

Plasma Proteins as Biomarkers of Mortality After Total Body Irradiation in Mice

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Abstract

During large-scale acute radiation exposure, rapidly distinguishing exposed individuals from nonexposed individuals is necessary. Identifying those exposed to high and potentially lethal radiation doses, and in need of immediate treatment, is especially important. To address this and find plasma biomarkers to assess ionizing radiation-induced mortality in the early stages, mice were administered a whole-body lethal dose of γ radiation, and radiation-induced damage was evaluated. Multiple blood biomarkers were screened using an antibody array, followed by validation using enzyme-linked immunoassay. The results revealed that irradiation (IR)-induced mortality in mice and caused body weight and blood platelet losses in deceased mice compared to surviving mice. The levels of certain proteins differed after IR between these 2 groups. Specific proteins in preirradiated mice were also found to potentiate radiosensitivity. Plasma levels of interleukin (IL)-22, urokinase, resistin, and IL-6 were associated with radiation-induced mortality in irradiated mice and may be useful as potential mortality predictors. Our results suggest that estimating the levels of certain plasma proteins is a promising alternative to conventional cytogenetic biodosimetry to accurately identify individuals exposed to high radiation doses and those at risk of death due to exposure. This strategy would facilitate the rapid triage of individuals requiring immediate and intensive medical treatment.

Keywords

radiation, plasma protein, biomarkers, mortality

Introduction

Exposure to high-dose ionizing radiation causes dose- and time-dependent multiple organ dysfunction, which is well characterized and potentially results in severe radiation injury and mortality. In incidents involving a large-scale radiological event or nuclear exposure, reliable bioassays are urgently required to rapidly identify exposed individuals and predict the radiation dose to which they were exposed for early triage decisions within hours or days after exposure.^{1,2} Furthermore, it is crucial to accurately evaluate individuals exposed to high-dose radiation who are at a high risk of mortality and need immediate and intensive special treatment.

Radiation exposure directly and indirectly triggers a complex and diverse set of molecular cascades associated with metabolism and inflammatory responses.^{3,4} Radiation-induced cytokines might serve as response biomarkers, and their levels have been well studied among survivors of atomic bomb explosions. Such high-level exposure chronically elevates levels of pro-inflammatory cytokines such as tissue necrosis factor (TNF)- α , interferon- β , interleukin (IL)-6, and IL-10, among others.⁵

Plasma proteins such as Flt3 ligand (Flt3L), serum amyloid A (SAA), and IL-6 have been identified as early biomarkers of radiation dose and injury for up to 1-week post-irradiation (IR) in mouse models^{6,7} and nonhuman primate models.^{8,9} Furthermore, proteomics-based technology has facilitated the identification of novel biomarkers for radiation biodosimetry in nonhuman primate models¹⁰ and humanized NOD/SCID/ γ

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mouse models.¹¹ However, to date, these radiation response proteins are mostly considered biodosimetric biomarkers to estimate the radiation dose and are considered a readout of clinically relevant responses. Thus, their potential to predict radiation-induced mortality remains unclear. Here, we used an antibody array to measure plasma protein levels in a mouse model of high-dose total body IR. Based on protein profiles, several molecules were selected for extended validation.

Materials and Methods

Experimental Animals

Male 6- to 8-week-old C57BL/6 mice were purchased from Vital River Experimental Animal Company (Beijing, China) and housed in a controlled environment under a 12-hour light/12-hour dark cycle. All animal experiments were approved by the Institutional Animal Care and Use Committee of Academy of Military Medical Sciences, China.

Radiation Exposure

Whole-body IR was performed using a ⁶⁰Co γ -ray source (Beijing Institute of Radiation Medicine, Beijing, China). Mice were randomly numbered and placed in a ventilated plexiglass cage and irradiated together. For antibody array analysis and survival evaluation, 50 mice were irradiated with a total dose of 9.0 Gy at 0.76 Gy/min, which was determined based on preliminary experiments to result in moderate mortality. After IR, the mice were returned to the animal facility and maintained routinely. General conditions and survival time were recorded daily for 30 days. For body weight and peripheral blood analysis experiments, the same radiation conditions were used, and the number of mice used has been indicated.

Antibody Arrays

Whole blood from the tail vein was sampled into EDTA-containing tubes 24 hours before and 24 hours after IR. Plasma was extracted from whole blood through centrifugation at $1000 \times g$ for 15 minutes (Heraeus Multifuge X1R; Thermo Scientific, Rockford, Illinois). Six mice that survived and 6 mice that died on day 13 were selected, and plasma samples were pooled for 2 animals (ie, 3 plasma samples were obtained from 6 mice that survived, and 3 plasma samples were obtained from 6 mice that died). Twenty microliters of plasma were used for the biotin label-based L-series mouse antibody array (AAM-BLG-1; Raybiotech, Atlanta, Georgia), according to the manufacturer's instructions.

