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Non-ionizing radiofrequency field induces unfolded protein response (UPR) in endoplasmic reticulum of mouse neuronal cells

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C/EBP homologous Protein (CHOP)

ABSTRACT

Objective: To examine whether exposure of mouse neuronal cells to radiofrequency fields used in mobile communication devices can induce stress in endoplasmic reticulum (ER) and activate unfolded protein response (UPR).

Methods: HT22 mouse hippocampus neuronal cells were exposed to continuous wave 900 MHz radiofrequency fields (RF) at 120 μW/cm^2 power intensity for 4 h/d for 5 consecutive days. The positive control cells were irradiated with 4 Gy of ^{60}Co γ-rays at a dose rate of 0.5 Gy/min (GR). Twenty-four hours after the last exposure, cells were collected, and the expressions of sensor transmembrane proteins were detected using Western blot analysis.

Results: The expression levels of Ire1, PERK, p-IRE1 and p-PERK, GRP78 and CHOP proteins were detected. There were no statistically significant differences in the expression levels of IRE1 and PERK proteins in control (CT), sham(SH)-, RF- and GR-exposed cells ($P < 0.05$). The phosphorylated protein levels of p-IRE1 and p-PERK were significantly increased in cells exposed to RF and GR ($P < 0.05$). The expression levels of GRP78 and CHOP were significantly increased in RF- and GR-exposed cells compared to CT and SH-exposed cells ($P < 0.05$). Cells treated with 1 μg/ml TM for 24 h showed significantly increased expression levels of GRP78 and CHOP proteins compared to controls ($P < 0.05$). In the presence of 2 mmol/L PBA, TM-induced increased levels of GRP78 and CHOP proteins were reduced ($P < 0.05$).

Conclusions: The exposure of non-ionizing 900 MHz RF was able to cause stress in HT22 mouse neuronal cells and activated UPR in ER. Since UPR plays an important role in both cell survival (when UPR is mitigated) and apoptosis/death (under unresolvable stress conditions), further studies are required to determine the fate of the cells exposed to RF.

1. Introduction

Non-ionizing radiofrequency fields (RF, 300 MHz to 300 GHz) are ubiquitous in environment. The number of people exposed to RF increased dramatically with the introduction of mobile communication systems. The biological and health effects of RF exposure have been investigated for several decades. Recently, the International Agency for Research on Cancer(IARC) classified RF as a possible carcinogen.[1] However, the few reports on the effect of RF exposure on endoplasmic reticulum (ER) have not received much attention.[2–4] ER is a multifunctional organelle in cells. It is crucial for the synthesis, folding and processing of secretory and transmembrane proteins which are required for various cellular functions.[5] Exposure to environmental agents, excess generation of reactive oxygen species (ROS), viral infection, inflammation, glucose deprivation, protein and lipid overload, etc. perturbs the homeostasis in ER and causes stress which leads to accumulation of unfolded proteins.[6–11] To adapt or to alleviate the stress, ER activates a cascade of complex signaling pathways/network, referred as unfolded protein response (UPR), which is regulated by 3 master sensor transmembrane proteins: (i) inositol-requiring element 1 (IRE1), (ii) protein-kinase-like endoplasmic reticulum kinase (PERK) and (iii) activating transcription factor 6 (ATF6).[12,13] The cells will survive when UPR is mitigated. Under unresolvable stress conditions, UPR promotes apoptosis/cell death.[14] In this context, there have been inconsistent reports of

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increased apoptosis in cells exposed to RF: some studies showed increased levels while the others did not observe the same.[1,15,16] The role of UPR in the induction of apoptosis was not delineated.

