Research article

Serum sSelectin-L is an early specific indicator of radiation injury

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ABSTRACT

Objective: It's crucial to identify an easily detectable biomarker that is specific to radiation injury in order to effectively classify injured individuals in the early stage in large-scale nuclear accidents.
Methods: C57BL/6J mice were subjected to whole-body and partial-body γ irradiation, as well as whole-body X-ray irradiation to explore the response of serum sSelectin-L to radiation injury. Then, it was compared with its response to lipopolysaccharide-induced acute infection and doxorubicin-induced DNA damage to study the specificity of sSelectin-L response to radiation. Furthermore, it was further evaluated in serum samples from nasopharyngeal carcinoma patients before and after radiotherapy. Simulated rescue experiments using Amifostine or bone marrow transplantation were conducted in mice with acute radiation syndrome to determine the potential for establishing sSelectin-L as a prognostic marker. The levels of sSelectin-L were dynamically measured using the ELISA method.
Results: Selectin-L is mainly expressed in hematopoietic tissues and lymphatic tissues. Mouse sSelectin-L showed a dose-dependent decrease from 1 day after irradiation and exhibited a positive correlation with lymphocyte counts. Furthermore, the level of sSelectin-L reflected the degree of radiation injury in partial-body irradiation mice and in nasopharyngeal carcinoma patients. sSelectin-L was closely related to the total dose of γ or X ray. There was no significant change in the sSelectin-L levels in mice intraperitoneal injected with lipopolysaccharide or doxorubicin. The sSelectin-L was decreased slower and recovered faster than lymphocyte count in acute radiation syndrome mice treated with Amifostine or bone marrow transplantation.
Conclusions: Our study shows that sSelectin-L has the potential to be an early biomarker to classify injured individuals after radiation accidents, and to be a prognostic indicator of successful rescue of radiation victims.

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1. Introduction

A sudden nuclear incident can cause large number of casualties due to acute radiation injury. Treating individuals with different levels of acute radiation Syndrome (ARS) require distinct approaches [1]. On-site rescue is the primary component of nuclear medicine emergency response, and one of its core tasks is to accurately and rapidly assess whether the casualties have suffered radiation injuries requiring medical intervention and to classify the severity of these injuries [2].

Biological methods, such as chromosomal aberration analysis, are commonly used for assessing post-irradiation injuries [3]. Nevertheless, this method requires substantial time for sample processing and highly precise instruments.γ-H2AX foci analysis is another widely used method, but its limited detection window period and complex experimental procedure restrict its application [4, 5]. Irradiation causes a dose-dependent decrease in peripheral blood lymphocyte count, which has been used to classify radiation-injured victims within 12–48 h. However, this method is likely unsuitable for field applications as it necessitates specialized laboratory instruments [6]. Therefore, the discovery of early marker molecules specific to radiation injury and easy to detect is necessary for the early classification of injured in large-scale nuclear incidents [7]. These biomarkers also have potential in predicting complications in normal tissues resulting from radiation therapy, aiding in personalized treatment plans and monitoring treatment responses [8,9]. Implementing these biomarkers in practice have the potential in improving patient outcomes and minimizing adverse effects on normal tissue [10].

Selectin-L(CD62L), also known as leukocyte endothelial cell adhesion molecular-1, lymphocyte homing receptor or MEL-14, is a type-I transmembrane glycoprotein and cell adhesion molecule expressed on various leukocytes [11]. It exists in two forms: constitutive Selectin-L (cSelectin-L) on the surface of leukocyte and soluble Selectin-L (sSelectin-L) in serum [12]. cSelectin-L mediates leukocyte attach to endothelial cells during inflammation and regulates signaling pathways for adhesion and transmigration [13,14]. Shedding of cSelectin-L is important in modulating monocyte invasion and polarity. After the activation of leukocytes, the membrane-bound cSelectin-L is shed and released into the serum as sSelectin-L [15,16], which can be used as a plasma/serum biomarker to trigger leukocyte activity during acute or chronic inflammation, such as T lymphocyte leukemia and Alzheimer's disease [17–19]. Regarding radiation research, it has been observed that the expression of cSelectin-L on the surface of lymphocytes shows a time- and dose-dependent decrease following radiation therapy [20–22]. After radiation therapy, lung cancer patients showed a significant decrease in sSelectin-L levels in the serum [23]. This suggests that the concentration of sSelectin-L decreases after radiation injury could potentially serve as a supplementary method for existing radiation biological dosimeters in the early stages of radiation incidents. In addition, it holds promise for providing guidance in the early diagnosis of normal tissue damage caused by radiotherapy, as well as predicting the development of complications.

This study confirmed that serum sSelectin-L is a sensitive and reliable radiation indicator in mice within a specific dose range after total body irradiation (TBI). In both incidents and radiotherapy, individuals are local exposed to radiation primarily, rather than whole-body exposure. Therefore, partial body radiation (PBI) was performed on mice, revealing a significant correlation between the decrease of sSelectin-L and the severity of radiation-induced injury. The feasibility of sSelectin-L as a radiation biomarker was also be confirmed in serum from nasopharyngeal carcinoma patients receiving head and neck radiotherapy. Moreover, the decreased serum sSelectin-L level was closely correlated with the total dose of exposure and is not affected by bacterial infection or DNA-damaging drugs. Importantly, sSelectin-L can also serve as a prognostic indicator for severely radiation-injured mice after bone marrow transplantation (BMT).

2. Materials and methods

2.1. Animals

The experiment used 8-week-old C57BL/6 mice purchased from Beijing Speford Biotechnology Co. The mice weighed 19–21g each, and were housed 3 to 5 per cage in a temperature and humidity-controlled environment with a 12 and 12-h light and dark cycle. The mice were held in the Beijing Institute of Radiology (Beijing, China) for at least one week before the experiment. All experimental protocols were approved by the Institutional Animal Care and Use Committee of the Academy of Military Medical Sciences (AMMS) (Beijing. China), and were numbered as follows: IACUC-DWZX-2022-830. At the end stage of the experiment, mice were euthanized by exposure to CO_2. All methods were carried out in accordance with relevant guidelines and regulations. All experiments performed on male mice, excepting the analysis for gender difference of radiation response.

2.2. Total body irradiation (TBI)

The equipment utilized in our study is operated by trained professionals, with each irradiation preceded by calibration of the physical dose to ensure guarantee precision in irradiation dosage. Mice were confined in a transparent plexiglass box and subjected to single TBI using an AMMS ^{60}Coγray. Each group has 12 mice, and the administered doses were 0.5, 1, 2, 4, 6.5 and 10 Gy respectively, at a dose rate of 0.011 Gy s^{-1}. In the experiment on the effect of dose rate, the dose rate was controlled by adjusting the distance between the radioactive source and the mice. Specifically, 6 mice each group were placed at distances of 1.5 m (0.021 Gy s^{-1}), 2.5 m (0.008 Gy s^{-1}), and 4 m (0.005 Gy s^{-1}) respectively. 6.5Gy X rays irradiation was performed using RAD SOURCE X-RAY RS2000, 4 mice each group were placed in the bottom of the exposure chamber, the dose rate is 0.009 Gy s^{-1}, and the operating parameters are 160 kV and 10.19 mA. Detailed information for each irradiation is thoroughly described in Supplement Table 1. At −3, 1, 2, 3, 5, 7, 11, 14, 20, 25 and 30 days after irradiation, 10 μl blood were collected from the tail vein for blood cell counts and 40 μl for detecting

dynamic changes of sSelectin-L by ELISA [24]. The body weight and survival rate were also detected at the same time.

2.3. Partial body irradiation (PBI)

Mice were anesthetized with an intraperitoneal injection of 0.4 mg kg^{-1} pentobarbital sodium and then secured onto the plexiglass plate. Lead bricks (22 × 10 × 5 cm) were used as shields to expose and the diaphragm was used as the dividing line to divide the mouse body into upper and lower parts. The mice were divided into three groups: upper body irradiation, lower body irradiation, and total body irradiation, and each group have 6 mice. All groups were irradiated simultaneously at the same dose rate (0.011 Gy s^{-1}) and totally 6.5 Gy. Detailed information is thoroughly described in Supplement Table 1 Blood samples of 10 μl were collected from the tail vein for blood cell counts, and an additional 40 μl for detecting dynamic changes of sSelectin-L by ELISA, at −3, 1, 2, 3, 5, 7, 11, 14, 20, 25 and 30 days after irradiation. The body weight of the mice were also monitored during the same time intervals.