Body Weight and Peripheral Blood Analysis

Thirty mice were irradiated as mentioned previously herein, and body weight measurements and standard hematological testing for white blood cell (WBC), lymphocyte (LYM), and platelet (PLT) counts were conducted 24 hours before (pre-IR) and 10 days after IR (post-IR). Cell counts were

determined using a hematology analyzer (Celltac E, Nihon Kohden, Tokyo, Japan) and were summarized for mice that finally survived and died.

Enzyme-Linked Immunoassay

Plasma samples were analyzed by sandwich enzyme-linked immunoassay (ELISA) for IL-22, IL-1 α , urokinase (PLAU), resistin (RETN; Elabscience, Wuhan, China), IL-6 (R&D Systems, Minneapolis, Minnesota), and SAA (MultiSciences, Hangzhou, China) levels according to the manufacturers' instructions. Twenty microliters of plasma samples were used for each assay. In each assay, the absorbance at 450 nm was read using a plate reader (Model 680XR; Bio-Rad, Hercules, California).

Statistical Analysis

Statistical significance was assessed using a 2-sided Student *t* test for paired samples or 1-way analysis of variance with a Tukey posttest for multiple comparisons using GraphPad Prism 7.0 software (GraphPad, San Diego, California). A value of $P < .05$ was considered statistically significant.

Results

Radiation-Induced Damage Is More Severe in Mice That Die Than in Those That Survive

We irradiated mice with a lethal dose of 9.0 Gy and 58% of irradiated mice survived (Figure 1A). We then compared radiation-induced damage between mice that eventually died (mortality group) and those that survived (survival group), on day 10 post-IR (ie, before the mice in the mortality group started dying). We found that the average body weight in the mortality group on day 10 post-IR was significantly lower than that in the same mice pre-IR ($P < .01$), as well as that in the surviving group post-IR ($P < .0001$). This indicated that radiation might induce severe overall damage in the mortality group. Interestingly, the pre-IR body weight of mice in the mortality group was significantly lower than that in the survival group ($P < .05$; Figure 1B), indicating that underweight mice are more likely to die when exposed to lethal dose IR. Hematologic examination also revealed more severe injury in the mortality group than in the survival group, with lower peripheral blood PLT counts on day 10 post-IR. However, WBC and LYM counts were not different between the groups (Figure 1C). Collectively, these results indicated that lethal dose radiation induced more severe damage in the mortality group than in the surviving group.

Plasma Protein Levels Differ Between Irradiated Mice That Survive and Those That Die

Next, we evaluated pre- and post-IR plasma protein levels in the mortality and survival groups using mouse antibody arrays, which revealed a differential expression pattern (Figure 2A). In

