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Chemical chaperones reduce ionizing radiation-induced endoplasmic reticulum stress and cell death in IEC-6 cells

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ABSTRACT

Radiotherapy, which is one of the most effective approaches to the treatment of various cancers, plays an important role in malignant cell eradication in the pelvic area and abdomen. However, it also generates some degree of intestinal injury. Apoptosis in the intestinal epithelium is the primary pathological factor that initiates radiation-induced intestinal injury, but the mechanism by which ionizing radiation (IR) induces apoptosis in the intestinal epithelium is not clearly understood. Recently, IR has been shown to induce endoplasmic reticulum (ER) stress, thereby activating the unfolded protein response (UPR) signaling pathway in intestinal epithelial cells. However, the consequences of the IR-induced activation of the UPR signaling pathway on radiosensitivity in intestinal epithelial cells remain to be determined. In this study, we investigated the role of ER stress responses in IR-induced intestinal epithelial cell death. We show that chemical ER stress inducers, such as tunicamycin or thapsigargin, enhanced IR-induced caspase 3 activation and DNA fragmentation in intestinal epithelial cells. Knockdown of *Xbp1* or *Atf6* with small interfering RNA inhibited IR-induced caspase 3 activation. Treatment with chemical chaperones prevented ER stress and subsequent apoptosis in IR-exposed intestinal epithelial cells. Our results suggest a pro-apoptotic role of ER stress in IR-exposed intestinal epithelial cells. Furthermore, inhibiting ER stress may be an effective strategy to prevent IR-induced intestinal injury.

1. Introduction

Radiotherapy is used as a principal therapeutic modality for various cancers. During radiotherapy, radiation treatment is applied locally and in multiple fractions to minimize harmful side effects [1,2]. Nevertheless, damage to radiosensitive tissues and cell types with high rates of cell division cannot be avoided [3,4]. For example, pelvic and abdominal radiotherapy can generate some degree of intestinal injury [5,6]. Acute radiation enteritis represents the toxicity that develops in the intestine during radiotherapy [6–8]. One-half to three-quarters of patients undergoing radiotherapy develop acute symptoms of radiation enteritis [6]. Its symptoms include diarrhea, abdominal pain, nausea, and vomiting, which lead to significant malnutrition and weight loss. In addition, these symptoms limit the tolerability for patients taking cancer radiotherapy and can force the suspension or termination of the therapeutic treatment course [6]. Apoptosis in the intestinal epithelium is the primary pathological factor that initiates radiation-induced intestinal injury [7,9]. However, the mechanism by which ionizing radiation (IR) induces apoptosis in the intestinal epithelium is not clearly understood. To this date, no agent has been approved by the Food and Drug Administration (FDA) as a medical countermeasure for radiation-induced intestinal injury.

The endoplasmic reticulum (ER) regulates protein synthesis, protein folding, lipid and sterol synthesis, and intracellular calcium levels [10]. ER stress is triggered by the perturbation of the ER functions, which leads to the activation of the unfolded protein response (UPR) [11]. The UPR is an adaptive signaling cascade that is aimed at restoring ER homeostasis by facilitating the degradation of misfolded proteins and expanding the protein-folding capacity of the cell [12–14]. The UPR involves the transcriptional activation of *Grp78* [15]. It has three branches: inositol-requiring

Abbreviations: ER, endoplasmic reticulum; UPR, unfolded protein response; eIF2α, eukaryotic translation initiation factor 2α; ATF4, activating transcription factor 4; PERK, RNA-dependent-protein-kinase-like ER kinase; PBA, 4-phenylbutyric acid; siRNA, small interfering RNA; TUDCA, tauroursodeoxycholic acid.

