BPIFA2 as a Novel Early Biomarker to Identify Fatal Radiation Injury After Radiation Exposure

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Abstract

Background: Current dosimeters cannot cope with the two tasks of medical rescue in the early stage of nuclear accident, the accurate determination of radiation exposure and the identification of patients with fatal radiation injury. As radiation can cause alterations in serum components, it is feasible to develop biomarkers for radiation injury from serum. This study aims to investigate whether serum BPIFA2 could be used as a potential biomarker of predicting fatal radiation injury in the early stage after nuclear accident.

Methods: A rabbit anti-mouse BPIFA2 polyclonal antibody was prepared to detect the expression of BPIFA2. C57BL/6J female mice were exposed to total body radiation (TBI) at different dose and Partial body radiation (PBI) at lethal dose to detect the dynamic changes of BPIFA2 in serum at different time points after irradiation by Western blot assay.

Results: BPIFA2 in mice serum were significantly increased at 1–12 h post-irradiation at .5–10 Gy, and increased again significantly at 3 d after 10 Gy irradiation with associated with mortality closely. It also increased rapidly after PBI and was closely related to injury degree, regardless whether the salivary glands were irradiated.

Conclusions: The increase of serum BPIFA2 is a novel early biomarker not only for identifying radiation exposure, but also for fatal radiation injury playing a vital role in rational use of medical resources, and greater efficiency of medical treatment to minimize casualties.

Introduction

Prompt injury classification is the priority of medical rescue after nuclear accidents and also the key to reasonably allocate medical resources for more efficient medical treatment.[1] Treatments are different for patients with varying degrees of irradiation injury, and patients with fatal injury should be given priority in treatment. Therefore, there are two core tasks of medical rescue in the early stage after nuclear accidents, to accurately determine whether people are exposed and to identify fatal injury among the irradiated casualties.

The assessment of post-radiation injury mainly relies on physical and biological methods. Physical dosimeters are not

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always available to irradiated individuals after the emergency. Instead, biological methods including chromosome aberration analysis, lymphocyte count analysis and lymphocyte γ-H2AX analysis are often applied for injury classification of mass casualties after nuclear accidents.[2-6] Cytogenetic techniques such as chromosome aberration and micronuclei analysis, which were demonstrated as "golden standard" for determining exposure dose, have high accuracy and specificity in identifying irradiation at .5–6 Gy. In recent years, chromosome analysis had been automated in cell collection, culture and analysis, but it still requires 48 h to culture cells in vitro and the upper limit of detection is still 6 Gy.[7,8] Lymphocyte counts analysis is used for the classification of radiation-injured casualties, as it is fast and simple, but still fails to achieve injury classification within 12 h after irradiation.[9-11] As a marker of DNA double-strand break, γ-H2AX has been broadly studied as a biodosimeter for radiation injury due to its specificity and sensitivity.[12-14] The time- and dose-response to radiation has been studied in human and animals systematically, and the stable detection methods have been established. Moreover, high-throughput techniques have been implemented for early screening of irradiated individuals from a large number of casualties. However, the number of γ-H2AX foci starts to increase a few minutes after irradiation, peaks at 1 h and then gradually decreases. Thus, its application is greatly limited by such a short detection window.[15-17] In summary, the existing biodosimeterss cannot achieve timely detection at the accident sites without delicate instrument. Most importantly, none of the current radiation biodosimeters can realize the early identification of fatal radiation injury.

Serum, an important medium for cross-talking between cells, is a barometer of the body's health, and its detection is of great importance in clinical diagnosis. As an exogenous stimulus, ionizing radiation causes cells apoptosis and necrosis in hematopoietic, immune, intestinal and other radiosensitive tissues,[18] especially exposure to high doses will lead to transient damage and even rupture of the cytoplasmicmembrane. As a result, molecules normally found only in cells, leak out from cells into fluid circulation,[19] leading to changes in serum composition. Concentrations of Flt3 ligand (FL), which is involved in stem cell differentiation and proliferation, was increased dose-dependently at the second day after irradiation. Monitored FL concentration in plasma can be used as an indicator of radiation-induced marrow aplasia.[20] The time- and dose-dependent decrease of plasma citrulline level was used to quantify acute small bowel epithelial radiation damage after total body irradiation (TBI).[21] The above reports demonstrate the feasibility of searching for biomarkers of radiation damage from serum. Hence, whether an early biomarker of radiation and fatal injury can be identified from the change of serum constituents, so as to effectively provide guidelines for the rescue and treatment of severe injured patients is well worth exploring.