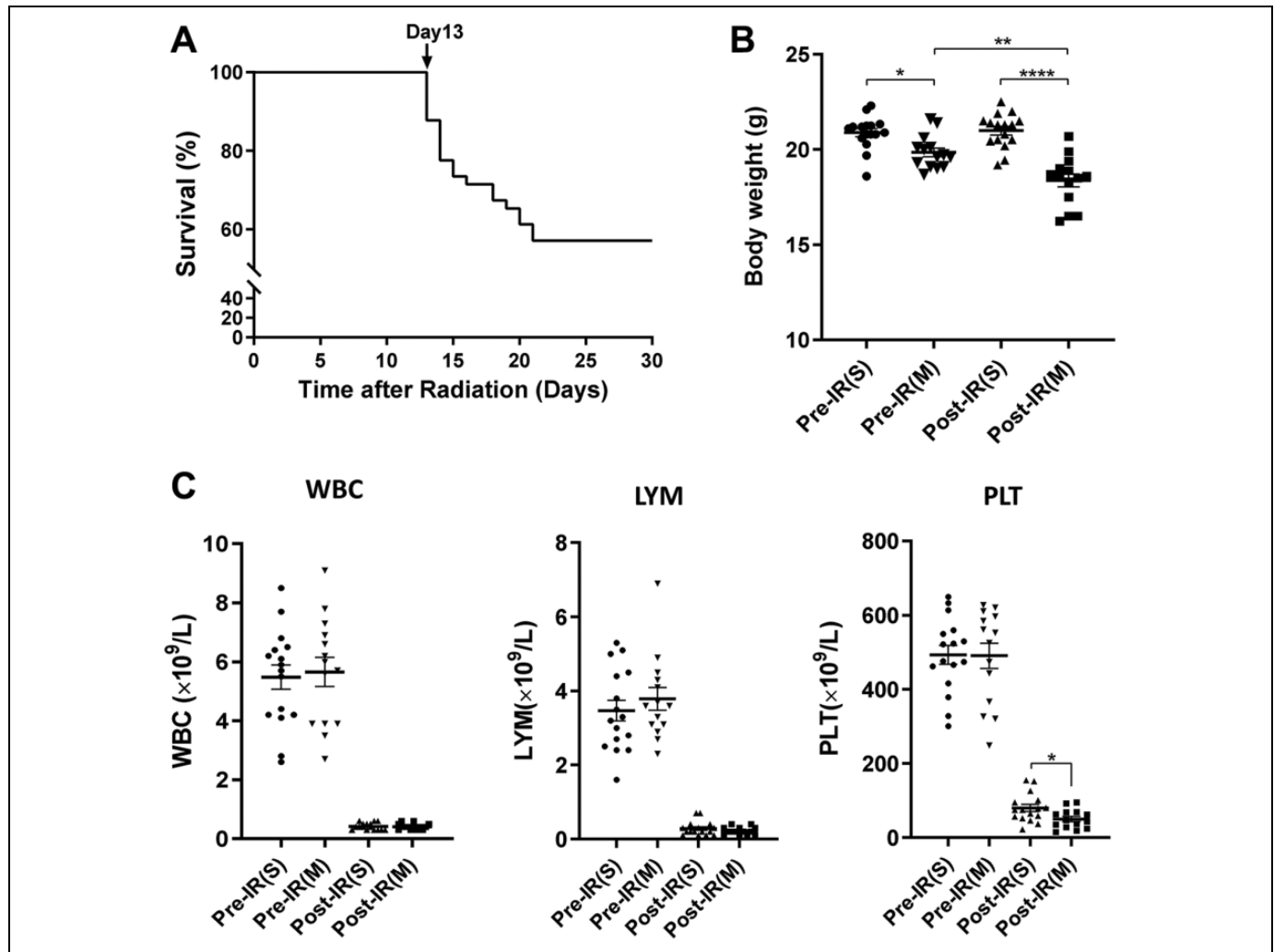


Figure 1. Mouse survival and damage analysis. A, Fifty mice were irradiated with 9.0 Gy at 76 cGy/min, and 21 mice died between day 13 and day 22. B, Body weight assessment at 24 hours before (pre-IR) and on day 10 after IR (post-IR) of mice who finally survived or died, in separate IR treatment groups. Data are presented as the mean \pm SEM; $n = 16$ for the survival group and 14 for the mortality group. * $P < .05$, ** $P < .01$, *** $P < .0001$ based on 2-tailed Student t test. C, Peripheral blood counts of WBCs, LYM, and PLT at 24 hours before and on day 10 after IR in the survival and mortality groups. Data are presented as the mean \pm SEM; $n = 16$ for survival group and 14 for the mortality group. * $P < .05$ based on 2-tailed Student t test. IR indicates irradiation; LYM, lymphocyte; M, mortality; PLT, platelet; S, survival; SEM, standard error of the mean; WBC, white blood cell.

the mortality group, IL-6, Fc fragment of IgG receptor II (FCGR2), and IL-21 were upregulated after IR, whereas secretory leukocyte peptidase inhibitor (SLPI) and lungkine (CXCL15) were downregulated (Figure 2B; fold-change >1.2 or <0.83 , $P < .05$). Considering that 3 samples were inadequate for the accurate assessment of protein levels, an exhaustive list of differentially expressed proteins ($P < .1$) is provided in Supplemental Table S1. In the surviving group, cardiotrophin-1 (CTF1), fibroblast growth factor receptor 3 (FGFR3), follistatin-like 1 (FSTL1), and FCGR2 were upregulated, whereas PLAU and SLPI were downregulated ($P < .05$; Figure 2C and Supplemental Table S2 for a complete list of changes in biomarkers with $P < .1$). Among these proteins, FCGR2 and SLPI were commonly altered in both the survival and mortality groups, suggesting that these proteins play

essential roles in radiation injury, albeit unrelated to mortality, and might be potential biomarkers of radiation biodosimetry.

Pre-IR Protein Profile in Mice Affects Susceptibility to Radiation-Induced Mortality

When we compared pre-IR plasma protein levels between the mortality and surviving groups, we found that CXCL15, C-X-C motif chemokine ligand 14 (CXCL14), and TNF receptor superfamily member 6 (TNFRSF6, also known as FAS) were higher, whereas IL-20R α , IL-18R α , and MIP-1 β (CCL4) were lower in the mortality group than in the survival group ($P < .05$; Figure 3C and Supplemental Table S3 for a complete list of changes in biomarkers with $P < .1$). These results suggested that the pre-IR levels of certain proteins might increase mouse