In view of the inconsistent reports of RF-induced apoptosis and paucity of RF investigations on UPR in ER, we have conducted the present study. Mouse HT22 neuronal cells were exposed to 900 MHz RF for 4 h/d for 5 d to examine if RF exposure can induce UPR in ER.[17] The rationale is that the mobile phone which transmits 900 MHz RF is held close to the head when used and thus, the brain is the target for RF exposure and, the exposure pattern mimics intermittent RF exposure in humans. Un-exposed (CT) and sham-exposed (SH) control cells as well as those exposed to an acute dose of ionizing/gamma radiation (GR) as positive control cells were included in the experiment. The expression levels of IRE1, PERK, ATF6, GRP78 (glucose-regulated protein) and CHOP (C/EBP homologous protein) proteins involved in UPR were examined. To verify the induction of UPR in HT22 cells, a known inducer (tunicamycin, TM) and an inhibitor (4-sodium phenylbutyrate, PBA) of UPR in ER[18] were also included in the study.

2. Materials and methods

2.1. Cells

HT22 cells, a sub-line derived from parent HT4 cells that were originally immortalized from mouse hippocampus neurons[17] were presented from Dr. Xingshun Xu, Institute of Neuroscience, Soochow University, Suzhou, China. They were cultured in Dulbecco's minimum essential medium (DMEM, Hyclone, Logan, Utah, USA) containing 10% fetal bovine serum (Gibco, Shanghai, China), 100 U/ml penicillin and 100 μg/ml streptomycin (Bio Basic, Hangzhou, China) in an incubator maintaining (37 ± 0.5)°C and, humidified atmosphere of 95% air and 5% carbon dioxide (Heal Force Bio-Meditech, Hong Kong, China). Cells in 2–3 passages were used for experiments. Several 100 mm petri dishes, each containing approximately 5×10^5 cells/ml (total 8 ml medium) were used for the following exposure conditions: (a) unexposed control cells kept in the incubator (CT); (b) 900 MHz RF, 120 μW/cm^2 power intensity; (c) sham-exposure (SH, kept in GTEM without RF transmission); (d) acute 4 Gy γ-ray radiation (GR, positive controls). Immediately after exposures, all cells were returned to the incubator. Twenty-four hours later, cells in each petri dish were collected to perform Western blot analysis. Each experiment was repeated 3 times.

2.2. RF/SH exposure

An in-house built RF exposure system was described in detail earlier.[19] It was installed in a room at (37 ± 0.5)°C, consisting of a Giga-hertz Transverse Electro-Magnetic chamber (GTEM, 5.67 ml, 2.83 mW, 2.07 mH), signal generator (SN2130J6030, PMM, Cisano sul Neva, Italy) and power amplifier (SN1020, HD Communication, Ronkonkoma, NY). The continuous wave 900 MHz RF signal was fed into GTEM through an antenna (Southeast University, Nanjing, Jiangsu, China). A field strength meter (PMM, Cisano sul Neva, Italy) was used to determine 120 μW/cm^2 power intensity at the precise position where the cells were exposed to RF inside the GTEM. The power was monitored and recorded every 5 min in a computer controlled data logging system. The temperature of the medium during exposure of cells to RF was (37 ± 0.5)°C(same as inside GTEM). At the input 120 μW/cm^2 power intensity and the direction of propagation of the incident field parallel to the plane of the medium, the estimated peak and average specific absorption rates (SARs) were 4.1×10^{-4} and 2.5×10^{-4} W/kg, respectively.[20] The same GTEM, without RF transmission, was used for SH-exposure of cells. The duration of RF/SH exposure was 4 h/day for 5 consecutive days. The culture medium was changed once during these 5 days. All experiments were conducted blindly so that the researchers performing laboratory protocols were not aware of the exposure conditions of the cells.

2.3. Validation of UPR in ER

Approximately 5×10^5/ml cells in 10 ml medium were placed in separate 100 mm petri dishes. Cells were exposed to 1 μg/ml TM (activator of UPR, SolarBio, Beijing, China) for 24 h at (37 ± 0.5)°C. (b) Cells were treated first with 2 mmol/L PBA (inhibitor of UPR, Sigma, St. Louis, MO, USA) for 5 h and then exposed to 1 μg/ml of TM for 24 h (i.e., PBA + TM together) at (37 ± 0.5)°C. The rationale is that PBA should be able to inhibit TM-induced UPR in ER. Cells exposed to 4 Gy ± PBA were also included in the experiment. All cells were washed 3 times with culture medium for use in Western blot analysis.

