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Original Article

Low dose radiation attenuates inflammation and promotes wound healing in a mouse burn model

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ABSTRACT

Background: Burn injuries are devastating traumas that functionally affect a variety of organ systems. As intensive inflammatory responses induced by burns can lead to multiple organ failures and impaired skin regeneration increases risk of infectious complex, multimodal therapeutic approaches are needed.
Objectives: To investigate the role of low dose radiation (LDR) treatment for regulation of excessive inflammation and wound healing after burn injury.
Methods: Mouse burn model was established by generating third-degree burn injury in dorsal skin and local LDR less than 100 mGy was delivered to the mice. After 3 or 12 days after burn injury, systemic inflammation in liver, lung, spleen, and kidney and skin wound healing were assessed. For investigation of molecular mechanisms, HaCaT keratinocytes were administrated with serum from mice with burn injury and alteration of viability and cornification biomarkers are assessed.
Results: In a mouse burn model, expression of proinflammatory cytokines, interleukin (IL)-1β, IL-6, and tumor necrosis factor-α, were downregulated by LDR in major organs and wound healing capacity was increased by LDR. In skin tissue, we observed the alleviation of reactive oxygen species generation and increased antioxidant gene expression by LDR. In addition, we found that treatment of serum from mice with burn injury and LDR increased proliferation and cornification in HaCaT cells through activation of focal adhesion kinase signaling pathway.
Conclusion: LDR could reduce proinflammatory signaling pathway and increase skin wound healing after burn injury. Therefore, the present study suggested LDR as a novel treatment for burn injury patients.

1. Introduction

Burn injuries are severe, traumatic, and physically devastating, often leading to a high risk of morbidity and mortality. According to the World Health Organization, about 180,000 deaths are caused by burns every year. The mortality rate of burn jury patients drastically increases according to the burned area and the rate overcomes 10% when burned area is wider than 30% of total body surface area [1]. Burns are not a single pathophysiological process, but an event affecting numerous organ systems structurally and functionally [2]. After burn injury, destruction of skin tissues results in malfunction of microcirculation, generation of oxidative stresses, and release of proinflammatory cytokines in the injured site [3]. Due to the lack of research about precise therapeutic targets, unimodal therapeutic approaches did not show successful outcomes in controlling burn injury. One of the major systemic damage response after burn injuries is caused by proinflammatory cytokines released by inflammatory and vascular endothelial cells [4].It was also reported that 45.3% of burn patients undergo multiple-organ dysfunction syndrome (MODS), and it is the leading cause of death in patients with severe burn injuries [5].

Abbreviations: CT, computed tomography; FAK, focal adhesion kinase; FABP4, fatty acid-binding protein 4; H&E, hematoxylin & eosin; HMOX1, heme oxygenase 1; HDR, high dose radiation; IL, interleukin; LDR, low dose radiation; MODS, multi-organ dysfunctions syndrome; ROS, reactive oxygen species; TNF-α, tumor necrosis factor α.

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Therefore, it is important to control excessive inflammatory responses after burn injuries to prevent MODS development and decrease the mortality rate.

Low dose radiation (LDR), which is typically defined as 1–100 mGy of ionizing radiation, is used for diagnostic purposes such as computed tomography (CT) ranging from 2.1 mGy for a head CT to 31 mGy for a multiphase abdomen and pelvis CT. In the radiation hormesis model, it was suggested that LDR is safe and beneficial to human health, opposing high dose radiation (HDR) [6].A previous study suggested that LDR induced miR-30a and miR-30b to reduce the aggressiveness of lung cancer acquired after radiotherapy [7]. The adaptive response is a mechanism wherein the LDR activates various cell defense signals including DNA repair and antioxidant production [8]. Another studies revealed that LDR elicited a decrease in proinflammatory markers in animal models [9]. Besides, the beneficial effects of LDR in wound healing was also suggested in the previous study describing the LDR induced keratinocyte differentiation and promoted skin regeneration [10,11]. A recent study suggested LDR promoted even fracture healing by induction of vasculogenesis signaling in wounded area [12]. However, to our knowledge, the effect of LDR on burn wounds has not yet been investigated.

The epidermis of the skin is the outermost layer, which is a stratified epithelium, and acts as the first protective barrier against infections and trauma. After a skin epidermis is damaged, wound repair follows a three-step process [13].The first stage, inflammation occurs immediately after skin damage. A blood clot is formed and inflammatory cells are recruited to prevent blood loss and remove dead tissues. The regenerative phase is the second stage during which most cells from the first stage migrate