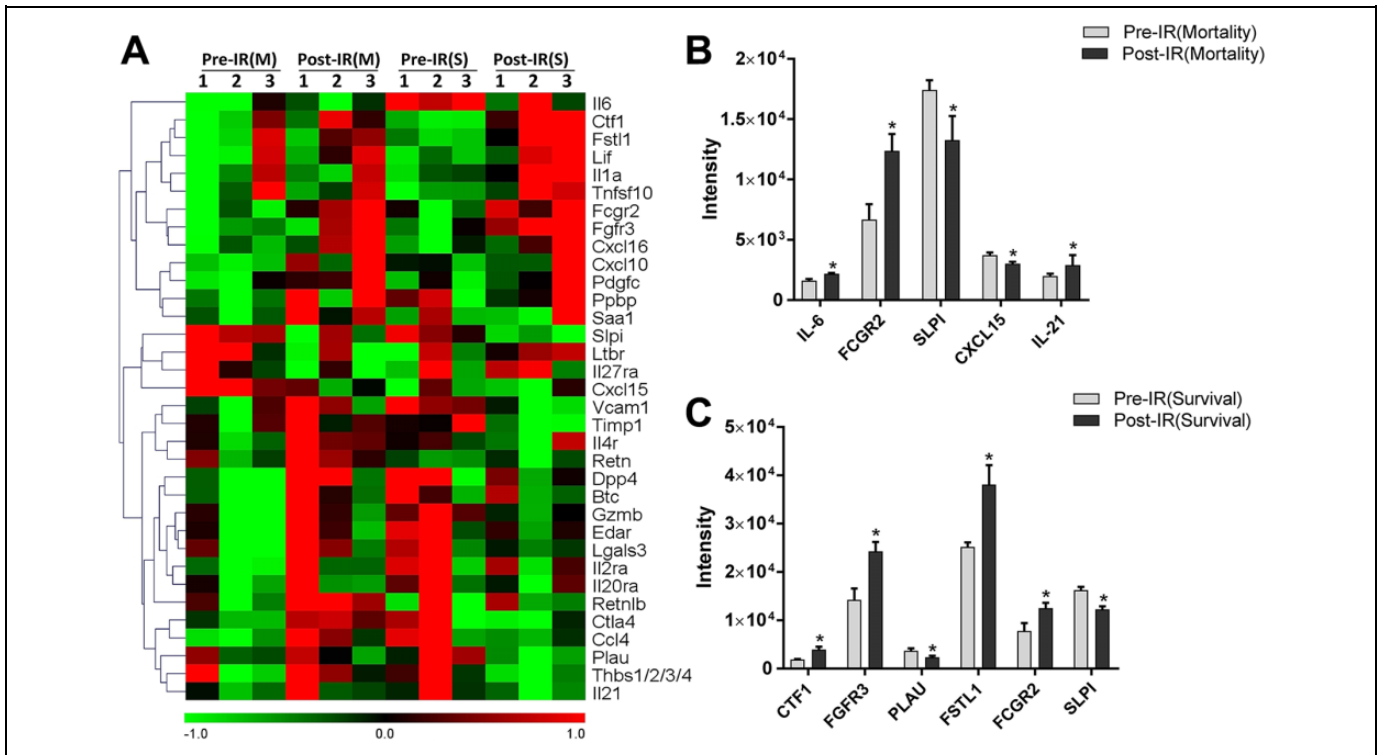


Figure 2. Comparison of plasma protein levels before and after high-dose radiation in the mortality and survival groups using RayBiotech antibody array. A, Heatmap of expression of plasma proteins between pre-IR and post-IR samples in either the survival or mortality group, with $P < .1$. The heatmap was drawn using MultiExperiment Viewer (4.9.0). B, Plasma protein levels differed between pre-IR and post-IR samples in the mortality group. C, Plasma protein levels differed between pre- and post-IR plasma samples in the survival group. Data are expressed as the mean \pm SEM; $n = 3$, fold-change >1.2 or <0.83 , $*P < .05$. SEM indicates standard error of the mean.

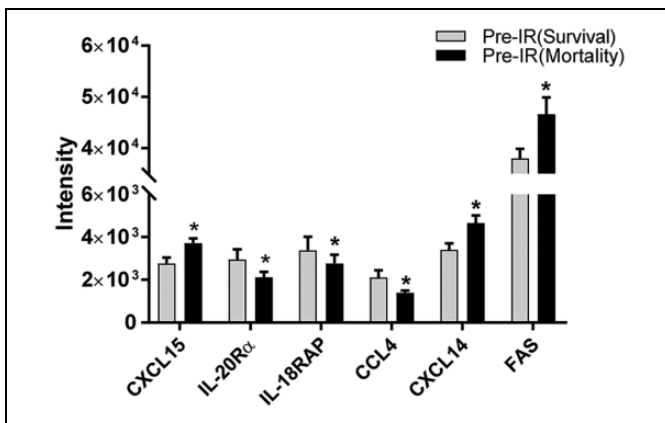


Figure 3. Comparison of plasma protein levels in pre-IR samples between the mortality group and survival group. Data are expressed as the mean \pm SEM; $n = 3$, fold-change >1.2 or <0.83 , $*P < .05$. SEM indicates standard error of the mean.

sensitivity to mortality in response to lethal dose radiation and might be related to radiation-induced damage.

