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Original Research

Fenofibrate Attenuates Radiation-Induced Oxidative Damage to the Skin through Fatty Acid Binding Protein 4 (FABP4)

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Abstract

Background: Radiation facilities and radioactive materials have been widely used in military, industry, medicine, science and nuclear facilities, which has significantly increased the potential of large-scale, uncontrolled exposure to radiation. The skin is one of the radiosensitive organ systems and radiation-induced skin injury remains a serious concern after ionizing radiation exposure. Our previous report indicates the involvement of the peroxisome proliferator-activated receptor pathway in the response of skin tissues to ionizing radiation. PPARα is a member of the PPAR nuclear hormone receptor superfamily, which can be activated by fibrate ligands. However, the protection of fenofibrate against ionizing radiation in skin keratinocytes and fibroblasts has not been described. **Methods**: The PPARα mRNA levels in irradiated and nonirradiated skin tissues of rats were determined by real-time assay. The expression of PPARα, and FABP4 were evaluated by western blot and IHC assay. The cell proliferation was detected by colony formation. The γH2AX foci and ROS levels in irradiated WS1 cells with FABP4 overexpression than in control cells were performed by Immunofluorescence assay. **Results**: We found that PPARα expression was lower in the irradiated skin tissues of mouse, rat, monkey, and human patients than in their nonirradiated counterparts. PPARα fenofibrate significantly decreased radiation-induced ROS and apoptosis in a dose-dependent manner in human keratinocyte HaCaT and skin fibroblast WS1 cells. Moreover, fenofibrate significantly decreased radiation-induced ROS and malondialdehyde (MDA) levels in electron beam irradiated skin tissues of rats. Mechanistically, the proximal promoter of fatty acid binding protein 4 (FABP4) harbored three binding sites of PPARα and fenofibrate stimulated the transcription of FABP4 in skin cells. FABP4 overexpression decreased radiation-induced ROS and γH2AX foci. FABP4 inhibitor BMS309403 abrogated the ROS-eliminating activity as well as the lipid-accumulating role of fenofibrate, indicating that FABP4 mediates the radioprotective role of fenofibrate. In addition, FABP4 overexpression significantly decreased radiation-induced oxidative damage *in vivo*. **Conclusions**: These results confirm that fenofibrate attenuated radiation-induced oxidative damage to the skin by stimulating FABP4.

Keywords: ionizing radiation; radiation-induced skin injury; peroxisome proliferator-activated receptor α (PPARα); fenofibrate; fatty acid binding protein 4 (FABP4)

1. Introduction

Radiation facilities and radioactive materials are used extensively in the military, industrial, medical and scientific fields, greatly increasing the possibility of large-scale, uncontrolled exposure to radiation [1,2]. As a constantly renewing organ with rapidly proliferating and maturing cells, the skin is sensitive to radiation [1,2]. Ionizing radiation promotes the production of reactive nitrogen and reactive oxygen species (RNA/ROS) due to the radiolysis of water and direct ionization of target molecules; this increased production leads to oxidative damage and skin injuries [3,4]. Approximately 95% of cancer patients treated with radiation develop some form of radiation dermatitis, including erythema, dry desquamation, and moist desquamation [5,6]. Radiation-induced skin damage has a negative impact on the effectiveness of radiation therapy and the quality of life of patients [7]. Despite significant improvements in radiation technology, radiation-induced skin toxicity remains a problem [5–8].

Peroxisome proliferator-activated receptors (PPARs) are ligand-inducible transcriptional factors that belong to the hormone nuclear receptor superfamily. Three members of the PPAR family (PPARα, PPARβ/δ and PPARγ) with a high degree of sequence homology have distinct physiolog-

ical roles, ligand specificity, and tissue distribution [9,10]. PPARα is a vital regulator of fatty acid oxidation in a wide variety of tissues [11,12]. Fibrates are synthetic PPARα ligands, and they serve as first-line drugs for reducing serum triglyceride levels [13,14]. When activated, nuclear-localized PPARα heterodimerizes with the retinoid X receptor and binds to PPAR-responsive elements (PPREs), which consequently stimulate the transcription of an extensive array of target genes associated with lipid metabolism, cell differentiation, inflammation and many other biological processes [15,16]. PPARα agonists have been shown to confer protection against various tissue injuries in a variety of radiation-induced injury models, including radiation-induced brain injury and heart injury [17,18]. In addition, previous research has confirmed that PPARα agonists would ameliorate the proinflammatory responses seen in the microglia following *in vitro* radiation [19].

Fenofibrate, a specific ligand for PPARα, has long been used to treat hypercholesterolemia, hypertriglyceridemia, diabetes and cardiovascular diseases [14,20]. Fenofibrate reduces low-density lipoprotein (LDL), very low-density lipoprotein (VLDL), and triglyceride levels and increases high-density lipoprotein (HDL) levels [14, 20]. PPARα also has antioxidant and anti-inflammatory properties [13]. Fenofibrate confers cytoprotective effects against myocardial ischemia-reperfusion (I/R) injury in rats by suppressing cell apoptosis and attenuating age-related renal injury by activating AMPK and SIRT1 signaling pathways [20,21].

We have recently reported the beneficial effect of fenofibrate against radiation-induced skin injury in animal models and human patients [22]. However, its underlying mechanisms remain unknown. In this study, we demonstrated that fenofibrate-induced PPARα activation conferred protection against ionizing radiation to the skin. We identified fatty acid binding protein 4 (FABP4) as a key effector for fenofibrate-mediated protection against radiation-induced ROS production and lipid accumulation. These results suggest that fenofibrate protects against radiation-induced skin damage through FABP4.

2. Materials and Methods

2.1 Reagents

Dimethylsulfoxide (DMSO) and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) were purchased from Solarbio (Beijing, China). Fenofibrate and the FABP4 inhibitor BMS309403 were obtained from Sigma-Aldrich (St. Louis, MO, USA). 4'-6-diamidino-2-phenylindole (DAPI) and Hoechst stains were purchased from Beyotime Biotech (Nantong, China). A SmartFlare uptake control probe (positive control) and FABP4 mRNA-specific SmartFlare probe were obtained from Millipore (Billerica, MA, USA). BODIPY fluorophore 493/503 for lipid droplets was obtained from Molecular Probes (Eugene, OR, USA). Adenoviruses (Ad-NC and Ad-FABP4) were obtained from HanBio (Shanghai, China).