2.4. Amifostine pre-treatment

Mice were pretreated with Amifostine (H20010403, Dalian, China) (150 mg kg^{-1} body weight of the mice) injected intraperitoneally 0.5 h before 6.5 Gy ^{60}Co γ ray irradiation at a dose rate of 0.011 Gy s^{-1}. There were 6 mice each group. Detailed information is thoroughly described in Supplement Table 1. Mouse tail vein blood was collected at −3, 1, 3, 7, 11, and 14 days post-radiation, 10 μl for blood cell counts and 30 μl for sSelectin-L concentration detection by ELISA. Their body weight were also observed.

2.5. LPS and DOX intraperitoneal injection

C57BL/6J mice were injected intraperitoneally with lipopolysaccharide (LPS) (Escherichia colial0111: B4, Sigma Aldrich, Steinheim, 1 mg kg^{-1}, Darmstadt, Germany), doxorubicin (DOX) (APExBIO.A3966, 10 mg kg^{-1}, Houston, USA), or normal saline as placebo. Each cohort has 5 mice. Detailed information is thoroughly described in Supplement Table 1. Mouse tail vein blood was collected at −3, 1, 3, and 7 days post-injection, 10 μl for blood cell counts and 30 μl for sSelectin-L concentration detect by ELISA. Their body weight were also observed.

2.6. Bone marrow transplantation (BMT)

Whole bone marrow cells from femurs of the donor mice were flushed into D-HANK's Balanced Salt Solution and filtered using a 40 μm cell strainer (151040, BIOLOGIX, Jinan, China). We selected the number of transplanted cells according to the literature [25,26]. Bone marrow cells (8 × 10^6 cells) in 200 μl D-HANK's containing 10 % FBS were intravenously injected into recipient mice at 1 day after irradiation. There were 4 groups and each group has 4 mice. Detailed information is thoroughly described in Supplement Table 1. Blood samples were collected from the mouse tail vein 3 days before irradiation and 1, 2, 3, 5, 7, 11, 14 and 20 days after irradiation to detect the dynamic of sSelectin-L by ELISA. Survival rate was also observed for 30 days post irradiation.

2.7. Patients serum

Human serum was collected from 18 nasopharyngeal cancer (NPC) patients who underwent radiation therapy without concurrent chemotherapy. The nasopharynx and its adjacent areas, including the nasopharyngeal fossa, selected paranasal sinuses, pharyngeal gaps, and skull base, received irradiation using a collimated photon beam emitted by a 6 MV linear accelerator at doses of 70 Gy or 63 Gy. Venous blood samples were collected before radiation therapy (3–7 days) and after radiation therapy (1–4 days), and serum was then separated. All the patients signed informed consent at the Nanfang Hospital, China. All specimens were obtained from the Clinical Research Project (LC 2016 PY015) of Southern Medical University funded by the High-level University Construction Fund of Guangdong Province, and are available at https://www.clinicaltrials.gov. These samples were also utilized in a previously published study conducted by Huang Jinfeng [24].

2.8. Tissue sample collection

Another group of mice (4 mice per group) was subjected to 0 Gy, 2 Gy, and 6.5 Gy irradiation (0.011 Gy s^{-1}). On the 3rd day after irradiation, they were anesthetized with pentobarbital sodium at a dosage of 50 mg kg^{-1} via intraperitoneal injection. Blood samples of 0.5 ml were collected from the eye socket for white blood cell isolation. The mice were then euthanized, and organs including the heart, liver, spleen, lungs, kidneys, thymus, small intestine, colon, testes, brain, and bone marrow were collected and stored at −80 °C for analysis.

2.9. Protein extraction and western blot analysis

Organs were collected and lysed with Radio Immunoprecipitation Assay (RIPA) Lysis Buffer. The concentration of protein was detected by bicinchoninic acid (BCA) assay (P0010, Beyotime Biotechnology, Shanghai, China). Samples were denatured in loading buffer (P0015L, Beyotime Biotechnology, Shanghai, China). Total protein extracts were separated by Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE) and transferred to nitrocellulose (NC) membranes. The membranes were blocked in 5 %
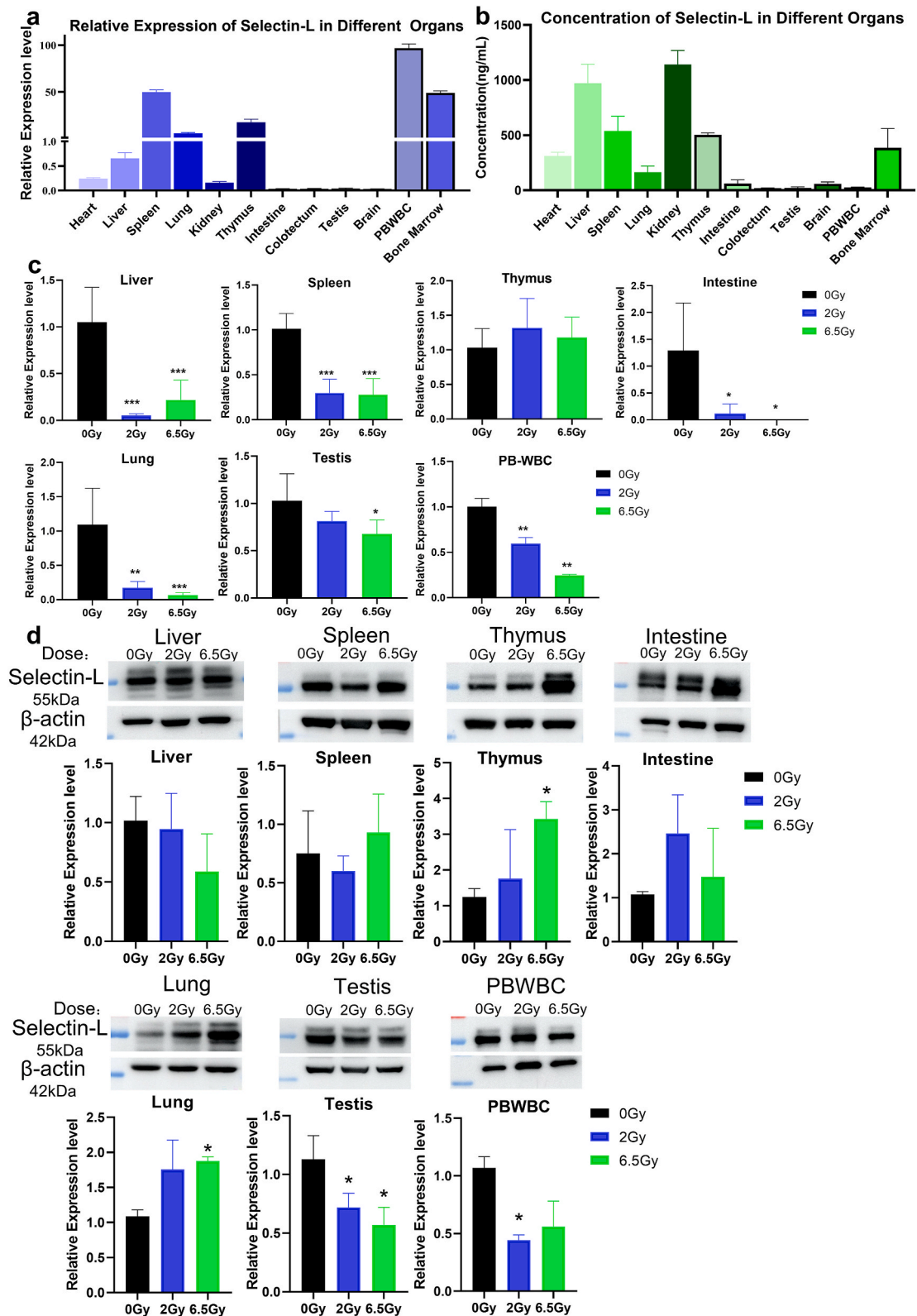
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Fig. 1. Expression of Selectin-L in mouse organs before and after radiation. **a.** Relative expression of selectin-L mRNA in normal mouse organs. **b.** Concentration of Selectin-L in normal mouse organs. **c.** Relative expression of selectin-L mRNA in radiation sensitive organs of mice at 3 days after 2 Gy, 6.5 Gy radiated. (2 Gy Blue, 6.5 Gy Green) **d.** Level of selectin-L protein in radiation sensitive organs of mice at 3 days after 2 Gy, 6.5 Gy radiated. The density of Western blots was measured by gray value analysis, and all band densities were normalized to beta actin. All data is shown as mean ± SD, n = 4, *P < 0.05, **P < 0.001, ***P < 0.0001.

