Biochemical and Biophysical Research Communications 434 (2013) 861–866

Contents lists available at SciVerse ScienceDirect

Biochemical and Biophysical Research Communications

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

Testicular hyperthermia induces Unfolded Protein Response signaling activation in spermatocyte

Jung-Hak Kim [a,1], Sun-Ji Park [a,1], Tae-Shin Kim [b], Hyo-Jin Park [a], Junghyung Park [a], Bo Kyung Kim [a], Gyeong-Ryul Kim [a], Jin-Man Kim [c], Song Mei Huang [c], Jung-Il Chae [d], Choon-Keun Park [e], Dong-Seok Lee [a,*]

[a] College of Nature Sciences, Kyungpook National University, Daegu 702-701, Republic of Korea
[b] Department of Reproductive Medicine, University of California, San Diego, 9500 Gilman Drives, La Jolla, CA 92093, USA
[c] Cancer Research Institute and Department of Pathology, College of Medicine, Chungnam National University, Daejeon, Republic of Korea
[d] Department of Oral Pharmacology, School of Dentistry and Institute of Oral Bioscience, BK21 Project, Chonbuk National University, Jeonju, Republic of Korea
[e] Division of Animal Biotechnology, College of Animal Life Science, Kangwon National University, Chuncheon 200-701, Republic of Korea

ARTICLE INFO

Article history:
Received 28 March 2013
Available online 20 April 2013

Keywords:
Testicular hyperthermia
Unfolded Protein Response signaling pathway
ER stress
Spermatocyte
Apoptosis

ABSTRACT

The testes of most mammals are sensitive to temperature. To survive and adapt under conditions that promote endoplasmic reticulum (ER) stress such as heat shock, cells have a self-protective mechanism against ER stress that has been termed the "Unfolded Protein Response" (UPR). However, the cellular and molecular events underlying spermatogenesis with testicular hyperthermia involved in the UPR signaling pathway under ER stress remain poorly understood. In the present study, we verified that UPR signaling via phospho-eIF2α/ATF4/GADD34, p90ATF6, and phospho-IRE1α/XBP-1 is activated with testicular hyperthermia (43 °C, 15 min/day) and induced ER stress-mediated apoptosis associated with CHOP, phospho-JNK, and caspase-3 after repetitive periods of hyperthermia. Levels of phospho-eIF2α protein of mouse spermatocytes in the testis were rapidly increased by one cycle of testicular hyperthermia. ATF4/GADD34 and p90ATF6 expression gradually increased and decreased, respectively, with repetitive cycles of hyperthermia. Spliced XBP1 mRNA as a marker of IRE1 activity was increased after one, three cycles of hyperthermia and decreased by five cycles of hyperthermia. Although the levels of anti-apoptotic phospho-JNK (p54) were gradually decreased after three cycles of hyperthermia, CHOP expression was rapidly increased. After five cycles of testicular hyperthermia, the levels of cleaved caspase-3 and TUNEL-positive apoptotic spermatocytes cells were significantly increased. Our data demonstrated that testicular hyperthermia induces UPR signaling and repetitive cycles of hyperthermia lead to apoptosis of spermatocytes in mouse testis. These results suggest a link between the UPR signaling pathway and testicular hyperthermia.

1. Introduction

The testes of most mammals are sensitive to temperature. Normal spermatogenesis requires a lower temperature than that of the abdomen [1], and exposure of the testis to body temperature or above results in increased germ cell death [2]. On the other hand, mild testicular hyperthermia has been established as a safe and reversible approach for suppressing spermatogenesis [3]. These finding suggest the theoretical viability of testicular hyperthermia as a reversible method of male contraception in humans. However, the cellular and molecular events underlying the effect of testicular hyperthermia on germ cells remain poorly understood.

The endoplasmic reticulum (ER) is an important organelle required for cell survival and development [4]. ER stress may be caused by various environmental stresses such as heat shock [5]. To survive and adapt under ER stress condition, cells have a self-protective mechanism against ER stress that has been termed the "Unfolded Protein Response" (UPR) [6]. The UPR of mammalian cells is initiated by three ER transmembrane proteins: activating transcription factor 6 (ATF6), inositol-requiring enzyme 1 (IRE1) and protein kinase-like ER kinase (PERK). These factors act as proximal sensors of ER stress. Under normal conditions, the luminal domains of these sensors are occupied by the ER chaperone protein Grp78/Bip. In the presence of ER stress, sequestration of Grp78/Bip by unfolded proteins activates these sensors by inducing the phosphorylation and homodimerization of IRE1α and PERK/eIF2α as well as relocalization of ATF6 to the Golgi where it is cleaved by S1P and S2P, leading to activation as a transcriptional factor. The UPR is fundamentally a cytoprotective response, but

* Corresponding author. Fax: +82 53 943 6925.
E-mail address: lee1@knu.ac.kr (D.-S. Lee).
[1] The first two authors contributed equally to this work.

