4 - 54 Inhibiting Autophagy Enhances the Anti-tumor Effect of High-LET Carbon Ions via Promoting ER Stress-related Apoptosis

Zheng Xiaogang, Jin Xiaodong and Li Qiang

Autophagy is an evolutionarily conserved catabolic process directly related to human health and various diseases. Autophagy helps cells under stress to cope with severe metabolic demand by degradation of basic cellular components. Diverse physiological or pathological changes may lead to endoplasmic reticulum (ER) stress. In response to ER stress, BiP disassociates from sensor proteins PERK, ATF6, and IRE1 and then the unfolded protein response (UPR) is activated. The UPR essentially aims at reestablishing proper ER homeostasis and eventually induces autophagy and apoptosis during acute or persistent ER stress.

In this work, by using the S180 mouse sarcoma cell line, combined *in vitro* and *in vivo* experiments after irradiation with high linear energy transfer (LET) carbon ions (CI) or low-LET X-rays, we examined how inhibition of autophagy induced by ER stress enhances apoptosis and increases the anti-tumor effect of ionizing radiation. We obtained the following results:

The combination of ionizing radiation and chloroquine (CQ) could inhibit the proliferation of S180 cells, promote cell apoptosis, and inhibit the growth of transplanted tumor. ER stress induced by ionizing radiation elicited autophagy via the IRE1/JNK/p-Bcl-2/Beclin-1 pathway, which can alleviate ER stress and maintain the proliferation of tumor cells and tumor growth. After the inhibition of autophagy by CQ, intracellular unfolded or misfolded proteins cannot be cleaned quickly and effectively, leading to apoptosis of tumor cells and enhancement of the radiosensitivity of tumor cells and xenografts. CI radiation could induce UPR signaling, and IRE1 upregulated the expression of pro-apoptotic protein CHOP, which led to the down-regulation of the anti-apoptotic mitochondrial protein Bcl-2 and increased the expression of apoptotic protein Bax. Meanwhile, X-rays exerted no effect on CHOP expression, which may be one of the reasons CI provides a greater advantage in tumor suppression, compared with X-rays. CI radiation combined with continuous administration of CQ could more effectively suppress tumor.

In summary, this work showed that high-LET CI combined with CQ could enhance the anti-tumor effect of CI radiation via the aggravation of ER stress-related apoptosis, thereby increasing the radiosensitivity of tumor cells.

4 - 55 Biological Effects of Iron Ion Radiation in Mice*

Zhang Hong, Li Hongyan and Yan Jiawei

Cosmic radiation and microgravity are the two main factors of the space environment affecting the health of astronauts[1]. The high background level of ionizing radiation is particularly dangerous during missions of long duration[2], since exposure to cosmic radiation causes oxidative damage that induces DNA lesions, cancer, cell death[3] and other adverse effects[4]. Protons and ions of high atomic number and energy (HZE) particles are the main source of radiation that astronauts are exposed to[5], and the high ionization density of heavy ions in particular causes complex DNA damage that is more difficult to repair than conventional radiation-induced damage from X-rays and γ-rays[6]. Heavy ion radiation (HIR) kills cells by a combination of direct and indirect actions. Direct actions involve a direct hit on a biologically important target by a particle or beam, and this causes more damage than indirect actions that involve water-derived free radicals[7]. Therefore, heavy ion radiation is effective at killing cells with minimal dependence on cell-cycle or oxygen levels[6], and can trigger cell death via multiple mechanisms including apoptosis, necrosis, autophagy, premature senescence, accelerated differentiation and/or delayed reproductive cell death[8]. Iron ions are of special interest in space radiation research[9], and ^{56}Fe is probably the most important ion regarding radiation exposure[10], while carbon ions are the preferred heavy ion for cancer radiotherapy[11].

As the development of manned space flight continues, the duration and distance of shuttle missions extend from those in past years. However, it has also increased the risks of central nervous system (CNS) damage which is attributed to exposure to solar particles and cosmic rays. In general, these solar particles and cosmic rays mainly consist of high linear energy transfer (LET) ions such as protons and high (H) atomic number (Z) and high-energy (E) ions[12]. Although HZE particles are a small part of cosmic rays, these highly diverse charged ions contribute a dominant share of the effective dose and they also possess a strong ability for oxidative damage which induces impairment of DNA and some other biological molecules[13]. It is known that whole-body exposure of mice to HZE particles may induce significant deficits in the CNS. Although the CNS is the most important system in the