skimmed milk and probed overnight at 4° Celsius with the primary antibodies (anti-mouse L-selectin monoclonal antibody, Santa Cruz Biotech, sc-13505, 1:1000, California, USA; β-Actin Monoclonal Antibody, 66009-1-Ig, proteintech, Chicago, USA), then probed with secondary antibody (Anti-mouse IgG (H + L) Antibody, 5220-0341; Anti-rabbit IgG (H + L) Antibody, 5220-0337, KPL, Maryland, USA). Membranes were washed in Tris-Buffered SalineTween-20 (TBST) and protein bands were visualized using the ImageQuant 800 imager (Amersham ImageQuant 800, GE Healthcare, USA). To enable quantitative analysis of the Western blot results, we employed Gray Value analysis for normalizing the beta-actin blots.

2.10. Total RNA extraction and quantitative real-time PCR analysis

Total RNA was extracted using TRIzol (T9424, Sigma, USA). The cDNA was synthesized with the PrimeScrip RT kit (RR047A, Takara, Japan). Real-time quantitative PCR was performed on the BioRad CFX96 using the iTaq Universal SYBR Green SuperMix (172–5124, BioRad, USA). Primers are listed as follows:Selectin-L (sense 5′-CATGAACTGGGAAAATGCT-3′, antisense 5′-GGCTTTTGGGCAATGTAT-3′); β-Actin (sense 5′-AAGATCAAGATCATTGCTCCTCC-3′, antisense 5′- GACTCATCGTACT CCTGCTTGC -3′). We selected beta-actin as the endogenous reference. Normalization was achieved through the calculation of the mean value of ΔCq (Cq (Selectin-L) minus Cq (β-actin)) across various organs.

2.11. Enzyme linked immunosorbent assay (ELISA)

The quantified detection of serum sSelectin-L concentration performed using the mouse SELECTIN-L-EK0504 kit (mouse Selectin-L: EK0504, Boster, Wuhan, China) for mice, and SELECTIN-L-EK0503 kit (human Selectin-L: EK0503, Boster, Wuhan, China) for humans according to the instructions. Serum was diluted for 1:100–1:400 before detection. The absorbance was measured at 450 nm wavelength using a microplate reader (TECA, SUNRISE, Switzerland), and the concentration was calculated using the cvxpt 32 software.

2.12. Statistical analysis

The animal numbers were determined using the resource equation (E = N–K═K(n-1)) and were cross-referenced with relevant research articles [26]. Statistical analysis was performed using the Microsoft Excel X or IBM SPSS 22, and graphs were generated using GraphPad prism software with data expressed as mean ± standard deviation. Statistical differences were analyzed by the student t-test. When it comes to more than 2 dependent variables, a mixed ANOVA test was used to exam whether an interaction exists between time and dose, then a post hoc *t*-test was proceeded to compare which of the groups were significantly different from another. A p-value of <0.05 was considered statistically significant.

3. Results

3.1. Selectin-L is a lymphocyte-specific radiosensitive protein

To investigate the response of Selectin-L to ionizing radiation in different tissues, we detected the baseline of Selectin-L mRNA expression in different organs by real-time Q-PCR (Fig. 1a) and protein level by ELISA (Fig. 1b). The result showed that Selectin-L mRNA was mainly expressed in peripheral blood white blood cell (PBWBC), bone marrow, spleen and thymus, and the highest concentrations of Selectin-L protein were found in the liver, kidney and lymphoid tissues. Because kidney has abundant blood flow and a certain number of resident lymphocytes, the organ can also play an important role in immunity [27,28]. Therefore, Selectin-L is mainly present in hematopoietic and lymphatic tissues.

Then, the mice were treated with 2 Gy and 6.5 Gy TBI, and the radiation sensitive organs were collected at 3 days after irradiation to detect the expression of Selectin-L. We found the transcription levels of Selectin-L were decreased significantly in liver, spleen, small intestine, lung, testis and PBWBC after radiation injury, while there was no significant change in thymus. (Fig. 1c–Supplemental Table 2). The Western blot showed that, Selectin-L protein decreased significantly in testis and PBWBC 3 days after irradiation, but increased in lung and thymus, but no significant changes in spleen and small intestine (Fig. 1d, Supplemental Fig. 1). In addition, the proportion of cell counting in different types has changed, which was caused by the massive death of Selectin-L negative cells. (Supplemental Fig. 2).

3.2. Serum sSelectin-L decrease effectively classifies radiated mice

Mice were exposed at 0 Gy (control), 0.5 Gy, 1 Gy, 2 Gy, 4 Gy, 6.5 Gy (sub-lethal) and 10 Gy (lethal) γ-irradiation to analyze the dynamic changes of serum sSelectin-L concentration in mice after irradiation. sSelectin-L concentration and peripheral blood
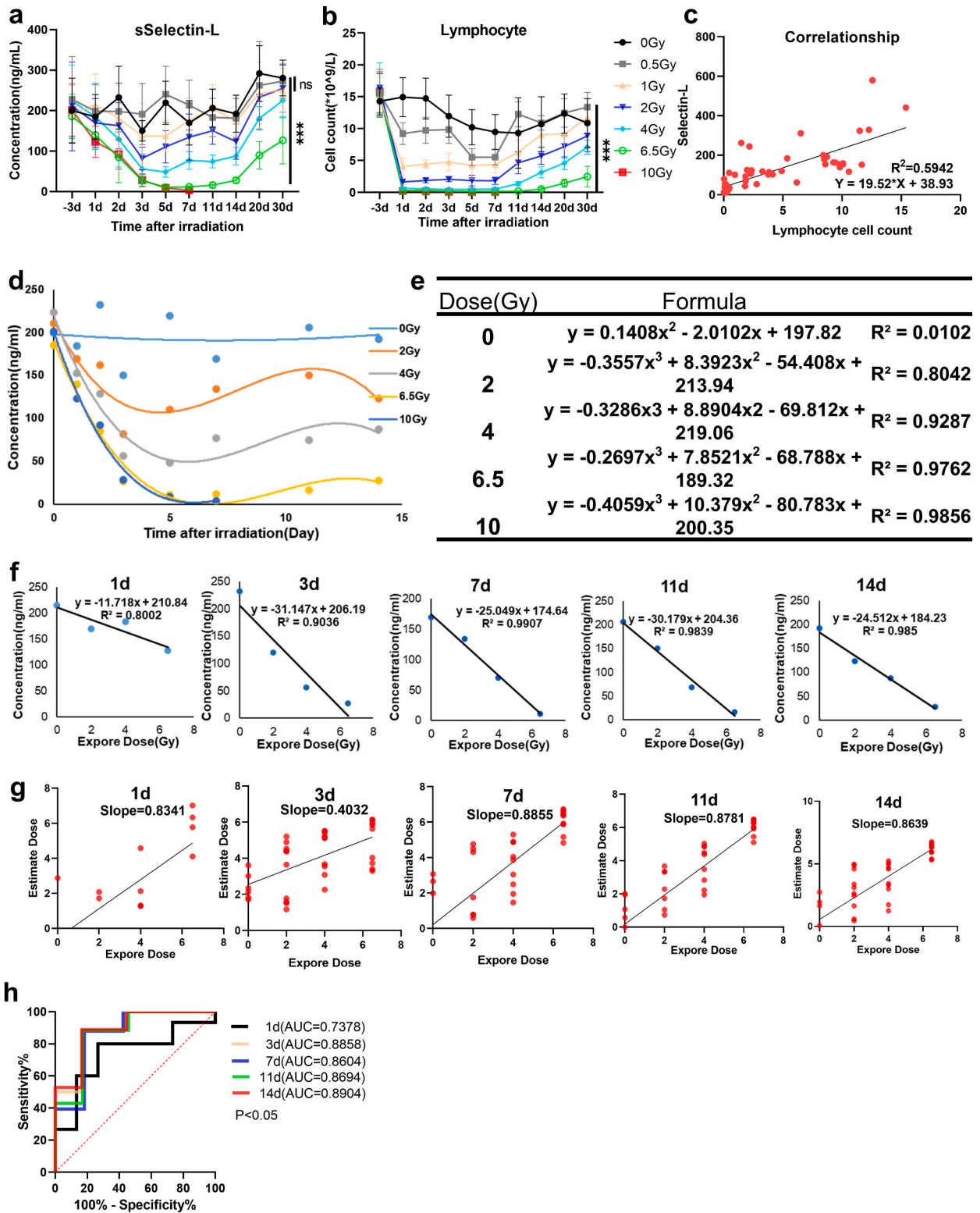
Fig. 2. Analysis of dose effect of serum sSelectin-L and evaluation of exposure dose in radiated mice. **a.** Dynamics of sSelectin-L, **b.** lymphocyte counts, **c.** the correlationship between Selectin-L and lymphocyte in radiated mice. (0 Gy black, 0.5 Gy Gy, 1 Gy orange, 2 Gy blue, 4 Gy cyan, 6.5 Gy green, 10 Gy red) **d.** Equation fitting was performed using the levels of serum sSelectin-L in mice after different doses of irradiation. (0 Gy cyan, 2Gy orange, 4 Gy Gy, 6.5Gy light orange, 10Gy blue) **e.** the formula of sSelectin-L dynamic change fitting curves. **f.** The dose estimation equation was fitted by sSelectin-L concentration and radiation dose at 1 day, 3 days, 7 days, 11 days and 14 days after irradiation. **g.** Verification of the dose estimation equation. **h.** The ROC curve showed that sSelectin-L was an appropriate biomarker to predict exposure dose at 1 day, 3 days, 7 days, 11 days and 14 days after irradiation. All data is shown as mean ± SD, n ≥ 6, *P < 0.05, **P < 0.001, ***P < 0.0001; ns, not significant. (1d black, 3d orange, 7d blue, 11d green, 14d red).