2.4. γ-ray radiation

The positive control cells were exposed to an acute dose of 4 Gy at a dose rate of 0.5 Gy/min from ^{60}Co γ-rays source (Nordion, Ottawa, ON, Canada). Hence, there was an interval of about 10 min between GR exposure and transport of the cells to the laboratory to perform Western blot analysis.

2.5. Western blot analysis

The radio-immuno-precipitation assay was used for Western blot analysis. Total protein was extracted from each cell lysate (Solarbio, Beijing, China) with 1 mmol/L phenylmethanesulfonyl fluoride (Beyotime, Shanghai, China) and the concentration was determined using the bicinchoninic acid protein assay kit (Beyotime, Shanghai, China). From each sample, equal amount of protein (30 μg per lane) was loaded, separated by 10% sodium dodecyl sulfate–polyacrylamide gel which was then transferred to polyvinylidene difluoride membrane (Millipore Corporation, Billerica, MA, USA). The membranes were blocked for 2 h in 5% non-fat powdered milk (Sangon Biotech, Shanghai, China) containing Tween 20-Tris-buffered saline and incubated overnight at 4°C with primary rabbit monoclonal antibodies for anti-Ire1, anti-pho-Ire1 (phosphorylated IRE1, p-IRE1), anti-PERK, anti-pho-PERK (phosphorylated PERK, p-PERK), anti-ATP-6, anti-pho-ATP6 (phosphorylated, ATP6, p-ATF6), anti-GRP78, anti-CHOP, and anti-β-actin (house-keeping gene) (all obtained from Abcam, Cambridge, UK). Then, the membranes were washed and further incubated with horseradish peroxidase-conjugated antibodies (Beyotime, Shanghai, China) for 2 h at room temperature. This was followed by washing the membranes three times with Tris buffer. The immunoreactive proteins on the membranes were detected using an enhanced chemiluminescence reagent (Millipore Corporation) using G: BOX Chemi XRQ (Syngene, UK). The blots were quantified by densitometry and normalized for β-actin to correct for differences in loading of the proteins in various groups of exposed cells.[21] The results presented were average ± standard deviation from three independent experiments.

2.6. Statistical analysis

All data were analyzed using statistical analysis system software for windows version 9.4. The results were subjected to One-way analysis of variance (ANOVA) to test differences between exposure groups. The data were also evaluated using Tukey's test. A P value < 0.05 was considered as statistically significant difference among groups.

3. Results

The expression levels of ATF6 protein or its phosphorylated counterpart protein could not be detected by Western blot analysis. This result suggested that the ATF6 signal pathway was probably not involved in UPR in ER of HT22 mouse hippocampus neuronal cells.

The expression levels of Ire1, PERK, p-IRE1 and p-PERK, GRP78 and CHOP proteins were presented in Fig. 1. There were no statistically significant differences in the expression levels of IRE1 and PERK proteins in