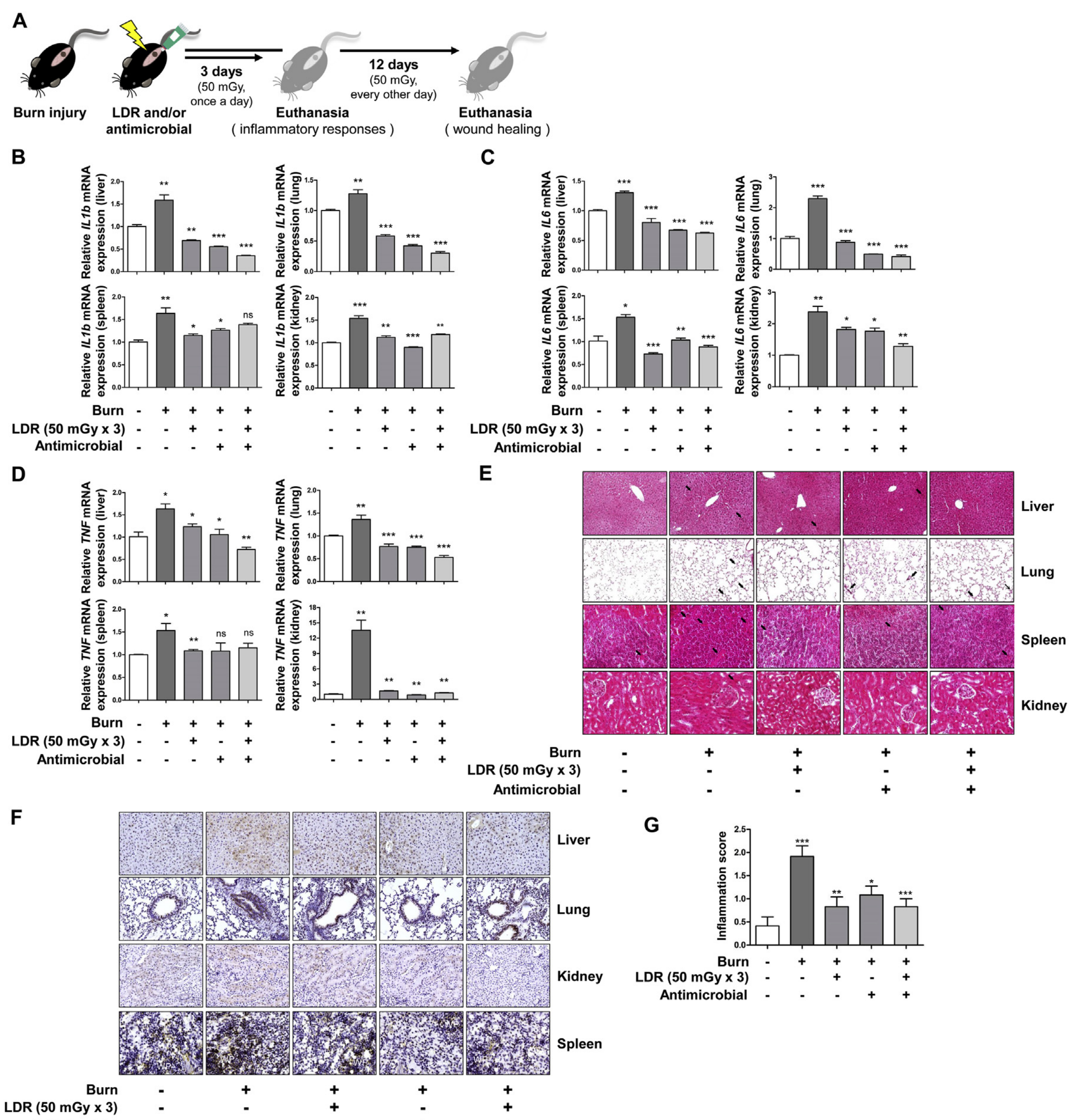

Fig. 1. LDR alleviates hyperinflammation after burn injury. (A) The experimental schedule for burn, LDR, and antimicrobial treatment to burn mouse model. (B–D) Expression of proinflammatory cytokines (IL-1β, IL-6, and TNF-α) in the liver, lung, spleen, and kidney of burn mouse model was assessed by real time qRT-PCR. (E) H&E staining results showed that LDR or antimicrobial treatment alleviated burn-induced inflammation. Arrows indicate inflammatory infiltrates (magnification: X200). (F) IHC analysis for CD3 showed infiltration of $CD3^+$ T cell in the organs (for liver, lung, kidney, magnification: X200, for spleen, magnification: X400). WP; white pulp. (G) Inflammation scores were evaluated based on the inflammatory infiltration observed in histological analysis (0–5). $^*p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, ns, not significant. The burn-only group was compared to control, and burn + LDR and/or antimicrobial groups were compared to the burn-only group.

away from the wound and new blood vessels form. The last stage of wound healing is remodeling, which lasts for a year or longer. Epidermis, dermis, and the extracellular matrix are remodeled. A wide variety of signaling molecules are involved in the wound repair. The epidermal growth factor is released by platelets, macrophages, and fibroblasts, and it stimulates re-epithelialization of the wound [14]. In the context of treating burn-injured patients, enhancing skin regeneration is also emphasized to prevent infection and occurrence of complications.

This study was conducted to investigate the potential of LDR treatment in alleviation of inflammation and wound healing after burn. We established burn mouse model and assessed the role of LDR as an adjuvant treatment for burn injury and its underlying molecular mechanisms. Our findings provide a possible explanation on how burn wound repair is promoted in response to LDR.

2. Materials and methods

2.1. Animal care protocol and establishment of burn mouse model

Seven-week-old female C57BL/6 mice (Orient Bio Inc.,Seongnam, Republic of Korea) were used for *in vivo* experiments. The protocols used were approved by the Institutional Animal Care and Use Committee of Pusan National University (Busan, Republic ofKorea), and experiments were performed in accordance with provisions of the NIH Guide for the Care and Use of Laboratory Animals. The mice were maintained in animal care facilities in a temperature-regulated room (23 ± 1 °C) with a 12 h light/dark cycle. All animals were fed water and standard mouse chow ad libitum.

For burn injury, mice (n = 5) were anesthetized and the dorsal hairs were clipped. The full thickness burn wound was generated

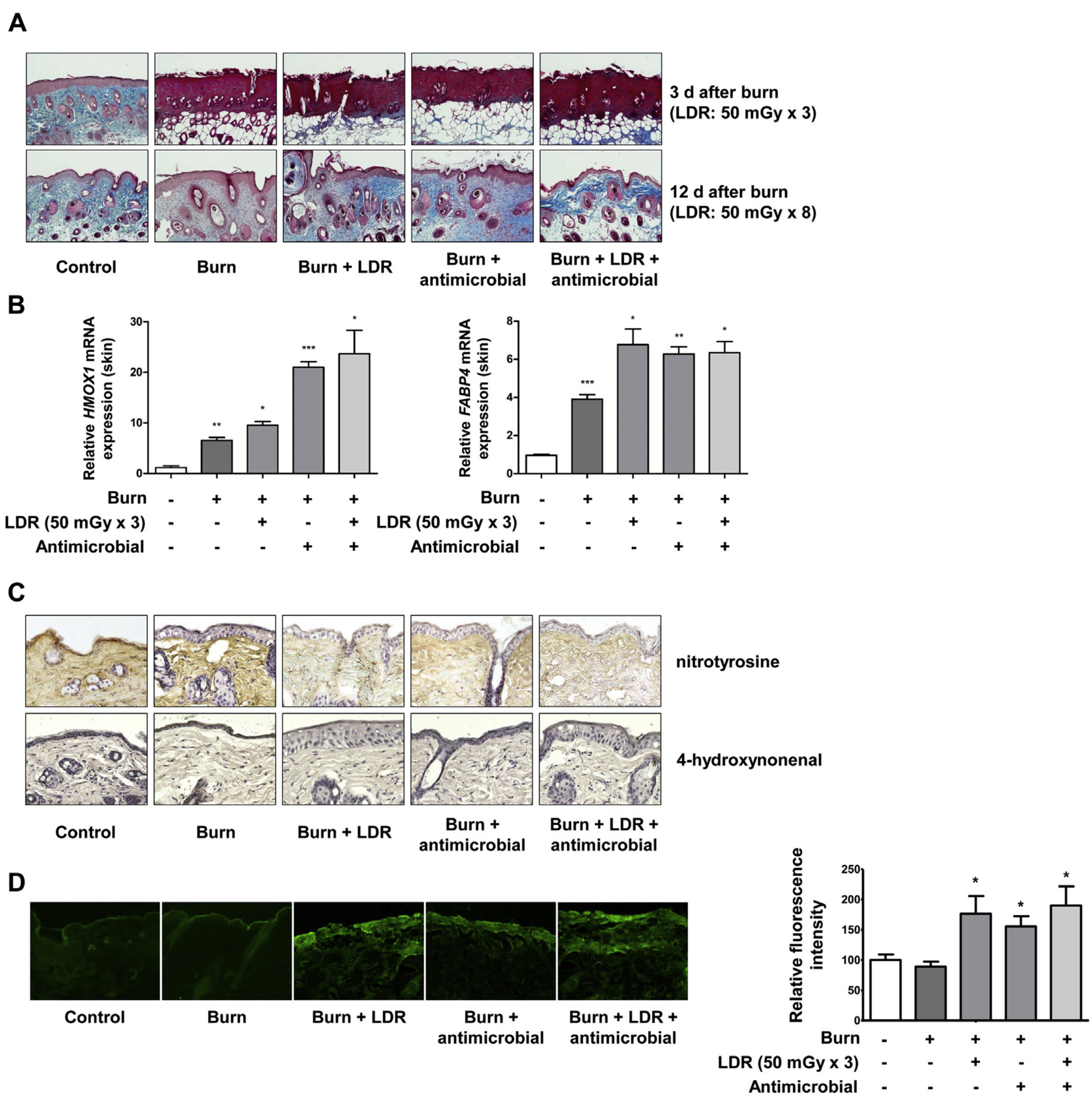

Fig. 2. LDR accelerates skin wound healing after burn injury. (A) The wound healing capacity of LDR was confirmed via Masson's trichrome staining (blue: collagen, red: cytoplasm, brown to black: cell nuclei). (B) HMOX1 (antioxidant protein) and FABP4 (an adipogenesis marker) expression in the skin of burn-treated mice was verified by real time qRT-PCR (time point: 3 d after burn). (C) IHC results of the two ROS markers, nitrotyrosine and 4-hydroxynoneal, showed decreased ROS levels by LDR in the skin of burn mouse model. (D) The effect of LDR treatment on the alteration of actin distribution was measured by phalloidin staining. *p < 0.05, **p < 0.01, ***p < 0.001. The burn-only group was compared to control, and burn + LDR and/or antimicrobial groups were compared to the burn-only group.

by 9 s of contact with a 10-mm-diameter brass rod heated to 95 °C. Mice were then resuscitated intraperitoneally with 1 mL of sterile isotonic saline to prevent shock. Mice subjected to LDR were exposed to X-rays employing an X-ray generator M-150WE at a dose rate of 0.025 Gy/min with 0.4 mm of Cu filter (Softex Co., Ltd., Tokyo, Japan). As an antimicrobial, 0.05 ml of Bactroban ointment (2% mupirocin in a polyethylene glycol base) was topically applied to the burn wound. Mupicrocin inhibits isoleucyl-transfer RNA and protein synthesis of bacteria and showed therapeutic effects after burn injury in previous study [15,16].