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enzyme 1 (IRE1), protein kinase RNA-like ER kinase (PERK), and activating transcription factor (ATF6). In response to ER stress, IRE1, PERK, and ATF6 initiate signal transduction processes to promote the expression of genes that are involved in the reestablishment of ER homeostasis [12–14]. However, when ER stress is prolonged or excessive, these UPR signaling pathways lead to the apoptotic death of stressed cells. The initial triggers leading to the accumulation of unfolded proteins in the ER involve oxidative stress, alterations in calcium homeostasis, and bacterial infection [11]. In addition, IR elicits ER stress and induces the UPR signaling pathway in various cell types. For example, the PERK/eukaryotic translation initiation factor 2α (eIF2α) branch of the UPR regulates radiosensitivity in breast cancer cells [16–18] and SV40-immortalized mouse embryonic fibroblast cells [16] in response to IR. Recently, IR has also been shown to induce moderate ER stress, which leads to the selective activation of the IRE1 and ATF6 branches of the UPR without activating PERK/eIF2α/ATF4 signaling in intestinal epithelial cells [19]. However, the consequences of the IR-induced activation of the UPR signaling pathway on radiosensitivity in intestinal epithelial cells remain to be determined.

In this study, we investigated the role of ER stress responses in IR-induced intestinal epithelial cell death.

2. Materials and methods

2.1. Cell culture

The IEC-6 cell line was purchased from American Type Culture Collection (Manassas, VA, USA), and cultured in Dulbecco's Modified Eagle Medium (DMEM) with 10% fetal bovine serum. IEC-6 cells originate from normal rat intestinal crypt cells. They display a normal karyotype and are not tumorigenic. Experiments were performed using the distribution stocks of IEC-6 cell lines up to ten population doublings, and the cells were in the logarithmic growth phase at the initiation of experiments.

2.2. Materials and irradiation

Tunicamycin, thapsigargin, and 4-phenylbutyric acid (PBA) were purchased from Sigma–Aldrich (St Louis, MO, USA). Tauroursodeoxycholic acid (TUDCA) was purchased from TCI America (Portland, OR, USA). Irradiation was performed using a ^{137}Cs gamma-ray source (Atomic Energy of Canada) at a dose rate of 3.51 Gy/min at room temperature.

2.3. Caspase 3 activity assay

The activity of caspase 3 was measured by using the CaspACE Assay System (Promega, WI, USA), according to the manufacturer's protocol. Briefly, cell lysates (100 μg) were incubated with the colorimetric substrate, DEVD-p-nitroanilide, which was cleaved by caspase 3 to release yellow p-nitroanilide. The caspase 3-catalyzed release of p-nitroanilide was measured by absorbance at 405 nm.

2.4. DNA fragmentation assay

Internucleosomal DNA fragmentation was quantitatively determined by using the Cell Death Detection ELISA^{PLUS} kit (Roche Diagnostics), according to the manufacturer's instructions. Briefly, IEC-6 cells were seeded in 96-well plates at a density of 1×10^3 cells per well and then treated with IR. Twenty-four hours after IR treatment, the cells were resuspended in 200 μl of the lysis buffer that was supplied by the manufacturer. After incubating for 30 min at room temperature, 20 μl of the extract was used in the enzyme-linked immunosorbent assay (ELISA), according to the manufacturer's protocol. Finally, upon incubating with a peroxidase substrate for 15 min, the absorbance at 405 nm with a reference wavelength at 492 nm was determined with a microplate reader (Bio-Tec Instruments, Winooski, VT, USA).

2.5. Quantitative real-time reverse transcription-polymerase chain reaction (qRT-PCR)

Total RNA was isolated from cells using a Hybrid-R Kit (GeneAll Biotechnology, Seoul, Korea). RNA (1.0 μg) was subjected to reverse transcription with the PrimeScript RT master mix, according to the manufacturer's protocol (Takara, Tokyo, Japan). PCR was performed in triplicate using the CFX96TM Real-Time system (Bio-Rad, Hercules, CA, USA) and qPCR SYBR Green master mix (m.biotech, Gyeonggi, Korea). Primers for qRT-PCR were designed using the AmplifX program (http://crn2m.univ-mrs.fr), and they purchased from Integrated DNA Technologies (Coralville, IA, USA): glucose-regulated protein, 78kD (*Grp78*), forward 5′–GACTTGGGGACCACCT ATTCCTGCG-3′, reverse 5′-CATTCAGTCCAGCAATAGTGCCAGC-3′; *Atf6*, forward 5′-TTCTCAGCTGATGGCTGTCCAGTA-3′, reverse 5′-T GCAGCTCACTCCCAGAATTCCTA-3′.