body and has been fully researched, it remained uncertain how ionizing radiation exposure affected the CNS[14]. The underlying mechanisms will certainly be multifaceted and several mechanisms are considered to play a role in the radiation-induced deficits[15], but oxidative stress may represent the direct and most important mechanistic explanation for it since studies shows that oxidative damage of nucleic acids, proteins, and lipids is directly correlated with neurodegeneration, aging, cardiovascular diseases and pathologies of some carcinomas[16]. Interactions between DNA and reactive oxygen species (ROS) induced by radiation in damaged cells lead to DNA strand-breaks and base modification which can be quantitatively estimated with 8-hydroxy-2′-deoxyguano-sine (8-OHdG) produced by the reaction of ROS on guanine ordinarily in animal organs and in human samples. We investigated the question of whether the brain can be adversely aff ected after 4 weeks by whole-body exposure to different doses (600 MeV/u, 0.5, 1 and 2 Gy) of ^{56}Fe ion irradiation. Experiments show that exposure to ^{56}Fe beam resulted in significant impairment of cognitive performance. To further study the causes of cognitive impairment, oxidative stress and DNA damage, which may relate to cogni-tive deficits, were evaluated by the levels of malondialdehyde (MDA), glutathione (GSH) and 8-OHdG. In addition, some other histological and biochemical experiments were carried out. Finally, the studies imply that these doses of ^{56}Fe ion irradiation compromise cognitive performance through mechanisms involving changes of oxidative stress and oxidative DNA damage in brain tissue[17].

The testis is a highly radiosensitive organ comprising many proliferating cells[18], and HIR affects testicular development and spermatogenesis[19]. Studies have confirmed that radiation-induced chromosomal aberrations in spermatogonia and spermatocytes can be transmitted into the developing spermatozoa, causing asthenospermia, hypospermia, and teratospermia[20]. Thus, it is important to ascertain whether exposure to cosmic radiation induces spermatogenesis dysfunction so that we can understand the possible effects on the reproductive potential of astronauts and space travellers. Owing to the paucity of robust human studies, our knowledge of biological effects of space radiation is dependent on in vitro studies with human or animal cells and in vivo research using animal models. Most of our current knowledge of the health effects of cosmic radiation exposures has been obtained from ground-based experiments at accelerators. We monitored some essential reproductive parameters of male mice at 2, 5 and 8 weeks after IIR (Table 1), and we utmost mimic the dynamic changes in reproductive systems of male after long space missions returned to earth. Moreover, this time was through differential period of mice that from puberty (from 5 to 7 weeks of age) to adult (from 8 to 30 weeks of age), it can more reasonably simulate to reflect the reproductive health statuses of different ages when young astronauts returned to earth.

Table 1 Comparison of body weight, reproductive organ indexes, epididymal weight, epididymal count and sperm motility in mice.

Time	Body weight/g	Testes weight/g	Testis ratio/%	Epididymides weight/g	Epididymides ratio/%	Epididymal count ($\times10^6$)	Sperm motility/%
2WCK(n=4)	31.49±1.203	0.1601±0.014 9	0.507 6±0.046 6	0.060 7±0.007 6	0.190 3±0.008 4	1.44±0.149	74.88±5.23
IIR(n=4)	25.03±0.050 1**	0.079 6±0.005 3**	0.337 1±0.021*	0.036 8±0.008 8**	0.147 2±0.013 8*	0.68±0.058*	30.87±9.49***
5WCK(n=5)	37.15±0.985 1	0.246 4±0.013 3	0.681±0.046	0.078 9±0.004 5	0.217 5±0.005 6	3.584±0.161 5	86.57±4.9
IIR(n=5)	36.33±0.802 7	0.159 3±0.006***	0.428 8±0.013 5*	0.069 3±0.006 8	0.186 7±0.008*	1.064±0.213 5***	40.35±2.85***
8WCK(n=5)	42.9±1.715	0.288 1±0.010 8	0.672 7±0.168	0.107 2±0.016 1	0.249 5±0.011	7.488±0.531 4	80.51±9.54
IIR(n=5)	38.14±0.973 9	0.219 4±0.018 6*	0.579 6±0.060 9*	0.080 1±0.011 2	0.209 8±0.011 2*	6.82±0.430 5	65.8±8.3

Values represent averages ±S.E.M. Asterisks denote values that are different from controls, $^*P<0.05$, $^{**}P<0.01$ and $^{***}P<0.001$ with Student's t–test analysis.