2.2. Histological analysis

At the end of the treatment period, animals were euthanized and liver, lung, spleen, kidney, or skin samples were harvested. The samples were then fixed with formalin, dehydrated, and embedded in paraffin. Next, sections were cut to 4 μm and utilized for H&E, Masson's trichrome staining, or immunohistochemistry (IHC) following standard procedures. Inflammation scores were obtained as previously described [17]. Stained sections were observed under an Axio Lab.A1 microscope (Zeiss, Germany).

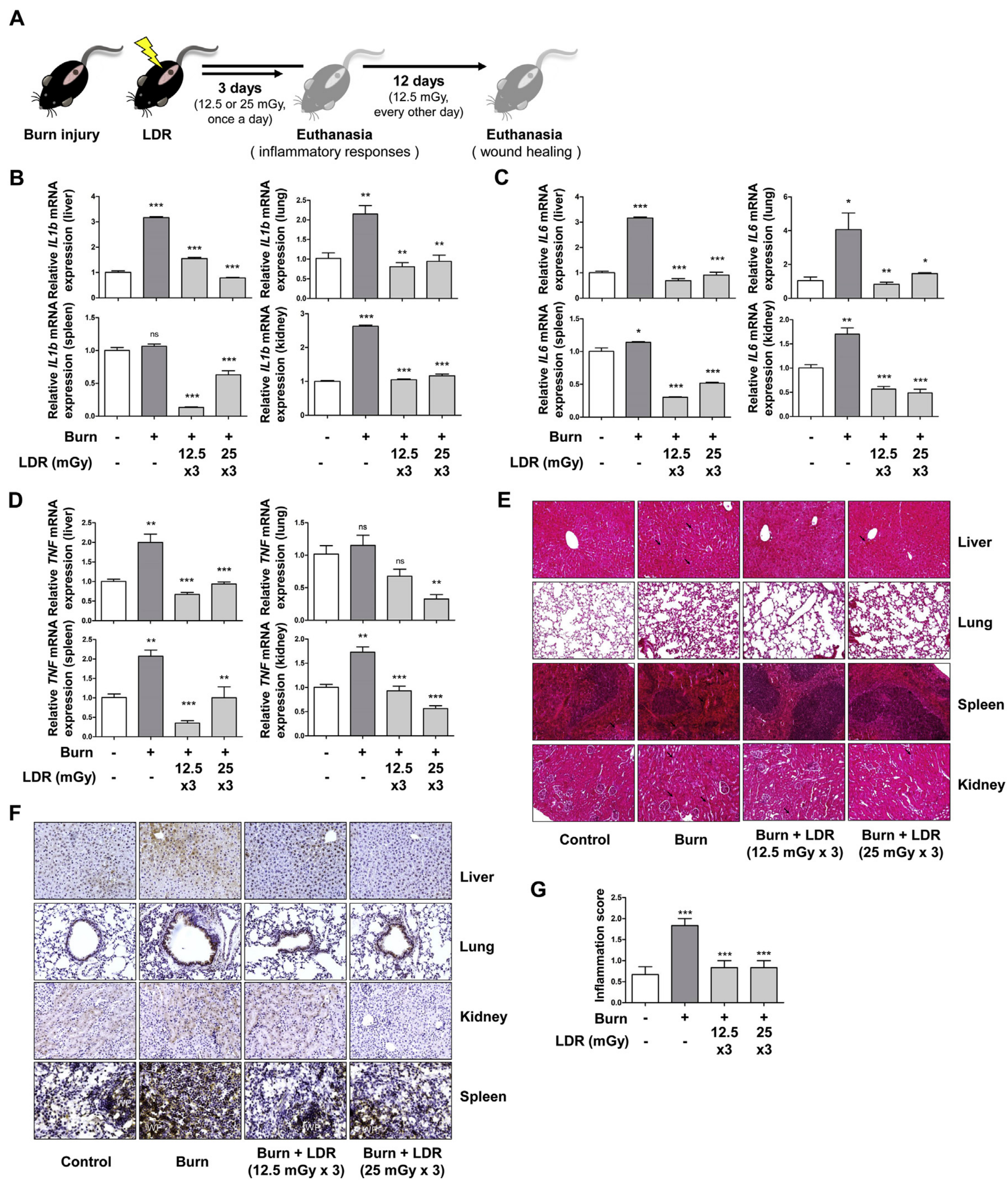

Fig. 3. Accumulative LDR less than 100 mGy alleviates hyperinflammation after burn injury.(A) The experimental schedule for burn and LDR (a total dose no more than 100 mGy) treatments to burn mouse model. (B–D) The mRNA expression of IL-1β, IL-6, and TNF-α was downregulated by LDR treatment. (E) H&E staining results indicated decreased inflammation in major organs of LDR-treated mice (magnification: X200). Arrows indicate inflammatory infiltrates. (F) IHC analysis for CD3 showed infiltration of $CD3^+$ T cell in the organs (for liver, lung, kidney, magnification: X200, for spleen, magnification: X400). WP; white pulp. (G) Inflammation scores were evaluated based on the inflammatory infiltration observed in histological analysis (0–5). *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ns, not significant. The burn-only group was compared to control, and burn + LDR groups were compared to the burn-only group.

2.3. Cell culture

The culture methods of HaCaT cells were described in previous study [18]. In detail, HaCaT cells were purchased from Korean Cell Line Bank (Seoul, Republic of Korea) and cultured in DMEM medium containing 10% of fetal bovine serum and antibiotics (all from Thermo Fisher Scientific, Waltham, MA) at 37 °C and 5% CO_2 with humidification. HaCaT cells were treated with sera from mice with burn injury and/or LDR for at least 24 h and utilized for further experiments.

2.4. Statistical analysis

All numerical data were presented as the means ± standard error of the mean from at least three independent experiments. For quantifications, data were analyzed using *t*-tests or ANOVA. The Prism 5 software (GraphPad Software, San Diego,CA, USA) was used to perform all statistical analyses. A p-value< 0.05 was considered statistically significant.

Detailed information about the materials and methods in this study is described in Supplementary materials and methods.

