Space Life Science of China in 2013

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ABSTRACT

In the past two years, space life science research in China is characterized by a wide area of basic researches for providing foundation for the future China Space Station. The effect of microgravity and radiation was further studied from physiology phenomena to the level of bio-molecule mechanisms. Chinese space life science is maturing in a new era of comprehensive development. Here, we review and summarize researches on space life sciences which were contributed by Chinese scientists.

KEY WORDS

Space life sciences, Simulated microgravity, Ionizing radiation

1 Research on Biological Effect of Ground-based Simulated Microgravity

Research in microgravity is indispensable to disclose the impact of gravity on biological processes and organisms. Ground-based microgravity simulators are valuable tools for preparing spaceflight experiments. The various microgravity simulators that are frequently used by gravitational biologists are based on different physical principles. The effects of ground-based microgravity on biological specimens were investigated by using simulators[1]. In this section, current heterogeneous researches are discussed critically, and a summary is given for comprehensive understanding of the influence.

1.1 Underlying Mechanisms in Simulated Microgravity Conditions

The research group from Institute of Zoology, Chinese Academy of Sciences found that LPS-induced tumor necrosis factor-α (TNF-α) expression, but not interleukin-1β expression, in mouse macrophages was significantly

suppressed under simulated microgravity. The molecular mechanism studies showed that LPS-induced intracellular signal transduction including phosphorylation of IKK and JNK and nuclear translocation of NF-κB in macrophages was identical under normal gravity and simulated microgravity. Meanwhile, TNF-α mRNA stability did not decrease under simulated microgravity. Finally, they found that Heat Shock Factor-1 (HSF1), a known repressor of TNF-α promoter, was markedly activated under simulated microgravity. This can be concluded that short-term treatment with microgravity caused significantly decreased TNF-α production. Microgravity-activated HSF1 may contribute to the decreased TNF-α expression in macrophages directly caused by microgravity, while the LPS-induced NF-κB pathway is resistant to microgravity[2].

Another group from Harbin Institute of Technology found that simulated microgravity can alter the structure of spindle microtubules, and stimulate the formation of multipolar spindles together with multicentrosomes, which causes the overexpression of SAC proteins to block the abnormal cells in metaphase, thereby inhibiting cell proliferation. By clarifying the relationship between cell proliferation inhibition, spindle structure and SAC changes under simulated microgravity, the molecular mechanism and morphology basis of proliferation inhibition induced by microgravity is revealed, which will give experimental and theoretical evidence for the mechanism of space bone loss and some other space medicine problems[3].

In order to investigate the potential triggers of cardiovascular dysfunction induced by microgravity, research group from Fourth Military Medical University reported that, simulated microgravity can cause enhancement of autophagosome formation, increasing of LC3 and beclin-1 expression, and the conversion of LC3-I to LC3-II in human umbilical vein endothelial cells (HUVECs)[4].

Another group from Fourth Military Medical University found that, simulated microgravity inhibited Cbfa1 activity, affected the responsiveness of Cbfa1 to cytokine BMP2, and caused a thinning and dispersed distribution of microfilament. Under normal gravity, cytochalasin B significantly attenuated BMP2 induction to Cbfa1 activity as well as DNA binding activity of Cbfa1 to OSE2. The addition of JAS (Jasplakinolide, microfilament-stabilizing agent) reversed the inhibitory effects of microgravity on the responsiveness of Cbfa1 to BMP2[5].

