HH, Kim MY, Chung S, Kim YS, Chang YS, Park CW, Lee JH. Aggravation of diabetic nephropathy in BCL-2 interacting cell death suppressor (BIS)-haploinsufficient mice together with impaired induction of superoxide dismutase (SOD) activity. *Diabetologia*. 2014;57:

- 214-223.
33. **Ankar J, Sistonen L.** Regulation of HSF1 function in the heat stress response: implications in aging and disease. *Annu Rev Biochem.* 2011;80:1089-1115.
 34. **Gidalevitz T, Prahlad V, Morimoto RI.** The stress of protein misfolding: from single cells to multicellular organisms. *Cold Spring Harb Perspect Biol.* 2011;3. pii: a009704.
 35. **Liao Q, Ozawa F, Friess H, Zimmermann A, Takayama S, Reed JC, Kleeff J, Büchler MW.** The anti-apoptotic protein BAG-3 is overexpressed in pancreatic cancer and induced by heat stress in pancreatic cancer cell lines. *FEBS Lett.* 2001;503: 151-157.
 36. **Gamerding M, Kaya AM, Wolfrum U, Clement AM, Behl C.** BAG3 mediates chaperone-based aggresome-targeting and selective autophagy of misfolded proteins. *EMBO Rep.* 2011;12: 149-156.