Protective Effects of IGFBP-3 Protein on Heavy Ion Radiation-Induced Injury in Mice

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Date: 2024-05-06T00:00:00+00:00

Abstract

China' s manned spaceflight and nuclear technology applications are undergoing rapid development, radiation and nuclear safety will continue to be major national priorities, and the continued development of novel radiation protection molecular targets and related drugs holds significant value. Previous studies have found that the level of insulin-like growth factor binding protein 3 (IGFBP-3) in mouse blood significantly increases after ionizing radiation exposure; however, the function of IGFBP-3 protein and the impact of changes in its blood level on radiation injury in mice remain unclear. In this study, Igfbp3 gene overexpression and knockdown cell models were established in mouse hepatic macrophages (Kupffer cells, MKC), and CCK-8, EdU incorporation, colony formation, and microsphere phagocytosis assays were employed to assess the proliferative viability, DNA replication activity, and phagocytic capacity of different cell models after carbon ion irradiation; in mouse models, recombinant IGFBP-3 protein was administered via tail vein injection 2 hours before irradiation to elevate blood IGFBP-3 protein levels in advance, and the survival curve of mice after lethal-dose (5 Gy) whole-body carbon ion irradiation was examined. The results showed that overexpression of IGFBP-3 protein significantly enhanced the DNA replication activity, cell viability, colony formation rate, and microsphere phagocytic capacity of MKC cells after radiation exposure; conversely, knockdown of IGFBP-3 protein expression decreased these measured indicators. Tail vein injection of IGFBP-3 protein prior to carbon ion radiation exposure could significantly delay the time to death in mice with acute radiation injury. These results demonstrate at both cellular and animal levels that IGFBP-3 protein possesses radiation injury-mitigating effects and great potential as a radiation protection drug target. Enhancing the radiation resistance and phagocytic capacity of Kupffer cells in mice to reduce infection risk after radiation exposure may be one of the mechanisms through which IGFBP-3 protein exerts its radiation protection effects.

Full Text

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Abstract

With China' s manned spaceflight and nuclear technology applications entering a period of rapid development, radiation and nuclear safety will remain a critical national priority for the foreseeable future, making the continued discovery of novel radiation protection molecular targets and related therapeutics highly valuable. Previous studies have demonstrated that circulating insulin-like growth factor binding protein 3 (IGFBP-3) levels increase significantly in mouse blood following ionizing radiation exposure. However, the functional role of IGFBP-3 and the impact of its altered blood levels on radiation injury remain unclear. In this study, we established Igfbp3 gene overexpression and knockdown cell models in mouse Kupffer cells (MKC) and assessed proliferation, DNA replication activity, and phagocytic capacity after carbon ion irradiation using CCK-8, EdU incorporation, colony formation, and microsphere phagocytosis assays. In the mouse model, recombinant IGFBP-3 protein was administered via tail vein injection two hours before irradiation to elevate blood IGFBP-3 levels, and survival curves were monitored following lethal-dose (5 Gy) whole-body carbon ion irradiation. The results showed that IGFBP-3 overexpression significantly enhanced DNA replication activity, cell viability, colony formation efficiency, and microsphere phagocytosis in MKC cells after radiation exposure. Conversely, IGFBP-3 knockdown reduced all these measured parameters. Pre-irradiation injection of IGFBP-3 protein significantly delayed mortality in mice with acute radiation injury. These findings at both cellular and animal levels demonstrate that IGFBP-3 protein can mitigate radiation damage and holds great potential as a target for radiation protection. One possible mechanism may involve reducing post-irradiation infection risk by enhancing the radiation resistance and phagocytic capacity of Kupffer cells in mice.