Certain Proteins in Post-IR Mice Might Serve as Indicators of Radiation-Induced Mortality

Since some proteins showed differences in expression in survival and mortality groups, even pre-IR, to minimize the

variation in protein levels in preirradiated mice and practically identify post-IR markers of radiation-induced mortality, the pre-IR plasma protein profiles of all mice—reflecting the mean protein levels in the total population—were compared to post-IR plasma samples from both the mortality and survival groups. As shown in Figure 4 (Supplemental Table S4 for a complete list of changes in biomarkers with $P < .1$), several proteins including IL-22, FSTL1, IL-1 α , and GFR receptor α 4 were significantly upregulated ($P < .05$), whereas insulin-like growth factor-binding protein 5 (IGFBP5) and PLAU were downregulated, in the survival group ($P < .05$); however, these levels were unchanged in the mortality group. Moreover, IL-6, RETN, and SAA were upregulated in the mortality group ($P < .05$). We then validated plasma IL-22, PLAU, IL-1 α , IL-6, RETN, and SAA levels with a commercially available ELISA kit. The results confirmed the correlation between the levels of these proteins and mouse mortality after high-dose ionizing radiation (Figure 5). For example, after IR, IL-22 and RETN were significantly increased in the survival and mortality group, respectively ($P < .05$), PLAU was significantly decreased in the mortality group ($P < .01$), and all 3 proteins exhibited significant differences between the 2 groups at 24 hours post-IR ($P < .05$). Interleukin-6 and SAA were upregulated in both post-IR groups when compared with mean pre-IR levels in the total population; however, IL-6 was significantly increased ($P < .001$) in the mortality group compared to

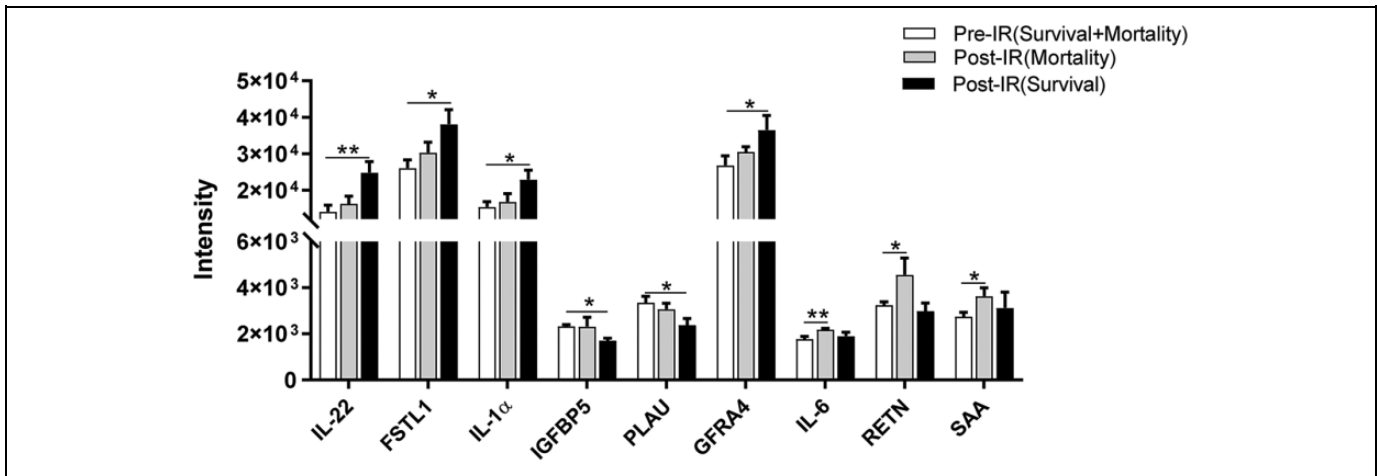


Figure 4. Comparison of plasma protein levels in either the post-IR survival or the post-IR mortality group relative to those in the combined pre-IR survival plus mortality group. Data are expressed as the mean \pm SEM; $n = 6$ for pre-IR group and $n = 3$ for post-IR groups, fold-change >1.2 or <0.83 , $*P < .05$, $**P < .01$ for either post-IR survival or post-IR mortality groups over pre-IR group, based on 1-way analysis of variance (ANOVA) with Tukey posttest. SEM indicates standard error of the mean.

levels in the survival group (Figure 5D). In contrast, SAA levels did not differ between these 2 groups (Figure 5E). Furthermore, no significant difference was found in IL-1 α between the mortality and survival groups, although IL-1 α was significantly upregulated in the survival group (Figure 5F). Meanwhile, the repeatability of these results was confirmed by capturing plasma IL-22 and RETN in a separate repeated experiment (Supplemental Figure S1). Taken together, these results indicated that certain plasma proteins including IL-22, PLAU, RETN, and IL-6 are potential indicators of mortality after exposure to ionizing radiation.

Discussion

The radiation-induced mortality rate is positively correlated with radiation dose and dose rates.¹² Shortly after radiation and radiation-induced injury, macrophages produce numerous hematopoietic cytokines and growth factors, which are associated with a wide range of systemic responses including the dramatic expression of acute-phase proteins. Plasma protein levels reportedly increase during acute radiation syndrome,^{9,13} thereby providing early diagnostic information regarding acute radiation exposure.¹⁴ C-reactive protein, Flt3L, SAA, IL-6, and granulocyte colony-stimulating factor (GCSF) are early biomarkers for radiation dose assessment and radiation injury.⁶⁻⁹ The present study focused on the association between plasma proteins and radiation-induced mortality and hematopoietic injury and revealed that specific proteins were preferentially altered in either one or both of the survival and mortality groups. We identified several proteins for which pre-IR serum levels might predict higher sensitivity to radiation-induced mortality, as well as others for which post-IR serum levels might be used as potential predictors of mortality.