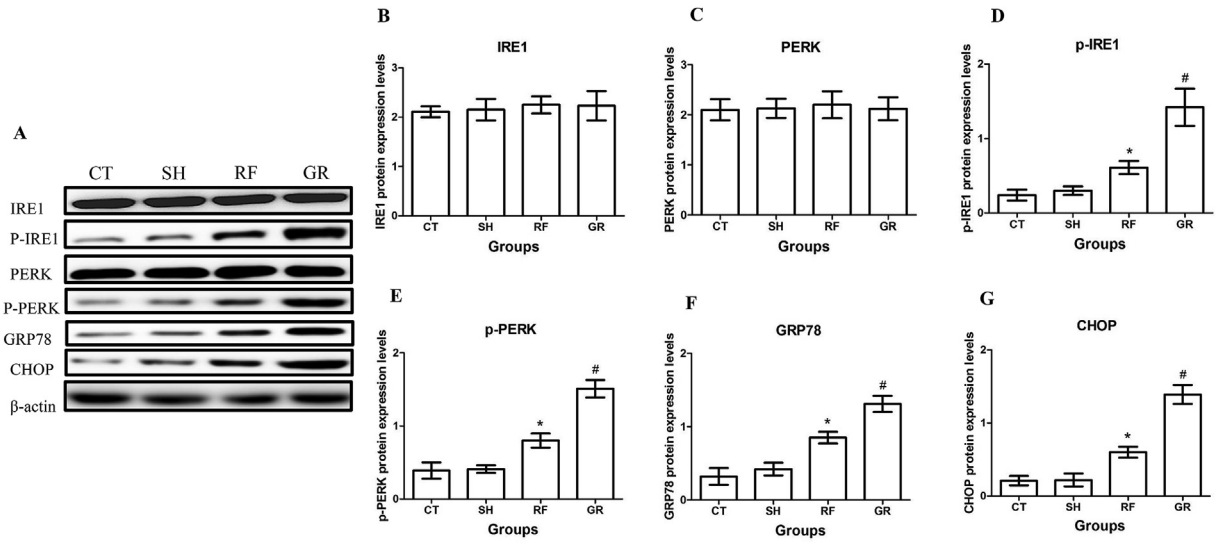


Fig. 1. Western blot analysis on the expression of GRP78, CHOP, IRE1, p-IRE1, PERK, p-PERK in HT22 cells.

A. Western blot analysis of UPR-related proteins in mouse HT22 neuronal cells: CT: Unexposed controls kept in the incubator. RF: Exposed to 900 MHz radio frequency fields at 120 μW/cm^2 power intensity of 4 h/d for 5 consecutive days. SH: sham exposed without RF transmission. GR: Positive control cells exposed to 4 Gy gamma radiation.

B. Relative expression on level of inositol-requiring element 1 (IRE1) protein by Western blot analysis in mouse HT22 neuronal cells. See text for other details. *, #: significantly different from CT and SH ($P < 0.05$).

C. Relative expression level of protein-kinase-like endoplasmic reticulum kinase (PERK) protein by Western blot analysis in mouse HT22 neuronal cells. *, #: significantly different from CT and SH ($P < 0.05$).

D. Relative expression level of phosphorylated inositol-requiring element 1 (p-IRE1) protein by Western blot analysis in mouse HT22 neuronal cells. *, #: significantly different from CT and SH ($P < 0.05$).

E. Relative expression level of phosphorylated protein-kinase-like endoplasmic reticulum kinase (p-PERK) protein by Western blot analysis in mouse HT22 neuronal cells. *, #: significantly different from CT and SH ($P < 0.05$).

F. Relative expression level of glucose-regulated protein 78 (GRP78) protein by Western blot analysis in mouse HT22 neuronal cells. *, #: significantly different from CT and SH ($P < 0.05$).**G**. Relative expression level of C/EBP homologous protein (CHOP) protein by Western blot analysis in mouse HT22 neuronal cells. *, #: significantly different from CT and SH ($P < 0.05$).

CT, SH-, RF- and GR-exposed cells ($P > 0.05$). The phosphorylated protein levels of p-IRE1 and p-PERK were significantly increased in cells exposed to RF and GR ($P < 0.05$). The expression levels of GRP78 and CHOP were significantly increased in RF- and GR-exposed cells compared to CT and SH-exposed cells ($P < 0.05$). These results indicate that both RF exposure and positive control γ-radiation could not significantly increase the inactive protein level of IRE1 and PERK, however could induce the autophosphorylation of these two UPR-related proteins (p-IRE1 and p-PERK), increase the expression levels of GRP78 and CHOP, hence UPR via IRE1 and PERK signal pathway to facilitate the restoration of homeostasis in ER of HT22 cells.