0006-291X/$ - see front matter Crown Copyright © 2013 Published by Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.bbrc.2013.04.032

an excessive or prolonged UPR can result in cell death, predominantly by the induction of apoptosis [7,8]. CHOP, JNK, and caspase-12 have been implicated in apoptotic signaling in response to ER stress. In mice, procaspase-12 is cleaved and activated specifically upon ER stress [9–11]. The activation of caspase-12, an ER resident protein, causes activation of cytoplasmic caspase-3 during ER stress-induced apoptosis.

The objective of this study was to verify whether the UPR signaling pathway is involved in the effects of testicular hyperthermia. In addition, we determined whether repetitive cycles of testicular hyperthermia induce an excessive UPR and promote severe ER stress, thereby leading to apoptosis in mouse testis. We speculated that the present study would suggest a link between UPR signaling and testicular hyperthermia.

2. Material and methods

2.1. Animals

Male C57/BL6 mice (9–10 weeks of age) were purchased from Central Animal Laboratory (Korea) and maintained in accordance with the institutional guidelines of the Institutional Animal Care and Use Committee of the Korea Research Institute of Bioscience and Biotechnology (KRIBB, Korea).

2.2. Induction of testicular hyperthermia

To induce testicular hyperthermia, mice were subjected to one or three or five cycles of hyperthermia at 43 °C for 15 min per day. After the heat treatment, the animals were dried, returned to their cages, and allowed to recover from the effect of anesthesia. Animals were then sacrificed 12 h after testicular hyperthermia.

2.3. Western blot analysis

Testes lysates were prepared in ice-cold PRO-PREP™ Protein Extraction Solution (iNtRON, Korea). Testes lysate were separated by 12% SDS–PAGE and transferred onto nitrocellulose membranes (Pall Life Sciences, NY). The blots were incubated with the following antibodies: anti-GRP78/Bip, anti-phospho-eIF2α, anti-phospho-SAPK/JNK, anti-caspase-3 (Cell Signaling, MA), anti-GADD153/CHOP, anti-GADD34, anti-β-actin, anti-caspase-12, anti-ATF6α/p90ATF6, anti-CREB-2/ATF4 (Santa Cruz Biotechnology, CA), anti-phospho-IRE1α (Abcam, MA), and anti-HSP70 (AbFrontier, Korea). Thereafter, HRP-conjugated anti-goat, anti-rabbit, and anti-mouse IgG antibodies (Thermo Scientific, MA) were used as secondary antibodies. The signal was developed using the enhanced chemiluminescence detection method (Advansta, CA).

2.4. Analysis of XBP-1 mRNA splicing

Total RNA was isolated from testis tissues using TRIzol reagent (Invitrogen, CA) according to the manufacturer's instructions. The cDNA was synthesized using 1ug of total RNA and RT-PCR Premix (Bioneer, Korea). PCR was carried out using 2× PCR Premix (Enzynomics, Korea) containing specific primers for splicing XBP-1 (Forward: AAACAGAGTAGCAGCGCAGACTGC, Reverse: TCCTTCTGGGT AGACCTCTGGGAG). The PCR products were digested with *Pst*1 and then separated by electrophoresis in a 2% agarose gel.

2.5. Immunohistochemistry

The testes were fixed in formalin, embedded in paraffin, and cut into 3-μm thick sections. The sections were deparaffinized and briefly heated. The sections were then treated with a protein block solution (Dako, CA) and incubated with antibodies: anti-phospho-eIF2α (Cell Signaling, MA). After washing with 0.1 M TBS containing 0.01% Tween-20 (TBST), the sections were incubated with anti-rabbit polymer (Dako, CA). Peroxidases bound to the antibody complex were visualized by treatment with a 3,3′-diaminobenzidine (DAB) chromogen substrate solution (Dako, CA). The DAB reaction was monitored under a microscope to determine the optimal incubation time and stopped with several washes of 0.1 M TBS. The immunolabeled sections were dehydrated in a graded ethanol series, defatted in xylene, and mounted. The sections were examined with an Olympus BX51 microscope (Olympus, Japan) under a bright field and images were acquired with an Olympus DP 70 camera (Olympus, Japan).

2.6. TUNEL assay

Apoptotic cells in paraffin sections of the testis were subjected to a TUNEL assay using a commercially available kit (Apop-Tag Peroxidase In Situ Apoptosis Detection Kit; Millipore, Inc.) according to the manufacturer's protocol.

2.7. Statistical analysis

All measurements were made in triplicate and all values are presented as the mean ± standard error of the mean (SEM). The results were subjected to a one-way analysis of variance (ANOVA). *P*-values <0.05 were considered significant. All calculations were carried out using the GraphPad Prism 2.0 software package (GraphPad Software, CA). All experiments were independently performed at least three times.