We found that the body weights of mice in the mortality group but not in the survival group decreased rapidly in

response to IR, which might indicate severe overall damage in this group; however, we observed this phenomenon on day 10 after IR. Interestingly, pre-IR body weights were lower in the mortality group than in the survival group, indicating that lower body weights might enhance the effects of IR on mice of the same age. However, it has been reported that there is no universal correlation between age or body weight and radiation-induced mortality, except that weight reflects a particular age range.¹⁵ Specifically, a negative association between body weight and mortality was found in 60-day-old mice exposed to lethal radiation, as mortality decreased from 27% with a body weight of 19 g to 15% with that of 23 g, suggesting that lower body weight is related to susceptibility to lethal radiation only within a narrow age range.¹⁵ This concurs with our results showing that pre-IR body weight was lower in the mortality group than in the survival group within this age range.

Changes in the peripheral hemogram might also indicate radiation-induced tissue damage^{16,17}; however, most peripheral blood cells including WBCs and LYMs were not found to significantly differ between the mortality and survival groups. This suggests that the general loss of peripheral blood or immune cells might not be the major reason for lethality. Only PLT counts were lower, and thus potentially related to increased hemorrhage, in the mortality group. Collectively, these findings indicate that lethal dose IR causes more severe damage to mice that eventually die than in those that survive. Nonetheless, further experimental investigations are required to elucidate the underlying physiological and molecular mechanisms.

Cytokine levels can change in response to multiple factors including metabolism and growth.¹⁸ However, as important regulators of the immune system, certain cytokines in the plasma were highly induced by IR, irrespective of their levels in various tissues and organs, rendering them potential