2.2 Animal Studies

Protocols for experiments involving animals were approved by the Animal Experimentation Ethics Committee at Soochow University (Suzhou, China). Male Sprague-Dawley (SD) rats (4 weeks old) and male C57 mice (4 weeks old) were purchased from the Shanghai SLAC Laboratory Animal Co., Ltd. (Shanghai, China). For irradiation, the rats were anesthetized with an intraperitoneal injection of ketamine (75 mg/kg) and xylazine (10 mg/kg), and the hair on the rat buttocks was shaved using a razor. A 3-cm-thick piece of lead was used to shield the rats and localize the radiation field (3 cm $\times$ 4 cm). A single dose of 45 Gy irradiation [23–25] was administered to the hindlimb region of the SD rats at a dose rate of 750 cGy/min using a 6-MeV electron beam accelerator (Clinac 2100EX, Varian Medical Systems, Inc., CA, USA). This dose was selected because it can significantly induce skin injury [23–26]. For the treatment, the rats were then randomly assigned to receive treatments by subcutaneous injection of DMSO, fenofibrate, or adenovirus [26].

2.3 RNA Extraction and Real-Time PCR Analysis

Total RNA was extracted from cells and tissues with Trizol reagent (Invitrogen, Carlsbad, CA, USA). *PPARα* and *FABP4* mRNA levels were quantified by quantitative real-time PCR as reported previously [27]. The primers used are listed in **Supplementary Table 1**.

2.4 Human Skin Samples

Human skin samples were obtained from a victim of an iridium radiation accident as reported previously [28]. The skin samples were obtained 160 days after irradiation from the right limb, which was exposed to iridium-192 (^{192}Ir) metal chain (with an activity of 966.4 GBq or 26.1 Ci). Normal skin tissues were obtained when performing skin grafting from the dorsal myocutaneous flap. Informed consent for sample collection was obtained from the patient.

2.5 Immunohistochemistry (IHC)

Skin tissues from mouse, rat and monkey were obtained as reported previously [24]. Mouse skin tissues were irradiated with 35 Gy electron beam. Rat skin tissues were irradiated with 45 Gy electron beam. The skin tissues of monkeys were treated with 0 or 20 Gy irradiation. Skin tissues were fixed in 10% neutral-buffered formalin and embedded in paraffin. Three-micrometer paraffin sections were deparaffinized and heat treated with citrate buffer (pH 6.0) for 7 min following an epitope retrieval protocol. Three-micrometer paraffin sections were incubated with a rabbit anti-PPARα antibody (Abcam, Cambridge, MA, USA, #ab 8934) at 4 °C overnight, followed by incubation with an anti-rabbit biotinylated secondary antibody

(Beyotime, Nantong, China), diaminobenzidine substrate detection, washing, hematoxylin staining, dehydration, and mounting.

2.6 Malondialdehyde (MDA) Concentration Measurement

Tissue MDA levels were determined using thiobarbituric acid (TBA) assays as reported previously [24]. MDA levels were normalized to those of the control group.

2.7 ROS Generation Assay

ROS levels were determined using the ROS-sensitive dye 2,7-dichlorofluorescein diacetate (DCF-DA) (Nanjing Jiancheng Bioengineering Institute, Nanjing, China). The cells were washed with PBS and incubated with DCF-DA (10 μM) for 30 min. Skin tissues were trypsinized into single cell suspension according to the manufacturer's instructions. The level of DCF fluorescence, which reflects the ROS concentration, was observed with a fluorescence microscope. DCF fluorescence levels in skin cells and tissues were quantified at 488 nm using a 96-well plate reader.

2.8 Cell Culture and Irradiation

Human keratinocyte HaCaT cells, human fibroblast WS1 cells [24–26] and primary skin fibroblasts were maintained in Dulbecco's modified Eagle's medium (DMEM). All culture media was supplemented with 10% FBS (Gibco, Grand Island, NY, USA). Cells were grown at 37 °C in 5% CO_2 incubators. The cells were exposed to different dosages of ionizing radiation using an X-ray linear accelerator (Rad Source, Suwanee, GA, USA) and a fixed dose rate of 1.15 Gy/min.

2.9 Cell Viability Assay

Cells were incubated with DMSO or fenofibrate. Cell viability was measured using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) assays. The cells were incubated for 4 h with 200 μg/mL MTT (Sigma, St Louis, MO, USA). The reagent was dissolved in DMSO (Solon, OH, USA). The absorbance values were measured at 490 nm using a 96-well plate reader. The experiments were performed in triplicate.

2.10 Immunostaining

Cells were fixed in 4% paraformaldehyde, washed with PBS, and permeabilized with 1% Triton X-100 in PBS. The cells were then blocked with blocking buffer (PBS, 1% Triton X-100, and 5% BSA) and incubated at 4 °C with a PPARα (Abcam, #ab 8934) or γH2AX antibody (Abcam; #ab 81299) overnight. Next, a rhodamine-conjugated goat anti-rabbit antibody (1:100) was added for 30 min at room temperature. The nuclei were counterstained with DAPI.

2.11 Luciferase Reporters and Luciferase Assay

The luciferase reporter with four PPREs in *luciferase* promoter was a kind gift from Dr. Zengpeng Li (Third Institute of Oceanography, State Oceanic Administration, Xiamen, China). The plasmid was verified by sequencing. Cells were transfected with the constructed vectors using Fugene HD transfection reagent (Promega, Madison, WI, USA). For each transfection, 50 ng pRL-TK (Promega) was used to enhance the transfection efficiency. Measurement of luciferase activity using the dual luciferase reporter assay system (Promega). Promoter activity was expressed as the ratio of firefly luciferase activity to Renilla luciferase activity.