Keywords: IGFBP-3 protein; heavy ions; radiation protection; Kupffer cells

Introduction

With the completion of China's space station and the advancement of manned lunar exploration programs, future space missions will feature significantly longer durations and greater complexity, exposing astronauts to more severe space radiation risks [1, 2]. As a nation with substantial demands for nuclear industry and technology facilities, China also faces immense challenges in radiation safety. Radiation exposure exceeding safe dose limits poses serious health hazards. Acute radiation syndrome (ARS), also known as acute radiation sickness, can occur when the body receives high-dose ionizing radiation (generally >2 Gy) within a short period. ARS primarily results from the depletion of immature parenchymal stem cells in specific tissues, manifesting as hematopoietic syndrome that may progress to gastrointestinal, neurovascular, and cutaneous syndromes depending on exposure severity [3]. Single or prolonged exposure to low-dose radiation may also induce stochastic effects, including increased cancer and genetic disease risks, as well as compromised hematopoietic and immune functions [4]. Among radiation types, heavy ion radiation warrants particular attention due to its high linear energy transfer (LET) and relative biological effectiveness (RBE), which cause more complex DNA double-strand breaks and clustered damage that are difficult to repair [5].

In response to radiation safety challenges, nations worldwide continue to expand their toolkit for managing radiation exposure and injury, including radiation dosimeters and protective agents. In 2021, the Seventy-fourth World Health Assembly called on countries to establish national stockpiles of drugs and supplies for human radiation exposure. On January 27, 2023, the World Health Organization (WHO) released the report "National Stockpiles for Radiological and Nuclear Emergencies: Policy Advice," updating key measures and drug inventories for nuclear emergencies. Blood contains abundant biomolecules, including RNA, proteins, and small metabolites, that not only respond actively to environmental stress but also offer advantages in detection convenience and timeliness. Recent studies have identified blood proteins, microRNAs, and tRNA fragments as promising candidates for ionizing radiation biodosimetry, becoming a research hotspot in radiation biology [6-8]. Our previous research confirmed that circulating insulin-like growth factor binding protein 3 (IGFBP-3) levels increase significantly in mice after exposure to carbon ions, protons, or X-rays. IGFBP-3 is a key component of the growth hormone–insulin-like growth factors–IGF binding proteins (GH–IGFs–IGFBPs) hormonal regulatory axis and is closely associated with cell proliferation and differentiation, glucose and lipid metabolism, bone formation, cardiovascular function, and hematopoietic and immune systems [9]. IGFBP-3 can bind to IGFs to form feedback regulation that modulates IGF functions, or act independently of IGFs through specific IGFBP receptors, playing important roles in promoting DNA double-strand break repair, stimulating cell proliferation, and inducing apoptosis [10, 11]. These findings suggest that the

radiation-induced increase in blood IGFBP-3 may represent not merely a stress response but an active defense mechanism against radiation injury. However, no experimental evidence currently supports this hypothesis, and the functional role of IGFBP-3 and its impact on radiation injury remain unclear.

Blood IGFBP-3 is primarily secreted by liver Kupffer cells [12]. Kupffer cells are macrophages located on the luminal surface of liver sinusoids that mainly function to phagocytose pathogens and foreign particles. Reports indicate that Kupffer cells can clear approximately 80% of blood-borne bacteria accumulating in the liver, representing a major barrier against infection caused by ARS [13]. In this study, we used mouse liver Kupffer cells as a model to establish Igfbp3-overexpressing and Igfbp3-knockdown cell lines, then examined differences in proliferation, DNA replication activity, and phagocytic capacity after carbon ion irradiation. Additionally, we elevated blood IGFBP-3 levels by tail vein injection of recombinant IGFBP-3 protein two hours before irradiation and monitored survival curves after lethal-dose (5 Gy) whole-body carbon ion exposure. These investigations aimed to clarify the role of IGFBP-3 in radiation injury protection and provide experimental evidence for blood IGFBP-3 as a novel radiation protection target.