3. Results

3.1. LDR alleviates hyperinflammation after burn injury

To investigate the potential protective effect of LDR in burn injuries, we established a mouse burn model by generating a third-degree burn and observed inflammatory response and wound healing (Fig. 1A) [19]. The mice were applied with LDR (50 mGy) or an antimicrobial for 3 days at 24 h intervals to the wounds after burn injury. As activation of inflammatory signaling is an initial step of wound healing, we assessed inflammation 3 days after burn injury. Based on the previous study, we examined the expression level of IL-1β, IL-6, and TNF-α, which crucially involved in inflammation-induced organ failure, in the burn-treated C57BL/6 mice [20]. The results showed that the cytokines were upregulated upon burn treatment in major organs, including the liver, lung, spleen, and kidney (Fig. 1B-1D). However, mice treated with LDR, antimicrobials, or both with burn exhibited decreased cytokine expressions in the four organs. The results also suggested that LDR had effects on regulating hyperinflammatory response after burn injury and they were comparable to those of antimicrobials application. In addition, regulation of these cytokines by LDR suggested the significant roles of LDR in prevention of organ failure after burn injury. We next investigated histological differences between experimental groups. According to hematoxylin and eosin (H&E) staining results, inflammatory infiltrates and pulmonary interstitial edema were reduced by LDR and/or antimicrobial treatment (Fig. 1E). For precise understanding of the activation of inflammatory response in the organs, we performed IHC to detect CD3. As shown in Fig. 1F, we observed that burn-induced increase of $CD3^+$ T cell infiltration was alleviated by antimicrobial and/or LDR. In the case of spleen, we found the increase of $CD3^+$ T cells in red pulp, not in white pulp (WP). In addition, inflammation score was assessed from 0 to 5 following the previous study, and a significant inflammatory regulation by antimicrobial and/or LDR was also confirmed (Fig. 1G) [17]. Taken together, these findings suggest the role of LDR in reducing hyperinflammation induced by burn injury.

3.2. LDR accelerates skin wound healing after burn injury

To determine if the wound healing process (second step) is promoted by LDR, a Masson's trichrome staining was performed in the skin of the mouse burn model. The formation of collagen layer showed the degree of proliferation in wounded site. As shown in Fig. 2A, until 3 days after the burn treatment, LDR or antimicrobials has little effect on healing of burn-induced skin destruction. Instead, we observed the formation of collagen (stained blue in the figures) increased by antimicrobial and/or LDR treatment at 12 days after burn injury (Fig. 2A, lower). We further determined the expression of heme oxygenase 1 (HMOX1) and fatty acid-binding protein 4 (FABP4) in the skin of the mouse burn model. HMOX1 derived from skin fibroblasts has antioxidant and tissue-protective actions, and FABP4 derived from adipocytes is a marker of adipogenesis, which reportedly helps skin regeneration after injury through fibroblast migration and extracellular matrix production [21–24]. In Fig. 2B, the expression of HMOX1 and FABP4 was increased by burn injury and antimicrobial and/or LDR showed further elevated FABP4 and HMOX1 levels. Previous studies demonstrated that the formation of ROS in burn wounds led to systemic inflammatory response syndrome and LDR could reduce oxidative stress-induced toxicity [25,26]. Therefore, we measured ROS levels through detection of nitrotyrosine (protein oxidation) and 4-hydroxynoneal (lipid peroxidation) via immunohistochemistry. In Fig. 2C, ROS level increased by burn injury, was ameliorated by antimicrobials and/or LDR.The skin wound healing was also validated through assessment of filamentous actin distribution through phalloidin staining to validate formation of fibroblast layer [27]. As shown in Fig. 2D, sole burn treatment did not change the filamentous actin distribution, antimicrobials and/or LDR significantly enhanced actin distribution in injured skin tissue at 3 days after burn injury. Collectively, these results indicate that the LDR promoted post-burn wound healing through regulation of ROS generation and increase actin distribution in burn-injured skin.

3.3. Accumulative LDR less than 100 mGy alleviates hyperinflammation after burn injury

LDR is generally defined as a dose of 100 mGy or less, and the presence of biological effect of LDR less than 100 mGy is still controversial [28].However, some previous studies suggested the effects of LDR is present by accumulation of low dose-rate radiation exposure [21]. Therefore, we next determined whether LDR of a total dose less than 100 mGy elicits similar effects as an LDR of a total dose more than 100 mGy as shown in Figs. 1 and 2. As shown in Fig. 3A, 3 fractions of 25 mGy or 8 fractions of 12.5 mGy LDR was suggested as a condition for short term or long term LDR exposure, respectively. In advance of the effects of LDR on regulation of hyperinflammation after burn injury, we assessed effects of LDR-only on regulation of systemic inflammation. As shown in Figure S1, the evident role of LDR in alleviating inflammation was not present. In mouse burn model, comparable to the results of Fig. 1, both of 3 fractions of 25 mGy and 12.5 mGy LDR reduced the mRNA expression of proinflammatory cytokines (IL-1β, IL-6, and TNF-α) in the liver, lung, spleen, and kidney of the mouse burn model (Fig. 3B–D). Furthermore, the H&E staining and IHC results suggested that burn-induced inflammatory infiltrates decreased upon LDR treatment in major organs (Fig. 3E–G). These findings demonstrated that LDR of a total dose of less than 100 mGy also reduced hyperinflammation caused by the burn wound as shown by single LDR less than 100 mGy.

3.4. LDR-induced alleviation of systemic inflammation enhances wound healing in keratinocytes

To verify the underlying molecular mechanisms of LDR-mediated wound healing in mouse models, we conducted *in vitro* experiments utilizing HaCaT keratinocytes. As a local exposure of LDR alleviated systemic inflammation in mouse model, we hypothesized that pro-inflammatory signaling progressing the burn injury is transferred via blood circulation. To verify this hypothesis, we treated the cells with serum from mice with burn injury and/or LDR at a total dose less than 100 mGy following the previous burn research [29]. The cells were treated with serum for up to 72 h and we assessed the alteration in viability and gene expressions related with wound healing, proliferation, and cornification. In the results of MTT assay, treatment of serum from mice with burn injury reduced proliferation of HaCaT cells and serum from mice with both burn injury and LDR abolished the inhibitory effects on proliferation of HaCaT cells (Fig. 4A). In addition, we assessed alteration of expressions of cornification biomarkers including Involucrin, Filaggrin, Transglutaminase-1, Loricrin, and Kertain 10 through qRT-PCR and Western blot. As shown in Fig. 4B and C, we observed that expressions of cornification biomarkers were slightly or non-significantly increased by treatment of serum from mice with burn and the expression was further increased by treatment of serum from mice with burn and LDR. In consistent with Fig. 4A, expression of p21, which slows cell cycle progression, was reduced by serum from mice with burn and LDR. In consistent with proinflammatory response in mouse burn model (Fig. 1 and 3), HaCaT cells treated with burn serum also showed increased expression of IL-1β, IL-6, and TNF-α, which wasreduced by serum from mice treated with burn injury and LDR treatment (Fig. 4D). As a upstream signaling pathway involved in skin wound healing, another study proposed the important involvement of activation of focal adhesion kinase (FAK) in re-epithelialization and actin cytoskeletal remodeling [30]. We next verified whether LDR activates FAK signaling. Although slight increase of phosphorylation (Y397) of FAK was induced by serum from mice with burn, serum from mice with burn and LDR showed further increase of FAK activation and filamentous actin distribution in HaCaT cells (Fig. 4E and 4 F). Taken together, the effects of LDR in enhancing wound healing in skin tissue was mediated by factors transferred through blood circulation and LDR also accelerated proliferation and cornification through activation of FAK signaling pathway.