lymphocyte cell counts were detected dynamically within 30 days and linear regression analyze was performed to analysis their correlation. Firstly, we detected the basic physiological indicators after irradiation (Supplemental Figs. 3a–d). It was found that all mice lost weight and died started from 6th day after 10 Gy irradiation, and there was no left at 10th, and there was no death in other dose groups (Supplemental Figs. 3a and b). Then, we compared the dynamic changes of sSelectin-L concentration and lymphocyte cell count in mice exposed to different dose of TBI. As Fig. 2a, sSelectin-L concentration decreased significantly and dose dependently from 1 day after irradiation higher than 1 Gy. sSelectin-L decreased to the minimum at 3rd day after 1 and 2 Gy irradiation and almost completely recovered at the 7th day. sSelectin-L decreased to the minimum at 3rd day after 1–2 Gy irradiation and almost completely recovered at the 7th day. In 4 Gy group, the minimum appeared in 3–5 days after irradiation, then gradually recover but still couldn't recover to the normal level until the 30th day. The concentration of sSelectin-L dropped rapidly after 6.5 Gy and 10 Gy irradiation,
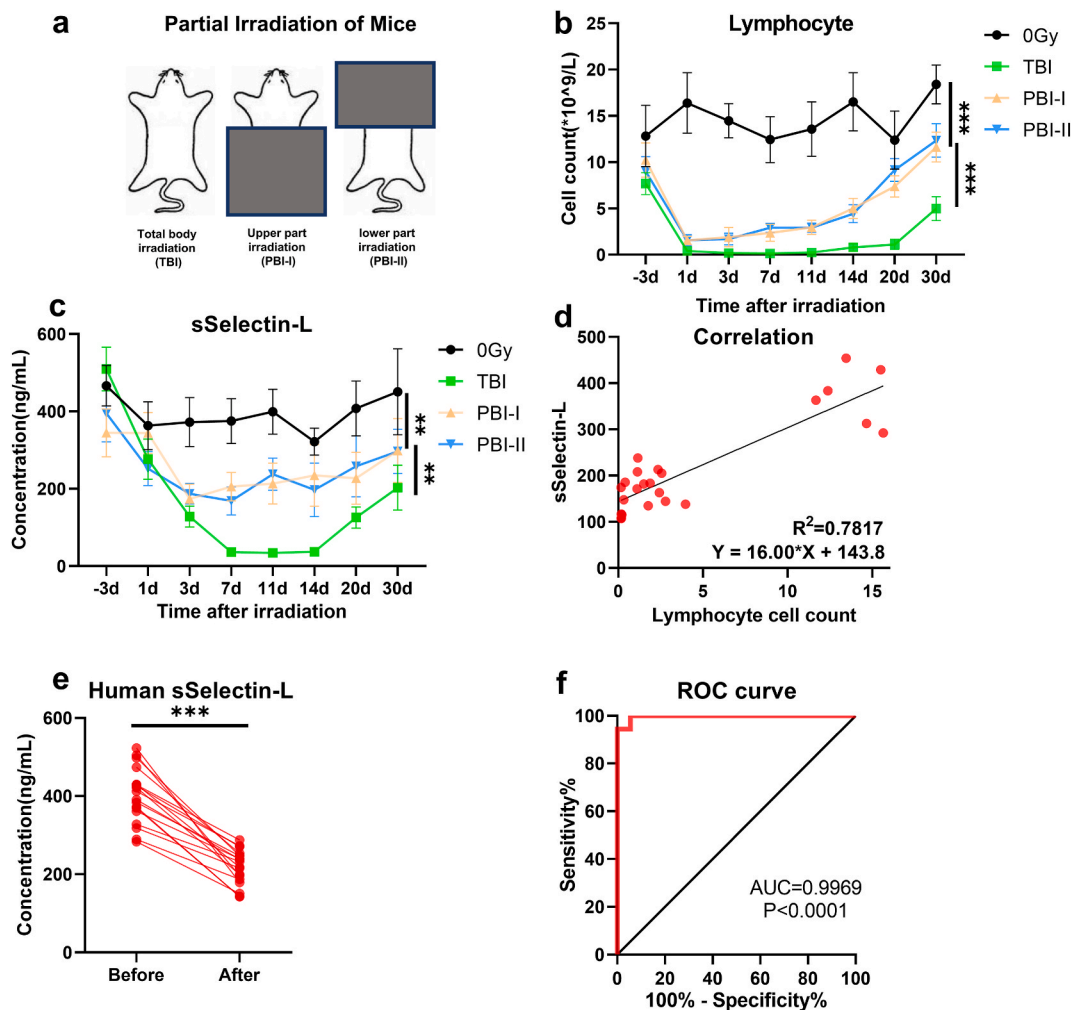

Fig. 3. Changes of sSelectin-L concentration after PBI. **a.** Schematic of PBI of mice. Mice were shielded lower or upper body in PBI-I or PBI-II group. (TBI green, PBI-I orange. PBI-II cyan) **b.** Lymphocyte counts of PBI mice. **c.** sSelectin-L of PBI mice. **d.** Correlation analysis of sSelectin-L and lymphocyte count. **e.** Changes in serum sSelectin-L in patients with NPC after radiotherapy. **f.** The ROC curve shows that sSelectin-L was used as a biomarker to predict radiation exposure in patients with NPC. All data is shown as mean ± SD, n = 6, *P < 0.05, **P < 0.001, ***P < 0.0001; ns, not significant. PBI, partial body irradiation.

remained at the lowest level on the 5th to 7th day. Then, it recovered slowly and reached the half of the normal level at 30 days after irradiation in 6.5 Gy group (Supplemental Table 3). Variation of sSelectin-L response to radiation between male and female was not found (Supplemental Figs. 3e–h). Lymphocyte counts decreased dose dependently to the minimum at 1 day after irradiation more than 0.5 Gy, and maintained until the 7th day, then began to recover (Supplemental Table 4). It almost recovered to normal in 30 days after 2 Gy and lower doses (Fig. 2b), and the decrease of Selectin-L and lymphocyte cell count was positively correlated (Fig. 2c). As shown in Supplemental Fig. 3c, the leukocyte cell counts decreased dose dependently in 1 day after irradiation, and started recover after 7 days, the leukocyte cell counts in 6.5 Gy irradiated group didn't fully recover at 30th day while other groups did. Neutrophil counts decreased significantly after irradiation, but there was no observable dose effect.