For this purpose, we analyzed the proteomics data of in mice serum after irradiation with different doses, found the concentrations of BPIFA2 (BPI Fold-Containing Family A Member 2) was increased significantly in mouse serum after lethal irradiation. BPIFA2, belonging to the PLUNC protein family, is a soluble salivary protein secreted in the extracellular region of the salivary glands, especially parotid gland.[22-29] The encoding gene containing 10 exons is located on human chromosome 20, with 750 bp CDS (protein coding region) region encoding 250 amino acid protein sequence. In mice, BPIFA2 containing 9 introns are located on chromosome 2, with 708 bp CDS region encoding 236 amino acid protein sequences. BPIFA2 is a saliva surfactant which maintains the surface tension of saliva, and inhibits bacterial proliferation through binding to LPS.[30-33] Recent studies have found that BPIFA2 can be used as a biomarker for some diseases. For example, the expression level of BPIFA2 in saliva of periodontitis patients is lower;[34] however, it was increased in patients infected with cytomegalovirus or mycobacterium[35] In 2017, BPIFA2 expression levels were found to be increased in both renal tissue and plasma of patients with acute kidney injury.[36] In 2019, BPIFA2 was found not only to be a useful biomarker for the prognosis of acute kidney injury induced by sepsis but also to prevent the progression of early fibrosis and the development of chronic kidney disease.[37] Therefore, this study aims to investigate whether BPIFA2 could be used as a potential early biomarker molecule of fatal radiation injury post accidents.

In this study, we prepared a polyclonal antibody to mice BPIFA2. Then, we found BPIFA2 concentrations in mouse serum was increased significantly at 1 h, continued to 12 h and then decreased after .5–10 Gy of TBI, with potential to be an early biomarker for the identification of irradiated patients, especially at 12 h. More importantly, BPIFA2 protein concentrations in mouse serum irradiated at lethal doses showed a significant re-elevation at 3 days post-irradiation and correlated with survival rate closely, indicating its feasibility as an early biomarker of lethal radiation injury. Subsequent results in partial irradiated mice showed serum BPIFA2 also increased rapidly after partial irradiation and correlated with the degree of injury, further confirming the radiosensitivity of BPIFA2.

Methods

Experimental Animals

SPF Japanese female rabbits (6 month) and C57BL/6j female mice (8-week-old) were purchased from SPF (Beijing) Biotechnology Co., LTD. and housed at the Experimental Animal Center of Military Medical Research Institute, with 12 h light/dark cycle, controlled temperature (18–23°C) and humidity (40–60%), all experimental manipulations are in accordance with the standards of the Animal Health Care Institution and the Beijing Regulations on the Administration of Laboratory Animals. All animal experiments were approved by the Animal Care and Use Committee of Beijing Institute of Radiation Medicine(NO.IACUC-DWZX-2020-548).

Irradiation

Total body irradiation (TBI). 56 mice were randomly divided into 7 groups and confined to a transparent plexiglass box three meters away from the ^{60}Co γ-ray source, and exposed to a single dose of 0, .5, 1, 2, 4, 6, 8, and 10 Gy radiation at a dose rate of 63.88 cGy/min using ^{60}Co γ-ray. Blood was collected from tail vein at −4 d, 1 h, 2 h, 3 h, 4 h, 6 h, 8 h, 12 h, 1 d, 3 d, 5 d, and 7 d after irradiation.

Partial body radiation (PBI). 30 mice were anesthetized with 0.4 mg/kg chloral hydrate by intraperitoneal injection and divided into 3 groups randomly to receive irradiation whole body (TBI), partial head (PBI-1) and shielded head (PBI-2) respectively at a single dose of 10 Gy using ^{60}Co γ-ray (dose rate is 63.88 cGy/min), using lead bricks as shields. Blood was collected from tail vein at -4 d, 3 h, 6 h, 12 h, and 1 d after irradiation.

Sample Processing

20 µl blood samples were collected from the tail vein into tube containing 480 µl of diluent (M-52 D diluent, Mindray, China), mixed well and counted by AUTO HEMATOLOGY ANALYZER (BC-5000, Mindray, China).

40 µl blood was collected from the tail vein and stored at 4°C for 1 h. After centrifugation at 5000 r/pm for 10 min, the serum transferred to another tube and stored at −80°C.

Construction of Recombinant Plasmid

Refer to the sequence in the NCBI database, PCR amplification primers for mice BPIFA2 were designed: BPIFA2-F1.GAGAACCTATACTTCCAAGGACCCTCTGAAGCTG TCCCTCAGAACCTG; BPIFA2-R: ATGATGCGGCCGC TCGAGGAGGGCAAGTTGTACCTGTCCTGC.

The target gene was amplified by Gloria Nova HS 2X HF Master Mix (RK20715, ABclonal): 94°C for 4 min; 94°C for 40 s, 58°C for 30 s, 72°C for 1 min, a total of 35 cycles. PCR products were identified by 1% agarose gel electrophoresis and then purified and retrieved (AFTSpin Multifunction DNA Purification Kit, RK30100, ABclonal).

Target sequence was ligated to the pGEX-4T-AB1 vector through homologous recombination (2X MultiF Seamless Assembly Mix, RK21020, ABclonal, the carrier was purchased from ABclonal) and determined by Jinkairui Bioengineering Co., LTD.

Induction of Prokaryotic Protein Expression

E. coli Rosetta transformed with pGEX-4T-AB1-BPIFA2 recombinant plasmid was cultured in LB medium containing Kana until logarithmic growth stage, and then induced by 0.8 mM IPTG at 37°C for 4 h. The solution was broken by ultrasound, and the supernatant and precipitation were collected, respectively, to detect the recombinant protein by SDS-PAGE electrophoresis and Coomassie brilliant blue staining.