Materials and Methods

2.1 Materials and Instruments Forty-eight Kunming mice (1:1 male-to-female ratio) weighing 18–20 g were purchased from the Lanzhou Veterinary Research Institute of the Chinese Academy of Agricultural Sciences. Mice were housed at room temperature with free access to water and food under normal circadian rhythms. Reagents included recombinant IGFBP-3 protein (ABclonal, China), Trp53 siRNA (RiboBio, China), negative control siRNA (RiboBio, China), Lipofectamine™ 2000 transfection reagent (Invitrogen, USA), CCK-8 kit (Yisheng Bio, China), Cell-Light™ EdU Apollo In Vitro Kit (BBI, China), and polystyrene red fluorescent microsphere solution (Yiyuan Bio, China). Instruments included the Heavy Ion Research Facility in Lanzhou (HIRFL, Institute of Modern Physics, Chinese Academy of Sciences), cell counter (Z2, Beckman, USA), refrigerated high-speed centrifuge (Centrifuge 5424R, Eppendorf, USA), fluorescence microscope (RVL-100-G, ECHO, USA), multimode microplate reader (Tecan Infinite M200 Pro, TECAN, Switzerland), and flow cytometer (Amnis FlowSight, Merck Millipore, Germany).

2.2 Animal Handling and Treatment Mice were divided into four groups (n=12 each, half male and half female): control group (saline injection), control+drug group (4 g recombinant IGFBP-3 [rhIGFBP-3] protein), irradiation group (saline injection before irradiation), and irradiation+drug group (4 g rhIGFBP-3 protein before irradiation). For tail vein injection, the drug groups received 200 L of rhIGFBP-3 protein solution (20 g/mL in saline) two hours before irradiation, while control groups received an equal volume of sterile saline. Irradiation was performed using a carbon ion beam ($^{12}C^{6+}$) from the shallow

terminal TR4 of HIRFL at 80 MeV with an LET of 30 keV/ m. The terminal-calibrated absorbed dose was 5 Gy delivered at 2 Gy/min. All animal experiments were approved and supervised by the Animal Ethics Committee of the Institute of Modern Physics, Chinese Academy of Sciences (Approval No.: 2020-012).

2.3 Survival Curve Measurement Mouse survival was monitored continuously for 30 days starting from the irradiation time point. Cessation of feeding or lack of movement upon human stimulation was considered the endpoint. Survival curves were plotted using Kaplan-Meier analysis.

2.4 Cell Culture Immortalized mouse Kupffer cells (MKC), Igfbp3-overexpressing MKC cells (MKC-OE-Igfbp3), and empty vector-transfected MKC cells (MKC-OE-Blank) were preserved and provided by the Biological Radiation Effects Laboratory of the Institute of Modern Physics, Chinese Academy of Sciences. MKC-OE-Igfbp3 and MKC-OE-Blank are stable cell lines with GFP tags. IGFBP-3 overexpression efficiency was verified by Western blot and GFP fluorescence detection, as detailed in Figure 5 [Figure 5: see original paper]-4 of Dr. Hao Bai' s doctoral dissertation [14]. MKC cells were cultured in RPMI-1640 medium supplemented with 10% fetal bovine serum and 1% penicillin-streptomycin at 37°C in a humidified incubator with 5% CO_2.

2.5 Cell Transfection and Irradiation Cells were seeded at 5$×10^{5}$ cells per 35 mm dish and cultured for 24 hours before co-transfection with Igfbp3 siRNA (si-Igfbp3) or negative control siRNA (si-NC) using Lipofectamine™ 2000 at a final siRNA concentration of 30 nM. Fresh medium was replaced 5 hours post-transfection, and cells were irradiated 24 hours later using carbon ion beams from HIRFL at a dose rate of 2 Gy/min.

2.6 Cell Proliferation and DNA Replication Activity Assays Immediately after irradiation, cells were digested and counted using a cell counter, then seeded in 96-well plates at 20,000 cells per well for culture until the designated time points. Cell viability was measured using the CCK-8 kit according to the manufacturer' s instructions, with absorbance (OD_{450}) measured to assess proliferation at 24, 48, and 72 hours post-irradiation. DNA synthesis activity was detected by EdU incorporation using the Cell-Light™ EdU Apollo In Vitro Kit. After staining, cells were immediately photographed using an ECHO fluorescence microscope, and EdU-positive cell rates were quantified at 24 hours post-irradiation.