The expression levels of GRP78 and CHOP protein in cells exposed to TM and PBA were presented in Fig. 2. Cells treated with 1 μg/ml TM for 24 h showed significantly increased expression levels of GRP78 and

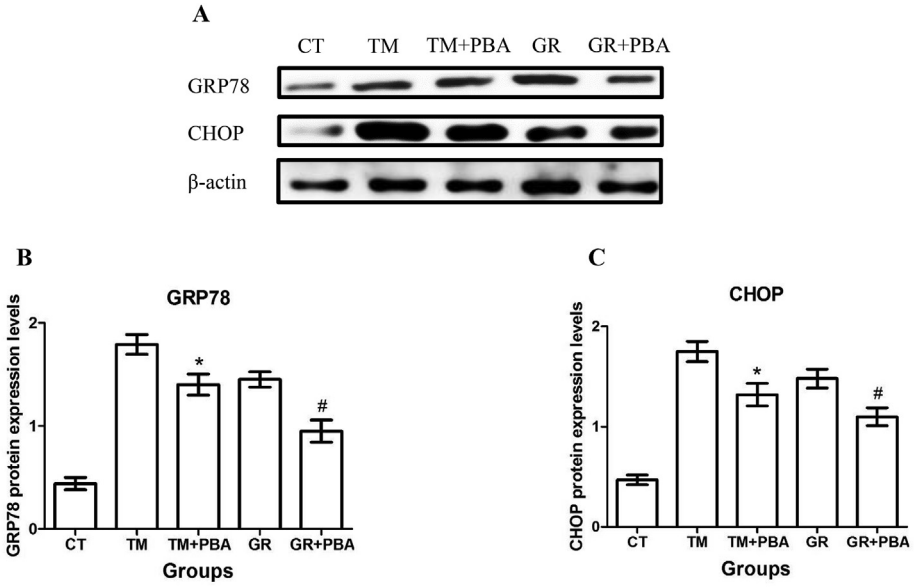


Fig. 2. Western Blot analysis on the expression of GRP78 and CHOP in HT22 cells exposed to TM and PBA.

A. Western blot analysis to validate activation of UPR in mouse HT22 neuronal cells exposed to tunicamycin (TM) and TM + 4-sodium phenylbutyrate (PBA), 4 Gy γ-ray radiation as positive control (GR) and GR + PBA.

B. Relative expression level of glucose-regulated protein 78 (GRP78) protein by Western blot analysis in mouse HT22 neuronal cells. *: significantly different from TM alone. #: significantly different from GR alone ($P < 0.05$).

C. Relative expression level of C/EBP homologous protein (CHOP) protein by Western blot analysis in mouse HT22 neuronal cells. *: significantly different from TM alone. #: significantly different from GR alone ($P < 0.05$).

CHOP proteins compared to controls ($P < 0.05$). In the presence of 2 mmol/L PBA, TM-induced increased levels of these 2 proteins were reduced ($P < 0.05$). In this investigation, TM and PBA were used as activator and inhibitor of UPR in ER, respectively, the results further validated the UPR-induction capacity of RF exposure and γ-ray radiation.

4. Discussion

The overall observations in this study showed no significant differences between CT cells kept in incubator and SH-exposed cells. SH-exposed cells were considered more appropriate for comparison with RF-exposed cells (they were handled identical in GTEM) and hence, the differences between RF- and SH-exposed cells were discussed below. Also, the expression of one of the 3 UPR sensor proteins in ER, namely ATF6, was not detected in RF- and SH-exposed cells and hence, it was not discussed below. The other two sensor transmembrane proteins in ER, IRE1 and PERK, are reportedly inactive by being sequestered to Bip/GRP78 (immunoglobulin binding protein/glucose-regulated protein 78). When ER is under stress, the increased concentrations of unfolded proteins binds completely to BiP/ GRP78resulting in the release and activation of IRE1 and PERK which initiate the UPR signal transduction through autophosphorylation.[12,13,21] The results obtained in this study suggested that RF exposure might have caused stress in ER leading to accumulation of unfolded proteins, and the presence of unfolded proteins might have released IRE1 and PERK from GRP78 and initiated UPR by autophosphorylation (p-IRE1 and p-PERK). The increased expression level of GRP78 protein in RF-exposed cells could be due to the release of sequestration of IRE1 and PERK.