We performed curve fitting for the dynamics of sSelectin-L levels in mice serum after 0 Gy, 2 Gy, 4 Gy, 6.5 Gy and 10 Gy irradiation within 14 days. The results were shown in Fig. 2d and e. The changes of sSelectin-L could effectively discriminate mice exposed to 2 Gy, 4 Gy and 6.5 Gy irradiation during 14 days post-irradiation. Subsequently, we fitted the dose-effect curves of sSelectin-L and analyzed the correlation between sSelectin-L concentration and radiation dose at the 1st, 3rd, 7th, 11th and 14th days after irradiation by linear regression. As shown in Fig. 2f, all the correlation coefficient was more than 0.9, except the first day was 0.8002. That means the decreased level of sSelectin-L concentration was closely correlated to the injury degree. Afterwards, we verified the dose curves at different timepoints (Fig. 2g). The horizontal axis is exposure dose and the vertical axis is the estimated dose. The slope factor values were 0.8341, 0.4032, 0.8855, 0.8781 and 0.8639 at the 1st, 3rd, 7th, 11th and 14th day. It informs that sSelectin-L concentration could effectively estimate actual exposure dose in mice within 14 days after irradiation. Finally, the receiver operating characteristic curve (ROC) was fitted to evaluate the sensitivity and specificity of the dose-effect curves. The area under the ROC curve (AUC) was more than 0.7 at the 1st day, and above 0.8 at the other days, $P < 0.05$ (Fig. 2h). It is strongly suggested that decrease of sSelectin-L concentration may be a potential indicator for the classification of irradiation injury.

3.3. sSelectin-L decrease accurately reflects the degree of radiation injury

As cSelectin-L is mainly expressed in leukocytes, its response to ionizing radiation does not have tissue specificity. Therefore, PBI was performed (Fig. 3a) including 6.5 Gy TBI, 6.5 Gy PBI-I (partial irradiation at upper part of the body) and 6.5 Gy PBI-II (partial irradiation at lower part of the body). We compared the body weight and other basic physiological index of mice after TBI and PBI, and the results are shown in Supplemental Fig. 4. Compared with control group, the body weight was significantly decrease in TBI mice during 3–14 days, but not in PBI mice (Supplemental Fig. 4a). Leukocyte counts of both PBI-I and PBI-II mice significantly decreased, but obviously higher than that of TBI mice (Supplemental Fig. 4b). Neutrophil, platelet also showed a similar downtrend (Supplemental Figs. 4c and d).
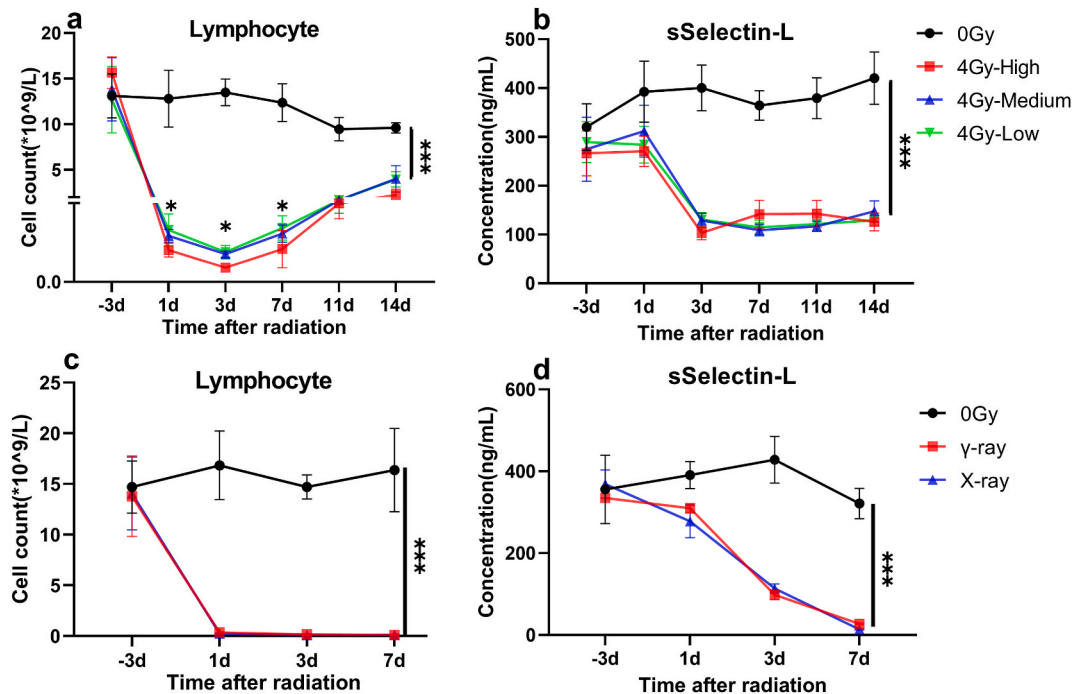

Fig. 4. The response of sSelectin-L to different dose rates and radiation sources. Dynamics of lymphocyte count (a) and sSelectin-L concentration (b) of mice after 4 Gy γ ray radiation at different dose rates. (high red, medium blue, low green). Changes in lymphocyte counts (c) and sSelectin-L concentration (d) of mice after 6.5 Gy γ or X ray irradiation at same dose rate. (γ ray red, X ray blue) All data are shown as mean ± SD, n = 4, *P < 0.05, **P < 0.001, ***P < 0.0001; ns, not significant.

The dynamic analysis of peripheral blood lymphocyte counts showed that lymphocytes decreased significantly after TBI and PBI. There was no disparity in lymphocyte counts between PBI-I and PBI-II mice, which was significantly higher than that of TBI mice (P < 0.05) (Fig. 3b). Similarly, sSelectin-L concentration in PBI mice was significantly higher than TBI mice (P < 0.05) and positively correlated with lymphocyte counts ($R^2 = 0.7817$) (Fig. 3c and d). More importantly, the decreased level of sSelectin-L concentration in PBI mice was approximately half of in TBI animals (Supplemental Table 0.5), that was completely consistent with the exposed area. So the decreased sSelectin-L could be used as an auxiliary or substitute indicator of lymphocyte counts to reflect the degree of radiation injury more accurately.

As shown above, sSelectin-L is a sensitive and robust radiation indicator in a dose range after TBI and PBI in mouse models. We then investigated whether it could be used as an indicator in human. So sSelectin-L concentrations were estimated after head and neck radiotherapy in 18 patients with nasopharyngeal cancer (NPC) (Supplemental Table 6). The sSelectin-L concentration of NPC patients after radiotherapy significantly decreased, from 400.8 ng ml^{-1} down to 218.0 ng ml^{-1} (P < 0.001) (Fig. 3e). We used ROC curves to analyze the capability of sSelectin-L as a diagnostic biomarker for radiotherapy patients. As shown in Fig. 3f, the AUC was 0.9969, P < 0.0001 which means that serum sSelectin-L has the potential to distinguish the patients before and after radiotherapy. However, more experiments are needed to further confirm it.