3. Results

3.1. Grp78/Bip is strongly expressed in mouse testis

As a trigger of the Unfolded Protein Response (UPR), activity of Grp78/Bip is controlled by perturbation of protein folding through binding-release of the ER stress transducers PERK/elF2α, ATF6, and IRE1α [12]. It is also well known GADD153/CHOP is an effector of ER stress-mediated apoptosis signaling [13]. In the present study, we performed a Western blot analysis to measure the expression of Grp78/Bip and GADD153/CHOP in testis and other organs such as brain, liver, pancreas, and kidney. As shown in Fig. 1, Grp78/Bip was more strongly expressed in the mouse testes than the other tissues. However, GADD153/CHOP expression was hardly detected. Interestingly, GADD153/CHOP was highly expressed in the liver and kidney. These results suggest that Grp78/Bip, but not GADD153/CHOP, may be involved in physiological regulatory activities in the mouse testis.

3.2. UPR signaling is involved in responses to testicular hyperthermia

Xu et al. reported that hyperthermia in variant cell line activates the ER stress pathway [14]. To survive and adapt under ER stress, cells have developed self-protective mechanisms such as the UPR [6]. Testicular hyperthermia was demonstrated to have a significant effect on testicular functions [15]. However, the detailed molecular mechanism governing responses to testicular hyperthermia remains poorly understood. Therefore, we determined whether activities of factors involved in the UPR, such as PERK/phospho-elF2α, ATF6, and phospho-IRE1α, are induced and altered after one or more (three or five) cycles of testicular hyperthermia once per d. HSP70 expression as used as a control hyperthermia stress marker after one round of testicular hyperthermia. Although

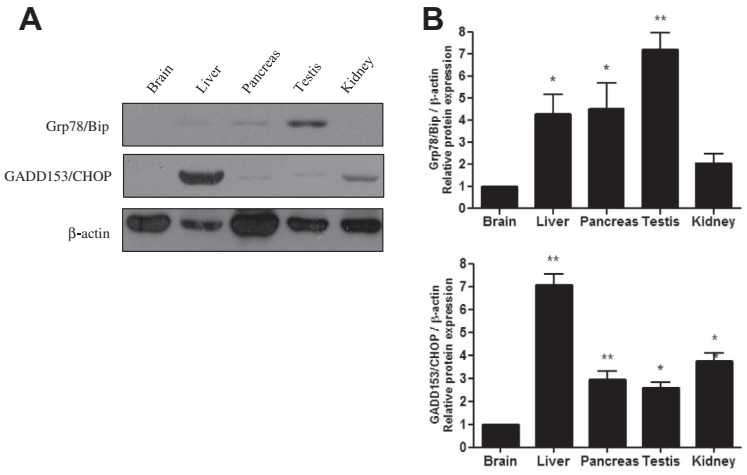

Fig. 1. Expression of ER stress proteins in different murine tissues. (A) Expression of ER stress marker proteins (GRP78/Bip and GADD153/CHOP) in representative organs and testis measured with Western blotting ($n \geqslant 3$). (B) Relative levels of GRP78/Bip and GADD153/CHOP protein expression. $^{**}P < 0.01$ and $^{*}P < 0.05$ compared to brain.

HSP70 expression was initially increased, it was not detected 12 h after one cycle of testicular hyperthermia (Supplemental data Fig. 1A). However, HSP70 expression was continuously increased at 12 h after three cycles of hyperthermia over 3 d (Supplemental data Fig. 1B, and Fig. 2A and C).

As shown in Fig. 1A, Grp78/Bip as a trigger of UPR signaling was already expressed in the mouse testes under control physiological conditions (Fig. 2A). Although Grp78/Bip expression was slightly increased after one cycle of hyperthermia on the first day, the protein levels started to decrease thereafter (Fig. 2A and D). We also measured the levels of phospho-eIF2α, ATF4, and GADD34 protein. As shown in Fig. 2A and E, phospho-eIF2α levels were rapidly increased by one cycle of hyperthermia and then gradually decreased depending on repetitive cycles of testicular hyperthermia. On the other hand, the expression of ATF4 and GADD34 gradually increased by repeated cycles of testicular hyperthermia (Fig. 2A, F, and G). We next measured ATF6 protein levels. After repeated cycles of heat exposure, p90ATF6 expression was gradually decreased (Fig. 2A and H).

Another hallmark of the UPR is XBP1 mRNA splicing by activated IRE1α serving as an endoribonuclease. To evaluate whether repeated testicular hyperthermia is related to activation of IRE1α and alternative splicing of XBP1 mRNA, we performed Western blotting and RT-PCR to detect the phospho-IRE1α and spliced XBP1 mRNA, respectively. As shown in Fig. 2A and I, phospho-IRE1α expression was noticeably increased after one cycle of testicular hyperthermia. Additionally, IRE1α activity was also measured by comparing the amount of spliced XBP1 mRNA according to testicular hyperthermia. Results of the RT-PCR analysis showed that the appearance of spliced XBP1 mRNA increased with one to three cycles of hyperthermia. However, the level of splicing rapidly decreased after five cycles of testicular hyperthermia (Fig. 2B and J). Taken together, these results indicated that UPR signaling via phospho-eIF2α/ATF4/GADD34, p90ATF6, and phospho-IRE1α/