2.6. Western blot analysis

Total cell lysates were prepared using radioimmunoprecipitation assay buffer, and Western blot analyses were performed as described previously [20]. Primary antibodies against cleaved caspase 3 (cat #9661; Cell Signaling Technology, Danvers, MA, USA) and β−actin (cat #A1978; Sigma–Aldrich) were used.

2.7. siRNA and cell transfection

siRNA oligonucleotides were designed using the siRNA design tool (Dharmacon Research, Lafayette, CO, USA). Oligonucleotide sequences were: 5′-GCUGUUGCCUCUUCAGAUU-dTdT-3′ (*Xbp1* siRNA), 5′-GCACAUGAGACUUACGAAA-dTdT-3′ (*Atf6* siRNA), and 5′-AAUCAACUGACUCGACCAC-dTdT-3′ (scrambled siRNA). Transfections were carried out using the RNAiMAX protocol provided by Invitrogen (Carlsbad, CA, USA). After 24 h, the transfected cells were used for the experiments.

2.8. Statistical analysis

The results were expressed as the means ± standard deviation (SD) of three to four experiments that were performed independently. The normality distribution of the variables was assessed by the Kolmogorov–Smirnov test. The paired Student's *t*-test was performed where indicated, and *P*-values less than 0.05 were considered statistically significant.

3. Results

3.1. IR activated the UPR, which preceded caspase 3 activation

Treatment of IEC-6 cells with IR triggered the UPR, which was evidenced by the enhanced expression of *Grp78* transcripts (Fig. 1A). As shown in Fig. 1A, increased mRNA levels of *Grp78* are observed within 8 h of IR treatment. However, caspase 3 activation was evident at 16 h after IR treatment (Fig. 1B), and subsequent internucleosomal DNA fragmentation was observed at 24 h (Fig. 1C). Thus, the UPR preceded caspase activation, suggesting that the UPR may play a role in the process of IR-induced caspase activation and cell death.

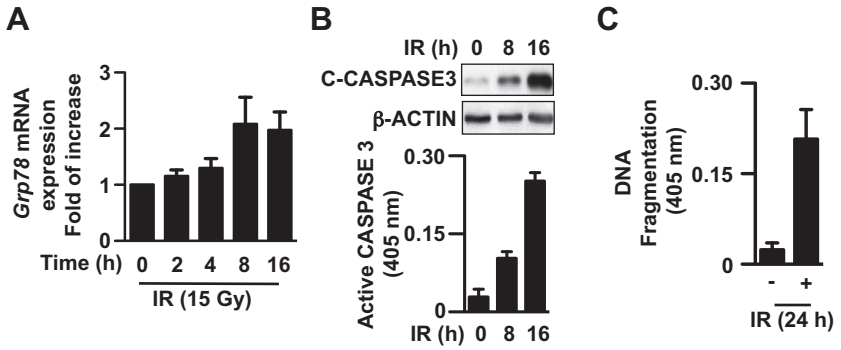

Fig. 1. Ionizing radiation (IR) activates the unfolded protein response (UPR), which precedes caspase 3 activation. (A) Relative amounts of *Grp78* mRNA were quantified by real-time PCR at the indicated time points after treatment with 15 Gy ionizing radiation (IR) in IEC-6 cells. RNA levels were normalized to those of glyceraldehyde-3-phosphate dehydrogenase (*Gapdh*) and to non-IR-treated controls. (B) Cleavage of caspase 3 (C-caspase3) was determined by Western blotting at 16 h after 15 Gy IR treatment in IEC-6 cells (upper panel). Caspase 3 activity was measured at 16 h after treatment with 15 Gy IR by using the CaspACE Assay System (Promega) (lower panel). (C) Internucleosomal DNA fragmentation was measured at 24 h after treatment with 15 Gy IR in IEC-6 cells by using the Cell Death Detection ELISA^{PLUS} kit (Roche).