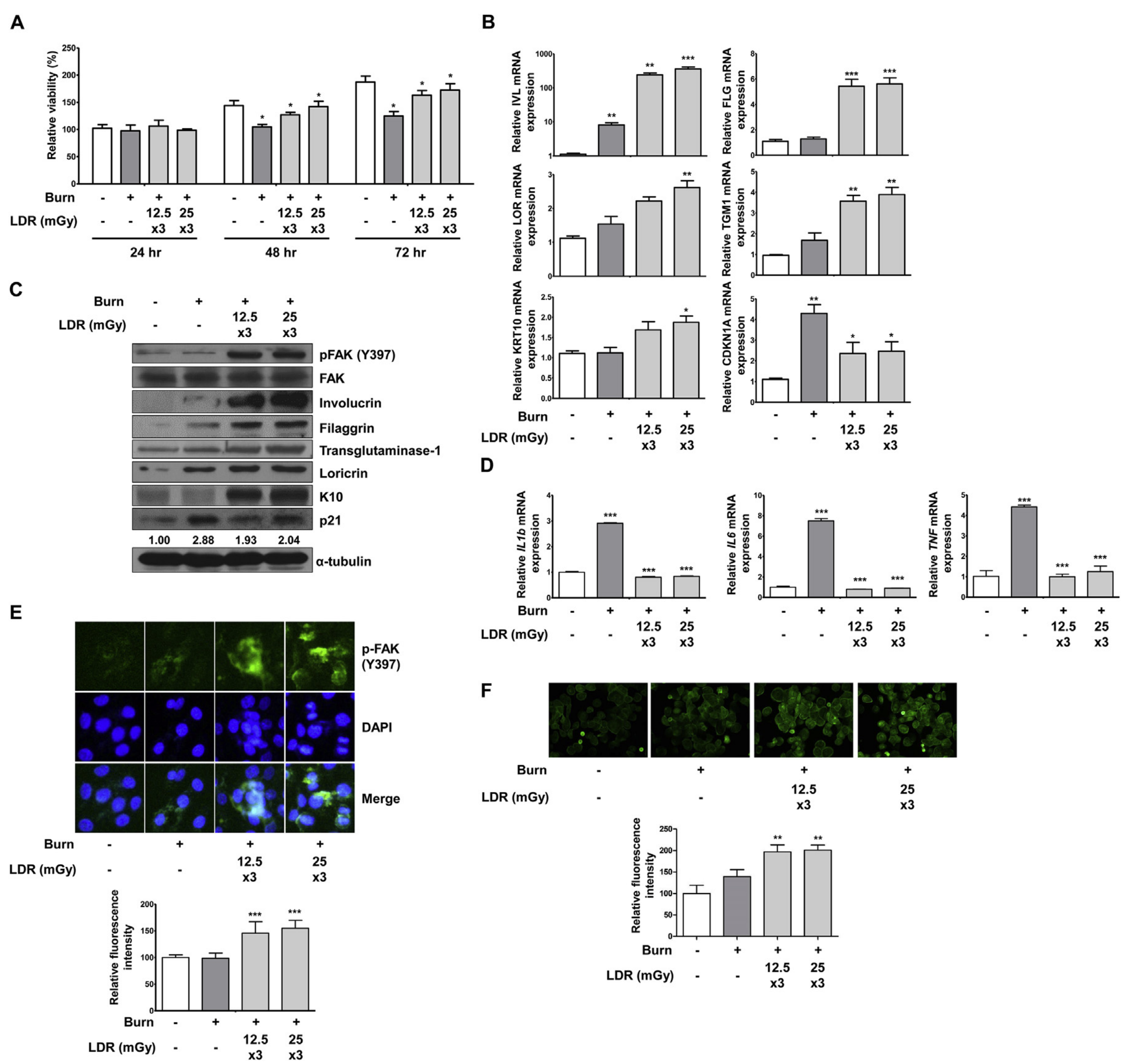

Fig. 4. LDR-induced alleviation of systemic inflammation enhances wound healing in keratinocytes. (A) Alteration of cell proliferation and viability was assessed by MTT assay. HaCaT cells were treated with serum from mice with burn injury and/or LDR for 24, 48, or 72 h. (B) The mRNA expression of IVL, FLG, LOR, TGM1, KRT10, and CDKN1A in HaCaT cells were assessed real time qRT-PCR. (C) The protein expression of cornification biomarkers, FAK activity, and p21 were assessed by western blot analysis (D) The mRNA expression of IL-1β, IL-6, and TNF-α in HaCaT cells was assessed by real time qRT-PCR.(E) Phosphorylation of focal adhesion kinase (FAK) in mouse serum-treated HaCaT keratinocytes was confirmed by immunofluorescence. (F) The distribution of filamentous actin in HaCaT cells was assessed by phalloidin staining. *p < 0.05, **p < 0.01, ***p < 0.001. The cells treated with serum from mice with burn-only group was compared to control, and those with burn + LDR groups were compared to the burn-only group.

3.5. Accumulative LDR less than 100 mGy accelerates skin wound healing after burn injury

To confirm the role of LDR on enhancing wound healing shown in HaCaT keratinocytes, we conducted a Masson's trichrome staining in the skin from the mice with burn and/or LDR. For 12 days, accumulative 100 mGy of LDR was delivered in 12 fractions and we observed that LDR treatment enhanced wound healing and epidermis formation in mouse skin tissue, which was not shown in 3 days after burn injury (Fig. 5A). Moreover, the expression of HMOX1 and FABP4 were elevated upon 3 fractions of 12.5 or 25 mGy LDR to the burn wound (Fig. 5B). In consistent with Fig. 2, we also observed that 3 fractions of 12.5 mGy or 25 mGy of LDR could reduce ROS generation in skin tissue. In addition, the FAK activation was also increased by LDR treatment in mouse skin tissues (Fig. 5D) and increased filamentous actin distribution by 3 fractions of 25 mGy or 12.5 mGy LDR was assessed via phalloidin staining, which was also verified in *in vitro* skin models (Fig. 5E). Collectively, our results indicate that LDR alleviates both proinflammatory signaling and ROS generation and promotes wound healing via FAK signaling pathway, which resulted in enhanced wound healing and skin regeneration in mouse burn model.

4. Discussion

Controlling burn wounds and their complications is one of the global health concerns. This study suggested LDR as a novel regulator of inflammatory imbalance caused by burn injuries. By establishing the burn mouse model, we focused on the histological modifications of major organs, including liver, lung, spleen, kidney, and skin. LDR with a single or total dose less than 100 mGy decreased inflammatory infiltrates and ROS generation through antioxidant gene expression, and augmented skin regeneration through FAK signaling activation (Fig. 6). Overall, this study provides the first evidence that LDR treatment could be an effective method to mitigate burn injuries.

In this study, we utilized HaCaT cells to elucidate the molecular events in skin tissue after burn injury and LDR exposure. In Fig. 4, we showed that treatment of serum from mice with burn and LDR showed further increased proliferation and cornification markers expression. In wound healing process, proliferation leads to filling the destructed sites and restore barrier function of skin, and cornification of outermost keratinocytes leads to establishment of structured dermal barrier [31,32]. Therefore, the results from HaCaT cells showed suggested pre-clinical meaning of LDR to enhance proliferation and cornification in skin as an *in vitro* model for skin tissue. Proliferation and cornification may be mutually exclusive, but these two events are not clearly distinguished and complexly regulated by environmental components. A previous study about the keratinocytes suggested that activation of keratinocytes resulted in induction of both proliferation and differentiation markers [33,34]. Therefore, we interpreted that LDR treatment activated HaCaT cells to enhance both proliferation and differentiation for successful wound healing through factors in serum. As for a serum factor for activating keratinocytes, previous studies suggested the involvement of inflammatory cytokines including IL-1α, IL-6, Monocyte Chemoattractant Protein-1, and C-reaction protein [35,36]. The involvement of cytokines was also consistent with our results about regulation of inflammation by