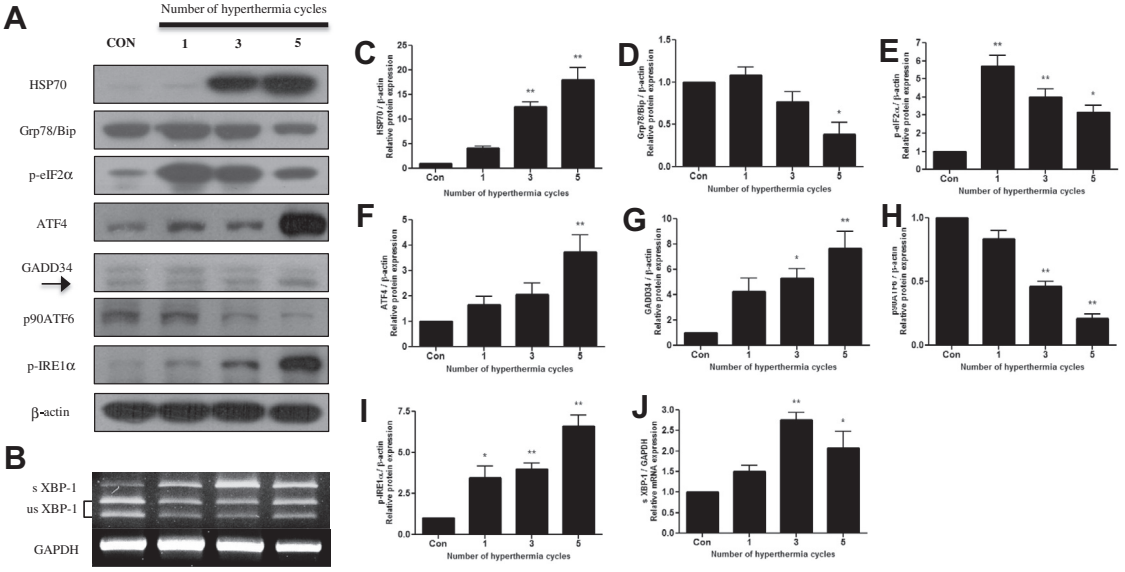

Fig. 2. Testicular hyperthermia induces UPR protein expression. Mouse testes were heated for the indicated number of times. (A) Expression of HSP70, GRP78/Bip, phospho-eIF2α, ATF4, GADD34, p90ATF6, phospho-IRE1α, and β-actin were examined by Western blot analysis. (B) XBP-1 mRNA splicing was evaluated by reverse transcription (RT)-PCR. (C–J) Relative levels of each UPR protein expression are shown. β-Actin was used to demonstrate equal protein loading while GAPDH mRNA ensured that all RNA signals were correctly quantified. $^{**}P < 0.01$ and $^{*}P < 0.05$ compared to the untreated control.

XBP-1 is activated by testicular hyperthermia. In particular, phospho-eIF2α levels were rapidly increased by one round of testicular hyperthermia.

3.3. Repetitive cycles of testicular hyperthermia induce ER stress-mediated apoptosis signaling

Szegezdi et al. reports have mentioned that severe ER stress leads to activation of c-Jun *N*-terminal kinase (JNK) and induction C/EBP homologous protein (CHOP) [16]. ER stress also leads to the activation of caspase-12 [17]. Upon activation, caspase-12 translocates from the ER to cytosol where it in turn activates caspase-3 [18]. In the present study, we determined whether repeated cycles of testicular hyperthermia induced the activity of factors associated with severe ER stress-mediated apoptosis signaling such as CHOP, phospho-JNK, caspase-12, and caspase-3.

As shown in Fig. 3A and B, CHOP expression was rapidly increased from after three cycles of testicular hyperthermia over 3 d. Anti-apoptotic phospho-JNK (p54) levels were slightly increased by one cycle of testicular hyperthermia and then gradually decreased after three cycles (Fig. 3A). In contrast, the levels of pro-apoptotic phospho-JNK (p46) were increased from after three cycles of testicular hyperthermia (Fig. 3A and C). Procapase-12 was cleaved into active caspase-12 after only one cycle of testicular hyperthermia (Fig. 3A and D). Finally, the level of cleaved caspase-3 was significantly increased after five rounds of testicular hyperthermia (Figs. 2D, 3A and E). These results indicated that repetitive cycles of testicular hyperthermia induced ER stress-mediated apoptosis signaling.

3.4. Phospho-eIF2α expression and apoptosis are induced in spermatocytes by repetitive testicular hyperthermia

As shown in Fig. 2, we observed changes in UPR signaling and apoptosis by Western blot analysis. In particular, phospho-eIF2α levels were rapidly increased by one cycle of testicular hyperthermia. However, the localization of phospho-eIF2α expression in mouse testis was unclear. Using immunohistochemistry, we discovered that phospho-eIF2α expression was localized in the spermatocytes after one cycle of hyperthermia (Fig. 4B). In addition, a TUNEL assay showed that apoptotic spermatocytes also appeared after five cycles of hyperthermia (Fig. 4D). This result confirmed that testicular hyperthermia induces UPR signaling and repetitive cycles of hyperthermia promote apoptosis of spermatocytes in mouse testes.