3.2. ER stress enhanced IR-induced cell death in IEC-6 cells

To investigate the role of the UPR in IR-induced cell death, IEC-6 cells were treated with tunicamycin, which induces ER stress by inhibiting N-glycosylation [21]. As shown in Fig. 2A, treatment of IEC-6 cells with tunicamycin significantly activates the UPR. However, no further increases in the degree of the tunicamycin-induced UPR were observed in combination with IR. Although the co-treatment with IR and tunicamycin showed no additive effects with regard to the degree of the UPR, it exerted clear additive effects on caspase 3 activity (Fig. 2B). Thapsigargin, which is another inducer of ER stress [22], also enhanced IR-induced caspase 3 activation (Fig. 2C). These data clearly indicate that the UPR plays a pro-apoptotic role in irradiated intestinal epithelial cells.

3.3. IR induced pro-apoptotic UPR

To confirm the role of the IR-induced UPR in IR-triggered intestinal epithelial cell death, we utilized siRNAs that targeted *Xbp1* and *Atf6* transcripts (Fig. 3A). As shown in Fig. 3B, *Xbp1* or *Atf6* knockdown inhibits IR-induced caspase 3 activation, and co-knockdown of *Xbp1* and *Atf6* does not have additive effects on the degree of caspase activity. This suggests that the XBP1 and ATF6 signaling pathways may share common mechanisms to regulate caspase 3 activation.

3.4. Chemical chaperones inhibited IR-induced cell death

Given that the IR-induced UPR played a pro-apoptotic role in irradiated intestinal epithelial cells (Fig. 3), we examined whether ER stress inhibitors could ameliorate IR-induced cell death in IEC-6 cells. Chemical chaperones, such as TUDCA and PBA, stabilize protein conformation to inhibit ER stress, thereby inhibiting the UPR [23,24]. As shown in Fig. 4A, PBA and TUDCA significantly ameliorate IR-induced ER stress, as evidenced by the inhibition of the IR-induced up-regulation of *Grp78* mRNA. PBA and TUDCA treatment resulted in the dose-dependent inhibition of IR-induced caspase 3 activation (Fig. 4B and C). In addition, PBA and TUDCA blocked IR-induced apoptosis (Fig. 4D). These findings suggest that the enhancement of ER function with chemical chaperones to cope with IR-induced ER stress could be a strategy to manage IR-triggered intestinal epithelial cell death.

4. Discussion

The UPR signaling pathway has primarily been associated with cell survival under various stressors. UPR signaling is activated in various tumors and is essential for tumor cells to survive unfavorable alterations, such as nutrient and oxygen deprivation and low pH, in their microenvironment [25–27]. IR can trigger ER stress,