Proteomic analysis of human SH-SY5Y neuroblastoma cells under simulated microgravity were performed by the researchers from Beijing Institute of Technology. Based on the (18)O labeling technique, they investigated the up-regulated proteins and down-regulated proteins in SH-SY5Y under simulated microgravity. Twenty-two differentially abundant proteins were quantified. The cell microfilament network was disrupted under simulated microgravity, which was determined by the immunocytochemistry. The concentration of reactive oxygen species, malondialdehyde, and free Ca^{2+} ion significantly increased, and the level of ATP significantly decreased under simulated microgravity. However, there was no obvious cell apoptosis observed under simulated microgravity[6]. As is known, microgravity severely halts the structural and functional cerebral capacity of astronauts especially affecting their brains due to the stress produced by cephalic fluid shift. This group employed a tail-suspension rat model to substant iate simulated microgravity. Comparative mass spectrometry was applied in order to demonstrate the differential expression of 17 specific cellular defense proteins. Gamma-enolase, peptidyl-prolylcis-transisomerase A, glial fibrillary acidic protein, heat shock protein HSP 90-alpha, 10 kDa heat shock protein, mitochondrial, heat shock cognate 71 kDa protein, superoxide dismutase 1 and dihydropyrimidinase-related protein 2 were found to be upregulated. Furthermore, five differentially expressed proteins including 60 kDa heat shock protein, mitochondrial, heat shock protein HSP 90-beta, peroxiredoxin-2, stress-induced-phosphoprotein, and UCHL-1 were found to be upregulated. In addition, down regulated proteins include cytochrome C, superoxide dismutase 2, somatic, and excitatory amino acid transporter 1 and protein DJ-1 were found. This study will not only help to understand the neurochemical responses produced under microgravity but also will give future direction to cure the proteomic losses and their after effects in astronauts[7]. Microgravity generates oxidative stress in central nervous system, causing distortion of various vital signaling cascades involved in many homeostatic functions. By wide proteomic analysis, total of 35 and 97 significantly differentially expressed proteins were found. Among the total of 132 proteins quantified, 25 proteins were found related to various signaling cascades. Protein Thy-1, 14-3-3 gamma, 14-3-3 epsilon, 14-3-3 theta, 14-3-3 eta, and 14-3-3 beta/alpha proteins, calmodulin and calcium/calmodulin-dependent protein kinase type-II subunit beta were found upregulated under the influence of simulated microgravity. These proteins were found involved in disrupting homeostatic pathways like sleep/wake cycle, drinking behavior, hypothalamic-pituitary-adre-

nocortical regulation and fight and/or flee actions under stress. These results will serve as means to understand the mechanism of action of microgravity and further reference for future detailed study of consequences of microgravity on astronauts and their possible counter-measures[8].

Researchers from Beihang University identified that seven miRNA were involved in long-term simulated microgravity response in Solanum lycopersicum. The expressions of six of the seven miRNAs were up-regulated, especially by long-term simulated microgravity. Gene ontology analysis showed that most of the predicted targeted genes were involved in transcription regulation, signal transduction and stress response, implying a complicated relationship among the external signal, internal transduction and final phenotype. This work can help reveal the regulation mechanism mediated by miRNAs under simulated microgravity condition and adaptation to Earth's gravity[9].

Findings from Chinese PLA General Hospital suggested that simulated microgravity reduced the metastatic potential of human lung adenocarcinoma cells by altering the expression of MKI67 and MMP2, thereby inhibiting cell proliferation, migration, and invasion, which may provide some clues to study cancer metastasis in the future.

Another cell line, vascular smooth muscle cells were proved that simulated microgravity suppressed cultured Rat Aortic Smooth Muscle Cells (RASMCs) prolife-ration and migration, enhanced cell apoptosis, stimulated NO release, and destroyed the original well-organized cytoskeleton. Moreover, at the mRNA level, long-time exposure (≥72 h) to simulated microgravity induced a contractile phenotype tendency by up-regulating smMHC expression. All the findings suggest that the phenotype modulation of vascular smooth muscle cells may be gravity dependent[10].

In addition, investigation about combined effect of simulated microgravity and Carbon Ion Rrradiation (CIR) was also performed in research institutions of China. Based on the experimental platform of Heavy Ion Research Facility in Lanzhou (HIRFL), researchers from Chinese Academy of Sciences elaborated the combined effect of spermatogenic cell apoptosis and sperm DNA damage to assess the risk associated with space environment. Their findings demonstrated that simulated microgravity and CIR can induce spermatogenic cell apoptosis and sperm DNA damage. Sperm DNA damage may be one of the underlying mechanisms behind male fertility decline under space environment[11]. This work may provide a scientific basis for protecting astronauts and space traveler's health and safety.

Interestingly, researchers from Wuhan University found that, reproductive and locomotory capacities of Caenorhabditis elegans cultured in standard agar-based nematode growth medium were not affected by sim-ulated variable gravities and spaceflight during the Shenzhou-8 mission. Alteration in either brood size of immediate progenies from post-flight nematodes or locomotory behavior, including speed of locomotion, frequency of reversals, and rate of body bends of space-flown nematodes collected directly from nematode test units were not detected[12].