There are some reports indicating that upon activation, PERK phosphorylates eukaryotic initiation factor 2 (eIF2α) which concurrently increases translation efficiency of activating transcription factor 4 (ATF4), which in turn regulates a transcriptional program that contributes to both pro-survival and pro-apoptotic death pathways.[22,23] Pro-survival transcripts include amino acid transporters and regulators of redox control while a key apoptotic transcript directly activated by ATF4 is CHOP/-GADD153 (C/EBP family member linked to cellular apoptosis). The regulation of CHOP expression appears to play a key role in cellular commitment to apoptosis.[24,25] The results obtained in this study showed elevated expression of CHOP protein in RF-exposed cells compared to SH-exposed cells. Whether or not the increased CHOP in RF-exposed cells influences cell survival or apoptosis needs further investigation. The observations in positive control cells exposed to GR in our study were essentially similar to those in RF-exposed cells. These results were similar to those in several reports indicating that ionizing radiation induced ER stress and activates UPR.[19,26,27] The results from the experiments using TM and PBA (activator and inhibitor of UPR in ER, respectively) validated the observations in RF-exposed cells. Cells treated with TM alone showed increased expression levels of GRP78 and CHOP while pre-treatment with PBA was able to significantly reduce the TM-induced response ($P < 0.05$).

To the best of our knowledge, there were very few reports examining the effect of RF exposure on UPR in ER. Nicolaz et al[2,3] exposed human astrocytoma cells exposed to 60 GHz RF (millimeter waves) at a maximum power intensity of 0.14 mW/cm^2 for 24 h and observed no changes in mRNA expression of BiP/GRP78 (RT-PCR technique) and protein synthesis/folding. They concluded that ER homeostasis did not undergo any modification at molecular levels. Le Quement et al[4] exposed A375 human malignant melanoma cells to 60.4 GHz RF at 1, 2.5, 5, 10, 15 and 20 mW/cm^2 power intensity for 20 min, 6, 16 or 24 h. The mRNA expression levels of two ER-resident chaperons, BiP and ORP150, showed no changes in basal levels while RF co-exposure with thapsigargin (TG, known ER-stress inducer) inhibited TG-induced over-expression of both chaperones. When the data were compared with SH-exposed controls, the inhibition was linked to the thermal effect resulting from RF exposure. In our study, 900 MHz RF exposure at 120 μW/cm^2 power intensity was conducted at (37 ± 0.5)°C and the results showed activation of UPR in ER which was also confirmed in cells treated with TM and PBA. However, whether the difference in RF frequency, power intensity, temperature and cell type used in our study contributed to the differential response in eliciting UPR in ER is not clear.

Whether or not activated UPR in RF-exposed cells poses any health consequences is not clear. However, it is known that if UPR is mitigated, it helps in cell survival or under unresolvable stress conditions, UPR promotes apoptosis.[14] In this context, it is important to mention several reports implicating UPR in human diseases, including but not limited to vascular dysfunction in diabetes, pancreatic β-cell dysfunction, insulin resistance, inflammation, hypoxia/ischemia, cystic fibrosis, liver diseases, cancer, aging and age-related neurodegenerative diseases such as Alzheimer's disease.[28–34]

Homeostasis in ER is crucial and critical for normal functioning of cells. There observations in this study suggested that exposure of the HT22 neuronal cells to 900 MHz RF caused stress and activated of UPR in ER which was also verified in cells exposed to TM and PBA, known inducer and inhibitor of stress in ER, respectively. Positive control cells exposed to γ-ray radiation, which is known to induce stress in ER, also showed activation of UPR. Since UPR is important for cell survival (when UPR is mitigated) and death/apoptosis (under unresolvable stress conditions), further studies are required to determine the fate of the cells exposed to RF. The overall paucity of research on UPR in ER in cells exposed to RF at frequencies used in mobile wireless communications systems and the implication of UPR in human diseases justifies detailed investigations on UPR in ER in animal and human cells.

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Declaration of competing interest

None.