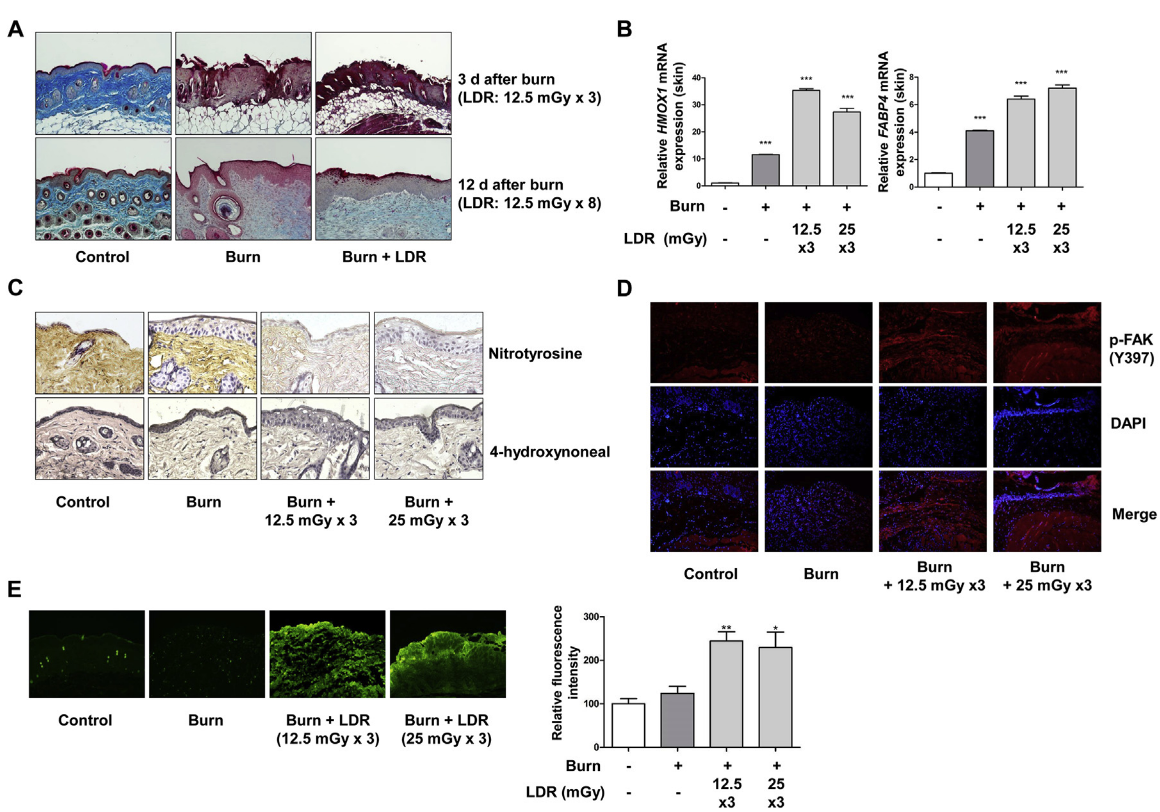

Fig. 5. Accumulative LDR less than 100 mGy accelerates skin wound healing after burn injury. (A) Masson's trichrome staining results suggested that a total 100 mGy of LDR promoted burn wound healing 12 d postburn (blue: collagen, red: cytoplasm, brown to black: cell nuclei). (B) The HMOX1 and FABP4 expression levels in the skin sample were assessed by real time qRT-PCR. (C) IHC results of the two ROS markers, nitrotyrosine and 4-hydroxynoneal, showed decreased ROS levels by LDR in the skin of burn mouse model. (D and E) The effect of LDR treatment to the burn wound of FAK activation and actin remodeling was measured by immunofluorescence and phalloidin staining, respectively. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$. The burn-only group was compared to control, and burn + LDR groups were compared to the burn-only group.

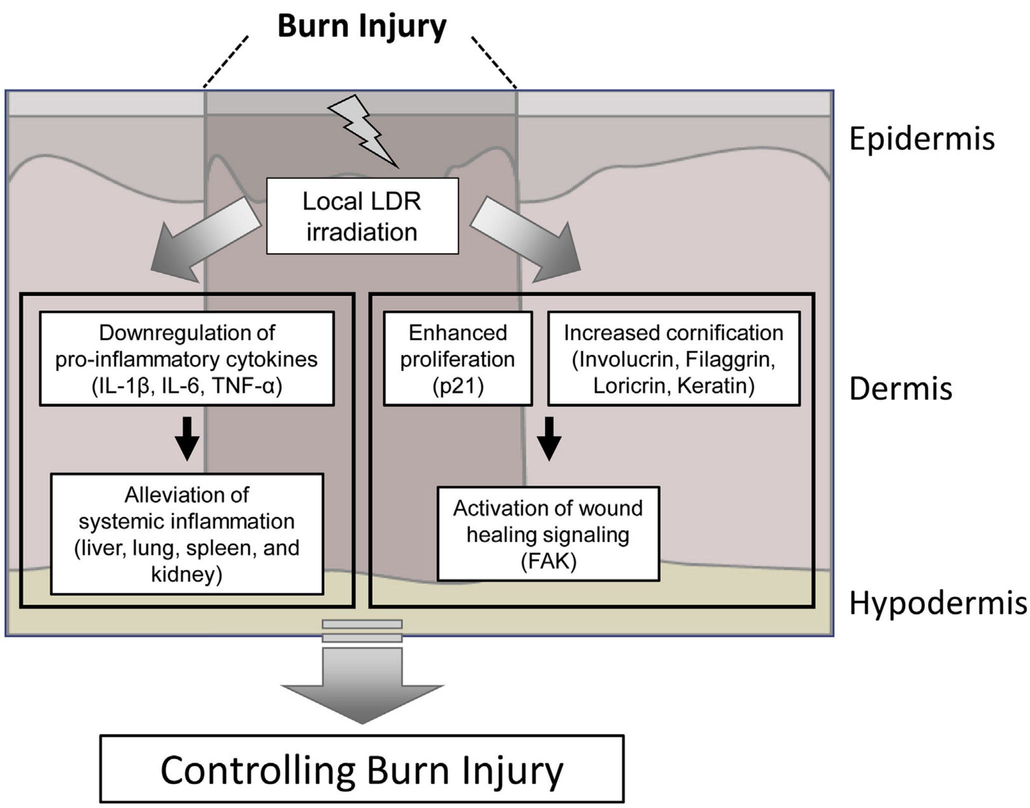

Fig. 6. Schematic summary of LDR-induced burn wound healing. A diagram described the effects of LDR in alleviation of systemic inflammation and skin wound healing upon burn injury.

LDR (Fig. 1 and 3). It can be summarized that serum proinflammatory cytokines regulated by LDR may be critical regulator for skin regeneration.

The minimum dose of ionizing radiation with biological significance has been controversial in various studies. Ionizing radiation less than 100 mGy was classically supposed to be non-effective dose, while a recent study suggested that the threshold and effects of LDR can vary according to the cells, tissues, organs, and even organisms [37]. As shown in our data, we proposed the biological effects of LDR both with single dose less than 100 mGy and accumulative dose less than 100 mGy. Especially, the significance of LDR with accumulative dose less than 100 mGy provided information about the effects of chronic LDR exposure to the organisms, which was also supported by previous studies stating thatboth short and long term exposure of LDR less than 100 mGy reduced ROS generation and increased antioxidant gene expression [26,38]. To verify the underlying mechanisms of the distinguishing effects of LDR from those of HDR, a study adopted an adaptive response induced by low level stress against high level stress [26]. Another study suggested the LDR specifically activated NF-κB-mediated cell survival signaling pathway without genomic instability [39]. However, further studies are still needed to validate these principles and our data could provide basic information about LDR effects.