Animal Immune

The recombinant protein purified by affinity chromatography column was injected subcutaneously into experimental rabbits as immunogen at the dose of 0.3 mg with Complete Freud's adjuvant for the first time. On the 12th, 26th, 40th, and 54th days after the first immunization, the rabbits were injected at the dose of .15 mg with incomplete Freund's adjuvant. Blood was collected at 66th day, and serum was separated to test the titers of antibody. Details are in supplementary Table 1.

Enzyme-Linked Immunosorbent Assay

The titer of BPIFA2 polyclonal antibody was tested with ELISA. The purified protein was diluted to 2 µg/ml with urea and added to a 96-well reaction plate at 100 µl per well, then incubated overnight at 4°C. The liquid was discarded and 150 µl BSA solution was added to each well, and then incubated at 30°C for 2 h. Following three washings with PBST, 100 µl of rabbit antibody (1:8000 dilution, 111-035-045, Jackson ImmunoResearch, USA) was added to each well and incubated at 37°C for 45 min. After five washes, TMB (P0209-100 ml, Beyotime Biotechnology, China) was added to each well. After incubation at 37°C for 10 min, stop solution was added to each well. Then the titer of BPIFA2 polyclonal antibody was measured at 450 nm.

Protein Extraction and Western Blot Analysis

Parotids, thymuses, livers and kidneys were collected and lysed by RIPA. The concentration of protein was detected by BCA (P0010, Beyotime Biotechnology, China). Samples were denatured in loading buffer (P0015 L, Beyotime Biotechnology, China). Total protein extracts were separated by SDS-PAGE electrophoresis. After electrophoresis, the proteins on the gel were either stained with the Coomassie brilliant blue dye (P0017, Beyotime Biotechnology, China) or transferred to NC membranes. The membranes were blocked in 5% skimmed milk and probed overnight at 4°C with the primary antibodies (prepared BPIFA2 antibody, 1:1000; β-Actin Monoclonal Antibody, 66009-1-Ig, proteintech). Then probed with secondary antibody (Anti-mouse IgG (H+L) Antibody, 5220-0341; Anti-rabbit IgG (H+L) Antibody, 5220-0337, KPL, USA); membranes were washed in TBST and protein bands were visualized using the ImageQuant 800 imager (Amersham ImageQuant 800, GE Healthcare, USA).

Data and Statistical Analysis

The intensity of bands on Western Blot was obtained by Image J (NIH, USA). All images were processed with Photoshop. And statistics analysis was fer-formed with SPSS18.0 and GraphPad Prism 8.0. All experiments were confirmed with at least three independent experiments. The quantitative results were presented as the mean ± SD. The significant difference
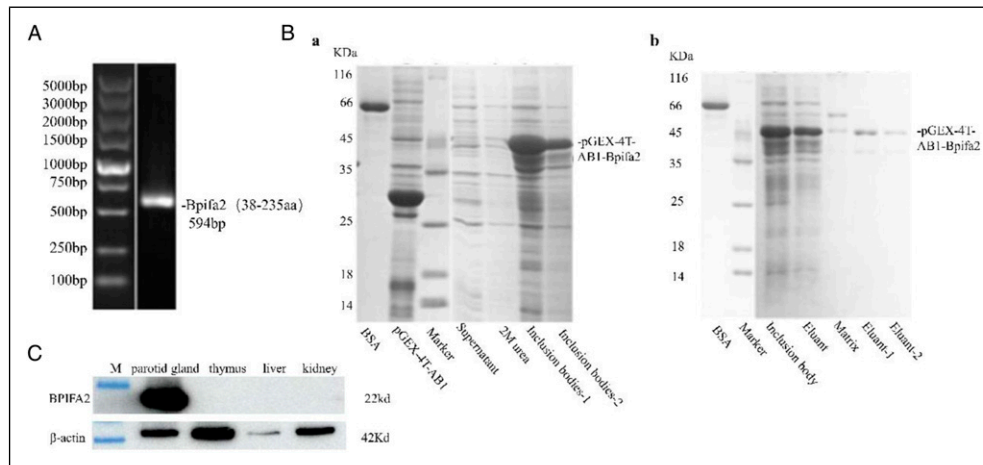
Figure 1. BPIFA2 antibody preparation and validation. **A:** Successful amplification of the target gene was confirmed by agarose gel electrophoresis. **B:** Identification and purification of recombinant proteins. (a) Bacteria induced by 0.8 mM IPTG was broken to detect the expression of recombinant BPIFA2 by Western Blot. The samples were loaded in order of: BSA, pGEX-4T-AB1, Marker, supernatant, 2 M urea lysis inclusion, inclusion bodies-1 (8 M urea lysis inclusion followed by 2-fold dilution),and inclusion bodies-1 (8 M urea lysis inclusion followed by 10-fold dilution). (b) The inclusion-body protein was purified by affinity chromatography column and detected by Western Blot. The samples were loaded in order of: BSA, Marker, inclusion bodies, eluant, matrix, eluant-1 (150 mM imidazole eluant 2-fold dilution), and eluant-2 (300 mM imidazole eluant 2-fold dilution). **C:** Western Blot assay was performed to verify BPIFA2 antibody in mouse parotid, thymus, liver and kidney tissues.

was evaluated with heteroscedasticity t-test. *P < .05, **P < .01, or ***P < .001 were considered as significant.