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References

1. International Agency on Research on Cancer. *Non-ionizing Radiation, Part 2: Radiofrequency Electromagnetic Fields. Monograph Series*. Geneva: World Health Organization; 2013.
2. Nicolaz CN, Zhadobov M, Desmots F, et al. Absence of direct effect of low-power millimeter-wave radiation at 60.4 GHz on endoplasmic reticulum stress. *Cell Biol Toxicol*. 2009;25:471–478. https://doi.org/10.1007/s10565-008-9101-y.
3. Nicolaz CN, Zhadobov M, Desmots F, et al. Study of narrow band millimeter-wave potential interactions with endoplasmic reticulum stress sensor genes. *Bioelectromagnetics*. 2009;30:365–373. https://doi.org/10.1002/bem.20481.
4. Le Quément C, Nicolaz CN, Habauzit D, et al. Impact of 60-GHz millimeter waves and corresponding heat effect on endoplasmic reticulum stress sensor gene expression. *Bioelectromagnetics*. 2014;35:444–451. https://doi.org/10.1002/bem.21864.
5. Braakman I, Bulleid NJ. Protein folding and modification in the mammalian endoplasmic reticulum. *Annu Rev Biochem*. 2011;80:71–99. https://doi.org/10.1146/annurev-biochem-062209-093836.
6. Yen YP, Tsai KS, Chen YW, et al. Arsenic induces apoptosis in myoblasts through a reactive oxygen species-induced endoplasmic reticulum stress and mitochondrial dysfunction pathway. *Arch Toxicol*. 2012;86(6):923–933. https://doi.org/10.1007/s00204-012-0864-9.
7. Cheng G, Feng Z, He AB. Herpes simplex virus 1 infection activates the endoplasmic reticulum resident kinase PERK and mediates eIF-2α dephosphorylation by the γ_1 34.5 protein. *J Virol*. 2005;79(3):1379–1388. https://doi.org/10.1128/JVI.79.3.1379-1388.
8. Zhang K, Kaufman RJ. From endoplasmic reticulum stress to the inflammatory response. *Nature*. 2008;454(7203):45562. https://doi.org/10.1038/nature07203.
9. Kozutsumi Y, Segal M, Normington K, et al. The presence of malfolded proteins in the endoplasmic reticulum signals the induction of glucose-regulated proteins. *Nature*. 1988;332:462–464. https://doi.org/10.1038/332462a0.

