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Lack of GCN5 remarkably enhances the resistance against prolonged endoplasmic reticulum stress-induced apoptosis through up-regulation of Bcl-2 gene expression

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ABSTRACT

The endoplasmic reticulum (ER), a complex membrane structure, has important roles in all eukaryotic cells. Catastrophe of its functions would lead to ER stress that causes various diseases such as cancer, neurodegenerative diseases, diabetes and so on. Prolonged ER stress could trigger apoptosis via activation of various signal transduction pathways. To investigate physiological roles of histone acetyltransferase GCN5 in regulation of ER stress, we analyzed responses of homozygous GCN5-deficient DT40 mutants, ΔGCN5, against ER stress. GCN5-deficiency in DT40 caused drastic resistance against apoptosis induced by pharmacological ER stress agents (thapsigargin and tunicamycin). Pharmaceutical analysis using specific Bcl-2 inhibitors showed that the drastic resistance against prolonged ER stress-induced apoptosis is, in part, due to up-regulation of Bcl-2 gene expression in ΔGCN5. These data revealed that GCN5 is involved in regulation of prolonged ER stress-induced apoptosis through controlling Bcl-2 gene expression.

1. Introduction

Endoplasmic reticulum (ER) is an important organelle for lipid synthesis, drug metabolism, calcium storage and protein synthesis (translation, folding and maturation) in eukaryotic cells [1–3]. Catastrophe of these functions would lead to ER stress that causes various diseases such as cancer [4], neurodegenerative diseases (e.g. Alzheimer's disease, amyotrophic lateral sclerosis and Parkinson's disease) [5,6] and diabetes [7,8]. Prolonged ER stress could trigger apoptotic cell death through various signal transduction pathways [5,9,10]. Upon chronic ER stress, caspase-12, the ER membrane associated protease belonging to the caspase family, is activated, followed by promoting apoptosis via initiating caspase-mediated limited proteolysis cascade [11]. On the other hand, protein kinase RNA-like ER kinase (PERK) promotes apoptosis through translation of activating transcription factor 4 (ATF4) [12]. Sequentially, ATF4 induces expression of C/EBP-homologous protein (CHOP) gene, resulting in apoptosis progression via suppressing gene expression of anti-apoptotic B-cell lymphoma 2 (Bcl-2) and activating that of pro-apoptotic B-cell lymphoma-associated X (Bax) [13]. The CHOP gene is also up-regulated by various unfolded protein response-related transcription factors such as activating transcription factor 6 (ATF6) and X-box binding protein-1 (Xbp-1) [10,14]. In addition, unmitigated ER stress also activates apoptosis

Abbreviations: AFT4, activating transcription factor 4; AFT6, activating transcription factor 6; Bax, B-cell lymphoma-associated X; Bcl-2, B-cell lymphoma 2; Bcl-xL, B-cell lymphoma extra-large; CHOP, C/EBP-homologous protein; ER, endoplasmic reticulum; GRP78, glucose-regulated protein 78; IRE1, inositol-requiring protein 1; PERK, protein kinase RNA-like ER kinase; SAGA, Spt-Ada-GCN5 acetyltransferase; Xbp-1, X-box binding protein-1.

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signaling pathway through interaction of inositol-requiring protein 1 (IRE1) with tumor necrosis factor receptor-associated factor 2 and apoptosis signal-regulating kinase [15]. In contrast, glucose-regulated protein 78 (GRP78), also called as binding immunoglobulin protein, protects cell against ER stress-induced apoptosis [16,17]. GRP78 plays an extremely important role in the cell survival against ER stress, while CHOP acts as the principal executor of various pro-apoptotic events upon severe ER stress. Studies on ER stress-induced apoptosis are rapidly developing. However, the mechanisms of ER stress-induced apoptosis remain poorly understood even now, from the viewpoint of epigenetic regulation such as histone acetylation catalyzed by histone acetyltransferases.

To investigate the physiological roles of GCN5, a prototypical histone acetyltransferase [18], we generated homozygous GCN5-deficient DT40 mutants, ΔGCN5, by gene targeting techniques [19]. The techniques used are excellent methods to study physiological roles of various genes including histone modification-related genes [20,21]. Our previous studies have revealed that GCN5-deficiency caused not only delayed growth rate, suppressed cell cycle progression at G1/S phase transition, and down- or up-regulated various G1/S phase transition-related genes [19], but also drastic resistance against B cell receptor-mediated apoptosis associated with negative selection of immature B cells [22]. In addition, GCN5 activated phosphatidylinositol 3-kinase/Akt survival pathway in cells exposed to oxidative stress via controlling gene expressions of tyrosine protein kinases Syk and Btk [23], promoted the superoxide-generating system in leukocytes via controlling the gp91-phox gene expression [24] and participated in tolerance against UV-irradiation via controlling gene expression of DNA polymerase η [25]. Recently, we showed that GCN5 is essential for interferon regulatory factor-4 gene expression followed by transcriptional activation of Blimp-1 during B cell development [26].

In this study, we investigated effects of GCN5-deficiency on prolonged ER stress-induced apoptosis. Our results revealed that GCN5 is involved in regulation of prolonged ER stress-induced apoptosis through controlling Bcl-2 gene expression.