1.2 Discovery and Mechanism Investigation of Candidate Drugs

Researchers from Chinese Academy of Sciences, Lanzhou University and Fudan University found that, simulated microgravity increased heavy ion radiation-induced cell apoptosis, mediated by a ROS-sensitive signal pathway in human B lymphoblasts. And the antioxidants NAC and quercetin, especially NAC, could reverse the phenomenon and was regarded as one of good candidate drugs for protecting astronauts' and space travelers' health and safety[13]. Synchronously, in School of Life Science, Beijing Institute of Technology, the researchers for the first time investigated the effect of Drageny I, which is a kind of rare Chinese Traditional Medicine herbs on the blood rheology and the protection on oxidative damage in myocardium in tail-suspended rats. In the experiment, blood rheology of veinal blood and oxidative damage (the level of H_2O_2, MDA and T-SOD) in myocardium were examined in the SD rats after 5 d and 21 d tail-suspension, respectively. Compared with the tail-suspension group, the hematocrit and the level of myocardium MDA in drug administration group significantly decreased after 5 d of tail-suspension and after 21 d of tail-suspension, whole blood viscosity of the drug administration group decreased apparently while red blood cell maximum deformation index increased remarkably. Other indexes showed recovery tendency. That means that the Chinese Traditional Medicine Dragney I has a protective effect on the myocardium from oxidative stress in rats under the simulated microgravity condition and also can be one of the candidate drugs for protecting astronauts' and space travelers' health and safety.

As to the NK cells of human, several processes, including apoptosis, receptor expression, and cytokine secretion were investigated under SMG. Human NK cells were sensitive to SMG. Further studies proved that

polysaccharides play a protective role from simulated microgravity-induced functional inhibition. This work was contributed by researchers from Northwestern Polytechnical University of China. Their results demonstrated that polysaccharides could markedly promote the cytotoxicity of NK cells by enhancing IFN-γ and perforin secretion and increasing the expression of the activating receptor NKp30 under normal conditions. But, polysaccharides can enhance NK cell function under SMG conditions by restoring the expression of the activating receptor NKG2D and reducing the early apoptosis and late apoptosis/necrosis. They also proved that CR3 receptor may play a critical role in the polysaccharides induced human NK cells activation[14].

1.3 Generation of Large-scale Organ and Biological Function Improvement Using Microgravity Simulator

It is known that Embryonic Stem (ES) cells are considered a potentially advantageous source of organs for both transplantation and artificial development. Researchers from Third Military Medical University successfully generated large quantities of high-quality hepatocytes in a rotating bioreactor *via* embryoid bodies formation. They found that, during the rotating culture, most of the EB (embryoid bodies)-derived cells gradually showed the histologic characteristics of normal hepatocytes. And the expression of hepatic genes and proteins was detected at a higher level in the differentiated cells from the bioreactor culture than in cells from a static culture. On further growing, the EBs on tissue-culture plates, most of the EB-derived cells displayed the morphologic features of hepatocytes, as well as albumin synthesis. In addition, the EB-derived cells grown in the rotating bioreactor exhibited higher levels of liver-specific functions, such as glycogen storage, cytochrome P450 activity, low-density lipoprotein, and indocyanine green uptake, than did differentiated cells grown in static culture. When the EB-derived cells from day-14 EBs and the cells' culture supernatant were injected into nude mice, the transplanted cells were engrafted into the recipient livers[15].

In the Fourth Military Medical University, studies shown that simulated microgravity could enhance multipotential differentiation capacity of BMSCs (bone marrow mesenchymal stem cells). They found that the pluripotency marker OCT4 was up-regulated in the SMG condition especially after SMG of 72 h. Endothelium oriented differentiated BMSCs expressed higher VWF and CD31 in the SMG group than in the NG group. This may be relevant to the changes of cytoskeleton and the stem cell marker OCT4[16].

In vitro culture of pancreatic islets reduces their immunogenicity and prolongs their availability for transplantation. Both SMG and a PGA (polyglycolic acid scaffold) are believed to confer advantages to cell culture. Researchers from Peking Union Medical College Hospital evaluated the effects of SMG combined with a PGA on the viability, insulin-producing activity and morphological alterations of pancreatic islets. Under PGA-SMG conditions, the purity of the islets was ⩾ 85%, and the islets had a higher survival rate and an increased ability to secrete insulin compared with islets cultured alone in the static, SMG, or PGA conditions[17]. These results suggest that PGA-SMG co-culture has the potential to improve the viability and function of islets in vitro and provides a promising method for islet transplantation.