2.12 Western Blotting Analysis

Detailed descriptions are given as previously described [25]. Briefly, the membranes were blotted with antibodies against PPARα (Abcam, Cambridge, MA, USA, #ab227074), GAPDH (Abcam, #ab181602), and FABP4 (Abcam, #ab 92501).

2.13 Measurement of Cell Apoptosis

Cells were pretreated with DMSO or fenofibrate and then exposed to irradiation. Apoptosis was measured using a 7-AAD/Annexin-V double staining apoptosis kit (BD Biosciences, Franklin Lakes, NJ, USA) and flow cytometry (BD Biosciences, CA, USA). The Annexin-V+/7-AAD- cells indicated early apoptosis, and the Annexin-V+/7-AAD+ cells indicated late apoptosis. The percentages of both types of cells were counted.

2.14 Electromobility Shift Assay (EMSA)

WS1 cell nuclear protein was extracted using a nuclear protein isolation kit (Beyotime). The sequences for the double-stranded oligonucleotide probes (**Supplementary Table 2**) were synthesized and labeled with biotin by Shanghai Sangon Biotech Co. Ltd. (Shanghai, China). EMSAs were performed according to the LightShift EMSA Kit instructions (Pierce, Rockford, IL, USA).

2.15 Statistical Analysis

The data are expressed as the mean $\pm$ SEM of at least three independent experiments. The results were evaluated via one-way ANOVA to determine statistical significance. The statistical analyses were performed using Prism 8 (GraphPad software, San Diego, CA). The differences were considered significant at $p < 0.05$.

3. Results

3.1 Ionizing Radiation Decreases Cutaneous PPARα Expression

We firs0074 analyzed the response of PPARα to ionizing radiation in multiple animal models. Rats were irradiated with a 45 Gy electron beam as reported previously [26,27]. The real-time PCR analysis results showed that *PPARα* mRNA levels in the irradiated skin tissues were 26.62% of those in the nonirradiated skin tissues. This result is consistent with our RNA-Seq data (GEO database

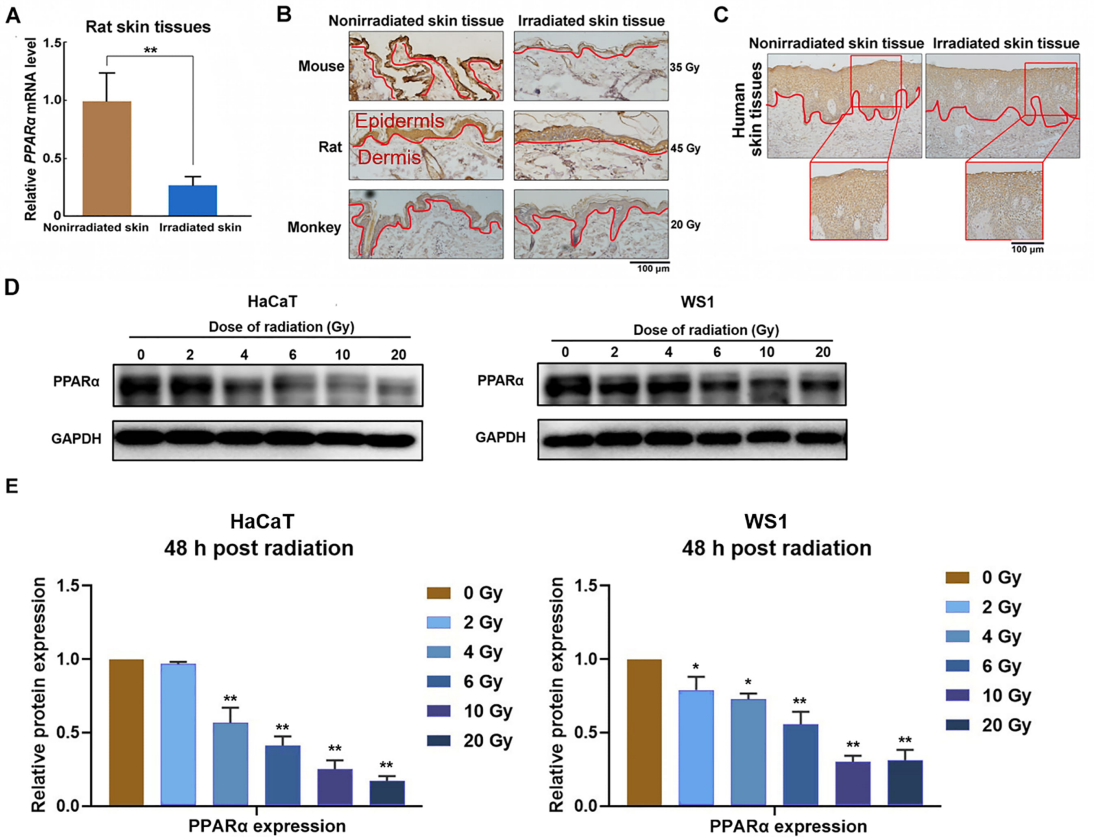

Fig. 1. Ionizing radiation decreases the expression of *PPARα* expression in skin tissues. (A) *PPARα* mRNA levels in irradiated and nonirradiated skin tissues of rats (n = 5). *PPARα* mRNA expression was measured by real-time PCR. The data are shown as the mean ± SEM. (B) The expression of PPARα in irradiated and nonirradiated skin tissues of mouse, rat and monkey. Skin tissues were collected three days after indicated radiation doses. PPARα expression was measured by IHC as described in the Materials and Methods section. (C) The expression of PPARα in nonirradiated and irradiated human skin tissues. (D) Western blotting analyses of PPARα expression at different doses of radiation in WS1 and HaCaT cells. (E) Quantitative analysis of Western blotting assay. Data are depicted as the mean ± SD from three independent experiments. * $p < 0.05$; ** $p < 0.01$, compared with the control group.

accession number GSE86252) [28]. Next, we attempted to confirm the expression of PPARα in skin tissue after irradiation by immunohistochemistry in different animal models. The results showed that the expression of PPARα in the skin tissues of mice, rats, and monkeys after irradiation was significantly lower than that of the nonirradiated control group (Fig. 1B). Moreover, in the irradiated epidermis of a human patient, the expression of PPARα was decreased, with pronounced distribution from the nucleus to the cytosol (Fig. 1C), indicating PPARα inactivation in irradiated skin cells. In addition, ionizing radiation downregulated PPARα protein levels in a dose-dependent manner in human skin fibroblast WS1 and human keratinocyte HaCaT cells (Fig. 1D).