4. Discussion

A number of studies have documented the adverse effects of heat on spermatogenesis in diverse mammal species [2,19,20]. Nevertheless, the cellular and molecular events underlying the effects of testicular hyperthermia and subsequent ER stress on spermatogenesis remain poorly understood.

Data from the present study showed that one cycle of testicular hyperthermia (43 °C for 15 min) induces UPR signaling and repetitive cycles of hyperthermia leads to ER stress-mediated apoptosis of spermatocyte in mouse testes. Interestingly, although the expression of Hsp70 as a control heat exposure stress marker rapidly increased after one round of testicular hyperthermia and disappeared after 12 h, repetitive cycles of hyperthermia led to continuously sustained Hsp70 expression. Hsp70 is specifically related to spermatocyte apoptosis, and the temporal Hsp70 expression is closely associated with increased numbers of TUNEL-positive cells [21]. However, our results demonstrated that Hsp70 expression increased by one cycle of hyperthermia completely disappears after 12 h as evidence of recovery from heat shock (Supplemental data Fig. 1A, and Fig. 2A and C). Consequently, Hsp70 expression may not be related to spermatocytes apoptosis we observed by Western blot analysis. Although we have not identified a mechanism underlying recovery from heat shock, more recovery time is needed for testes subjected to greater numbers of hyperthermia cycles. Therefore, the dramatic increase of Hsp70 expression after heat stress might be responsible for the protection of cells from hyperthermia.

Hsp70 family members like Grp78/Bip have been found to play critical roles in mouse spermatogenesis. In both mouse and human testes, strong Grp78/Bip staining has been observed in pachytene spermatocytes and postmeiotic germ cells but not in spermatogonium or other cell types [22]. Our results also indicated that Grp78/Bip was more strongly expressed in mouse testes compared to other tissues (Fig. 1). These observations indicated that Grp78/Bip may be involved in spermatogenesis. The expression of Grp78/Bip, a key mediator of ER stability, could be an adaptive response evolved in mammals to protect endothelial cells against

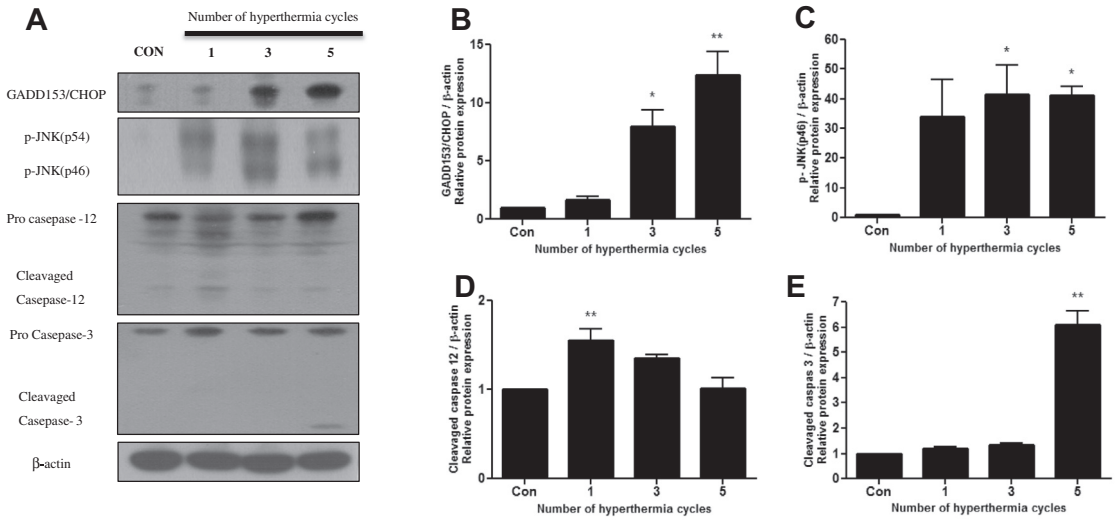

Fig. 3. Testicular hyperthermia-induced ER stress associated with pro-apoptotic proteins expression. Mouse testes were treated with heat for the indicated number of times. (A) Expression of GADD153/CHOP, phospho-JNK, caspase-12, and caspase-3 was measured by Western blot analysis. (B–E) Relative levels of ER stress associated with pro-apoptotic proteins expression are shown. β-actin was used as a loading control. $^{**}P < 0.01$ and $^{*}P < 0.05$ compared to the untreated control.