2.7 Colony Formation Assay Immediately after irradiation, cells were digested and counted, then seeded in 35 mm dishes at 2,000 cells per dish. After 8 days of culture, cells were washed twice with 1×PBS, fixed with 75% ethanol for 5 minutes at room temperature, stained with 0.5% crystal violet for 5 minutes,

rinsed under running water, air-dried, and counted (colonies with >50 cells were considered valid).

2.8 Cell Phagocytosis Assay Cells were irradiated with 2 Gy carbon ions, with a control group (0 Gy). Time zero was defined as the irradiation time point. Cells were counted at 72 and 120 hours post-irradiation. Polystyrene red fluorescent microspheres (300 nm diameter) served as phagocytic targets. For each 10^6 cells, 20 g of microspheres in 1640 medium (15% serum) were added, and cells were incubated at 37°C with 5% CO_2 for 30 minutes in the dark. After incubation, medium was removed, cells were washed three times with 0.01 M PBS, resuspended in 2 mL fresh medium, and observed under a fluorescence microscope to quantify microsphere uptake.

2.9 Flow Cytometry Analysis After 30 minutes of microsphere phagocytosis, MKC cells were washed with PBS to remove unphagocytosed microspheres. Cells were digested, collected in 1.5 mL EP tubes, centrifuged at 2,000 rpm for 5 minutes, and resuspended in 300 L PBS. Amnis imaging flow cytometer acquired 10,000 single cells per sample. Data were analyzed using IDEAS Application v6.0 (Amnis, Merck Millipore, Germany) and FlowJo v10 (Tree Star, Ashland, USA), with quantitative analysis of fluorescence intensity in the microsphere channel.

Statistical Analysis

Data are presented as mean ± standard deviation. One-way ANOVA was used for multi-group comparisons, and Student' s t-test for two-group comparisons. *$p<0.05$ was considered statistically significant;* ***$p<0.01$ and*** $p<0.001$ indicated highly significant differences. Data analysis was performed using GraphPad Prism 8 (GraphPad Software, USA), and graphs were plotted using OriginPro 2018 (OriginLab Corporation, USA).

Results

3.1 Effect of IGFBP-3 Overexpression on DNA Replication, Proliferation, and Colony Formation in MKC Cells After Carbon Ion Irradiation To investigate the effect of IGFBP-3 expression on cell proliferation after heavy ion irradiation, Igfbp3-overexpressing MKC cells (MKC-OE-Igfbp3) were irradiated with 1 Gy and 2 Gy carbon ions, alongside control groups including wild-type MKC cells (MKC-Wild type) and empty vector-transfected negative controls (MKC-OE-Blank). DNA replication activity, proliferation viability, and colony formation capacity were assessed by EdU incorporation, CCK-8 assay, and colony formation assay, respectively. As shown in Figure 1 [Figure 1: see original paper], carbon ion irradiation significantly reduced DNA replication activity and cell viability in control groups, with EdU-positive rates decreasing from 45% to 15% and proliferation viability dropping by approximately 30% at 2 Gy. MKC-OE-Igfbp3 cells exhibited significantly higher

DNA replication activity than MKC-Wild type and MKC-OE-Blank cells, with EdU-positive rates increasing by approximately 30% at 2 Gy (Figures 1a, 1b). Moreover, MKC-OE-Igfbp3 cell proliferation viability was significantly higher than the MKC-OE-Blank group at 24, 48, and 72 hours post-irradiation (Figure 1c). Colony formation assays revealed significantly higher colony numbers in MKC-OE-Igfbp3 cells compared to MKC-OE-Blank after carbon ion irradiation ($p<0.05$) (Figures 2a, 2c). Furthermore, IGFBP-3 knockdown via siRNA-Igfbp3 transfection followed by 2 Gy carbon ion irradiation resulted in significantly reduced colony formation capacity compared to controls (MKC-Wild type and MKC+si-NC) ($p<0.001$) (Figures 2b, 2d). These results demonstrate that elevated IGFBP-3 expression alleviates carbon ion radiation-induced reductions in DNA replication activity, proliferation viability, and colony formation capacity in MKC cells, indicating a radioprotective effect of IGFBP-3.