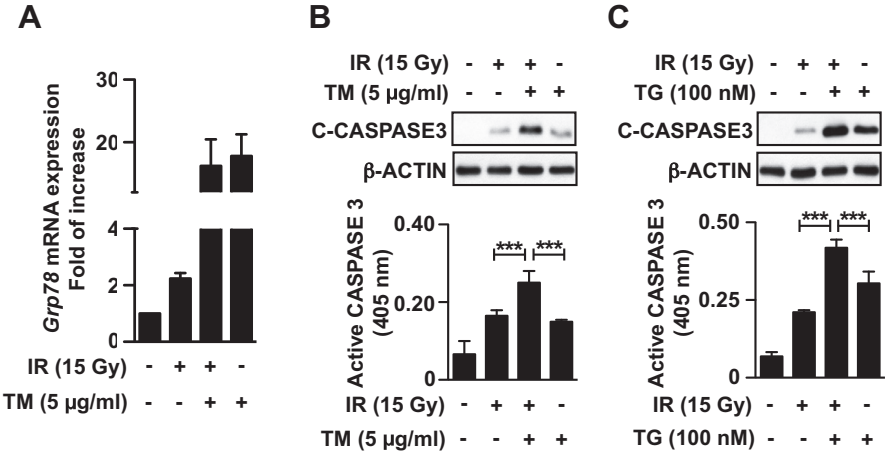

Fig. 2. Endoplasmic reticulum (ER) stress enhances IR-induced cell death in IEC-6 cells. (A) IEC-6 cells were pretreated for 1 h with or without 5 μg/ml tunicamycin (TM) and subsequently treated with or without 15 Gy IR for 8 h. Relative amounts of *Grp78* mRNA were then quantified by real-time PCR. RNA levels were normalized to those of *Gapdh* and to non-IR-treated controls. (B) IEC-6 cells were pretreated with or without 5 μg/ml TM for 1 h and subsequently treated with or without 15 Gy IR for 16 h. Cleavage of caspase 3 was then examined by Western blotting (upper panel), and caspase 3 activity was measured by using the CaspACE Assay System (Promega) (lower panel). (C) IEC-6 cells were pretreated with or without 100 nm thapsigargin (TG) for 1 h and subsequently treated with or without 15 Gy IR for 16 h. Cleavage of caspase 3 was then examined by Western blotting (upper panel), and caspase 3 activity was measured by using the CaspACE Assay System (Promega) (lower panel).

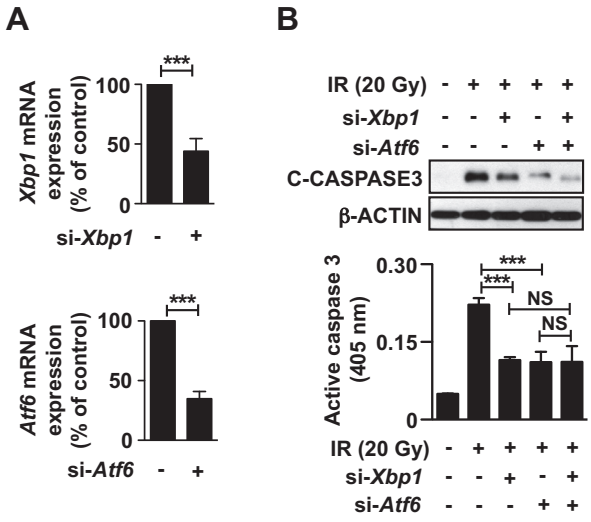

Fig. 3. IR induces pro-apoptotic UPR. (A) Twenty-four hours after transfection with scrambled siRNA, *Xbp*1 siRNA (si-Xbp1), or *Atf6* siRNA (si-Atf6), relative amounts of *Xbp1* (upper panel) and *Atf6* (lower panel) mRNA were quantified by real-time PCR in IEC-6 cells. RNA levels were normalized to those of *Gapdh* and to scrambled siRNA-transfected controls. (B) Twenty-four hours after transfection with si-Xbp1 or si-Atf6, or both, cleavage of caspase 3 (C-caspase3) was determined by Western blotting at 16 h after 15 Gy IR treatment in IEC-6 cells (upper panel), and caspase 3 activity was measured by using the CaspACE Assay System (Promega) (lower panel). (C) Twenty-four hours after transfection with si-Xbp1 or si-Atf6, or both, internucleosomal DNA fragmentation was measured at 24 h after treatment with 15 Gy IR in IEC-6 cells by using the Cell Death Detection ELISA^{PLUS} kit (Roche).

and the subsequent UPR signaling pathway causes the radioresistance of MDA-MB-231 breast cancer cells by increasing DNA damage repair signaling [17]. However, the UPR signaling pathway has the potential to contribute to cell killing in response to IR. For example, the UPR enhances radiosensitivity in SV40-immortalized mouse embryonic fibroblast cells by upregulating IR-induced autophagy [16], and it sensitizes human esophageal cancer cells to radiation [28]. However, the precise mechanism by which the UPR mediates cell survival or death in response to IR remains to be elucidated.