3.2 Fenofibrate-Mediated PPARα Activation Protects Skin Cells against Radiation

Because fenofibrate is a synthetic fibrate ligand of PPARα, we next explored its effect on PPARα activation and its influence on the radiosensitivity of cultured skin cells. HaCaT cells were exposed to fenofibrate and then subjected to immunofluorescence for PPARα detection. The results indicated that fenofibrate induces the translocation of PPARα into the nucleus (Fig. 2A). Moreover, the activity of the PPRE harboring luciferase reporter was significantly increased after fenofibrate addition; this result confirmed PPARα activation by fenofibrate (Fig. 2B).

Because ionizing radiation elicits cutaneous free radical reactions [3,4], we examined whether PPARα activation confers protection against radiation-induced oxidative damage. Fenofibrate concentrations of up to 50 μM did not significantly affect viability in HaCaT and WS1 cells (**Supplementary Fig. 1**). We first measured fenofibrate effects on cellular ROS elimination in human HaCaT keratinocytes, WS1 fibroblasts and primary human fibroblasts. HaCaT cells pretreated with 25 or 50 μM Fenofibrate significantly reduced radiation-induced ROS levels (Fig. 1C). Similar results were obtained in WS1 cells and human primary fibroblasts; in these cells, 50 μM fenofibrate exhibited the strongest antioxidative activity (Fig. 2D and **Sup-**