In this study, we conducted experiments in two separated tracks, one utilized LDR with a single dose less than 100 mGy, but a total dose over 100 mGy (Fig. 1 and 2), and the other utilized LDR with a total dose less than 100 mGy (Fig. 3–5). However, the it is hard to find a difference between two condition of LDR. Previous studies showed that an accumulation of LDR (0–100 mGy) increased mutation hazard and modulates cellular signalingin a dose-dependent manner [40,41]. This was also supported by an epidemiological research about leukemic hazard of nuclear workers in US [42]. Besides, fractionated LDR showed different biological effects even at the same total dose. 10 fractions of 50 mGy LDR showed stronger epigenetic effects than single fraction of 500 mGy [43,44]. Therefore, the LDR treatment as a burn therapy also needs to be optimized for practical application and it warrants the importance of further study.

Some previous studies were already reported that LDR have curative effects. A study confirmed that whole body 10 fraction of 75 mGy of LDR exposure with basic fibroblast growth factor and zinc accelerated healing of skin wounds in diabetic rats [45]. Other reports also demonstrated the acceleration of skin wound healing by LDR in a rat model [11]. In addition, whole body irradiation of LDR was reported to regulate systemic inflammation after diabetes, infectious sepsis, and asthma [21,46,47]. In this study, we firstly suggested alleviation of systemic inflammation and acceleration of wound healing after burn injuries by LDR treatment. However, further studies on the mechanisms in inflammation and ROS after burn trauma and related signaling pathways modulated by LDR are warranted.

The results of the present study including LDR-induced inflammation attenuation and enhanced wound healing after burn injury suggested the role in burn wound repair and the molecular mechanism underlying the protective effect of LDR. In burn wound mouse model, adipogenesis during tissue regeneration was promoted and redox signaling was activated by LDR. In addition, we provided new insights that adjuvant radioactive treatment can be utilized to attenuate burn trauma with pharmacological agents during burn treatment.

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Declaration of Competing Interest

The authors declare no conflict of interest.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.jdermsci.2019.10.004.

References

[1] A.B. Association, National Burn Repository 2019 Report Version 14.0, American Burn Association, Chicago, IL, 2019.

[2] A. Abdullahi, S. Amini-Nik, M.G. Jeschke, Animal models in burn research, Cell. Mol. Life Sci. 71 (17) (2014) 3241–3255.

[3] A.J. Singer, S.T. Boyce, Burn wound healing and tissue engineering, J. Burn Care Res. 38 (3) (2017) e605–e613.

[4] J. Zeng, X. Lin, H. Fan, C. Li, Hydrogen sulfide attenuates the inflammatory response in a mouse burn injury model, Mol. Med. Rep. 8 (4) (2013) 1204–1208.

[5] L.N. Nguyen, T.G. Nguyen, Characteristics and outcomes of multiple organ dysfunction syndrome among severe-burn patients, Burns 35 (7) (2009) 937–941.

[6] M. Doss, Linear No-Threshold model VS, Radiat. Horm. Dose-Resp. 11 (2013) 480–497.

[7] G. Park, B. Son, J. Kang, S. Lee, J. Jeon, J.H. Kim, G.R. Yi, H. Youn, C. Moon, S.Y. Nam, B. Youn, LDR-induced miR-30a and miR-30b target the PAI-1 pathway to control adverse effects of NSCLC radiotherapy, Mol. Ther. 27 (2) (2019) 342–354.

[8] W.M. Bonner, Low-dose radiation: thresholds, bystander effects, and adaptive responses, Proc. Natl. Acad. Sci. U. S. A. 100 (9) (2003) 4973–4975.

[9] D. Mathias, R.E. Mitchel, M. Barclay, H. Wyatt, M. Bugden, N.D. Priest, S.C. Whitman, M. Scholz, G. Hildebrandt, M. Kamprad, A. Glasow, Low-dose irradiation affects expression of inflammatory markers in the heart of ApoE -/- mice, PLoS One 10 (3) (2015)e0119661.

[10] H.J. Hahn, H.J. Youn, H.J. Cha, K. Kim, S. An, K.J. Ahn, Single Low-Dose Radiation Induced Regulation of Keratinocyte Differentiation in Calcium-Induced HaCaT Cells, Ann. Dermatol. 28 (4) (2016) 433–437.

[11] N. Jabbari, G.H. Farjah, B. Ghadimi, H. Zanjani, B. Heshmatian, Acceleration of skin wound healing by low-dose indirect ionizing radiation in male rats, Kaohsiung J. Med. Sci. 33 (8) (2017) 385–393.

[12] X.S. Song, X.Z. Zhou, G. Zhang, Q.R. Dong, L. Qin, Low-dose X-ray irradiation promotes fracture healing through up-regulation of vascular endothelial growth factor, Med. Hypotheses 75 (6) (2010) 522–524.

[13] G.C. Gurtner, S. Werner, Y. Barrandon, M.T. Longaker, Wound repair and regeneration, Nature 453 (7193) (2008) 314–321.

[14] I. Haase, R. Evans, R. Pofahl, F.M. Watt, Regulation of keratinocyte shape, migration and wound epithelialization by IGF-1- and EGF-dependent signalling pathways, J. Cell. Sci. 116 (Pt 15) (2003) 3227–3238.

[15] M.A. Parenti, S.M. Hatfield, J.J. Leyden, Mupirocin: a topical antibiotic with a unique structure and mechanism of action, Clin. Pharm. 6 (10) (1987) 761–770.

[16] L.L. Strock, M.M. Lee, R.L. Rutan, M.H. Desai, M.C. Robson, D.N. Herndon, J.P. Heggers, Topical Bactroban (mupirocin): efficacy in treating burn wounds infected with methicillin-resistant staphylococci, J. Burn Care Rehabil. 11 (5) (1990) 454–459.

[17] A. Seno, T. Maruhashi, T. Kaifu, R. Yabe, N. Fujikado, G. Ma, T. Ikarashi, S. Kakuta, Y. Iwakura, Exacerbation of experimental autoimmune encephalomyelitis in mice deficient for DCIR, an inhibitory C-type lectin receptor, Exp. Anim. 64 (2) (2015) 109–119.

[18] J. Kang, W. Kim, H. Seo, E. Kim, B. Son, S. Lee, G. Park, S. Jo, C. Moon, H. Youn, B. Youn, Radiation-induced overexpression of transthyretin inhibits retinol-mediated hippocampal neurogenesis, Sci. Rep. 8 (1) (2018) 8394.

[19] H. Karimi, A. Soudmand, Z. Orouji, E. Taghiabadi, S.J. Mousavi, Burn wound healing with injection of adipose-derived stem cells: a mouse model study, Ann. Burns Fire Disasters 27 (1) (2014) 44–49.

[20] D.L. Maass, J. White, J.W. Horton, IL-1beta and IL-6 act synergistically with TNF-alpha to alter cardiac contractile function after burn trauma, Shock (Augusta, Ga.) 18 (4) (2002) 360–366.

[21] K.H. Song, S.Y. Jung, S.H. Kho, S.G. Hwang, H. Ha, S.Y. Nam, J.Y. Song, Effects of low-dose irradiation on mice with Escherichia coli-induced sepsis, Toxicol. Appl. Pharmacol. 333 (2017) 17–25.

[22] C.F. Lima, C. Pereira-Wilson, S.I. Rattan, Curcumin induces heme oxygenase-1 in normal human skin fibroblasts through redox signaling: relevance for anti-aging intervention, Mol. Nutr. Food Res. 55 (3) (2011) 430–442.