In the present study, treatment with ER stress inducers enhanced IR-induced caspase 3 activation, which was preceded by a significant activation of the UPR, in intestinal epithelial cells. Moreover, knockdown of *Xbp1* or *Atf6* with siRNA inhibited IR-induced caspase 3 activation, suggesting a pro-apoptotic role of the UPR in IR-exposed intestinal epithelial cells. We now show that ER stress and the UPR signaling pathway can sensitize intestinal epithelial cells to radiation.

Chemical chaperones are small molecules that help to stabilize protein conformation and relieve ER stress [23]. The most prominent members are PBA and TUDCA. PBA is a short-chain fatty acid that has the ability to bind to the hydrophobic surfaces of unfolded proteins, thus protecting them from aggregation and assisting the refolding of unfolded proteins [23,24]. PBA protects against cerebral ischemia and liver ischemia–reperfusion injury by inhibiting ER stress-related apoptosis [29,30]. TUDCA is a derivative of an endogenous bile acid that prevents the formation of misfolded proteins and alleviates signs of ER stress in the liver and adipose tissue of obese mice [31]. In addition, TUDCA and PBA are safe in humans. TUDCA is approved by the FDA for the treatment of primary biliary

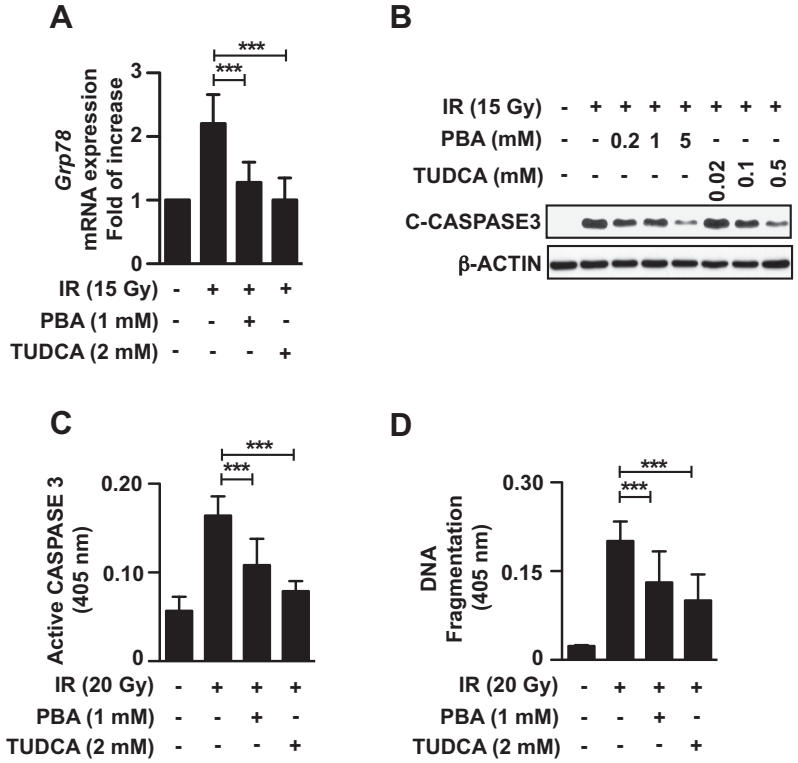

Fig. 4. Chemical chaperones inhibit IR-induced cell death. (A) IEC-6 cells were pretreated with or without 4-phenylbutyric acid (PBA) or tauroursodeoxycholic acid (TUDCA) for 1 h and subsequently treated with or without 15 Gy of IR for 8 h. Relative amounts of *Grp78* mRNA were quantified by real-time PCR. RNA levels were normalized to those of *Gapdh* and to non-IR-treated controls. (B) IEC-6 cells were pretreated with or without the indicated concentrations of PBA or TUDCA for 1 h and subsequently treated with 15 Gy of IR for 16 h. Cleavage of caspase 3 was examined by Western blotting. (C) IEC-6 cells were pretreated with or without the indicated concentrations of PBA or TUDCA for 1 h and subsequently treated with 15 Gy of IR for 16 h. Caspase 3 activity was measured with the CaspACE Assay System. (D) IEC-6 cells were pretreated with or without the indicated concentrations of PBA or TUDCA for 1 h and subsequently treated with 15 Gy of IR for 24 h. Internucleosomal DNA fragmentation was measured at 24 h after treatment with 15 Gy IR in IEC-6 cells by using the Cell Death Detection ELISA^{PLUS} kit (Roche).

cirrhosis, and PBA is approved by the FDA for the management of urea cycle disorders.