Compared with control, a significant differences in the body weight was just observed at 2 weeks, suggesting that the deleterious consequence of IIR on mice body weight can be decreased as time passed, however, a significant decrease of the testes weight and testes ratio were observed in all time points that indicating deleterious consequence of IIR on mice testis is a sustainable position, because previous studies demonstrated radiation can kill some spermatogenic cells and cause recovery is more gradual, however, the excessive damage could cause no recovery of the seminiferous epithelium in mice. At 2 weeks, a significantly decreased in epididymides weight and epididymides ratio showed that IIR was not just disturbed spermatogenesis, but also cause epididymis atrophy and deleterious that effects in sperm maturation and storage. Moreover, there is a relationship between decreased luminal sperm in cauda epididymis and damaged spermatogenesis. The reasons of decreasing in sperm count may be have two aspects, one is that IIR directly damages or kills some spermatogenic cells when radiation and cause recovery is more gradual. Other is that IIR indirectly increases spermatogenic cells apoptosis after radiation. The relationship between these two possibilities and sperm functions after radiation has been demonstrated, that radiation-induced spermatogenic cells damage or apoptosis can be transmitted into the sperm, causing asthenospermia, hypospermia, and teratospermia.

Moreover, Advances in proteomics and mass spectrometry have provided appropriate methods for measuring changes in sperm protein expression following irradiation. We used a comparative proteomics approach based on a

two-dimensional electrophoresis (2-DE) reference map to determine alterations in protein expression in the sperm of mice following exposure to (IIR). A significant proteome difference between the CK and IIR groups was observed (Table 2).

Table 2 Differential expression of proteins in mice sperm.

Protein spot	Protein name	Abbreviation	Accession (NCBI)	pI/Mw	Peptides Count	Protein Score	Average fold Change [a]
Spot 1	Serum albumin precursor [Mus musculus]	Alb	gi|163310765	5.75/70700.5	16	1100	0.23±0.06
Spot 2	Heat shock 70 kDa protein 1-like	Hspa1l	gi|124339838	5.9 /70992.3	13	299	0.157±0.046
Spot 3	Glycerol-3-phosphate dehydrogenase mitochondrial	Gpd2	gi|146345428	6.17/81415.7	5	340	0.41±0.121
Spot 4	Succinate dehydrogenase [ubiquinone] flavoprotein subunit, mitochondrial, partial	SDHA	gi|15030102	7.11/73363.5	1	113	0.313±0.084
Spot 5	60 kDa heat shock protein, mitochondrial	Hspd1	gi|51702252	5.91/61088.4	9	1000	0.453±0.035
Spot 6	T complex polypeptide 1	Tcp1	gi|201725	6.25/57951.5	4	378	0.47±0.136
Spot 7	Cytochrome b-c1 complex subunit 1, mitochondrial	Uqcrc1	gi|46593021	5.81/53445.7	16	892	0.51±0.062
Spot 8	Alpha-enolase	ENO1	gi|158853992	6.37/47453.3	2	756	0.52±0.02
Spot 9	Testis-specific phosphoglycerate kinase	Pgk2	gi|200326	6.63/45281.4	8	775	0.033±0.006
Spot 10	Aconitate hydratase, mitochondrial	Aco2	gi|60391212	8.08/86151.3	5	773	0.016±0.006
Spot 11	Creatine kinase M-type	Ckm	gi|6671762	6.58/43245.9	7	622	4.9±0.38
Spot 12	Unnamed protein product		gi|12845061	6.5799/43231.9	7	24	4.31±0.326
Spot 13	Keratin, type I cytoskeletal 18	Krt18	gi|254540068	5.22/47509.2	4	901	2.87±0.429
Spot 14	Glial fibrillary acidic protein, partial	Gfap	gi|72679940	5.09/48105.6	22	48	2±0.3
Spot 15	Creatine kinase B-type	Ckb	gi|10946574	5.4/42971.4	4	933	2.67±0.77
Spot 16	Phosphatidylethanolamine-binding protein 1	Pebp1	gi|84794552	5.19/20988.4	17	279	0.443±0.025

[a] Match between control and iron ion radiation sperm (gels of 3 × iron ion radiation *vs.* control; $n = 3$ in each group). Values shown are mean ± S.E.M. ($P < 0.05$).

A total of 16 differentially expressed proteins were found between the CK and IIR groups, the name and function of these proteins are described in Table 2. Among which spots 11, 12, 13, 14, 15 were upregulated (ratios of irradiated/control in Table 2), and spots 1, 2, 3, 4, 5, 7, 8, 9, 10, 16 were downregulated (ratios of irradiated/control in Table 2). The results were used to construct a network of differentially expressed proteins that may play an important role in sperm motility. Bioinformatics analysis identified ENO1 to be involved in the regulation of glycolysis in the sperm of mice, and its expression was correlated with sperm motility. Proteomics and bioinformatics analyses were integrated in an attempt to identify the underlying molecular mechanisms that determine sperm motility following exposure to IIR. Although the functions of many of the differentially expressed proteins requires further investigation, these results provide crucial information on the underlying mechanism of diminished sperm motility in astronauts enduring long space missions.

References

[1] D. J. Lugg, Avait Space Environ Med., 76(2005)B74.