Results

Successful Preparation of BPIFA2 Polyclonal Antibodies

In order to detect the expression of BPIFA2 accurately, we produced a polyclonal antibody to mice. We designed primers based on the sequence of mice BPIFA2 in NCBI database to amplify target gene by PCR and tested it by agarose gel electrophoresis. The results showed there was a bright and sharp band at about 600 bp (Figure 1A), consistent with the 594 bp (38–235aa) shown in the NCBI, proving the target gene was successfully amplified. The recombinant plasmid was then constructed by homologous recombination and amplified in *E. coli* DH5α.The DNA sequencing showed the recombinant plasmids have been constructed successfully. Details about sequence alignment are exhibited in the Supplementary Material (Figure S1(a) and Supplemental Result 1). Next, we produced the antigen protein. Small-scale induction of BPIFA2 expression in *E. coli* Rosetta showed there was a well-marked and sharp band at about 48 KD (see Figure S1(b)), demonstrating the recombinant plasmid was expressed as expected. The supernatant and inclusion bodies were isolated, to determine the expression and localization of the recombinant protein by SDS-PAGE electrophoresis. According to the result, pGEX-4T-AB1-BPIFA2 was mainly expressed in the inclusion bodies (Figure 1Ba). Further, we renatured and purified the protein from inclusion bodies (Figure 1Bb) to obtain a purified

BPIFA2 protein with a concentration of 1 mg/ml for animal immune experiment.

The rabbits were immunized five times at a dose of .3 mg for the first time, and .15 mg for subsequent 4 times. Serum was collected and diluted at a gradient of 1:1000 to 1:512,000 before testing the antibody titer in serum by ELISA assay. As the results exhibited, the OD value of E15846 was greater than .4 in the dilution of 1:1000–1:64,000, proving the serum was qualified at a dilution of 1:64,000 when encapsulated with 200 ng of antigen. The antibody E15847 had an OD value greater than .4 in the dilution of 1:1000–1:32,000, and the serum was found to be of lower potency than E15846 at 1:32,000. The results of the tests are shown in (Table S1and Figure S1(c)).

Finally, the parotid, thymus, liver and kidney of C57BL/6J female mice were collected to test the specificity of antibody. Firstly, BPIFA2 was specifically expressed in the parotid gland by PCR (Figure S1(d)). Subsequently, Western Blot showed BPIFA2 was detected in mouse parotid characteristically by prepared antibody (Figure 1(c)) with a band around 25Kd, which was consistent with the reported size of approximately 22Kd. There was no band detected in other tissues, consistent with the high specificity of BPIFA2 in parotid gland. So far, we have successfully prepared the rabbit anti-mouse BPIFA2 polyclonal antibody that can be used in Western Blot assays, creating favorable conditions for subsequent radiosensitivity studies of BPIFA2.

Serum BPIFA2 Is a Sensitive and Reliable Early Indicator for Radiation Damage

Serum BPIFA2 was found to increase significantly after 10 Gy lethal irradiation, through proteome analysis. In order to
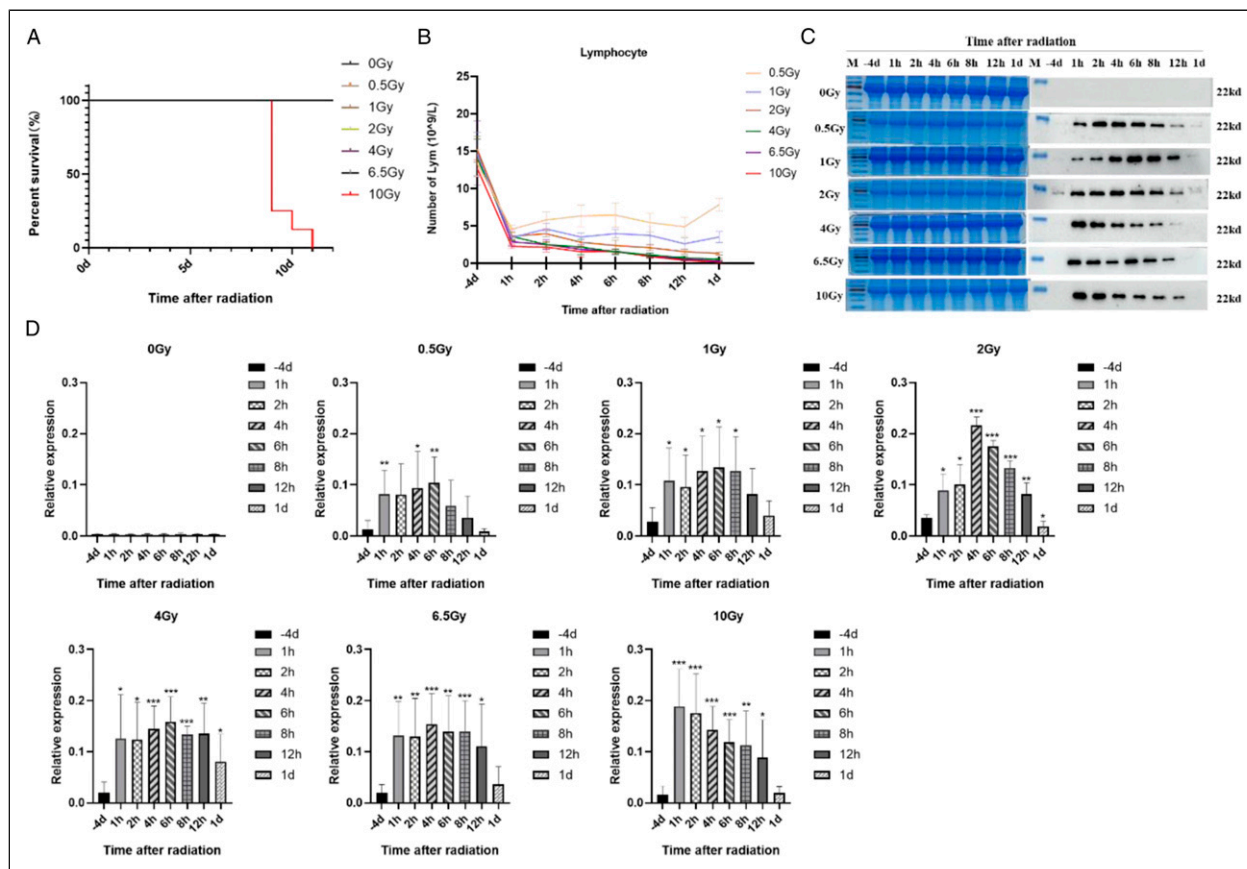
Figure 2. Serum BPIFA2 increased in mice after exposure to different doses of TBI. **A:** Survival rate of mice after different doses of TBI irradiation. **B:** Lymphocyte counts analysis at -4 d, 1 h, 2 h, 4 h, 6 h, 8 h, 12 h and 1 d after different doses of TBI irradiation. Data are mean ± SD of n ≥ 5 mice. **C:** BPIFA2 expression in serum were detected by Western blot at -4 d, 1 h, 2 h, 4 h, 6 h, 8 h, 12 h and 1 d after different doses of TBI irradiation, with Coomassie bright blue staining used as control. **D:** The density analysis of bands on Western Blot in **C,** where n ≥ 5, P < .05: *, P < .01: **, P < .001: ***.