3.4. The decrease levels of sSelectin-L were only affected by the total exposed dose

Further, we explored the correlation between the decline of sSelectin-L and the radiation dose rate or source. Mice were exposed to 4 Gy γ-radiation at different dose rate, include high dose rate (0.021 Gy s^{-1}), medium dose rate (0.008 Gy s^{-1}) and low dose rate (0.005 Gy s^{-1}). Compared with control groups, both leukocyte and platelet counts declined significantly after irradiation. But there was no significant difference among the high, medium and low dose rate groups (Supplemental Figs. 5a–c). The body weights of the three irradiated groups also have no significant difference. Results of ELISA showed consistent changes in sSelectin-L levels in mice after radiation among the high, medium and low dose rates groups (P > 0.05). It dropped dramatically from 1 day after irradiation, and achieved the lowest value at 3rd days, but no significant difference was noted among three irradiated groups. Compared with the control group, the lymphocyte counts significantly decreased within 2 weeks after exposure, especially in the high dose rate group, it was significantly lower than that in the other two groups (P < 0.05) (Fig. 4a). Hence, the decrease of serum sSelectin-L in mice with radiation injury was closely related to the total dose, without dose rate interference (Fig. 4b).

Then, we analyzed whether the reduction of serum sSelectin-L in mice was a specific response to different radiation sources. We exposed mice to 6.5 Gy TBI with γ or X ray (0.009 Gy s^{-1}). Results showed the consistent trend of leukocyte, weight and platelet decline
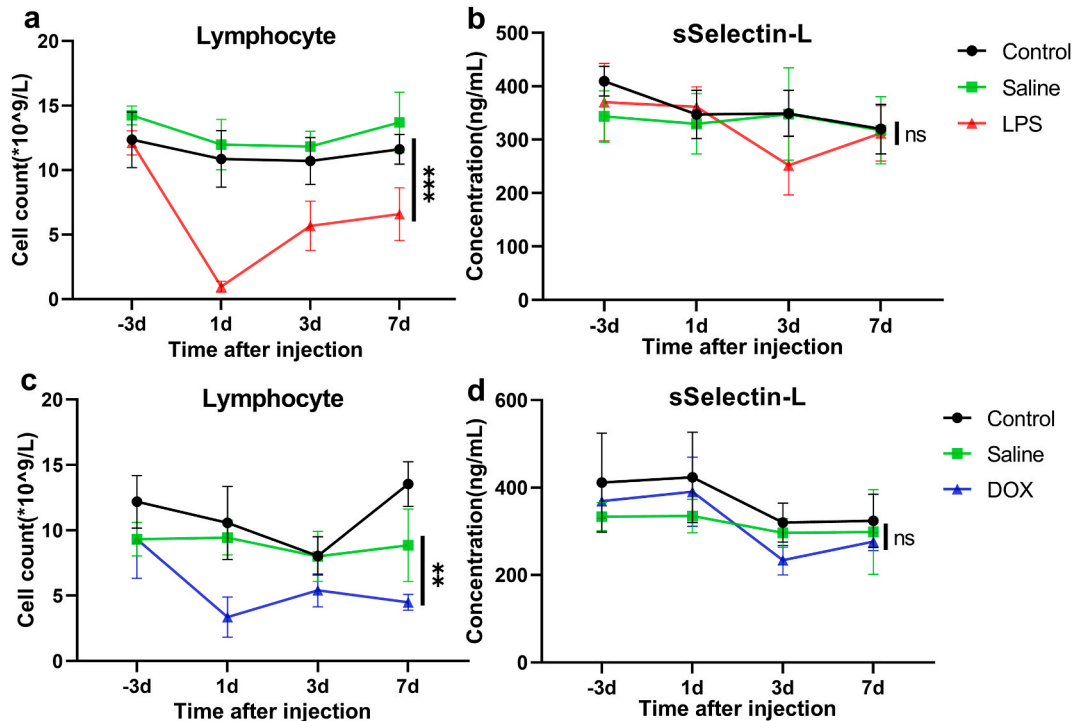
Fig. 5. Changes in sSelectin-L concentration after LPS and DOX injection. **(a)** lymphocyte count and **(b)** sSelectin-L concentration of mice after LPS intraperitoneal injection. **(c)** lymphocyte count and **(d)** sSelectin-L concentration of mice after DOX intraperitoneal injection. (Saline green, LPS red, DOX blue) All data is shown as mean ± SD, n = 5, *P < 0.05, **P < 0.001, ***P < 0.0001; ns, not significant. LPS Lipopolysaccharide, DOX doxorubicin.
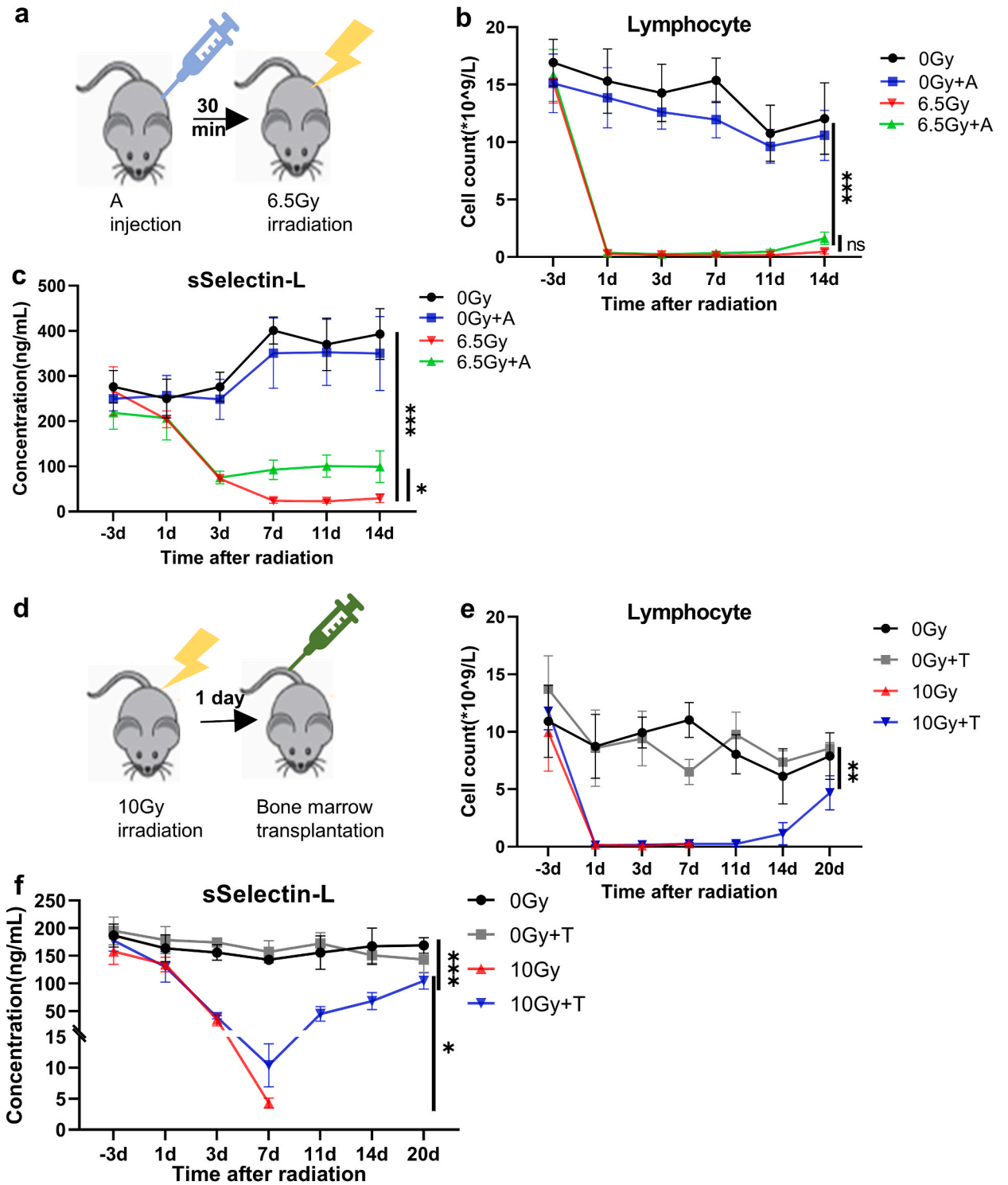
Fig. 6. sSelectin-L levels in irradiated mice with Amifostine pre-treatment or BMT. **a.** Schematic to describe intraperitoneal injection of Amifostine into mice 0.5 h before radiated. **b.** Lymphocyte count and **c.** sSelectin-L concentration was detected for 2 weeks after irradiation. (0 Gy + A blue, 6.5 Gy red, 6.5 Gy + A green) **d.** Schematic to describe transplantation of whole bone marrow into lethally irradiated mice. **e.** Lymphocyte count and **f.** sSelectin-L concentration was detected for 20 days after irradiation. (0Gy + T gray, 10Gy red, 10Gy + T blue) All data are shown as mean ± SD, n = 6, *P < 0.05,**P < 0.001,***P < 0.0001; ns, not significant. A, Amifostine, T, transplantation.

in γ and X ray irradiated mice (Supplemental Figs. 5d–e). Furthermore, we found that sSelectin-L and lymphocyte counts in mice decreased continuously from 1 to 7 days after X ray irradiation, without significant difference with γ ray irradiation ($P > 0.05$) (Fig. 4c and d). These outcomes indicated that sSelectin-L concentration in mice may be a good biomarker for either γ or X ray irradiation.