3.2 Effect of Carbon Ion Irradiation on MKC Cell Phagocytic Function Kupffer (MKC) cells are specialized macrophages in the liver that clear foreign particles and bacteria from circulating blood, representing a crucial component of innate immunity and a primary barrier against infection following radiation injury. However, the impact of carbon ion irradiation on MKC phagocytic capacity remains unclear. Using red fluorescent plastic microspheres as phagocytic targets, we irradiated MKC cells with 2 Gy carbon ions and assessed phagocytic capacity at 72 and 120 hours post-irradiation. The results showed that MKC cells possess strong phagocytic activity, which was significantly reduced by 2 Gy carbon ion irradiation (Figures 3a, 3b). Quantitative fluorescence intensity analysis revealed that 2 Gy irradiation decreased microsphere uptake by over 50% (Figures 3c, 3d). These findings indicate that carbon ion irradiation significantly suppresses the phagocytic ability of MKC cells.

3.3 Effect of IGFBP-3 Overexpression on MKC Cell Phagocytic Function After Carbon Ion Irradiation Igfbp3-overexpressing MKC cells (MKC-OE-Igfbp3) and control cells (MKC-Wild type and MKC-OE-Blank) were irradiated with 2 Gy carbon ions, and phagocytic microsphere numbers were quantified at 72 and 120 hours post-irradiation. Fluorescence microscopy revealed that MKC-Wild type and MKC-OE-Blank cells showed markedly reduced microsphere uptake after irradiation, with average phagocytosed microsphere numbers decreasing by 50–70%. In contrast, MKC-OE-Igfbp3 cells exhibited significantly higher microsphere uptake ($p<0.05$) (Figure 4 [Figure 4: see original paper]). These results demonstrate that IGFBP-3 overexpression significantly mitigates carbon ion radiation-induced suppression of MKC cell phagocytic capacity.

3.4 Effect of IGFBP-3 Knockdown on MKC Cell Phagocytic Function After Carbon Ion Irradiation To further validate the protective role of IGFBP-3, we knocked down Igfbp3 expression using siRNA-Igfbp3 and irradiated these cells (MKC+si-Igfbp3) alongside controls (MKC-Wild type and

MKC+si-NC) with 2 Gy carbon ions. Phagocytic capacity was assessed at 72 hours (Figure 5) and 120 hours (Figure 6 [Figure 6: see original paper]) post-irradiation. Carbon ion irradiation significantly reduced microsphere uptake in control groups, while IGFBP-3 knockdown further decreased phagocytic capacity ($p<0.01$) (Figures 5–6). These results confirm that inhibiting IGFBP-3 expression exacerbates carbon ion radiation-induced suppression of MKC cell phagocytosis.

3.5 Effect of Tail Vein IGFBP-3 Injection on Survival of Carbon Ion-Irradiated Mice To evaluate the protective efficacy of IGFBP-3 at the organismal level, female and male mice (n=6 each) received whole-body irradiation with a lethal dose (5 Gy) of carbon ions. Two hours before irradiation, each mouse was injected with 4 g recombinant IGFBP-3 protein (rhIGFBP-3) via tail vein to elevate blood IGFBP-3 levels; this 4 g dose was pre-screened as a safe, non-toxic dosage. Thirty-day survival curves were then plotted. As shown in Figure 7 [Figure 7: see original paper], 5 Gy carbon ion exposure caused 100% mortality within 5–6 days, whereas the IGFBP-3-treated group had 4/6 female and 3/6 male mice surviving at this time point, with maximum survival extending to 8–9 days post-irradiation. The difference between treated and control groups was statistically significant ($p<0.01$). These results indicate that elevating blood IGFBP-3 levels before radiation exposure can prolong survival time in lethally irradiated mice.