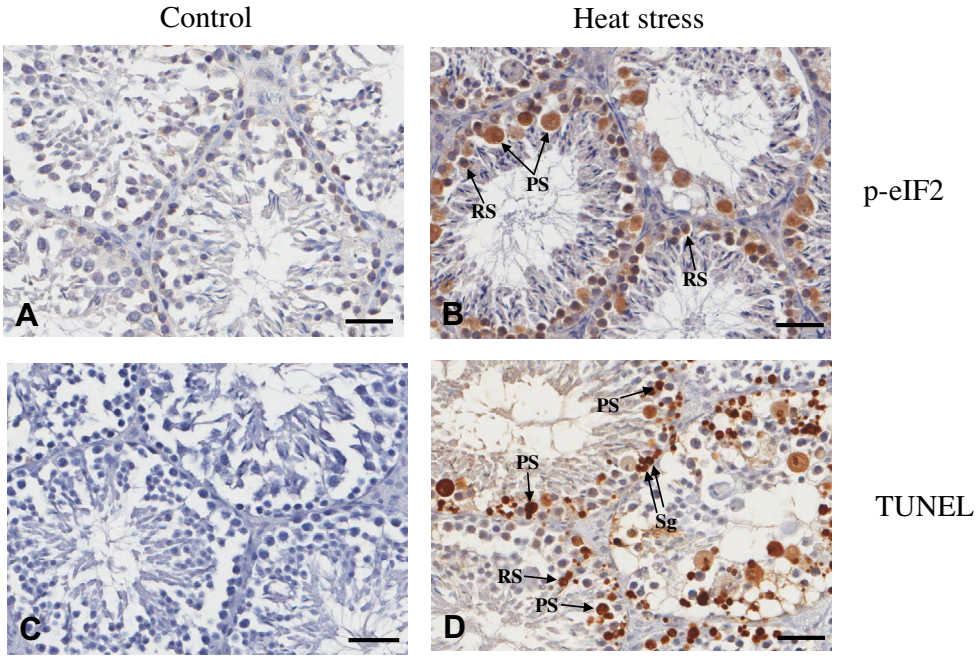

Fig. 4. Testicular hyperthermia induces eIF2α phosphorylation and apoptosis in germ cells. (A) Immunohistochemical staining for phospho-eIF2α was carried out for the control mouse testis and (B) testes exposed to five cycles of hyperthermia. Phospho-eIF2α protein was expressed in spermatocytes. (C) Visualization of TUNEL-positive apoptotic cells in sections of control testis and (D) testes exposed five times to hyperthermia. Scale bar = 20 μm. Sg: spermatogonia, PS: pachytene spermatocyte, RS: round spermatid.

stress-induced cell death [23]. The expression of Grp78/Bip as a trigger of the UPR was remarkably decreased after five cycles of hyperthermia over the course of 5 d (Fig. 2A and D). After this time, it appeared that the survival and adaptive response against repetitive cycles of hyperthermia in testis could not be sustained. It might be induced ER stress-mediated apoptosis signaling such as increased expression of cleavage caspase-3 by decreasing Grp78/Bip (Fig. 3A and E). Taken together, our observations suggested that testes generated adaptive signaling pathways, such as the UPR, in order to promote physiological regulation of mouse testis function in the presence of testicular hyperthermia.

We next evaluated changes in factors associated with three UPR pathways (PERK/phospho-eIF2α, ATF6, and phospho-IRE1α) after repetitive hyperthermia cycles (Fig. 2). In the ER, the transmembrane proteins PERK, IRE1α and ATF6 act as sentinels that sense increasing stress and transduce signal into the cytoplasm and nucleus. Upon activation, PERK phosphorylates the alpha subunit of eIF2α, resulting in translation attenuation, and selectively enhances the translation of transcription factor ATF4 [24]. In parallel, ATF4 expression is selectively enhanced along with the expression of downstream factors such as GADD34 and CHOP/GADD153 that help control the cellular redox status and cell death [25]. A recent study of eIF2α phosphorylation in heat-stressed testes suggests that male germ cells can be regulated by similar molecular mechanisms. These finding are consistent with our results showing that phospho-eIF2α expression rapidly increased after one cycle of hyperthermia (Fig. 2A and E) and was detected in spermatocytes (Fig. 4A). However, these levels gradually decreased along with ATF4 and GADD34 expression with more cycles of testicular hyperthermia (Fig 2A, F, and G). It seems that GADD34 may regulate ATF4, Bip, and CHOP. After five rounds of testicular hyperthermia, apoptotic spermatocytes were also observed with a TUNEL assay (Fig. 4D). Thus, eIF2α phosphorylation may serve as a trigger of cellular survival pathways in male germ cells subjected to testicular hyperthermia. On the other hand, GADD34 might contribute to the induction germ cell apoptosis by decreasing eIF2α phosphorylation.

We next assessed the expression patterns of the ATF6 protein as a representative of the second UPR signaling pathway. ATF6 is a constitutively expressed, ER membrane-anchored transcription factor that is activated by cleavage during ER stress [24]. Our previously generated data demonstrated that hCG-induced ER stress plays important roles in steroidogenic enzyme expression by modulating the ATF6 pathway as well as ER stress-mediated apoptosis in Leydig cells [26]. In the present study, the protein levels of full-length 90-kDa ATF6 (p90ATF6) were gradually reduced depending on the number of heat exposure cycles (Fig. 2A and H). Based on our findings, we were unable to determine whether the ATF6 pathway plays a role in cell survival with testicular hyperthermia. Therefore, we will perform future studies to confirm whether lentivirus-mediated transient expression of p50ATF6 reduces the expression of Hsp70 in the testis.