3.5. Serum sSelectin-L is a specific indicator for radiation injury

Both inflammation and DNA damage are significant biological markers of irradiation injury. LPS was validated for bacillosis model in several articles and DOX in clinical has been verified its efficacy in inhibiting DNA synthesis. So, a bacillosis mouse model and a DNA damage mouse model were constructed by intraperitoneally injecting LPS and DOX respectively. The distinct weight loss, with significant leukocyte decrease and neutrophil decrease after LPS injection suggesting the bacillosis model was constructed successfully (Supplemental Figs. 6a–c) [29,30]. The lymphocyte count decreased sharply on the first day after injection, and then began to recover, but it remained significantly lower than the control group until the 7th day ($P < 0.05$) (Fig. 5a). However, the sSelectin-L just showed a slight decreased on the 3rd day (not significant) ($P > 0.05$) (Fig. 5b), and returned to normal by the 7th day.

Relevant physiological indicators of DOX-induced DNA damage mice are shown in Supplemental Figs. 6d–e. One week after DOX injection, the rat's body weight and white blood cell count significantly decreased ($P < 0.05$), while the neutrophil count showed a slight increase (not statistically significant). After injection, lymphocyte counts significantly decreased compared to the control group on the first day and remained at a lower level over the following 7 days ($P < 0.05$) (Fig. 5c). However, the sSelectin-L in mice did not change significantly ($P > 0.05$) (Fig. 5d). Therefore, the decrease of serum sSelectin-L may specifically reflect the degree of radiation injury without interference from microbial infection or chemical-induced DNA damage.

3.6. Serum sSelectin-L is a molecular indicator for the effectiveness of prevention and treatment of radiation injury

Amifostine is a common radioprotectant, which is known to prolong survival in mice and in humans by reducing radiation-related cytotoxicity. Mice were pre-treated with Amifostine by intraperitoneal injection 30 min before TBI, then, basic physiological indices and sSelectin-L dynamic changes were recorded for 2 weeks. The weight loss began at the 1st day after exposure, and recovered from the 3rd day in 6.5 Gy + A group, while it continued to decrease in 6.5 Gy group ($P < 0.001$). Leukocyte counts decreased precipitously to extremely low levels after exposure in the 6.5 Gy radiation. In 6.5 Gy + A group, leukocyte counts began to recover after 3 days, which was significantly higher than those observed in 6.5 Gy group ($P < 0.001$). The recovery of neutrophil counts in 6.5 Gy + A group was also significantly earlier than that in 6.5 Gy group ($P < 0.05$) (Supplemental Figs. 7a–c). We believe that Amifostine can counteract the decrease of sSelectin-L in the serum and improve the damage induced by TBI. As Fig. 6c showed, the concentration of sSelectin-L in mice continued to decrease to the lowest within 1 week after exposure to 6.5 Gy TBI. Intraperitoneal injection of Amifostine can effectively inhibit this decrease. In the second week after irradiation, the serum concentration of sSelectin-L in mice injected with Amifostine is significantly higher than that in non-injected mice. ($P < 0.05$). However, the lymphocyte counts were not affected by Amifostine (Fig. 6b). These results further indicate that within a certain dose range, the serum concentration of sSelectin-L in mice is more sensitive to radiation damage than lymphocyte count, and can more effectively reflect the degree of radiation damage.

BMT is a recognized clinical treatment for severe radiation injury [31,32]. Our study showed that lethally-irradiated mice experience continued rapid weight loss over the course of one week, until their death. However, administering bone marrow transplants at 1d post-exposure can effectively mitigate weight loss and avert mortality in mice. The leukocyte counts of mice decreased rapidly after exposure to 10 Gy TBI, reached the minimum on the 3rd day, and continued to decline until death. The leukocyte counts of the BMT mice began to recover 11 days after exposure. Neutrophil counts in the 10 Gy + T group began to recover 3 days after irradiation, and were higher than normal values after the 11th day, while they decreased continually in the 10 Gy group (Supplemental Figs. 7d–g). The lymphocyte counts rapidly decreased to the lowest level within a week after lethal irradiation. There was no significant improvement in the lymphocyte count within one week after BMT, ($P > 0.05$) (Fig. 6e), but it began to recover from the 11th day after irradiation, and returned to normal by the 20th day. Since all the mice exposed to 10 Gy died within 7–10 days, we could not continue monitoring their weight, serum Selectin-L concentration, lymphocyte count and other blood cell parameters. The detection of sSelectin-L showed that BMT at 1 day after lethal irradiation could significantly inhibit the decrease of sSelectin-L and promote its recovery, while effectively protecting mice from death. sSelectin-L in the transplanted group were significantly higher than non-transplanted mice at the 7th day after irradiation, and returned to normal at the 20th day (Fig. 6f). These results indicated that sSelectin-L in mice with ARS decreased slower after BMT, and recovered rapidly after the extreme stage, which was earlier than lymphocytes, suggesting that serum sSelectin-L could be used as a prognostic indicator of BMT in patients with severe radiation injury [26,33].

4. Discussion

In the context of large-scale nuclear accidents, early classification diagnosis is of paramount importance in the field of medical rescue. However, the current methods for assessing biological doses are challenging to implement in such emergency environments. Therefore, there is an urgent requirement for a radiation-specific and easily detectable early marker molecule. This molecule is expected to overcome the limitations of existing methods and enable the early classification of injured individuals in the event of large-scale nuclear accidents.

DNA damage and cell apoptosis are important indicators of radiation injury. Proteins such as ATM, DNA-PKCs, 53BP1, and γ-H2AX are involved in radiation-induced DNA damage signals and can serve as markers of exposure. However, the accurate measurement of these proteins often requires specialized instruments. Some radiation-sensitive proteins can be found in serum, like Flt3-ligand

indicating bone marrow aplastic anemia [34], citrulline indicating intestinal injury severity [35]. and C-reactive protein showing dose-dependent upregulation post-irradiation [36,37]. However, the application of these proteins is limited by tissue specificity and potential interference from other factors. On the other hand, serum sSelectin-L, derived from lymphocytes, is a significant marker of lymphocyte activation [38]. Our research demonstrated that sSelectin-L decreased in a dose-dependent manner starting from 1 day after irradiation and correlated positively with lymphocyte counts ($R^2 = 0.5942$). This protein could be used for casualty classification within 14 days of a nuclear incident. The change between Selectin-L mRNA and protein in radiation-sensitive tissues can be attributed to the migration of Selectin-L positive leukocytes to the injured tissues after irradiation [15,39]. Notably, sSelectin-L has extensive homology between humans and mice, with similar concentrations (both $0.94\ \mu g\ ml^{-1}$) [39,40]. Moreover, only $2\ \mu l$ serum is needed for sensitive and easy detection of sSelectin-L. This makes it suitable for developing portable and quick self-examination technologies that can rapidly and accurately triage patients, with a focus on assessing serum sSelectin-L.