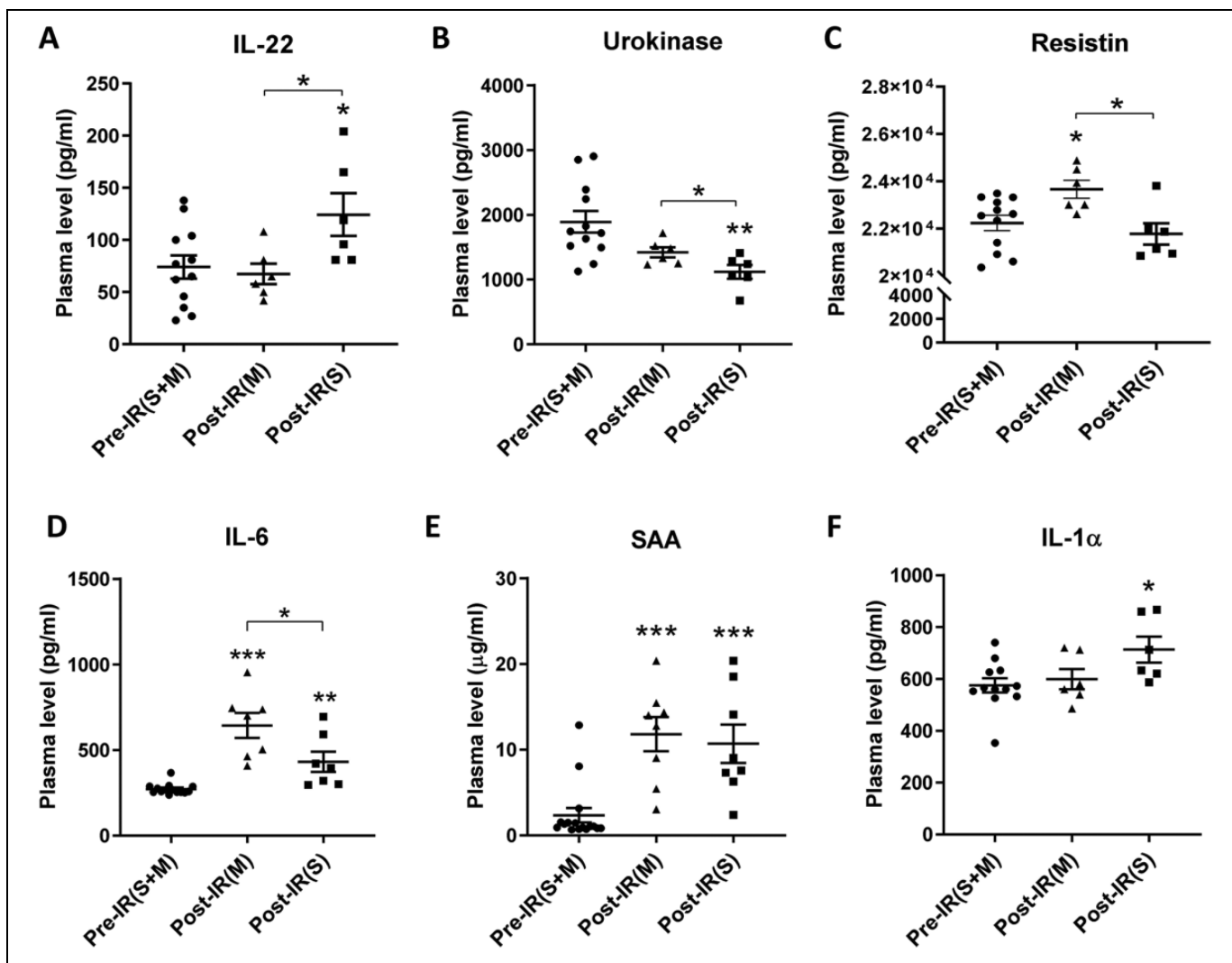


Figure 5. Associations between plasma protein levels, as determined by ELISA, and mouse mortality or survival. A, IL-22, (B) IL-1 α , (C) urokinase (PLAU), (D) resistin (RETN), (E) IL-6, and (F) SAA. Plasma levels of IL-22, PLAU, RETN, and IL-6 were found to serve as indicators of radiation-induced mortality in mice. Data are expressed as the mean \pm SEM; $n = 6-8$ for post-IR survival and post-IR mortality groups, and $n = 12-16$ for the combined pre-IR survival plus mortality group. * $P < .05$, ** $P < .01$, *** $P < .001$ based on 1-way ANOVA with Tukey posttests. ANOVA indicates analysis of variance; ELISA, enzyme-linked immunoassay; IL, interleukin; IR, irradiation; M, mortality; S, survival; SAA, serum amyloid A; SEM, standard error of the mean.

candidates for the evaluation of mouse susceptibility to lethal dose radiation. Our results showed that some proteins were preferentially changed in the survival group (CTF1, FGFR3, PLAU, FSTL1, FCGR2, and SLPI) or mortality group (IL-6, FCGR2, SLPI, CXCL15, and IL-21), or even in mice before IR (CXCL15, IL-20Ra, IL-18RAP, CCL4, CXCL14, and FAS). Among these proteins, FCGR2 and SLPI were commonly altered in both the survival and mortality groups, suggesting that these proteins play essential roles in radiation injury, albeit unrelated to mortality, and might be potential biomarkers of radiation biodosimetry.

A comparison of the protein profiles between pre-IR and postsurvival or postmortality plasma samples revealed that IL-22, PLAU, and IL-1 α levels were altered exclusively in the survival group, whereas IL-6, RETN, and SAA levels were

increased exclusively in the mortality group. Alterations in the levels of these proteins were confirmed by ELISA, except for IL-1 α and SAA, for which levels were not found to differ between postsurvival and postmortality groups. These results suggest that these markers potentially play roles in regulating radiation injury and might serve as indicators of radiation-induced mortality. Some of these proteins (IL-6 and SAA) were previously reported as biomarkers for radiation biodosimetry⁹ and are related to radiation-induced hematopoietic response events or acute-phase responses. In contrast, the others (IL-22, IL-1 α , IGFBP5, and RETN) were reported to be altered in response to radiation¹⁹⁻²² or to be involved in radiation-induced pulmonary fibrosis (FSTL1)²³ or CNS damage (PLAU).²⁴ Although the roles of most of these proteins in radiation injury are unclear, our study provides preliminary

evidence indicating that these biomarkers are associated with survival or mortality upon radiation exposure; however, further verification is warranted.

IL-6 and SAA displayed differences in the mortality group compared to levels in the pre-IR group based on antibody array findings; however, these differences were not consistent with ELISA results. Furthermore, some known early response cytokines including GCSF were not identified; perhaps, their levels were not significantly altered (such as FLT3L; Supplemental Table S4). These issues are probably due to the insufficient number of samples used for the antibody array or interindividual variation in protein levels resulting from hyper-radiosensitivity.¹³ This warrants further verification, particularly for proteins that showed changes with $P > .05$ and $< .1$. Further, this indicates that plasma proteins other than those identified in this study might be identified as potential radiation biomarkers in future studies.

In conclusion, our current data preliminarily indicate that certain plasma proteins can promote sensitivity to high-dose radiation and can be considered indicators of mortality immediately after radiation. Although some mortality-associated proteins might have been missed owing to interindividual variations in plasma proteins or complications associated with their pleiotropy, redundancy, and mutual interference,²⁵ our results provide novel insights into biomarkers that could be used to predict radiation-associated mortality. This could lead to the rapid triage of certain patients for medical treatment after exposure to high-dose total body ionizing radiation.

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Declaration of Conflicting Interests

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Supplemental Material

Supplemental material for this article is available online.