2. Materials and methods

2.1. Materials

Thapsigargin (Nacalai Tesque, Kyoto, Japan), tunicamycin (Calbiochem, San Diego, CA, USA), Bcl-2 inhibitor ABT-199 and HA14-1 (Selleck Chemicals, Houston, TX, USA), Trizol reagent (Invitrogen, Carlsbad, CA, USA), Ex *Taq* DNA polymerase (Takara Bio, Shiga, Japan), proteinase K (Wako, Osaka, Japan), RNase A (Sigma–Aldrich, St. Louis, MO, USA) and cDNA synthesis kit ReverTra Ace-α (Toyobo, Osaka, Japan) were obtained.

2.2. Cell cultures and apoptosis induction

Generation of ΔGCN5 was described in our previous report [19]. DT40 cells and all subclones were cultured essentially as described [19]. ER stress-mediated apoptosis was induced as follows: cells (1.5×10^6) in 10 ml of culture medium were incubated with 1 μM thapsigargin or 5 μg/ml tunicamycin at 37 °C up to 24 h. Treatment with Bcl-2 inhibitor ABT-199 [27] was carried out as follows. Cells (1.5×10^6) in 10 ml of culture medium were treated with ABT-199 (2 or 5 μM) at 37 °C for 30 min and subsequently incubated with 1 μM thapsigargin at 37 °C for 24 h in the presence of ABT-199. Viable cells were counted by trypan blue dye exclusion method as described [28]. DNA fragmentation assay was carried out as described [28]. In brief, cells were lysed in 50 mM Tris-HCl (pH 8.0) containing 2 mM EDTA, 100 μg/ml proteinase K and 1% SDS. DNA was extracted, re-suspended in 10 mM Tris-HCl (pH 7.5) containing 1 mM EDTA and 20 μg/ml RNase A, and incubated at 37 °C for 2 h. DNA samples were applied to 1.5% agarose gel electrophoresis.

2.3. Semiquantitative RT-PCR

Total RNA was isolated from DT40 and its subclones using Trizol reagent. RT was performed with a first strand cDNA synthesis kit at 42 °C for 20 min, followed by heating at 99 °C for 5 min. Semiquantitative RT-PCR was performed as described [19,22–26] using forward and reverse primers for appropriate genes listed in Supplementary Table S1, which were synthesized according to the sequence data deposited in GenBank. Chicken GAPDH gene was used as internal control. PCR products were subjected to 1.5% agarose gel electrophoresis. Data obtained by semiquantitative RT-PCR before reaching the plateau were analyzed by Multi Gauge software (densitometrical analysis mode) using a luminescent image analyzer LAS-3000 (Fujifilm, Tokyo, Japan).

2.4. Detection of spliced and unspliced Xbp-1 mRNA

To detect both spliced and unspliced chicken Xbp-1 mRNA, RT-PCR was performed using forward (5′-ATGTGAAGGAATCCCAGGTG-3′) and reverse (5′- CATTTCTGGGTCCAGACTGT-3′) primers. Chicken GAPDH gene was used as control. PCR products were subjected to 1.5% agarose gel electrophoresis. Data obtained by RT-PCR before reaching the plateau were analyzed as described above.

2.5. Statistical analysis

Results (viability and PCR) are presented as averages of three separate experiments. Error bars indicate standard deviation. Statistical differences were calculated with Student's *t* test.

3. Results and discussion

3.1. Effects of GCN5-deficiency on progression of pharmacological ER stress agents-induced apoptosis of DT40 cells

To know the effects of GCN5-deficiency on pharmacological ER stress agents-induced apoptosis, we first examined influences of thapsigargin treatment on apoptosis of ΔGCN5 and wild type DT40 cells (Fig. 1A). Thapsigargin is an inhibitor of sarcoplasmic/ER Ca^{2+} ATPases which act as calcium ion transporters [11]. The viability of both ΔGCN5 and DT40 remained unchanged in the absence of thapsigargin, but the viability of DT40 (~12% at 24 h) was remarkably affected as compared to that of three independent ΔGCN5 clones (~92% at 24 h) in the presence of 1 μM thapsigargin (Fig. 1A, upper graph). The DNA fragmentation in ΔGCN5 was more suppressive compared with accelerated level of that in DT40 (Fig. 1A, lower electrophoretic profile). The suppressive DNA fragmentation pattern was similarly obtained from two other independent ΔGCN5 clones (data not shown). Next, we examined effects of tunicamycin treatment on apoptosis of ΔGCN5 and DT40 (Fig. 1B). Tunicamycin is an inhibitor of N-linked protein glycosylation [11]. The viability of ΔGCN5 and DT40 was not altered in the absence of tunicamycin, but in the presence of this agent (5 μg/ml) the viability of DT40 (~42% at 24 h) was remarkably lower than that of three independent ΔGCN5 clones (~90% at 24 h) (Fig. 1B, upper graph) in almost the same manner as upon thapsigargin treatment. The DNA fragmentation in ΔGCN5 was also more repressive compared with prominent level of that in DT40 (Fig. 1B, lower electrophoretic profile). The repressive DNA fragmentation pattern was also observed from two other independent ΔGCN5 clones (data not shown). Analyses using these two agents with different modes of