2 Research in the Underlying Mechanisms of Radiation Effects

2.1 Studies on Radiation Cancer Therapy and Mechanisms of Radioresistance

Different cancers respond differently to radiation therapy. Radiation therapy is commonly applied to the cancerous tumor because of its ability to control cell growth. Ionizing radiation works by damaging the DNA of cancerous tissue leading to cellular death. The response of a cancer to radiation is described by its radiosensitivity. Highly radiosensitive cancer cells are rapidly killed by modest doses of radiation. Some types of cancer are notably radioresistant, that is, much higher doses are required to produce a radical cure than may be safe in clinical practice. In this section, representative studies related to clinical radiation cancer therapy and potential target for improvement of radiation therapy efficiency and safety are concluded and discussed.

2.2.1 Suppression of E. Multilocularis Hydatid Cysts after Ionizing Radiation Exposure

Heavy-ion therapy has an advantage over conventional radiotherapy due to its superb biological effectiveness and dose conformity in cancer therapy. It could be a potential alternate approach for hydatid cyst treatment. But there is no information currently available on the cellular and molecular basis for heavy-ion irradiation induced cell death in cystic echinococcosis. Research group from Department of Heavy Ion Radiation Medicine,

Institute of Modern Physics, Chinese Academy of Sciences found that, ionizing radiation induced sparse cytoplasm, disorganized and clumped organelles, large vacuoles and devoid of villi [18]. The initial mtDNA damage caused by ionizing radiation increased in a dose-dependent manner. The kinetic of DNA repair was slower after carbon-ion radiation than that after X-rays radiation. High dose carbon-ion radiation caused irreversible mtDNA degradation. Cysts apoptosis was pronounced after radiation. Carbon-ion radiation was more effective to suppress hydatid cysts than X-rays.

2.2.2 DNA-PKcs Inhibition Sensitizes Cancer Cells to Carbon-ion Irradiation *via* Telomere Capping Disruption

Critical shortening of telomeres can trigger DNA damage responses such as DSBs. Telomeres are very sensitive to oxidative stress such as ionizing radiation. The DNA-dependent protein kinase catalytic subunit (DNA-PKcs) is the central component in the Non-Homologous End Joining (NHEJ) repair complex and participates in telomere maintenance. Researcher from Department of Heavy Ion Radiation Medicine, Institute of Modern Physics, Chinese Academy of Sciences, found that MCF-7 and HeLa cells with DNA-PKcs inhibition were more susceptible to carbon-ion irradiation than those without DNA-PKcs inhibition. Even though NHEJ was inhibited by the DNA-PKcs specific inhibitor, NU7026, most DNA damage induced by carbon-ion irradiation was repaired within 24 hours after irradiation in both cell lines. However, potential lethal damage repair (PLDR) could not restore cellular inactivation in DNA-PKcs inhibited cells. MCF-7 cells showed extensive senescence and accelerated telomere length reduction, while HeLa cells underwent significant apoptosis after irradiation with NU7026 incubation. In addition, both cell lines with shorter telomere were more susceptible to carbon-ion radiation [19]. Their data suggested that DNA-PKcs inhibition could enhance cellular sensitivity to carbon-ion radiation via disturbing its functional role in telomere end protection. The combination of DNA-PKcs inhibition and carbon-ion irradiation may be an efficient method of heavy-ion therapy.

2.2.3 Effect of Carbon-ion Beam Irradiation on Anti-apoptosis ΔNp73 Expression in HeLa Cells

ΔNp73 has emerged as an interesting novel factor in cancer research. Researchers in Department of Heavy Ion Radiation Medicine, Institute of Modern Physics, Chinese Academy of Sciences, observed stronger G2/M phase arrest, more significant increase in apoptosis and more pronounced ΔNp73 degradation after exposure to high-LET (linear energy transfer) carbon beams. The observations indicated that there was a differential ΔNp73 expression in response to different LET radiations, and down-regulated ΔNp73 expression might play a critical role in promoting cycle arrest and apoptosis in cancer cells. This study highlighted the potential of ΔNp73 in radiotherapy [20].