further analyze the dynamic change of serum BPIFA2 levels before and after irradiation, we constructed total-body irradiation (TBI) mouse models at different single doses of 0 Gy (control group), 0.5 Gy, 1 Gy, 2 Gy, 4 Gy, 6.5 Gy (sublethal dose) and 10 Gy (lethal dose). The detection of basic physiological indicators after irradiation were showed in Figure 2A and Figure S2(a). The survival rate analysis showed that all mice exposed to lethal dose died within 11 d, while no mice died in other groups. The weight of the mice dropped sharply after the lethal dose until died, while the others started to recover at 7 d after irradiation. Then, we compared the dynamic changes of lymphocyte count and serum BPIFA2 level during 1 d after irradiation at different doses. The absolute number of lymphocytes in the irradiated mice was decreased dose-dependently (Figure 2B), compared to control group. Table S2 in the supplementary material shows the numbers of lymphocyte were decreased significantly at 1 h after irradiation in all mice, however, subsequent changes are different. The counts rebounded gently within 24 h post-radiation in mice of 0.5 Gy and 1 Gy groups, while continuous to decline in other groups.

BPIFA2 was almost not detected in control mice, while clear and specific bands were detected in all radiated mice serum from

1 h to 12 h after irradiation followed by a decrease at 24 h after irradiation (Figure 2C), indicating TBI induced a quick increase of BPIFA2 in serum within 24 h post-radiation. Comparing the intensity of bands on the Western Blot (Figure 2D), we found that the serum BPIFA2 levels in all the irradiated mice was increased significantly at 1 h after irradiation, then decreased at 1 d after irradiation. BPIFA2 expression was peaked at 4 h-6 h post-irradiation in mice radiated at 2 Gy or less, while keeping high level until 12 h after irradiation without any significant decrease in 4 Gy and 6 Gy groups. BPIFA2 levels rose sharply at 1 h after lethal irradiation, and then fell down slowly until 1 d post-radiation. The results proved that BPIFA2 had the potential to become an early biomarker of ionizing radiation, as it was highly sensitive to ionizing radiation, accompanied with a significant up-regulation at 1 h after irradiation.

Secondary Elevation of BPIFA2 Is an Early Prognostic Indicator of Fatal Radiation Injury