Injury to the intestine often represents the most important cause of radiation-induced side effects in patients undergoing pelvic and abdominal radiotherapy [5,6]. Therefore, there is a pressing need to develop safe and effective radiation countermeasure agents to prevent the harmful side effects of radiotherapy. Our results show that chemical chaperones can be utilized to ameliorate IR-induced apoptosis in intestinal epithelial cells by reducing ER stress.

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References

[1] M.M. Elkind, H. Sutton-Gilbert, W.B. Moses, T. Alescio, R.W. Swain, Radiation response of mammalian cells grown in culture: v. temperature dependence of the repair of X-ray damage in surviving cells (aerobic and hypoxic), Radiat. Res. 25 (1965) 359.
[2] J.B. Little, G.M. Hahn, E. Frindel, M. Tubiana, Repair of potentially lethal radiation damage in vitro and in vivo, Radiology 106 (1973) 689–694.
[3] W.K. Sinclair, R.A. Morton, X-ray sensitivity during the cell generation cycle of cultured Chinese hamster cells, Radiat. Res. 29 (1966) 450.
[4] W.K. Sinclair, Cyclic X-ray responses in mammalian cells in vitro, Radiat. Res. 33 (1968) 620.
[5] H.J.N. Andreyev, Gastrointestinal problems after pelvic radiotherapy: the past, the present and the future, Clin. Oncol. (R. Coll. Radiol.) 19 (2007) 790–799.
[6] M. Hauerjensen, J. Wang, J. Denham, Bowel injury: current and evolving management strategies, Semin. Radiat. Oncol. 13 (2003) 358–371.
[7] W.K. MacNaughton, Review article: new insights into the pathogenesis of radiation-induced intestinal dysfunction, Aliment. Pharmacol. Ther. 14 (2000) 523–528.
[8] J. Andreyev, Gastrointestinal complications of pelvic radiotherapy: are they of any importance?, Gut 54 (2005) 1051–1054
[9] C.S. Potten, A. Merritt, J. Hickman, P. Hall, A. Faranda, Characterization of radiation-induced apoptosis in the small intestine and its biological implications, Int. J. Radiat. Biol. 65 (1994) 71–78.
[10] K. Araki, K. Nagata, Protein folding and quality control in the ER, Cold Spring Harb. Perspect. Biol. 3 (2011). a007526-a007526.
[11] S.G. Fonseca, J. Gromada, F. Urano, Endoplasmic reticulum stress and pancreatic β-cell death, Trends Endocrinol. Metab. (2011).
[12] S. Wang, R.J. Kaufman, The impact of the unfolded protein response on human disease, J. Cell Biol. 197 (2012) 857–867.
[13] C. Hetz, The unfolded protein response: controlling cell fate decisions under ER stress and beyond, Nat. Rev. Mol. Cell Biol. (2012).
[14] C. Hetz, F. Martinon, D. Rodriguez, L.H. Glimcher, The unfolded protein response: integrating stress signals through the stress sensor IRE1, Physiol. Rev. 91 (2011) 1219–1243.
[15] A.S. Lee, The ER chaperone and signaling regulator GRP78/BiP as a monitor of endoplasmic reticulum stress, Methods 35 (2005) 373–381.
[16] K.W. Kim, L. Moretti, L.R. Mitchell, D.K. Jung, B. Lu, Endoplasmic reticulum stress mediates radiation-induced autophagy by perk-eIF2α in caspase-3/7-deficient cells, Oncogene 29 (2010) 3241–3251.