10. Oyadomari S, Yun C, Fisher EA, et al. Cotranslocational degradation protects the stressed endoplasmic reticulum from protein overload. *Cell*. 2006;126(4):727–739. https://doi.org/10.1016/j.cell.2006.06.051.
11. Fang DL, Wan Y, Shen W, et al. Endoplasmic reticulum stress leads to lipid accumulation through upregulation of SREBP-1c in normal hepatic and hepatoma cells. *Mol Cell Biochem*. 2013;381(1–2):127–137. https://doi.org/10.1007/s11010-013-1694-7.
12. Ma Y, Hendershot LM. The unfolding tale of the unfolded protein response. *Cell*. 2001;107:827–830. https://doi.org/10.1016/s0092-8674(01)00623-7.
13. Ron D, Walter P. Signal integration in the endoplasmic reticulum unfolded protein response. *Nat Rev Mol Cell Biol*. 2007;8:519–529. https://doi.org/10.1016/j.molcel.2017.06.017.
14. Jäger R, Bertrand MJ, Gorman AM, et al. The unfolded protein response at the crossroads of cellular life and death during endoplasmic reticulum stress. *Biol Cell*. 2012;104(5):259–270. https://doi.org/10.1111/boc.201100055.
15. Swedish Radiation Safety Authority. *Recent research on EMF and health risk: Tenth report from SSM's Scientific Council on electromagnetic fields*. Stockholm: Swedish Radiation Safety Authority; 2015.
16. Zhao L, Li J, Hao YH, et al. Microwave-induced apoptosis and cytotoxicity of NK cells through ERK1/2 signaling. *Biomed Environ Sci*. 2017;30:323–332. https://doi.org/10.3967/bes2017.043.
17. Liu J, Li L, Suo WZ. HT22 hippocampal neuronal cell line possesses functional properties. *Life Sci*. 2009;84:267–271. https://doi.org/10.1016/j.lfs.2008.12.008.
18. Lee ES, Lee HJ, Lee YJ, et al. Chemical chaperones reduce ionizing radiation-induced endoplasmic reticulum stress and cell death in IEC-6 cells. *Biochem Biophys Res Commun*. 2014;450:1005–1009. https://doi.org/10.1016/j.bbrc.2014.06.091.
19. Cao Y, Xu Q, Jin ZD, et al. Effects of 900-MHz microwave radiation on γ-ray-induced damage to mouse hematopoietic system. *J Toxicol Environ Health Part A*. 2010;73:507–513. https://doi.org/10.1080/15287390903523451.
20. He Q, Zong L, Sun Y, et al. Adaptive response in mouse bone marrow stromal cells exposed to 900 MHz radiofrequency fields: impact of poly (ADP-ribose) polymerase (PARP). *Mutat Res*. 2017;820:19–25. https://doi.org/10.1016/j.mrgentox.2017.05.007.
21. Harding HP, Novoa I, Zhang Y, et al. Regulated translation initiation controls stress-induced gene expression in mammalian cells. *Mol Cell*. 2000;6:1099–1108. https://doi.org/10.1016/s1097-2765(00)00108-8.
22. Ma Y, Brewer JW, Diehl JA, et al. Two distinct stress signaling pathways converge upon the CHOP promoter during the mammalian unfolded protein response. *J Mol Biol*. 2002;318(5):1351–1365. https://doi.org/10.1016/S0022-2836(02)00234-6.
23. Pavitt GD, Ron D. New insights into translational regulation in the endoplasmic reticulum unfolded protein response. *Cold Spring Harb Perspect Biol*. 2014;4(6):1–9. https://doi.org/10.1101/cshperspect.a012278.
24. Harding HP, Zhang Y, Zeng H, et al. An integrated stress response regulates amino acid metabolism and resistance to oxidative stress. *Mol Cell*. 2003;11:619–633. https://doi.org/10.1016/S1097-2765(03)00105-9.
25. Tabas I, Ron D. Integrating the mechanisms of apoptosis induced by endoplasmic reticulum. *Nat Cell Biol*. 2011;13:184–190. https://doi.org/10.1038/ncb0311-184.
26. Zhang B, Wang Y, Pang X, et al. ER stress induced by ionizing radiation in IEC-6 cells. *Int J Radiat Biol*. 2010;86:429–435. https://doi.org/10.3109/09553001003668014.
27. Panganiban R, Mungunsukh O, Day R. X-irradiation induced ER stress, apoptosis, and senescence in pulmonary artery endothelial cells. *Int J Radiat Biol*. 2013;89:656–667. https://doi.org/10.3109/09553002.2012.711502.
28. Yoshida H. ER stress and diseases. *FEBS J*. 2007;274:630–658. https://doi.org/10.1111/j.1742-4658.2007.05639.x.
29. Marchetti P, Bugliani M, Lupi R, et al. The endoplasmic reticulum in pancreatic beta cells of type 2 diabetes patients. *Diabetologia*. 2007;50:2486–2494. https://doi.org/10.1007/s00125-007-0816-8.
30. Garg AD, Kaczmarek A, Krysko O, et al. ER stress-induced inflammation: does it aid or impede disease progression. *Trends Mol Med*. 2012;18:589–598. https://doi.org/10.1016/j.molmed.2012.06.010.
31. Dara L, Ji C, Kaplowitz N. The contribution of endoplasmic stress to liver diseases. *Hepatology*. 2011;53:1752–1763. https://doi.org/10.1002/hep.24279.
32. Hetz C. The UPR as a survival factor of cancer cells: more than folding proteins? *Leuk Res*. 2009;33(7):880–882. https://doi.org/10.1016/j.leukres.2009.02.017.
33. Doyle KM, Kennedy D, Gorman AM, et al. Unfolded proteins and endoplasmic reticulum stress in neurodegenerative disorders. *J Cell Mol Med*. 2011;15:2015–2039. https://doi.org/10.1111/j.1582-4934.2011.01374.x.
34. Brown MK, Naidoo N. The endoplasmic stress response in aging and age-related diseases. *Front Physiol*. 2012;3:263. https://doi.org/10.3389/fphys.2012.00263.