To further explore the indicative role of the serum BPIFA2 for lethal radiation injury in mice, we constructed sublethal (6.5 Gy) and lethal dose (10 Gy) radiation injury mouse
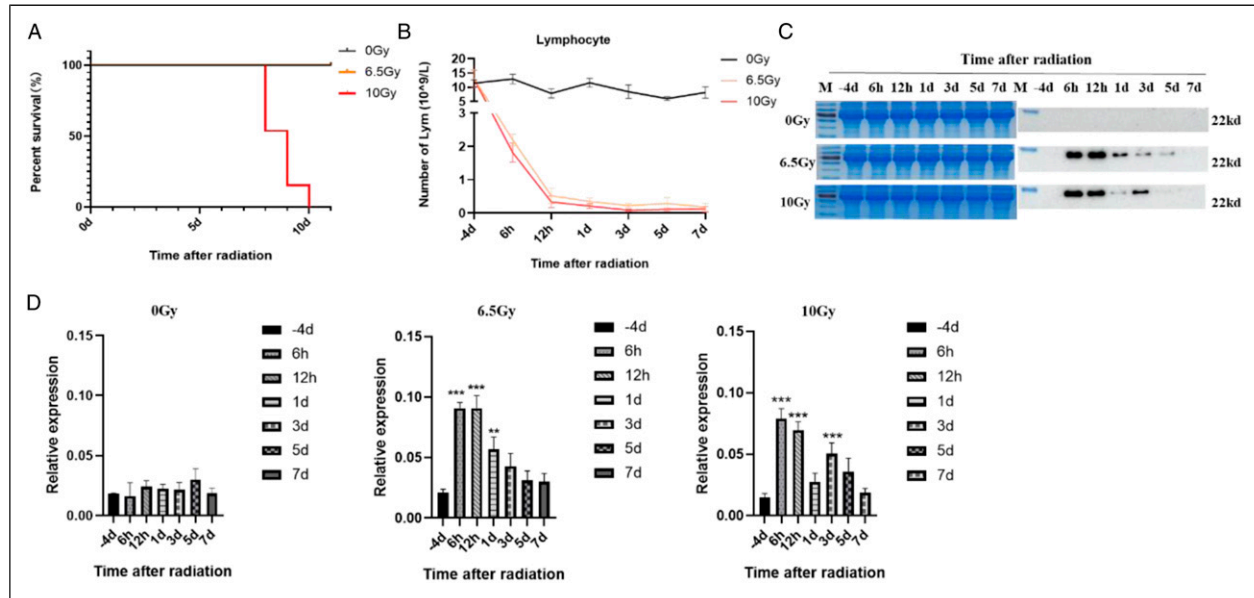
Figure 3. The second increase of serum BPIFA2 in mice after exposure to lethal radiation. **A:** Survival rate of mice after 0 Gy, 6.5 Gy and 10 Gy irradiation—4 d, 1 h, 2 h, 4 h, 6 h, 8 h, 12 h and 1 d.**B:** Lymphocyte counts analysis at 4 d, 12 h, 1 d, 3 d, 5 d, and 7 d after 0 Gy, 6.5 Gy and 10 Gy irradiation. Data are mean ± SD of n ≥ 5 mice. **C:** BPIFA2 expression in serum were detected by Western blot at 4 d, 12 h, 1 d, 3 d, 5 d, and 7 d after 0 Gy, 6.5 Gy and 10 Gy irradiation, with Coomassie bright blue staining as control. **D:** The density analysis of bands on Western Blot in **C**, where n ≥ 5, $P < .05$: *, $P < .01$: **, $P < .001$: ***.

models. The survival rate, body weight, lymphocyte count, and other basic physiological indexes were detected both before and after irradiation. The results showed that all 10 mice in the lethal radiated group died within 10 d, while the survival rate of mice in the sublethal group was 100% (Figure 3A). After exposure to lethal radiation, the weight of mice decreased significantly until death, whereas the one decreased lightly in sublethal group (Figure S2(b)). Then we analyzed the dynamic changes in lymphocyte counts and serum BPIFA2 levels after lethal and sublethal radiation. Lymphocyte counts were decreased dramatically in both lethal and sublethal irradiated mice, and there was no significant difference between them (Figure 3B), suggesting that was unprocurable to identify fatal radiation damage according to lymphocyte count decreasing.

Subsequently, we analyzed the dynamic changes of serum BPIFA2 levels in mice within 7 d after lethal and sublethal irradiation. As the WB assay shown in Figure 3C, serum BPIFA2 in mice exposed to both 6.5 Gy and 10 Gy radiation increased and then decreased within 1 d, consistent with the results in Figure 2C, however, showed different trends later. It began to decrease continuously until undetectably by WB at 7 d after irradiation; in contrast, significant secondary increase was observed at 3 d after 10 Gy irradiation, and then fall back again. The relative intensity analysis of bands on the Western Blot further showed the secondary increase of BPIFA2 in serum from lethal radiated (Figure 3D). These results fully demonstrated the feasibility of the second increase of serum BPIFA2 as an early biomarker of lethal radiation injury in mice.

Serum BPIFA2 Increase Reflects the Overall Degree of Injury Regardless Salivary Gland Exposure

Because BPIFA2 expressed specifically in salivary gland, we speculated serum BPIFA2 levels would be directly affected by irradiated salivary glands. So, we exposed mice to total body irradiation (TBI), only head irradiation (PBI-1) or head shielded irradiation (PBI-2) at 10 Gy (Figure 4A). All mice of TBI group continued to lose weight significantly after irradiation and died within 7 d–18 d after irradiation (Figure S2(c)); however, the mice stopped losing weight from 1 d post-radiation and 100% survival (Figure 4B). The absolute peripheral blood lymphocyte count decreased sharply after irradiation in TBI, PBI-1, and PBI-2 mice, among them, the number of lymphocytes in PBI-1 mice was slightly higher than the other two groups due to smaller area irradiated (Figure 4C).