References

1. Chaudhry MA. Biomarkers for human radiation exposure. *J Biomed Sci.* 2008;15(5):557-563.
2. Singh VK, Newman VL, Romaine PL, Hauer-Jensen M, Pollard HB. Use of biomarkers for assessing radiation injury and efficacy of countermeasures. *Expert Rev Mol Diagn.* 2016;16(1):65-81.
3. Pannkuk EL, Laiakis EC, Authier S, Wong K, Fornace AJ. Targeted metabolomics of nonhuman primate serum after exposure to ionizing radiation: potential tools for high-throughput biodosimetry. *RSC Adv.* 2016;6(56):51192-51202.
4. Di Maggio FM, Minafra L, Forte GI, et al. Portrait of inflammatory response to ionizing radiation treatment. *J Inflamm (Lond).* 2015;12(1):14.
5. Hayashi T, Morishita Y, Kubo Y, et al. Long-term effects of radiation dose on inflammatory markers in atomic bomb survivors. *Am J Med.* 2005;118(1):83-86.
6. Sproull M, Kramp T, Tandle A, Shankavaram U, Camphausen K. Multivariate analysis of radiation responsive proteins to predict radiation exposure in total-body irradiation and partial-body irradiation models. *Radiat Res.* 2017;187(2):251-258.
7. Ossetrova NI, Ney PH, Condliffe DP, Krasnopolsky K, Hieber KP. Acute radiation syndrome severity score system in mouse total-body irradiation model. *Health Phys.* 2016;111(2):134-144.
8. Blakely WF, Ossetrova NI, Whitnall MH, et al. Multiple parameter radiation injury assessment using a nonhuman primate radiation model-biodosimetry applications. *Health Phys.* 2010;98(2):153-159.
9. Ossetrova NI, Sandgren DJ, Blakely WF. Protein biomarkers for enhancement of radiation dose and injury assessment in nonhuman primate total-body irradiation model. *Radiat Prot Dosimetry.* 2014;159(1-4):61-76.
10. Byrum SD, Burdine MS, Orr L, et al. Time- and radiation-dose dependent changes in the plasma proteome after total body irradiation of non-human primates: Implications for biomarker selection. *PLoS One.* 2017;12(3):e0174771.
11. Lee Y, Pujol Canadell M, Shuryak I, et al. Candidate protein markers for radiation biodosimetry in the hematopoietically humanized mouse model. *Sci Rep.* 2018;8(1):13557.
12. Gengozian N, Taylor T, Jameson H, Lee ET, Good RA, Epstein RB. Radiation-induced hemopoietic death in mice as a function of photon energy and dose rate. *Radiat Res.* 1986;105(3):320-327.
13. Ossetrova NI, Condliffe DP, Ney PH, et al. Early-response biomarkers for assessment of radiation exposure in a mouse total-body irradiation model. *Health Phys.* 2014;106(6):772-786.
14. Marchetti F, Coleman MA, Jones IM, Wyrobek AJ. Candidate protein biodosimeters of human exposure to ionizing radiation. *Int J Radiat Biol.* 2006;82(9):605-639.
15. Abrams HL. Influence of age, body weight, and sex on susceptibility of mice to the lethal effects of X-radiation. *Proc Soc Exp Biol Med.* 1951;76(4):729-732.
16. Heylmann D, Rodel F, Kindler T, Kaina B. Radiation sensitivity of human and murine peripheral blood lymphocytes, stem and progenitor cells. *Biochim Biophys Acta.* 2014;1846(1):121-129.
17. Qian Q-Z, Cao X-K, Liu H-Y, et al. Analysis of hemogram of radiation workers in Tangshan, China. *J Clin Lab Anal.* 2016;30(5):682-688.
18. Spurlock ME. Regulation of metabolism and growth during immune challenge: an overview of cytokine function. *J Anim Sci.* 1997;75(7):1773-1783.

19. Xia F, Wu YJ, Lu ZZ, Xu KL, Pan B. The role of IL-22 in T cell reconstitution after thymus damage induced by ionizing radiation [in Chinese]. *Zhonghua Xue Ye Xue Za Zhi*. 2018;39(9):761-765.
20. Ahmad IM, Abdalla MY, Moore TA, Bartenhagen L, Case AJ, Zimmerman MC. Healthcare workers occupationally exposed to ionizing radiation exhibit altered levels of inflammatory cytokines and redox parameters. *Antioxidants (Basel)*. 2019;8(1):E12.
21. Kim KS, Kim JE, Choi KJ, Bae S, Kim DH. Characterization of DNA damage-induced cellular senescence by ionizing radiation in endothelial cells. *Int J Radiat Biol*. 2014;90(1):71-80.
22. Song J, Zhang H, Wang Z, et al. The Role of FABP5 in radiation-induced human skin fibrosis. *Radiat Res*. 2018;189(2):177-186.
23. Chen Z, Fang Y, Zhang S, et al. Haplodeletion of follistatin-like 1 attenuates radiation-induced pulmonary fibrosis in mice. *Int J Radiat Oncol Biol Phys*. 2019;103(1):208-216.
24. Rao JS, Rayford A, Yamamoto M, Ang KK, Tofilon P, Sawaya R. Modulation of fibrinolysis by ionizing radiation. *J Neurooncol*. 1994;22(2):161-171.
25. Schooltink H, Rose-John S. Cytokines as therapeutic drugs. *J Interferon Cytokine Res*. 2002;22(5):505-516.