At present, early stage dose assessment and classification of radiation-injured individuals still relies on lymphocyte counts, typically used within 0.5–10 Gy and with accuracy 12–48h after exposure [41]. However, the absolute value of lymphocytes in healthy individuals is influenced by factors such as age, gender, and ethnicity, making the baseline unstable. Thus, relying solely on lymphocyte count analysis leads to certain inaccuracies. Furthermore, constructing a dynamic lymphocyte count curve requires collecting blood samples multiple times with 2-h intervals, which increases workload and is not practical for a large number of patients in a mass radiation injury incident [42,43]. Our findings demonstrate that the peripheral blood lymphocyte count in mice significantly decreased after exposure to more than 0.5 Gy TBI. Furthermore, mice exposed to 0.5–10 Gy radiation could be distinguished based on lymphocyte counts from the 1st to 7th day post-irradiation. Similarly, sSelectin-L levels in mice decreased after irradiation, allowing the differentiation of radiated mice from the 1st to 7th day after 1–6.5 Gy radiation. Serum sSelectin-L displayed a similar time range and a limited dose range as a response to radiation compared to lymphocyte counts. Considering the ease of sSelectin-L detection, it is expected to be used as a complementary or alternative technology to lymphocyte count in the early stages of a radiation accident, particularly at the accident site, to enhance the efficiency and accuracy of triage.

During a nuclear accident, individuals at the scene may experience varying levels of injury. Our study examined the correlation between the decrease in sSelectin-L levels and injury severity using PBI and TBI. We observed that both lymphocyte counts and sSelectin-L levels were higher in the PBI group compared to the TBI group at the same exposure dose. Additionally, there was no significant difference in lymphocyte counts and sSelectin-L levels among the two PBI cohorts. These findings indicate a correlation between the decrease in sSelectin-L levels and the severity of injury. Secondly, the distance between an exposed individual and the radioactive source determines the exposed dose rate, which in turn affects the rate of radiation damage [44]. Our results showed the decrease in sSelection-L is consistent with the total radiation dose received, and is not influenced by the dose rate. This suggests that sSelection-L exhibits a stable response to radiation injury. The consistency of sSelection-L dynamics in mice following exposure to the same doses of γ- and X-rays further confirms its stability in response to ionizing radiation.

Inflammation is a common response to radiation, exacerbating the direct effects of ionizing radiation and leading to tissue damage. Patients with systemic inflammation from microbial infections or systemic lupus erythematosus also experience a decrease in peripheral blood lymphocyte count [45,46]. Factors like physical damage or endogenous processes can also cause a decline in lymphocyte count [47,48]. Recently, researchers found the expression level of sSelectin-L was similar between control and DOX administration [49]. In our study, mice were injected with LPS or DOX to investigate if the observed decline in sSelectin-L is specific to radiation. Within one day of LPS or DOX injection, lymphocyte counts significantly decreased compared to the control group, consistent with previous reports [30]. However, there was no significant difference in serum sSelectin-L before and after LPS or DOX injection, indicating that the alteration in sSelectin-L was more specific and accurate in response to radiation injury and not affected by bacillosis or drug-induced DNA damage.

BMT is an important clinical treatment for severe radiation injury [50]. The recovery of lymphocyte counts serves as the primary indicator for the success of transplantation and immune recovery in clinical treatment [50,51]. The migration and homing of hematopoietic stem cells after transplantation are crucial for blood cell regeneration and prognosis. Our study found that BMT effectively alleviated the decline of Selectin-L in mice with fatal radiation injury. The level of Selectin-L in transplanted mice have been significantly higher than that of non-transplanted group since the 7th day after radiation, and returned to normal by the 20th day (Fig. 6). However, the recovery of absolute lymphocyte counts occurred on 14 days after irradiation, later than Selectin-L. These results suggest that the recovery of Selectin-L is closely related to the prognosis of irradiated mice after BMT and can be a sensitive and effective indicator compared to lymphocyte counts.

Certainly, sSelectin-L also has its constraints as an early classification diagnostic indicator for radiation injury. sSelectin-L only demonstrates a dose-dependent decrease in the serum of mice exposed to whole-body irradiation ranging from 2 to 6.5 Gy, indicating that it cannot identify lethal radiation injury or exposures below 2 Gy in the early stage. Therefore, in the classification of injured individuals following accidents, it is crucial to consult additional indicators for accurate and comprehensive assessments. It is essential to acknowledge that our study was conducted solely on mice and patients with nasopharyngeal cancer after undergoing head and neck radiation therapy, which may limit the generalizability of our research findings to a broader population. Furthermore, as the immune system capabilities vary across different age groups (children, young adults, and elderly individuals), though we have established that gender does not impact the reduction of Selectin-L after irradiation, it is crucial to conduct further research to determine if there is consistency in the baseline levels of Selectin-L and its susceptibility to radiation damage in their sera. Additionally, if necessary, it would be prudent to develop Selectin-L -based radiation damage classification models specifically tailored to different populations. Moreover, Selectin-L plays significant roles in the adhesion, activation, and homing of lymphocytes and leukocytes [11]. The concentration of sSelectin-L fluctuates in various immune-related diseases, with elevated levels observed in patients with HIV, systemic sclerosis, or cancer [52–54]. Whereas, individuals diagnosed with Alzheimer's disease (AD) often demonstrate a notably reduced levels

of sSelectin-L[18]. Hence, it is imperative to undertake thorough investigations regarding the influence of various factors, including burns, chemotherapy, and diseases such as HIV, on sSelectin-L [48,52,55]. Consequently, a more extensive study involving a larger population is warranted to confirm and validate our findings.

In summary, our study found that serum Selectin-L levels decreased in mice and patients during early radiation injury stages. This decrease correlated with lymphocyte counts and showed specificity to ionizing radiation. It could serve as a marker for accurate prediction of the extent of radiation injury. The recovery of Selectin-L after bone marrow transplantation could also be an indicator of transplantation success and prognosis.

5. Ethical approval statement

This study was reviewed and approved by the Institutional Animal Care and Use Committee of the Academy of Military Medical Sciences (AMMS) (Beijing. China), with the approval number: IACUC-DWZX-2022-830.

All patients signed informed consent at the Southern Medical University of China. All specimens were obtained from the Clinical Research Project (LC 2016 PY015) of Southern Medical University funded by the High-level University Construction Fund of Guangdong Province, and are available at https://www.clinicaltrials.gov.

Data availability statement

The data that support the findings of this study are available on request from the corresponding author.

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Math formulae

Fig.2c dose fitting curve formula: 0Gy $y = 0.1408x^2 - 2.0102x + 197.82$, 2Gy $y = -0.3557x^3 + 8.3923x^2 - 54.408x + 213.94$, 4Gy $y = -0.3286x^3 + 8.8904x^2 - 69.812x + 219.06$, 6.5Gy $y = -0.2697x^3 + 7.8521x^2 - 68.788x + 189.32$, 10Gy $y = -0.4059x^3 + 10.379x^2 - 80.783x + 200.35$.

Fig.2d dose fitting curve at different time formula: 1d $y = -11.718x + 210.84$, 3d $y = -31.147x + 206.19$, 7d $y = -25.049x + 174.64$, 11d $y = -30.179x + 204.36$, 14d $y = -24.512x + 184.23$.

CRediT authorship contribution statement

Siyuan Li: Writing – review & editing, Writing – original draft, Visualization, Validation, Resources, Methodology, Investigation, Formal analysis, Data curation. **Wencheng zhang:** Writing – review & editing, Writing – original draft, Visualization, Validation, Resources, Methodology, Investigation, Formal analysis, Data curation. **Hong zhang:** Writing – review & editing, Writing – original draft, Visualization, Validation, Resources, Methodology, Investigation, Formal analysis, Data curation. **Ying Fan:** Validation, Investigation, Data curation. **Meng Jia:** Supervision, Project administration, Investigation, Data curation. **Zhenhua Qi:** Visualization, Validation, Investigation, Formal analysis, Data curation. **Liping Shen:** Validation, Investigation, Data curation. **Shuya He:** Writing – original draft, Resources, Project administration, Data curation, Conceptualization. **Zhidong Wang:** Writing – review & editing, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Qi Wang:** Writing – review & editing, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Yaqiong Li:** Writing – review & editing, Writing – original draft, Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e30527.

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