Unexpectedly, serum BPIFA2 levels were significantly elevated in all mice at 3 h, 6 h, and 12 h after irradiation, then fell back at 1 d, regardless of shielding or exposing the salivary gland (Figure 4D). AS shown in Figure 4E, serum BPIFA2 increase rapidly in all mice, and peaked at 6 h. BPIFA2 in serum of PBI-1 radiated mice were significantly lower than that of other two groups at 6 h and 12 h post-irradiation due to smaller area irradiated, but there was no significant difference between PBI-2 and TBI. This result further confirmed the radiosensitivity of BPIFA2 in mice serum, which was significantly increased within 12 h after irradiation. More importantly, the increase of serum BPIFA2
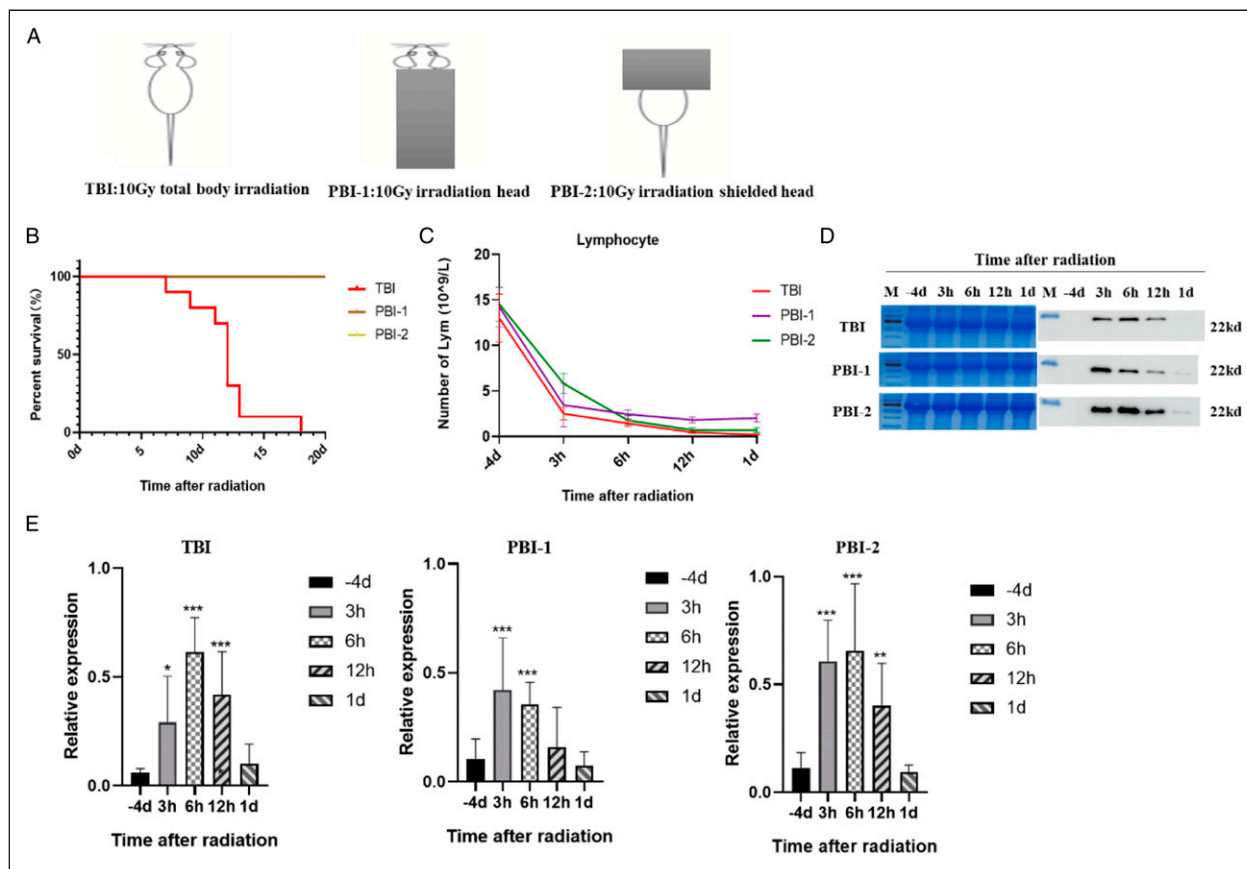
Figure 4. Serum BPIFA2 increased in mice after exposure to PBI irradiation. **A:** Mice exposed to total body irradiation (TBI), only head irradiation (PBI-1) or head shielded irradiation (PBI-2) at 10 Gy. **B:** Survival rate of mice at 4 d, 1 h, 2 h, 4 h, 6 h, 8 h, 12 h, and 1 d after PBI irradiation. **C:** Lymphocyte counts analysis at 4 d, 3 h, 6 h, 12 h, and 1d after PBI irradiation. **D:** BPIFA2 expression in serum were detected by Western blot at 4 d, 3 h, 6 h, 12 h, and 1 d after PBI irradiation, with Coomassie bright blue staining used as control. **E:** The density analysis of bands on Western Blot in **D**, where n ≥ 5, P < .05: *, P < .01: **, P < .001: ***.

Discussion

Radioactive contamination from radiation accidents poses a serious threat to people. Among the large number of wounded exposed to high doses of radiation, most people will experience an incubation period, but only a few will show observable clinical symptom, such as leucopenia, nausea, and vomiting, with high mortality rate. Due to the deficiency of effective identification methods at early stage, wounded exposed to lethal radiation cannot be diagnosed and treated in time, and thus these people might aggravate injuries or even die. Therefore, early, rapid and accurate triage is essential for medical treatment after nuclear accident.