2.2.4 Combining of Heavy Ion Radiation and Artificial MicroRNAs in Human Tumor Cells Killing

Studies from Provincial Hospital Affiliated to Shandong University showed that targeting either XRCC4 (NHEJ factor) or XRCC2 (HRR factor) sensitized the human tumor cells to X-rays, in vitro and the xenograft animal model, targeting only XRCC2 but not XRCC4 sensitized the human tumor cells to heavy ions in vitro and in the xenograft animal model. The research suggested that, combining heavy ions and artificial microRNAs (amiRs) to target homologous recombination repair (HRR) but not nonhomologous end-joining (NHEJ) were more efficient in killing human tumor cells[21].

2.3 Mechanisms of Radioresistance and Therapeutic Strategy Discovery

2.3.1 Genome-wide Analyses of Radioresistance-associated Expression Profile

Rapidly growing evidence suggests that microRNAs (miRNAs) are involved in a wide range of cancer malignant behaviours including radioresistance. Therefore, the study performed by Central South University investigated miRNA expression patterns associated with radioresistance in NPC. Their work provided an overview of miRNA expression profile and the interactions between miRNA and their target mRNAs, which will deepen current understanding of the important roles of miRNAs in NPC radioresistance[22].

Moreover, Ionizing Radiation (IR) is of clinical importance for glioblastoma therapy; however, the recurrence of glioma characterized by radiation resistance remains a therapeutic challenge. Research on irradiation-induced transcription in glioblastomas can contribute to the understanding of radioresistance

mechanisms. In the study, by using the total mRNA sequencing (RNA-seq) analysis, researchers from School of Life Science, Beijing Institute of Technology assayed the global gene expression in a human glioma cell line U251 MG at various time points after exposure to a growth arrest dose of γ-rays. They identified 1656 genes with obvious changes at the transcriptional level in response to irradiation, and these genes were dynamically enriched in various biological processes or pathways, including cell cycle arrest, DNA replication, DNA repair and apoptosis. Interestingly, the results showed that cell death was not induced even many proapoptotic molecules, including death receptor 5 (DR5) and caspases were activated after radiation. The RNA-seq data analysis further revealed that both proapoptosis and antiapoptosis genes were affected by irradiation. Namely, most proapoptosis genes were early continually responsive, while antiapoptosis genes were responsive at later stages. Moreover, HMGB1, HMGB2 and TOP2A involved in the positive regulation of DNA fragmentation during apoptosis showed early continual downregulation due to irradiation. Furthermore, targeting of the TRAIL/DR5 pathway after irradiation led to significant apoptotic cell death, accompanied by the recovered gene expression of HMGB1, HMGB2 and TOP2A. Taken together, these results revealed that inactivation of proapoptotic signaling molecules in the nucleus and late activation of antiapoptotic genes may contribute to the radioresistance of gliomas. Overall, this study provided novel insights into not only the underlying mechanisms of radioresistance in glioblastomas but also the screening of multiple targets for radiotherapy[23].

2.3.2 Potential Strategy about Cancer Radiotherapy

Researchers from Zhejiang University reported that, when combined with ionizing radiation, histone deacetylase inhibitors (HDIs) inhibited Bmi-1 expression in KYSE-150R cells and their ability to repair DNA damage. The results demonstrated the potential utility of HDIs in augmenting the efficacy of fractionated radiotherapy [24].

Long non-coding RNAs (lncRNAs) are aberrantly expressed and have important functions in pathological processes. By using microarrays, studies by researcher from College of Traditional Chinese Medicine, Southern Medical University showed that, curcumin significantly reversed the ionizing radiation-induced lncRNA and mRNA expression signatures, suggesting that lncRNAs have important functions in IR-induced radioresistance. Thus, curcumin could serve as a good radiosensitizer[25].