[23] M. Furuhashi, S. Saitoh, K. Shimamoto, T. Miura, Fatty Acid-Binding Protein 4 (FABP4): Pathophysiological Insights and Potent Clinical Biomarker of Metabolic and Cardiovascular Diseases, Clin. Med. Insights Cardiol. 8 (Suppl 3) (2014) 23–33.

[24] G. Rivera-Gonzalez, B. Shook, V. Horsley, Adipocytes in skin health and disease, Cold Spring Harb. Perspect. Med. 4 (3) (2014)a015271.

[25] A.S. Sahib, F.H. Al-Jawad, A.A. Al-Kaisy, Burns, endothelial dysfunction, and oxidative stress: the role of antioxidants, Ann. Burns Fire Disasters 22 (1) (2009) 6–11.

[26] N. Chen, L. Wu, H. Yuan, J. Wang, ROS/Autophagy/Nrf2 pathway mediated low-dose radiation induced radio-resistance in human lung adenocarcinoma A549 cell, Int. J. Biol. Sci. 11 (7) (2015) 833–844.

[27] M. Kaushik, R. Niranjan, R. Thangam, B. Madhan, V. Pandiyarasan, C. Ramachandran, D.-H. Oh, G.D. Venkatasubbu, Investigations on the antimicrobial activity and wound healing potential of ZnO nanoparticles, Appl. Surf. Sci. 479 (2019) 1169–1177.

[28] W. Ruhm, T. Azizova, S. Bouffler, H.M. Cullings, B. Grosche, M.P. Little, R.S. Shore, L. Walsh, G.E. Woloschak, Typical doses and dose rates in studies pertinent to radiation risk inference at low doses and low dose rates, J. Radiat. Res. 59 (suppl_2) (2018) ii1–ii10.

[29] X. Bai, L. Fan, T. He, W. Jia, L. Yang, J. Zhang, Y. Liu, J. Shi, L. Su, D. Hu, SIRT1 protects rat lung tissue against severe burn-induced remote ALI by attenuating the apoptosis of PMVECs via p38 MAPK signaling, Sci. Rep. 5 (2015) 10277.

[30] C. Shen, L. Sun, N. Zhu, F. Qi, Kindlin-1 contributes to EGF-induced re-epithelialization in skin wound healing, Int. J. Mol. Med. 39 (4) (2017) 949–959.

[31] M. Takeo, W. Lee, M. Ito, Wound healing and skin regeneration, Cold Spring Harb. Perspect. Med. 5 (1) (2015)a023267.

[32] K.H. Hanel, C. Cornelissen, B. Luscher, J.M. Baron, Cytokines and the skin barrier, Int. J. Mol. Sci. 14 (4) (2013) 6720–6745.

[33] M. Schmidt, M. Goebeler, G. Posern, S.M. Feller, C.S. Seitz, E.B. Brocker, U.R. Rapp, S. Ludwig, Ras-independent activation of the Raf/MEK/ERK pathway upon calcium-induced differentiation of keratinocytes, J. Biol. Chem. 275 (52) (2000) 41011–41017.

[34] D. Walker, T. Sun, S. MacNeil, R. Smallwood, Modeling the effect of exogenous calcium on keratinocyte and HaCat cell proliferation and differentiation using an agent-based computational paradigm, Tissue Eng. 12 (8) (2006) 2301–2309.

[35] A. Sehat, R.M. Huebinger, D.L. Carlson, Q.S. Zang, S.E. Wolf, J. Song, Burn serum stimulates myoblast cell death associated with IL-6-Induced mitochondrial fragmentation, Shock (Augusta, Ga.) 48 (2) (2017) 236–242.

[36] G. Zhao, Y.M. Yu, M. Kaneki, A.A. Bonab, R.G. Tompkins, A.J. Fischman, Simvastatin reduces burn injury-induced splenic apoptosis via downregulation of the TNF-alpha/NF-kappaB pathway, Ann. Surg. 261 (5) (2015) 1006–1012.

[37] F.R. Tang, W.K. Loke, Molecular mechanisms of low dose ionizing radiation-induced hormesis, adaptive responses, radioresistance, bystander effects, and genomic instability, Int. J. Radiat. Biol. 91 (1) (2015) 13–27.

[38] R.K. Kim, M.J. Kim, K.M. Seong, N. Kaushik, Y. Suh, K.C. Yoo, Y.H. Cui, Y.W. Jin, S.Y. Nam, S.J. Lee, Beneficial effects of low dose radiation in response to the oncogenic KRAS induced cellular transformation, Sci. Rep. 5 (2015) 15809.

[39] J.H. Yim, J.M. Yun, J.Y. Kim, S.Y. Nam, C.S. Kim, Estimation of low-dose radiation-responsive proteins in the absence of genomic instability in normal human fibroblast cells, Int. J. Radiat. Biol. 93 (11) (2017) 1197–1206.

[40] Y. Abe, M.A. Yoshida, K. Fujioka, Y. Kurosu, R. Ujiie, A. Yanagi, N. Tsuyama, T. Miura, T. Inaba, K. Kamiya, A. Sakai, Dose-response curves for analyzing of dicentric chromosomes and chromosome translocations following doses of 1000 mGy or less, based on irradiated peripheral blood samples from five healthy individuals, J. Radiat. Res. 59 (1) (2018) 35–42.

[41] M. Katsura, H. Cyou-Nakamine, Q. Zen, Y. Zen, H. Nansai, S. Amagasa, Y. Kanki, T. Inoue, K. Kaneki, A. Taguchi, M. Kobayashi, T. Kaji, T. Kodama, K. Miyagawa, Y. Wada, N. Akimitsu, H. Sone, Effects of chronic low-dose radiation on human neural progenitor cells, Sci. Rep. 6 (2016) 20027.

[42] R.D. Daniels, S. Bertke, K.M. Waters, M.K. Schubauer-Berigan, Risk of leukaemia mortality from exposure to ionising radiation in US nuclear workers: a pooled case-control study, Occup. Environ. Med. 70 (1) (2013) 41–48.

[43] J. Wang, Y. Zhang, K. Xu, X. Mao, L. Xue, X. Liu, H. Yu, L. Chen, X. Chu, Genome-wide screen of DNA methylation changes induced by low dose X-ray radiation in mice, PLoS One 9 (3) (2014)e90804.

[44] G. Silasi, R. Diaz-Heijtz, J. Besplug, R. Rodriguez-Juarez, V. Titov, B. Kolb, O. Kovalchuk, Selective brain responses to acute and chronic low-dose X-ray irradiation in males and females, Biochem. Biophys. Res. Commun. 325 (4) (2004) 1223–1235.

[45] H.F. Zhang, J. Cheng, Y. Lv, F.S. Li, G.Y. He, B. Wang, L. Cai, Repeated whole-body exposure to low-dose radiation combined with topical application of basic fibroblast growth factor and zinc accelerates wound healing in diabetic rats, Dose-Response 16 (3) (2018)1559325818789845.

[46] X. Xing, C. Zhang, M. Shao, Q. Tong, G. Zhang, C. Li, J. Cheng, S. Jin, J. Ma, G. Wang, X. Li, L. Cai, Low-dose radiation activates Akt and Nrf2 in the kidney of diabetic mice: a potential mechanism to prevent diabetic nephropathy, Oxid. Med. Cell. Longev. 2012 (2012)291087.

[47] J.S. Kim, Y. Son, M.J. Bae, S.S. Lee, S.H. Park, H.J. Lee, S.I. Lee, C.G. Lee, S.D. Kim, W. S. Jo, S.H. Kim, I.S. Shin, Continuous exposure to low-dose-Rate gamma irradiation reduces airway inflammation in Ovalbumin-Induced Asthma, PLoS One 10 (11) (2015)e0143403.