[17] D. Oommen, K.M. Prise, Down-regulation of PERK enhances resistance to ionizing radiation, Biochem. Biophys. Res. Commun. 441 (2013) 31–35.
[18] A. Nagelkerke, J. Bussink, A.J. van der Kogel, F.C.G.J. Sweep, P.N. Span, The PERK/ATF4/LAMP3-arm of the unfolded protein response affects radioresistance by interfering with the DNA damage response, Radiother. Oncol. 108 (2013) 415–421.
[19] B. Zhang, Y. Wang, X. Pang, Y. Su, G. Ai, T. Wang, ER stress induced by ionising radiation in IEC-6 cells, Int. J. Radiat. Biol. 86 (2010) 429–435.
[20] Y.B. Lim, S.S. Kang, T.K. Park, Y.S. Lee, J.S. Chun, J.K. Sonn, Disruption of actin cytoskeleton induces chondrogenesis of mesenchymal cells by activating protein kinase C-alpha signaling, Biochem. Biophys. Res. Commun. 273 (2000) 609–613.
[21] A. Heifetz, R.W. Keenan, A.D. Elbein, Mechanism of action of tunicamycin on the UDP-GlcNAc:dolichyl-phosphate Glc-NAc-1-phosphate transferase, Biochemistry 18 (1979) 2186–2192.
[22] T. Nakagawa, H. Zhu, N. Morishima, E. Li, J. Xu, B.A. Yankner, et al., Caspase-12 mediates endoplasmic-reticulum-specific apoptosis and cytotoxicity by amyloid-beta, Nature 403 (2000) 98–103.
[23] F. Engin, G.S. Hotamisligil, Restoring endoplasmic reticulum function by chemical chaperones: an emerging therapeutic approach for metabolic diseases, Diabetes Obes. Metab. 12 (Suppl. 2) (2010) 108–115.
[24] E. Papp, P. Csermely, Chemical chaperones: mechanisms of action and potential use, Handb. Exp. Pharmacol. (2006) 405–416.
[25] M. Bi, C. Naczki, M. Koritzinsky, D. Fels, J. Blais, N. Hu, et al., ER stress-regulated translation increases tolerance to extreme hypoxia and promotes tumor growth, EMBO J. 24 (2005) 3470–3481.
[26] J. Ye, M. Kumanova, L.S. Hart, K. Sloane, H. Zhang, D.N. De Panis, et al., The GCN2-ATF4 pathway is critical for tumour cell survival and proliferation in response to nutrient deprivation, EMBO J. 29 (2010) 2082–2096.
[27] J.D. Blais, C.L. Addison, R. Edge, T. Falls, H. Zhao, K. Wary, et al., Perk-dependent translational regulation promotes tumor cell adaptation and angiogenesis in response to hypoxic stress, Mol. Cell. Biol. 26 (2006) 9517–9532.
[28] X.-L. Pang, G. He, Y.-B. Liu, Y. Wang, B. Zhang, Endoplasmic reticulum stress sensitizes human esophageal cancer cell to radiation, World J. Gastroenterol. 19 (2013) 1736–1748.
[29] X. Qi, T. Hosoi, Y. Okuma, M. Kaneko, Y. Nomura, Sodium 4-phenylbutyrate protects against cerebral ischemic injury, Mol. Pharmacol. 66 (2004) 899–908.
[30] I. Ben Mosbah, I. Alfany-Fernández, C. Martel, M.A. Zaouali, M. Bintanel-Morcillo, A. Rimola, et al., Endoplasmic reticulum stress inhibition protects steatotic and non-steatotic livers in partial hepatectomy under ischemia-reperfusion, Cell Death Dis. 1 (2010) e52.
[31] U. Ozcan, Q. Cao, E. Yilmaz, A.-H. Lee, N.N. Iwakoshi, E. Ozdelen, et al., Endoplasmic reticulum stress links obesity, insulin action, and type 2 diabetes, Science 306 (2004) 457–461.