[2] F. Yatagai, N. Ishioka, Life Sciences in Space Research, 3(2014)76.

[3] J. Li, J. Xu W. Xu, et al., Int. J Mol. Sci., 16(2015)18938.

[4] C. E. Hellweg, C. Baumstark-Khan, Naturwis-senschaften, (2007)517.

[5] P. Todd, M. J. Pecaut, M. Fleshner, Mutat Res., 430(1999)211.

[6] N. Hamada, T. Imaoka, S. Masunaga, et al., J Radiat. Res., 51(2010)365.

[7] R. Hirayama, A. Ito, M. Tomita, et al., Radiat. Res., 171(2009)212.
[8] A. Jinno-Oue, N. Shimizu, N. Hamada, et al., Int. J Radiat. Oncol. Biol. Phys., 76(2010)229.
[9] D. Schulz-Ertner, H. Tsujii, Journal of Clinical Oncology, 25(2007)953.
[10] M. Durante, A. Kronenberg, Adv Space Res., 35(2005)180.
[11] Y. Jongen, Academie Royale de Medecine de Belgique, 16 (2008)471.
[12] C. Zeitlin, D. Hassler, F. Cucinotta, et al., Science, 340(2013)1080.
[13] M. Durante, K. Eighth Warren, Health Phys, 103(2012)532.
[14] V. K. Parihar, B. Allen, K. K. Tran, et al., Sci. Adv., 1(2015)e1400256.
[15] J. Raber, R. H. Olsen, W. Su, et al., Brain Res., 275(2014)146–149.
[16] W. Zhao, M. E. Robbins, Curr. Med. Chem., 16(2000)130.
[17] A. Collins, Free Radical Res., 32 (2000) 333.
[18] M. S. Baliga, S. Rao, J Cancer Res. Ther., 6(2010)255.
[19] C. X. Di, L. Han, H. Zhang, et al., Sci Rep., 5 (2015)16020.
[20] H. Zhang, W. Zhao, Y. Wang, et al., Mutat Res., 653(2008)109.

* Foundation item: Key Program of National Natural Science Foundation of China (U1432248), Ministry of Science and Technology National Key R&D project (2016YFC0904600), National Natural Science Foundation of China (Y660010GJ0), Western Talent Program of Chinese Academy of Sciences (Y560020XB0)

4 - 56 Combination Effects of Curcumin and Radiation on the Angiogenesis of Tumor*

Liu Yang, Yan Jianwei and Zhang Hong

Tumor angiogenesis is the formation of new blood vessels from the existing vascular bed to provide tumor cells with sufficient oxygen, nutrients, *etc.*, and consequently contributes to tumorigenesis, invasion and metastasis of solid tumor. Hence, Anti-angiogenesis is a desirable strategy for tumor therapy. Chinese herbal medicine (CHM) and their extracts are emerging as the noticeable choice for its multi-level, multi-target and coordinated intervention effects against tumor such as Danshen, Angelica, Chuan xiong, Apigenin, Silibinin, Wogonin[1−2]. Curcumin derived from the spice plant Curcuma longa, a powerful anti-cancer agent, has a strong anti-inflammatory and antioxidant functions. The aim of the current study was to explore the combinated application of curcumin and radiation in inhibition of angiogenesis of tumor. Our findings revealed that after combinated treatment with curcumin and radiation, the proliferation was suppressed ($P < 0.01$), and the apoptotic rate increased from 3.21% to 17.87% in human microvascular endothelial cells. Furthermore, endothelial cell motility was obviously reduced in the cells administrated with curcumin and radiation than in those treated with curcumin or radiation alone. Importantly, our data showed that endothelial growth factor (VEGF) secretion level was notably blocked when cells were treated with curcumin and radiation, which are important for the degradation of extracellular matrix as well as promotion of endothelial cell proliferation, migration and survival. To understand the underlying mechanisms for the reduced VEGF levels, hypoxia-inducible factor 1 (HIF-1), the major transcriptional regulator of hypoxia-induced angiogenesis through transactivation of genes that encode VEGF, was determined. HIF-1 protein level decreased in line with VEGF alteration. In summary, our data demonstrated that the combination effect of curcumin and radiation on retarding tumor angiogenesis is possibly related to the HIF-1/VEGF pathway.

Reference

[1] C. Liu, X. W. Li, L. M. Cui, et al., World J Gastroenterol, 17(2011)4875.
[2] X. Yang, X. Z. Wu, Mni. Rev. Chem., 15(2015)1011.

* Foundation item: National Natural Science Foundation of China (U1432248), Ministry of Science and Technology National Key R&D project (2016YFC0904600), National Natural Science Foundation of China (11575262)