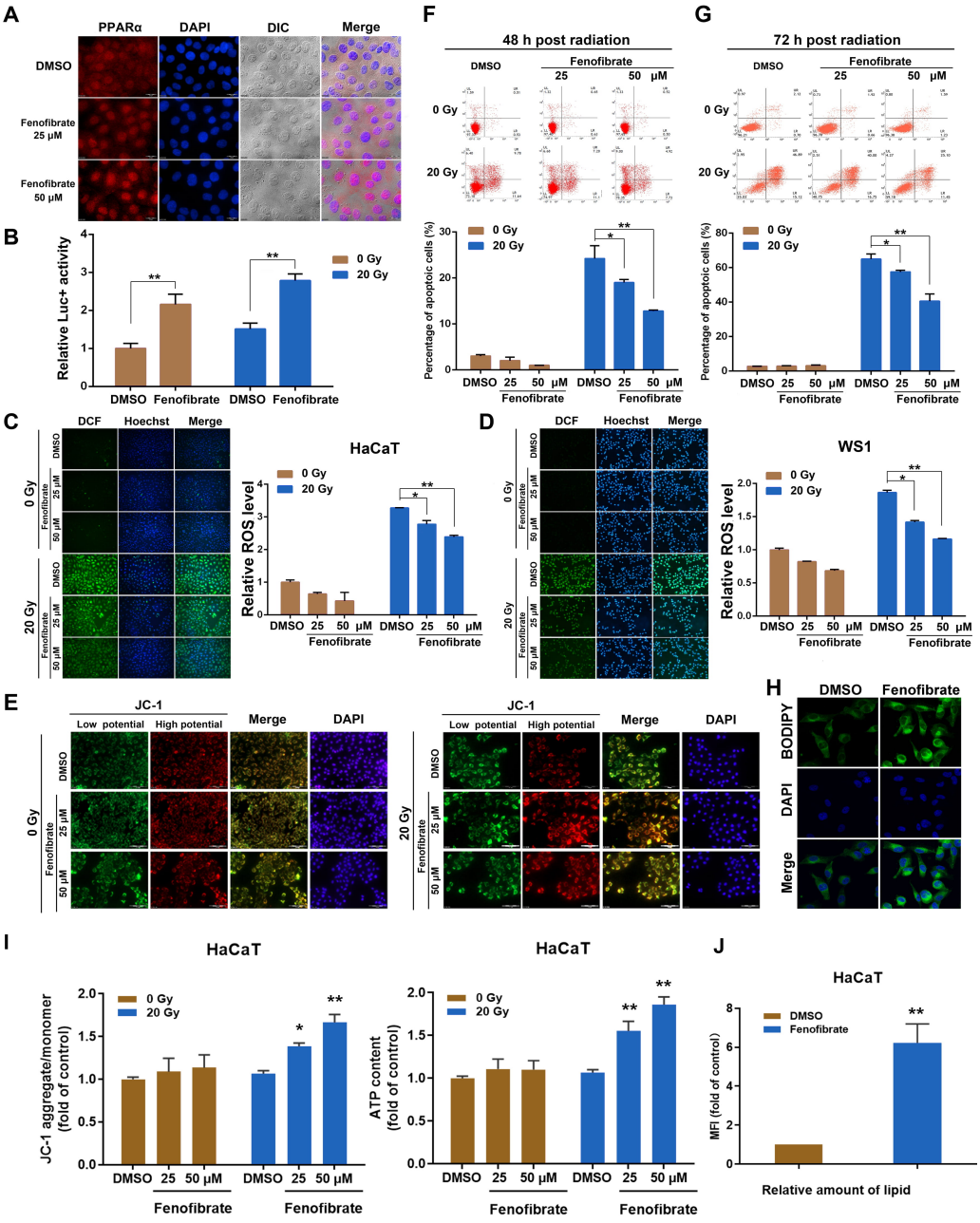

Fig. 2. Fenofibrate-meditated PPARα activation protects skin cells against radiation. (A) HaCaT cells were treated with 25 and 50 μM fenofibrate, and immunofluorescence was performed to investigate PPARα translocation. (B) The effect of fenofibrate on PPARα activity was measured with a PPRE luciferase reporter. Luciferase activity was assayed 24 h after transfection. The *firefly* luciferase activity of each sample was normalized to the *Renilla* luciferase activity. The final luciferase activity was normalized to that of the control group. (C) HaCaT and (D) WS1 cells were pretreated with fenofibrate and subjected to 0 or 20 Gy irradiation. One hour later, the cellular ROS levels of each group of cells were determined using a DCF-DA probe. Cellular fluorescence was observed using a fluorescence microscope. ROS levels were quantified with a microplate reader. (E) HaCaT cells were pretreated with DMSO or fenofibrate and then irradiated. Mitochondrial membrane potential was evaluated using JC-1 staining. (F) HaCaT cells were pretreated with 25 and 50 μM fenofibrate. Then, the cells were mock irradiated or irradiated with 20-Gy X-rays. Cell apoptosis rates were detected with Annexin-V/7-AAD staining at (F) 48 h and (G) 72 h after irradiation. The data are shown as the mean $\pm$ SEM of three independent experiments. (H) HaCaT cells were treated with DMSO or fenofibrate. Representative photomicrographs of BODIPY fluorophore 493/503 staining for lipid droplets. The cells were observed with a confocal microscope (Olympus, Tokyo, Japan). (I) Quantification of the ratio of JC-1 aggregate to JC-1 monomer and ATP contents. (J) Quantification of BODIPY fluorophore 493/503 staining for lipid droplets. Data are depicted as the mean $\pm$ SD from three independent experiments. *$p < 0.05$; ** $p < 0.01$, compared with the control group.

plementary Fig. 2).

Mitochondrial functional failure, involving mitochondrial membrane potential changes is considered to be one of the most important factors leading to cell death [29,30]. Nonirradiated HaCaT cells were stained with JC-1 to show red fluorescence, while a large number of cells switched to green fluorescence after irradiation. These results indicate a decrease in mitochondrial membrane potential. HaCaT cells treated with fenofibrate showed less fluorescence from red to green, suggesting that fenofibrate can maintain mitochondrial membrane potential after ionizing radiation (Fig. 2E). These results demonstrated that fenofibrate protects mitochondria from ionizing radiation.

We next explored whether fenofibrate was associated with decreased apoptosis in skin cells. As shown in Fig. 2E,F, fenofibrate did not affect apoptosis in HaCaT cells that were not exposed to irradiation. In comparison, treatment with both 25 and 50 μM fenofibrate significantly decreased apoptosis in HaCaT cells that were exposed to 20 Gy irradiation (Fig. 2G). These results demonstrated that fenofibrate-mediated PPARα activation reduces apoptotic cell death caused by irradiation in skin cells.

Because epidermal lipids and free fatty acids play important roles in cell growth, differentiation and permeability barrier function [31,32], we investigated whether fenofibrate-mediated PPARα activation modulated lipid accumulation in human keratinocytes. The results revealed that PPARα activation by fenofibrate increased cytoplasmic lipid accumulation in HaCaT cells (Fig. 2H).

3.3 Fenofibrate Ameliorates Radiation-Induced Skin Injury in Rat Model

Next, we sought to investigate whether fenofibrate could mitigate the progression of radiation-induced skin injury in animal models. A radiation-induced rat skin injury model (45 Gy electron beam irradiation) [25,26] was used to evaluate the role of clinically approved fenofibrate in oxidative damage. After exposure to 45 Gy of irradiation, rat skin was injected subcutaneously with DMSO or fenofibrate. To test whether fenofibrate affects radiation-induced lipid peroxidation, we measured ROS and MDA concentrations in skin tissues three days after 45 Gy of irradiation. As shown in Fig. 3A,B, both cellular ROS and MDA levels were significantly lower in fenofibrate-injected tissues than in DMSO-injected tissues. This result indicated that fenofibrate attenuated radiation-induced ROS and consequent lipid peroxidation.

3.4 Fenofibrate Stimulates FABP4 Expression in Skin Cells

Our results showed that fenofibrate-mediated PPARα activation promoted lipid accumulation in skin cells. This finding indicates a potential relationship between skin cells and lipid metabolism. Free fatty acids, which are relatively insoluble and potentially toxic, can be transported to other cells by noncatalytic binding proteins [33]. FABPs belong

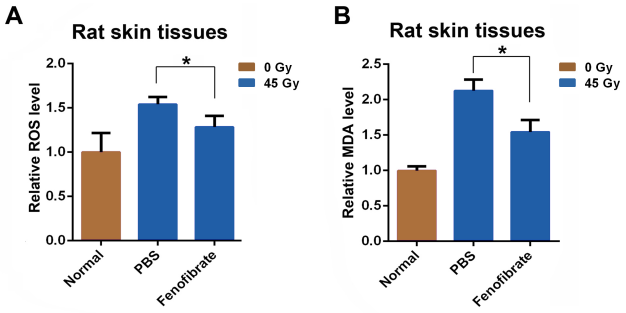

Fig. 3. Fenofibrate ameliorates radiation-induced skin injury in a rat model. Rat gluteal skin was unexposed or irradiated with a single dose from a 45-Gy electron beam. The rats were then randomly assigned to receive one of the following treatments (n = 4): (1) subcutaneous 110 μL DMSO injection (in 890 μL PBS); (2) subcutaneous 400 μg fenofibrate injection (in 110 μL DMSO and 890 μL PBS). (A) Relative ROS levels in the rat skin. Three days after irradiation, skin ROS levels were determined as described in the Materials and Methods section. (B) MDA concentration levels in rat skin from different groups at three days after irradiation. $p < 0.05$, compared with the control group.

to a family of intracellular proteins and exhibit a high affinity for non-covalent binding to long-chain fatty acids [34]. We, therefore, hypothesized that FABPs may be involved in the radioprotective role of fenofibrate. Among the 12 identified members of the human FABP family, three putative binding sites for PPARα (PPRE) in the proximal promoter of *FABP4* (Fig. 4A) were predicted by bioinformatics analysis. This result suggested transcriptional regulation by PPARα. FABP4 is an intracellular lipid-binding protein responsible for fatty acid transportation [35] and we have recently shown that FABP4-mediated the radioprotection of adipocytes [26]. We next performed EMSAs to investigate the binding of potential transcriptional factors. EMSAs revealed that oligonucleotides representing the predicted PPARα binding sites all formed a specific complex when incubated with WS1 nuclear extracts (Fig. 4B). Western blotting analyses showed that fenofibrate increased FABP4 protein levels in both HaCaT and WS1 cells (Fig. 4C). Using real-time PCR analyses, we found that fenofibrate increased *FABP4* mRNA levels in a dose-dependent manner (Fig. 4D). A FABP4 mRNA-based fluorescent probe, but not a SmartFlare uptake control probe, confirmed that fenofibrate upregulated *FABP4* transcripts specifically in WS1 and HaCaT cells (Fig. 4E and **Supplementary Fig. 3**). Taken together, these results clearly indicated that FABP4 is positively regulated by the PPARα agonist fenofibrate in skin cells.