Another primary hallmark of the UPR is splicing of XBP1 mRNA, which is then translated into a functional transcription factor [27]. IRE1α also induces pro-apoptotic JNK signaling in the presence of ER stress [28]. Our results demonstrated that the amount of spliced XBP1 mRNA increased with elevated phospho-IRE1α expression after one cycle of hyperthermia. However, it seems that spliced XBP1 remarkably decreased by increasing IRE1α phosphorylation at five cycles of testicular hyperthermia (as shown in Fig. 2A, B, I and J). In addition, the level of phospho-JNK increased after three cycles of testicular hyperthermia (Fig. 3A and C). These results imply that IRE1α activation plays a role in cellular survival pathways activated after testicular hyperthermia. However, increasing the number of times the testes are exposed hyperthermia may induce apoptosis. Altogether, our data indicated that UPR signaling via phospho-eIF2α/ATF4/GADD34, p90ATF6, and phospho-IRE1α/XBP-1 is involved in the response to testicular hyperthermia. Although the UPR mediates physiological regulation or homeostasis of the ER, it can also mediate apoptotic signaling pathways under excessive ER stress [29]. In particular, ATF6, IRE1α, and PERK, which are ER transmembrane proteins, serve as mediators of apoptosis due to ER stress [9,30]. CHOP, phospho-JNK, and caspase-3 are activated by PERK/ATF6, IRE1α/TRAF2/ASK1, and caspase-12

respectively, resulting in apoptosis [9]. In the present study, the levels of cleaved caspase-12 and phospho-JNK were slightly increased after one cycle of testicular hyperthermia (Fig. 3A, C and D). CHOP and phospho-JNK expression was increased from after three cycles of testicular hyperthermia (Fig. 3A–C). Finally, the levels of cleaved caspase-3 were significantly increased in mouse testis after five cycles of testicular hyperthermia (Fig. 3A and E). These findings indicate that repetitive cycles of testicular hyperthermia may have induced ER stress-mediated apoptosis signaling and disrupted spermatogenesis. Although it has been reported that testicular hyperthermia is a theoretically viable reversible method of male contraception in humans, our results showed that hot baths (over 43 °C) increase spermatocyte cell death with repetitive cycles of hyperthermia. Therefore, we suggest that frequent hot baths or trips to the sauna may have negative effects on spermatogenesis.

In conclusion, we demonstrated that the UPR signaling pathway can be fully activated or down-regulated in mouse testis as a consequence of hyperthermia. With hyperthermia, the UPR signal pathway may promote the survival and adaptation, but not ER stress-mediated apoptosis, of spermatocytes in mouse testis. However, we found that repetitive cycles of testicular hyperthermia induced ER stress-mediated apoptosis signaling. Our findings will be helpful for better understanding the basic mechanism that regulates spermatogenesis in the presence of testicular hyperthermia.

Acknowledgments

This research was supported by Kyungpook National University Research Fund 2012 and supported by Grant No. 20120000639 from the SRC program, by Grant No. 2012008880 from the National Research Foundation of Korea grant funded by the Korea government, by Grant No. 110056-03-3-HD110 and 112020-03-1-CG000 from the ARPC program of the Korea Institute of Planning and Evaluation for Technology in Food, Agriculture, Forestry and Fisheries.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bbrc.2013.04.032.