Discussion

Space radiation is compositionally complex, comprising protons, α particles, electrons, photons, and heavy ions, characterized by low dose rates, high energies, and stochastic properties that make complete shielding difficult [15]. With China' s space station now in regular operation and manned lunar missions planned, astronauts will face substantially increased space radiation health risks due to longer mission durations and frequent extravehicular activities. Currently, international manned space programs primarily rely on radiation-hardened spacecraft materials and spacesuits to mitigate space radiation effects, yet dedicated space radiation protection drugs remain lacking. Radiation exposure events such as nuclear leaks or warfare are typically accidental or uncontrollable, making radioprotective drugs crucial for life-saving and injury mitigation. Radiation countermeasures are classified by administration timing: radioprotectors (pre-exposure), radiomitigators (post-exposure but pre-symptom), and radiotherapeutics (post-symptom) [16]. Existing approved radiation countermeasures have significant limitations; traditional chemical radioprotectors and hormonal drugs such as amifostine (WR-2721), nitroxides (NIT2011), steroid hormones, and glucocorticoids frequently cause side effects including hypotension, nausea, vomiting, and fatigue [17, 18]. Active compounds from medicinal plants and animals, such as trans-resveratrol and thymol, have attracted research interest [19], but their complex mechanisms yield suboptimal efficacy when used alone [20]. Therefore, identifying safe and effective radiation protection molec-

ular targets and low-toxicity drugs remains a research priority. Cytokine-based radioprotectors have gained attention recently due to their ability to enhance cellular radiation resistance with minimal toxicity. Current cytokine radioprotectors include interleukins, growth factors, and colony-stimulating factors that exert protective effects by scavenging free radicals, restoring hematopoietic function, and modulating immune responses [21]. To date, the Food and Drug Administration (FDA) has approved five cytokine drugs: Filgrastim (Neupogen), Pegfilgrastim (Neulasta®), Sargramostim (Leukine), Romiplostim (Nplate), and Neulasta biosimilar (Udenyca) [22]. Our previous findings that blood IGFBP-3 levels increase significantly after various radiation exposures, combined with IGFBP-3' s role in the GH–IGFs–IGFBPs axis and its functions in promoting DNA double-strand break repair and cell proliferation, led us to hypothesize that radiation-induced IGFBP-3 elevation may represent a protective response, suggesting IGFBP-3 as a promising novel radiation protection target.

Previous studies have confirmed that radiation-induced elevation of blood IGFBP-3 originates primarily from liver Kupffer cells [12]. Therefore, we first used a Kupffer cell line (MKC) to investigate IGFBP-3' s effects on radiation-induced cellular damage. We established IGFBP-3-overexpressing and IGFBP-3-knockdown MKC cell models using overexpression plasmids and siRNA, respectively, then irradiated these cells with carbon ions and assessed key radiation biology endpoints including DNA replication activity, cell viability, proliferation capacity, and Kupffer cell-specific phagocytic function. Our findings revealed that elevated intracellular IGFBP-3 expression significantly mitigated carbon ion radiation-induced reductions in MKC cell DNA replication activity, viability, and proliferation capacity (Figures 1 and 2). Heavy ion radiation causes extensive DNA double-strand breaks and clustered damage that trigger apoptosis or other cell death pathways. Our results suggest IGFBP-3 may promote DNA damage repair, thereby enhancing post-irradiation DNA replication, viability, and proliferation. As liver-resident macrophages, Kupffer cells are the primary functional cells for purifying blood of foreign substances, dead cells, and bacterial antigens. However, immune cells are also among the most radiation-sensitive cell types, and we found that carbon ion irradiation significantly suppressed MKC phagocytic capacity. Through both upregulation and downregulation of IGFBP-3 expression, we demonstrated IGFBP-3' s critical role in preserving Kupffer cell phagocytic function after irradiation (Figures 4–6). These results suggest IGFBP-3 may function against ARS-induced infection by rescuing Kupffer cell survival and phagocytic capacity. To directly verify IGFBP-3' s protective effects at the organismal level, we conducted in vivo experiments examining mouse survival. Since ionizing radiation significantly elevates blood IGFBP-3 levels, we pre-elevated IGFBP-3 through pre-irradiation administration and found it significantly delayed mortality after lethal-dose carbon ion whole-body irradiation (Figure 7). Notably, IGFBP-3 treatment showed slightly better protection in female than male mice, possibly reflecting known sex differences in mouse radiation resistance, as female mice exhibit marginally greater resistance