3.5 FABP4 Protects Skin Cells from Radiation-Induced Damage

Next, we sought to investigate whether increased FABP4 expression could modulate radiation-induced dam-

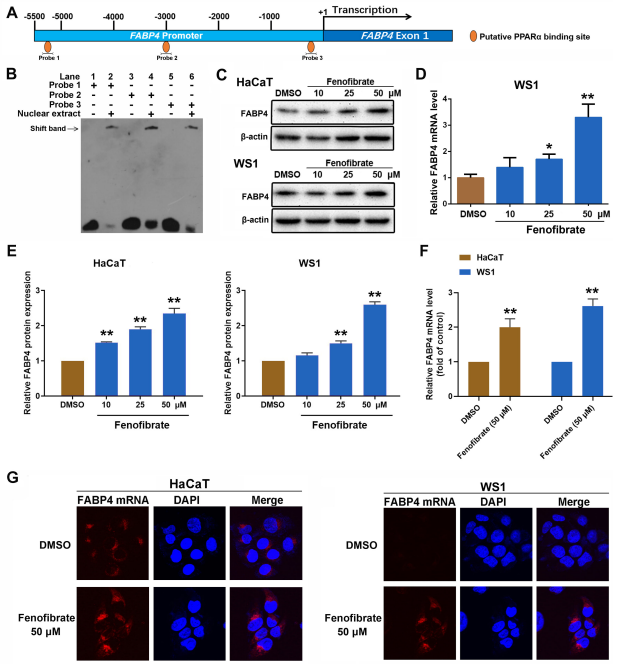

Fig. 4. Fenofibrate activates FABP4 expression in skin cells. (A) Bioinformatics analysis predicted three putative binding sites in the proximal promoter of FABP4. (B) EMSA using nuclear proteins from WS1 cells and oligonucleotides carrying the indicated probes. Lanes 1, 3 and 5 contain the probes without nuclear extracts. Lanes 2, 4 and 6 contain the oligonucleotide probes 1, 2 and 3, respectively. (C) HaCaT and WS1 cells were treated with 10–50 μM fenofibrate. FABP4 expression was measured by Western blotting analyses. (D) WS1 cells were treated with 10–50 μM fenofibrate, and *FABP4* mRNA was quantified by real-time PCR. (E) Quantitative analysis of Western blotting assay. (F) Quantification of FABP4-specific SmartFlare probe fluorophore microscope (G) HaCaT and WS1 cells were treated with 50 μM fenofibrate for 24 h, and FABP4 mRNA was detected with a FABP4-specific SmartFlare probe (Millipore, Billerica, MA, USA). Fluorescent signals reflecting the *FABP4* mRNA levels were observed using a confocal microscope. $p < 0.05$ and ** $p < 0.01$, compared with the control group.

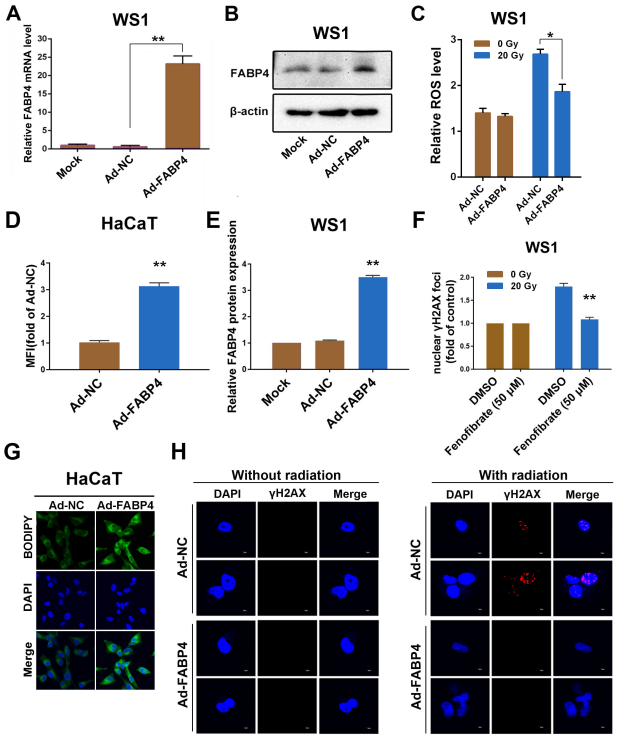

Fig. 5. FABP4 confers radioprotection to skin cells. WS1 cells were infected with the indicated adenoviruses. (A) FABP4 expression was measured by Western blotting analyses. (B) FABP4 expression was measured by Western blotting. (C) WS1 cells were infected with the indicated adenovirus, followed by 0 or 20 Gy irradiation. Cellular ROS levels for each group of cells were determined 1 h after radiation using a DCF-DA probe and quantified with a microplate reader. (D) Quantification of BODIPY fluorophore 493/503 staining for lipid droplets. (E) Quantification of Western blotting assay. (F) Quantification of nuclear γH2AX foci fluorescent signals. (G) The effect of FABP4 overexpression on lipid accumulation in HaCaT cells. Representative photomicrographs of BODIPY fluorophore 493/503 staining for lipid droplets. (H) WS1 cells were infected with Ad-NC or Ad-FABP4, and the dynamic repair process of DNA double-strand breaks (DSBs) was measured by detecting nuclear γH2AX foci after X-ray irradiation. $p < 0.05$ and ** $p < 0.01$, compared with the control group.

age in skin cells. Skin cells were pre-infected with a control adenovirus (Ad-NC) or FABP4 overexpression adenovirus (Ad-FABP4) and subjected to X-ray irradiation (Fig. 5A,B). The results showed that FABP4 overexpression reduced radiation-induced ROS levels (Fig. 5C). Moreover, FABP4 overexpression increased cellular lipid accumulation in HaCaT cells, which mimics the effect of fenofibrate (Fig. 5D). Immunofluorescence assays for γH2AX foci showed that fewer foci were present in irradiated WS1 cells with FABP4 overexpression than in control cells (Fig. 5E). These data suggested that FABP4 facilitated the repair of radiation-induced DNA damage.