References

[1] S. Danno, K. Itoh, T. Matsuda, J. Fujita, Decreased expression of mouse Rbm3, a cold-shock protein, in Sertoli cells of cryptorchid testis, Am. J. Pathol. 156 (2000) 1685–1692.
[2] R. Mieusset, L. Bujan, Testicular heating and its possible contributions to male infertility: a review, Int. J. Androl. 18 (1995) 169–184.
[3] F.R. Kandeel, R.S. Swerdloff, Role of temperature in regulation of spermatogenesis and the use of heating as a method for contraception, Fertil. Steril. 49 (1988) 1–23.
[4] I. Kim, W. Xu, J.C. Reed, Cell death and endoplasmic reticulum stress: disease relevance and therapeutic opportunities, Nat. Rev. Drug Discov. 7 (2008) 1013–1030.
[5] Y. Liu, H. Sakamoto, M. Adachi, S. Zhao, W. Ukai, E. Hashimoto, M. Hareyama, T. Ishida, K. Imai, Y. Shinomura, Heat stress activates ER stress signals which suppress the heat shock response, an effect occurring preferentially in the cortex in rats, Mol. Biol. Rep. 39 (2012) 3987–3993.
[6] J.W. Brewer, J.L. Cleveland, L.M. Hendershot, A pathway distinct from the mammalian unfolded protein response regulates expression of endoplasmic reticulum chaperones in non-stressed cells, EMBO J. 16 (1997) 7207–7216.
[7] M. Schroder, R.J. Kaufman, The mammalian unfolded protein response, Annu. Rev. Biochem. 74 (2005) 739–789.
[8] D. Ron, P. Walter, Signal integration in the endoplasmic reticulum unfolded protein response, Nat. Rev. Mol. Cell Biol. 8 (2007) 519–529.
[9] E. Lai, T. Teodoro, A. Volchuk, Endoplasmic reticulum stress: signaling the unfolded protein response, Physiology (Bethesda) 22 (2007) 193–201.
[10] T. Nakagawa, H. Zhu, N. Morishima, E. Li, J. Xu, B.A. Yankner, J. Yuan, Caspase-12 mediates endoplasmic-reticulum-specific apoptosis and cytotoxicity by amyloid-beta, Nature 403 (2000) 98–103.
[11] T. Nakagawa, J. Yuan, Cross-talk between two cysteine protease families. Activation of caspase-12 by calpain in apoptosis, J. Cell Biol. 150 (2000) 887–894.
[12] A. Bertolotti, Y. Zhang, L.M. Hendershot, H.P. Harding, D. Ron, Dynamic interaction of BiP and ER stress transducers in the unfolded-protein response, Nat. Cell Biol. 2 (2000) 326–332.
[13] S. Oyadomari, M. Mori, Roles of CHOP/GADD153 in endoplasmic reticulum stress, Cell Death Differ. 11 (2004) 381–389.
[14] X. Xu, S. Gupta, W. Hu, B.C. McGrath, D.R. Cavener, Hyperthermia induces the ER stress pathway, PLoS One 6 (2011) e23740.
[15] B.P. Setchell, The Parkes Lecture. Heat and the testis, J. Reprod. Fertil. 114 (1998) 179–194.
[16] E. Szegezdi, S.E. Logue, A.M. Gorman, A. Samali, Mediators of endoplasmic reticulum stress-induced apoptosis, EMBO Rep. 7 (2006) 880–885.
[17] T. Nakagawa, J. Yuan, Cross-talk between two cysteine protease families. Activation of caspase-12 by calpain in apoptosis, J. Cell Biol. 150 (2000) 887–894.
[18] N. Morishima, K. Nakanishi, H. Takenouchi, T. Shibata, Y. Yasuhiko, An endoplasmic reticulum stress-specific caspase cascade in apoptosis. Cytochrome c-independent activation of caspase-9 by caspase-12, J. Biol. Chem. 277 (2002) 34287–34294.
[19] J.W. Hand, H. Walker, S. Hornsey, S.B. Field, Effects of hyperthermia on the mouse testis and its response to X-rays, as assayed by weight loss, Int. J. Radiat. Biol. Relat. Stud. Phys. Chem. Med. 35 (1979) 521–528.
[20] R. Casady, R. Myers, J. Legates, The Effect of Exposure to High Ambient Temperature on Spermatogenesis in the Dairy Bull1, J. Dairy Sci. 36 (1953) 14–23.
[21] J.C. Rockett, F.L. Mapp, J.B. Garges, J.C. Luft, C. Mori, D.J. Dix, Effects of hyperthermia on spermatogenesis, apoptosis, gene expression, and fertility in adult male mice, Biol. Reprod. 65 (2001) 229–239.
[22] R. Huo, Y.F. Zhu, X. Ma, M. Lin, Z.M. Zhou, J.H. Sha, Differential expression of glucose-regulated protein 78 during spermatogenesis, Cell Tissue Res. 316 (2004) 359–367.
[23] A.S. Lee, The glucose-regulated proteins: stress induction and clinical applications, Trends Biochem. Sci. 26 (2001) 504–510.
[24] E.D. Lai, T. Teodoro, A. Volchuk, Endoplasmic reticulum stress: signaling the unfolded protein response, Physiology 22 (2007) 193–201.
[25] H.P. Harding, I. Novoa, Y. Zhang, H. Zeng, R. Wek, M. Schapira, D. Ron, Regulated translation initiation controls stress-induced gene expression in mammalian cells, Mol. Cell. 6 (2000) 1099–1108.
[26] S.J. Park, T.S. Kim, C.K. Park, S.H. Lee, J.M. Kim, K.S. Lee, I.K. Lee, J.W. Park, M.A. Lawson, D.S. Lee, HCG-induced endoplasmic reticulum stress triggers apoptosis and reduces steroidogenic enzyme expression through activating transcription factor 6 in Leydig cells of the testis, J. Mol. Endocrinol. 50 (2013) 151–166.
[27] H. Yoshida, T. Matsui, A. Yamamoto, T. Okada, K. Mori, XBP1 mRNA is induced by ATF6 and spliced by IRE1 in response to ER stress to produce a highly active transcription factor, Cell 107 (2001) 881–891.
[28] F. Urano, X. Wang, A. Bertolotti, Y. Zhang, P. Chung, H.P. Harding, D. Ron, Coupling of stress in the ER to activation of JNK protein kinases by transmembrane protein kinase IRE1, Science 287 (2000) 664–666.
[29] S.G. Fonseca, J. Gromada, F. Urano, Endoplasmic reticulum stress and pancreatic beta-cell death, Trends Endocrinol. Metab. 22 (2011) 266–274.
[30] E. Szegezdi, S.E. Logue, A.M. Gorman, A. Samali, Mediators of endoplasmic reticulum stress-induced apoptosis, EMBO Rep. 7 (2006) 880–885.