3 Application of HIRFL for Space Life Science Studies

Heavy Ion Research Facility in Lanzhou (HIRFL) was built in 1988 in the Institute of Modern Physics, Chinese Academy of Sciences (IMP-CAS), which generates carbon ion beam of high energy up to 120 MeV/u. Based on HIRFL, the National Laboratory of Heavy Ion Accelerator was founded in 1991. Cooler Storage Ring (HIRFL-CSR) passed quality evaluation in 2006, by which the carbon ions are accelerated to 1000 MeV/u. Key Laboratory of Heavy Ion Radiation Medicine of Gansu Province and Key Laboratory of Heavy Ion Radiation Biology and Medicine of CAS were founded in 2007 and 2010, respectively. The Heavy Ion Micro-beam Radiation Facility, which was constructed in 2011, is the micro-beam facility with the highest single particle energy in the world. The Ground-Based Experimental Platform for Space Radiation Research, which is composed of high-energy heavy ion irradiation terminal, high LET heavy ion irradiation terminal and space radiation research laboratories, was established in 2011. This is the second experimental platform specially for space radiobiological studies in the world besides NASA Space Radiation Laboratory (NSRL). Support team has been organized for the platform running, including sample irradiation, dose monitoring and biological experiment assistance.

On this platform, the researchers carried out a series of phenomenal and mechanismatic studies on space radiobiology, including genetic background-related carcinogenesis by radiation, lethal effects of heavy ion on cancer stem cells, biological effects of secondary radiation, and cellular radio-sensitivity. Meanwhile, the functions of radiation-related microRNAs and biogenesis of cancer stem cell were investigated.

3.1 Biological Effects of Heavy Ion Beams

3.1.1 Radio-sensitivity of Human Cells to Heavy Ion Radiation

Researchers from Institute of Modern Physics, Chinese Academy of Sciences found that cell cycle suspension happened in highly radio-sensitive human melanoma 92-1 cells after exposure to high dose irradiation, not in another melanoma cell line OCM-1. Further, they found that 92-1 cells in cell cycle suspension underwent senescence. Several makers in G2-M phase transition were found to decrease prematurely in this process at both the transcriptional and translational levels. Also,

they found that the expression levels of the G1-specific markers, Cyclin D1 and Caveolin-1, were distinctly increased, while S/G2-specific markers, Cyclin B1 and Aurora A, were significantly down-regulated. The findings collectively imply that long-term G2-arrested cells undergo senescence *via* G2 slippage into G1 phase[26].

Their further studies show that when cells with normal p53-p21 pathway are subjected to severe DNA damage, they refrain from cell division through G2 slippage and subsequent cell senescence. However, when cells with abnormal p53-p21 pathway are subjected to severe DNA damage, mitotic catastrophe and subsequent apoptosis happen. Therefore, the status of p53-p21 pathway is a key factor determining the fate of cells exposed to ionizing radiation[27].

3.1.2 Effects of Secondary Radiation

The high atomic number and high- energy particles (HZE particles), represented by high-energy iron ions, are major source of radiation damage confronted by the astronauts. The interaction of HZE particles with the spacecraft bulkhead or shielding materials generates a large number of secondary radiation particles. Study on the underlying mechanisms of secondary radiation is expected to be helpful for precise risk evaluation of space radiation.

The group from Institute of Modern Physics, Chinese Academy of Sciences investigated the biological effects of secondary radiation with cells exposed in different phases of the cell cycle by comparing the biological effects of a 200 MeV/u iron beam with a shielded beam, in which the energy of the iron ion beam was decreased from 500 MeV/u to 200 MeV/u with PMMA, polyethylene (PE), or aluminum. They found that beam shielding with PMMA resulted in increased induction of 53BP1 foci and micronuclei compared with the unshielded 200 MeV/u Fe ion beam and that G1 phase cells were more sensitive to secondary radiation compared with G0 phase cells and exponentially growing cells. Then, they compared damage effects of secondary radiation resulted from different shielding materials. It was suggested that all three shielding materials that the researchers used caused additional DNA damage in MRC-5 cells and shielding with aluminum appeared to produce lower yield of 53BP1 foci in comparison with PMMA and PE. However, secondary radiation from shielding has little impact on genome stability as evaluated by micronucleus assay[28].

The findings provide experimental proof that the biological effects of secondary particles resulting from the interaction between HZE particles and shielding materials should be not be neglected in shielding design. The damage level depends on both the experimental material and chosen biological endpoint.

3.1.3 The Lethal Effects of Heavy Ions on Cancer Stem Cells

The stemness of CD133 positive M059K glioblastoma cells by employing spheroid formation test in serum-free culture were studied. $CD133^-$ and $CD133^+$ M059K glioblastoma cells were exposed to X-ray irradiation or carbon ion beams of various doses. DNA damage was evaluated by immunofluorescence, Cell survival and proliferation were tested by colony-forming assay and soft-agar assay. The results indicated that carbon ion beams kill CD133+M059K glioblastoma cells more efficiently than X-rays. The results confirmed that the advantage of heavy ion radiotherapy and revealed cancer stem cell hypothesis as a new mechanism of tumor recurrence after conventional radiotherapy.