3.6 FABP4 Mediates the Radioprotective Role of Fenofibrate

To investigate whether FABP4 mediated the radioprotective role of fenofibrate, FABP4 inhibitor BMS309403 [36] was used. The results showed that the addition of BMS309403 exacerbated radiation-induced ROS in human skin fibroblasts. Moreover, the ROS-eliminating activity of fenofibrate was abrogated by BMS309403 (Fig. 6A). These results indicated that FABP4 was involved in antioxidant response and that FABP4 mediated the ROS eliminating the role of fenofibrate. Moreover, combined treatment with

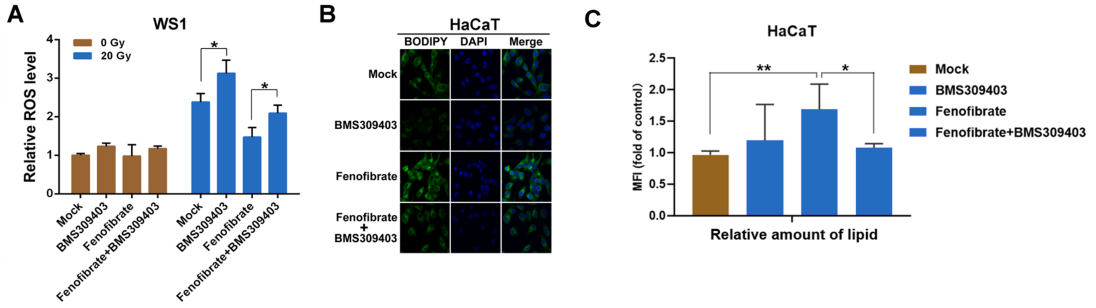

Fig. 6. FABP4 mediates the radioprotective role of fenofibrate. (A) WS1 cells were infected with the indicated adenovirus or treated with fenofibrate and/or BMS309403, followed by 0 or 20 Gy irradiation. Cellular ROS levels for each group of cells were determined 1 h after radiation using a DCF-DA probe and quantified with a microplate reader. (B) The FABP4 inhibitor BMS309403 abrogated fenofibrate-induced lipid accumulation in HaCaT cells. Representative photomicrographs of BODIPY fluorophore 493/503 staining for lipid droplets. (C) Quantification of BODIPY fluorophore 493/503 staining for lipid droplets. $p < 0.05$, compared with the control group.

fenofibrate and BMS309403 abrogated lipid accumulation activity of fenofibrate in keratinocytes, which suggested that FABP4 mediated fenofibrate-induced lipid accumulation (Fig. 6B). Taken together, these above results indicated that FABP4 was likely to mediate the radioprotective role of fenofibrate.

3.7 FABP4 Protects Skin from Radiation-Induced Damage In Vivo

Next, we investigated whether FABP4 overexpression could reduce radiation-induced skin damage *in vivo*. The buttock region of rats was irradiated with a 45 Gy electron beam to model the irradiation-induced skin injury in rats. Irradiation at 45 Gy significantly increased skin ROS levels at three days after treatment, as shown in Fig. 7A, ROS levels were significantly lower in tissues infected with Ad-FABP4 than in the control tissues. Moreover, FABP4 overexpression also reduced radiation-induced MDA levels (Fig. 7B). These results indicate that FABP4 overexpression attenuates lipid peroxidation resulting from radiation-induced oxidation.

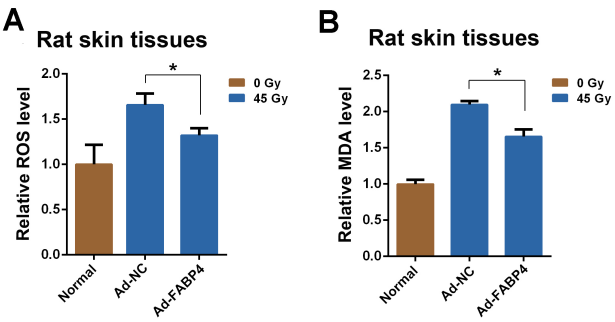

Fig. 7. FABP4 attenuates radiation-induced skin injury in a rat model. Rat gluteal skin was unexposed or irradiated with a single dose from a 45-Gy electron beam, followed by subcutaneous injection of Ad-NC (5×10^9 genomic copies of Ad-NC in a 200-μL volume) or Ad-FABP4 (5×10^9 genomic copies of Ad-FABP4 in a 200-μL volume) (n = 4). (A) Relative ROS levels in rat skin. Three days after irradiation, skin ROS levels were determined as described in the Materials and Methods section. (B) MDA concentration levels in rat skin from different groups at three days after irradiation. $p < 0.05$, compared with the control group.