to γ-rays than males [23]. Due to the high biological effectiveness of heavy ions, the 5 Gy carbon ion dose used in this study was substantial, preventing IGFBP-3 treatment from improving survival rates; future studies using lower doses such as LD_{50} may demonstrate enhanced survival.

Although this study provides preliminary evidence for IGFBP-3' s protective effects in MKC cells and acute radiation injury mouse models, the underlying molecular mechanisms remain unclear. IGFBP-3 exhibits high affinity for IGF proteins and normally forms complexes to maintain stable IGF levels [24, 25]. Reports indicate IGF-1 and IGF-2 play important roles in radiation injury responses [26, 27], suggesting IGFBP-3 may function through the IGFBP-3–IGFs regulatory axis. IGFBP-3 also has independent membrane receptors and can interact with EGFR, translocate to the nucleus under DNA damage signaling, and participate in DNA repair through the DNA-PK complex [11]. Additionally, IGFBP-3 expression is regulated by the tumor suppressor p53/Trp53 [12, 28], which is activated by DNA damage signals and participates in radiation damage and repair processes [29]. We therefore hypothesize that radiation-induced DNA damage activates p53 expression, with IGFBP-3 acting downstream of P53 in radiation damage and repair pathways.

As a member of the GH–IGFs–IGFBPs hormonal axis, IGFBP-3 offers good human safety and minimal toxicity profiles. Synthetic IGFBP-3 protein has been investigated for treating dry eye disease, childhood growth disorders, and bone injuries [30-33]. However, no reports have described IGFBP-3 as a radiation protection drug. Our findings indicate IGFBP-3 holds tremendous potential as a safe, effective novel ionizing radiation protectant or protection target, laying a foundation for future research and clinical translation. Nevertheless, IGFBP-3' s specific effects and mechanisms on key radiation-sensitive systems (hematopoietic/immune, gastrointestinal, reproductive) and cell types require further investigation. Our research has identified multiple radiation-sensitive blood molecules, such as IGFBP-3 and miR-342-3p, with promising radioprotective effects [34]. Extending this approach, the numerous radiation-sensitive biomolecules in blood represent a vast reservoir for discovering new radiation protection targets and developing protective drugs.

Conclusion

Developing new radiation protection molecular targets and mechanisms, along with related pharmaceuticals, is crucial for meeting national radiation and nuclear safety needs worldwide. Blood radiation-sensitive biomolecules represent a research frontier in radiation biology, yet their functions and mechanisms remain poorly defined. This study targeted the radiation-sensitive blood protein IGFBP-3 and demonstrated its radioprotective effects on Kupffer cells after carbon ion irradiation and its life-saving effects in acute radiation injury mouse models through combined in vitro and in vivo approaches. Our results suggest that enhancing Kupffer cell radiation resistance and phagocytic capacity to reduce post-exposure infection risk may be one mechanism underlying IGFBP-3'

s radioprotective effects. This work establishes IGFBP-3 protein as a promising new radiation protection molecular target and suggests that modulating blood IGFBP-3 levels may represent a safe and effective radiation protection strategy. Furthermore, the numerous radiation-sensitive blood molecules already identified may serve as a treasure trove for discovering novel radiation protection targets and developing protective drugs, warranting further exploration.

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Note: Figure translations are in progress. See original paper for figures.