3.1.4 Irradiation Effect on the Progeny of Bystander Primary Human Fibroblast

Recent study showed that, no significant level of inheritable interchromosomal aberrations were induced in the progeny of the bystander cell groups after alpha particle irradiation, while the fractions of gross aberrations (chromatid breaks or chromosomal breaks) significantly increased in some bystander cell groups[28]. The research suggested that genomic instability occurred in the progeny of the irradiation associated bystander normal fibroblasts exclude the inheritable interchromosomal aberration.

3.2 Functional Studies on Radiation-related MicroRNAs

There are increasing experimental evidences showing that a lot of microRNAs (miRNAs) respond to radiation by down-regulating the expression levels of their target genes. Up to now, many radiation-related miRNAs together with their target genes have been identified. However, there are still a large number of radiation-related miRNAs as well as their regulation signaling pathways waiting for intensive study due to the complexity of the cellular response. The researchers measured the miRNAome patterns in renal cell carcinoma, gastric cancer and established cell lines by adopting miRNA microarray and deep sequencing technologies and chose several miRNAs for functional studies.

3.2.1 miR-185 Regulates DNA Damage Response by Targeting ATR

Although ATR plays key roles in sensing and signaling of DNA damage in cellular stress response, the regulatory mechanisms of ATR expression is little known. In the study, it was identified ATR as a specific target of miR-185 and the over-expression of miR-185 enhances the radio-sensitivity of cancer cells.

It was verified the direct bind of miR-185 to 3'-UTR of ATR transcript and the down-regulation of both mRNA and protein levels of ATR by the over-expression of miR-185. Further studies revealed that X-ray irradiation significantly induced the expression of ATR-Chk1 pathway, whereas in cells transfected with miR-185 mimics, ATR was suppressed and both the expression and activation of its downstream Chk1 were decreased. These findings suggest that miR-185 over-expression inhibits the radiation damage repair pathways and enhance the radio-sensitivity of cancer cells[29].

3.2.2 miR-3928 Modulates Cellular Radiation Response by Targeting Dicer

Dicer, an endoribonuclease of RNase III family, is a key factor for the maturation of the majority of miRNAs. It plays crucial roles in miRNA regulation networks. A large body of evidence suggests that Dicer also works as a stress responsor. Dicer is down-regulated when cells are treated with H_2O_2, interferons, starvation or apoptosis inducers and the knockdown of Dicer causes DNA damage by activating transposons. However, its function in radiation responses is poorly understood.

By adopting deep sequencing, the researchers found that miR-3928 is obviously up-regulated in HeLa cells exposed to X-ray irradiation. It is predicted by online software that Dicer is a potential target of miR-3928. Since Dicer's critical functions in miRNA maturation and stress response, the authors speculate that miR-3928 may regulate cellular radiation response through targeting Dicer.

Dicer expression in HeLa cells either exposed to X-ray irradiation or transfected with miR-3928 mimics/inhibitors were detected and the result was that Dicer expression is negatively correlated with miR-3928. Over-expression of miR-3928 induced DNA damage, activated ATR, phosphorylated Chk1 accompanied by G1 arrest, and suppressed maturation of other miRNAs including miR-185, miR-300, and miR-663. Taken together, miR-3928 plays an important role in cellular response by targeting Dicer [30].

This study combined Dicer with miRNA-mediated cellular radiation response for the first time. On one hand, it replenishes Dicer signaling pathway and may facilitate the functional study of Dicer. On the other hand, it provided new experimental evidence for a deep understanding of mechanisms of cellular radiation response.

Functional studies on heavy ion radiation-related miRNAs and radiation systems biology will be the focus of the research group in the future. Study aims to deepen understanding of the underlying mechanisms of cellular response to heavy ion radiation. Besides that, the researchers will establish and optimize the mathematical model of the cellular response to ionizing radiation, and complete the expression database of proteins responding to radiation. In a word, the research team will focus on the space radiation research on both effect and mechanism, form epigenetics and systems biology perspectives, establish a personalized evaluation system for space radiation risk and develop efficient protection means.

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