4. Discussion

Radiation-induced skin damage remains a serious problem following exposure to ionizing radiation, including nuclear accidents, terrorist attacks, and radiation therapy. However, there are currently only limited effective treatments to prevent or mitigate radiation-induced skin damage [5–7]. Our previous report indicates the involvement of the PPAR pathway in the response of skin tissues to ionizing radiation [26]. The three different PPAR isotypes display distinct physiological and pharmacological functions depending on their target genes and tissue distribution [37,38]. Although PPARα as a target for radiation is well established in radiation research, especially in normal tissue injuries such as heart, skin, and brain injuries, PPARα agonists have been shown to confer tissue injury protection in a variety of radiation-induced injury models [17,18]. Previous research has established that persistent alteration of cardiac metabolism due to impaired PPARα activity contributes to the heart pathology after radiation [18]. We also have previously reported the beneficial effect of fenofibrate against radiation-induced skin injury in animal models and human patients [22], but its underlying mechanism remains elusive. In this study, we found that fenofibrate-mediated PPARα activation reduced radiation-induced ROS and apoptosis. Comparatively, equivalent amounts of the PPARγ agonist rosiglitazone [39] did not protect against radiation-induced cutaneous damage in our study (data not shown), indicating a PPARα-specific effect or that these specific rosiglitazone doses are ineffective for this disease. Compared with that of PPARγ, the function of PPARα has been reported to be more restricted to fatty acid

uptake and β-oxidation [10–14]. In addition, the antioxidant and anti-inflammatory roles of PPARα activators have also been reported in specific types of cells [21,22]. For example, the PPARα agonist WY14643 improves homeostasis and the skin barrier function [40]. Fenofibrate has been shown to reduce LPS-induced ROS through GCH1 in human umbilical vein endothelial cells (HUVECs) [41]. We also have previously shown that GCH1 overexpression reduces radiation-induced ROS by inhibiting NOS uncoupling in skin cells [25]. On the other hand, research has confirmed that expression of heme oxygenase-1 (HO-1) in human vascular cells is regulated by peroxisome proliferator-activated receptors [42]. Our previous reports have provided further evidence that increased HO-1 expression due to ionizing radiation suppressed ROS production and reduced radiation-induced skin injury [26,43]. In this study, it was found that PPARα agonist can regulate target protein the expression of FABP4, and it can regulate the expression of FABP4 through regulating lipid antioxidants to reduce ROS production. However, the specific mechanism remains to be further explored. Taken together, this study expands the beneficial application of fenofibrate in treating human diseases.

PPARα, together with RXR, binds to a specific PPRE DNA sequence element with a consensus sequence that consists of a direct repeat of the hexameric sequence AGG(A/T) CA separated by one less well-conserved spacer nucleotide [40]. In this study, we identified FABP4 as a direct target of PPARα activation in skin cells. This finding expands the list of PPARα-regulated targets. Of all the FABPs, FABP4 possesses a unique high affinity for both saturated and unsaturated fatty acids; this function has been well characterized in cellular metabolism homeostasis [34,35]. In addition, FABP4 has also been shown to promote cell growth and metastasis in multiple malignancies, partially through supplying fatty acids and energy [44,45]. We have previously shown that FABP4 facilitates cell migration and the repair of radiation-induced DNA breaks [26]. During wound healing, the skin often requires more energy from the body's energy stores to build new cells and restore the barrier function [46]. PPARα activates FABP4, which can facilitate cellular free fatty acid uptake, deliver essential fatty acids and provide an energy supply for damaged cells. In addition, the skin needs lipids for rapid cornification and the barrier function of the stratum corneum, which is present as a lipid double layer in a lipid matrix [30,31,47]. Therefore, increased levels of FABPs likely provide essential fatty acids for normal metabolism and skin barrier function. Herein, we confirmed that fenofibrate/FABP4 increased lipid accumulation in human keratinocytes. Another PPARα agonist, WY-14643, has been shown to increase cellular lipids in keratinocytes *in vitro* and *in vivo*, which is consistent with our finding [48]. Several skin diseases, such as psoriasis and atopic dermatitis, are associated with reduced skin lipids [49,50]. Therefore, these findings may have significance not only for radiation-induced skin injury but may represent one mechanism in cutaneous diseases. Moreover, we also found that FABP4 mediated the ROS scavenging role of fenofibrate. Thus, PPARα/FABP4 constitutes a novel strategy to ameliorate radiation-induced skin injury. However, the molecular mechanism of FABP4 in eliminating ROS warrants further investigation.

5. Conclusions

In summary, we found that PPARα agonist fenofibrate confers radioprotection by stimulating FABP4 in skin cells (Fig. 8). These findings provide a potential strategy for treating radiation-induced skin injury.

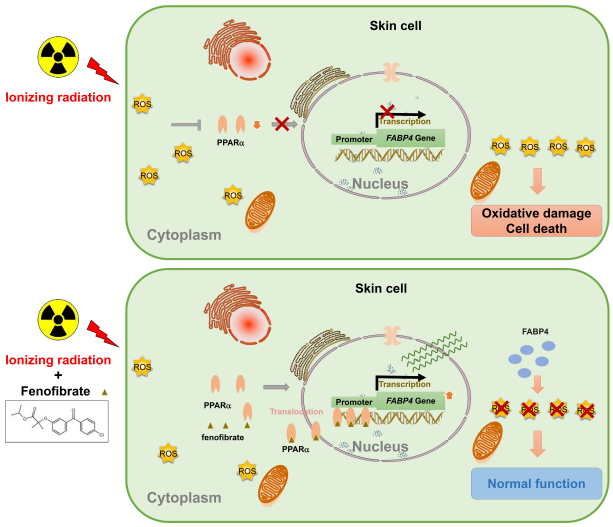

Fig. 8. Schematic representation of PPARα agonist fenofibrate confers radioprotection by stimulating FABP4 in skin cells. PPARα agonist fenofibrate induced PPARα expression in the irradiated skin cells, which results in the proximal promoter of fatty acid binding protein 4 (FABP4) harbored three binding sites recruitment of PPARα and stimulated the transcription of FABP4 in skin cells. FABP4 activation significantly decreased radiation-induced oxidative damage *in vivo*.

Abbreviations

PPARα, peroxisome proliferator-activated receptor α; ROS, reactive oxygen species; FABP4, fatty acid binding protein 4.

Author Contributions

SZ and JZ conceived and designed the study. CS, BS, and DY carried out the molecular biology studies. WS and FG performed the animal experiments. SZ and YJ drafted the manuscript and the figures. YJ, TY and KF performed statistical analyses. All authors read and approved the final

manuscript.

Ethics Approval and Consent to Participate

Ethical approval was obtained from the Ethics Committee of Soochow University (approval number: 2016-0101).

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Conflict of Interest

The authors declare no conflict of interest.

Supplementary Material

Supplementary material associated with this article can be found, in the online version, at https://doi.org/10.31083/j.fbl2707214.

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