Lymphocyte counting, lymphocyte chromosome aberration analysis and lymphocyte γ-H2AX analysis are mature technologies to estimate the dose of casualties after radiation accidents. Because of the simple and rapid features, lymphocyte counts were used to classify radiation casualties within 12–48 h post-exposure, but it is still difficult to distinguish irradiated person within 12 h.[9-11] Cytogenetic estimation analysis such as chromosomal aberrations and micronuclei is regarded as gold standard for dose estimation; however, it requires 48 h for cell culture.[7,8] The number of γ-H2AX foci responds to DNA double-strand breaks sensitively and dose-dependently, but it also responds to physical or chemical agents. In addition, short detection window period of 1–3 h greatly limits its application.[38] In conclusion, the existing biodosimeters can basically realize screening for a few people exposed, but it cannot achieve injury triage among a large number of casualties during the earlier stages post-irradiation. In this study, we found that BPIFA2 was increased at 1 h after irradiation, which persisted until 12 h, then falling back to different degrees at 1 d post-irradiation. Especially in mice irradiated at lethal dose (10 Gy), the serum BPIFA2 reached the peak at 1 h post-radiation. PBI experiments further confirmed the increase of BPIFA2 in serum have

after irradiation was closely related to the degree of injury induced by radiation, regardless whether the salivary gland was irradiated or not. This is an important factor of serum BPIFA2 as an early biomarker for ionizing radiation injury.

positive correlation with the degrees of injury, regardless whether the salivary glands were irradiated or not. Therefore, the serum BPIFA2 is strongly sensitive to irradiation, so that it can be used as an important biological indicator for determining whether a person is irradiated or not in a short time, especially within 12 h post-radiation. BPIFA2 was increased markedly at 1 h post-irradiation, which was neck and neck with conventional lymphocyte counts and γ-H2AX foci assays, 'what's more, it has a wider detection window than γ-H2AX foci. On the other hand, as a serum protein, BPIFA2 is easier to achieve rapid testing at accident scenes, making it more valuable for medical rescue after severe radiation accidents.

The upper limit of detected dose for chromosomal aberrations and micronuclei is 6 Gy, while γ-H2AX foci analysis is 5 Gy. Our mouse model also shows that lymphocyte count analysis cannot distinguish injury induced by lethal or sublethal doses (Figure S2(b)). Therefore, the search and development of potential biomarkers for lethal radiation has been an important issue of radiation biodosimetry. Through a mouse model, Acharya et al. found miR-30a-3p and miR-30c-5p were significantly increased in serum at 24 h after 8 Gy (lethal dose) irradiation, while miR-187-3p, miR-194-5p, and miR-27a-3p were reduced.[39] A previous study in our laboratory found a secondary increase in serum SAA1 (Serum amyloid A1) expression on days 5–7 after lethal irradiation, which could be used to identify fatal radiation injury.[40] We continuously monitored the dynamic changes of BPIFA2 in mice serum within 7 d after lethal or sublethal irradiation, found it increases at 1 h and then decreases within 24 h post-radiation in both groups. Later, it showed a significant increase again at 3 d after irradiation in the lethal radiated group, while in the sublethal radiated group, it continued to decrease until they were undetectable. It is suggested that the secondary elevation of serum BPIFA2 after irradiation can be used as an early biomarker for fatal radiation injury. It is easier to detect than the miRNA above-mentioned and its secondary elevation is earlier than SAA1, gaining valuable time for the diagnosis and treatment of severely injured patients irradiated.

In conclusion, this study explored the changes of BPIFA2 in mice serum during 7 d after irradiation through TBI and PBI mice, we found that a significant increase of BPIFA2 level has a positive correlation with the degrees of injury in serum during the early 12 h after radiation, suggesting that increased BPIFA2 in serum can be used to identify irradiated people accurately after a large-scale nuclear accident. On the 3rd day after lethal irradiation, a significant secondary increase of BPIFA2 in serum associated with mouse mortality closely indicated that serum BPIFA2 was a novel early biomarker for accurate identification of casualties with fatal radiation injury. The dynamic changes of serum BPIFA2 after irradiation would effectively contribute to the diagnosis and classification of casualties with acute radiation syndrome (ARS). That would play a vital role in allocating medical resources rationally, achieving the goal of more efficient medical rescue and minimizing the numbers of casualties.

Conclusion

This study showed that increased BPIFA2 in serum can be used as a novel early biomarker of irradiated personnel after a large-scale nuclear accident. And the secondary increase of BPIFA2 in serum three days after irradiation is an early biomarker of fatal radiation injury.

Author contributions

ZL, YL, JC and PZ contributed to the conception and design of the study. LH, SZ, WL and SL contributed to the samples and data collection. LH, SZ, ZQ and QW. contributed to the acquisition, analysis, or interpretation of data. LH, SZ, YL and ZW drafted and revised the manuscript. All authors read and approved the final manuscript.

Declaration of Conflicting Interests

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Supplemental Material

Supplemental material for this study is available online.

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