Advances in Radiation Oncology (2016) 1, 170-181

advances
in radiation oncology

www.advancesradonc.org

Biology Contribution

FGF18 signaling in the hair cycle resting phase determines radioresistance of hair follicles by arresting hair cycling

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Received 7 January 2016; received in revised form 24 May 2016; accepted 27 May 2016

Abstract

Purpose: Telogen (resting phase) hair follicles (HFs) are more radioresistant than their anagen (growth phase) counterparts. Fibroblast growth factor (FGF) 18 is strongly expressed in telogen HFs to maintain the telogen phase, whereas several other FGFs exert radioprotective effects; however, the role of FGF18 in the radioresistance of HFs remains unknown. This study focused on clarifying the role of FGF18 in the radioresistance of telogen HFs and its potential as a radioprotector.

Methods and materials: BALB/c mice with telogen or plucking-induced anagen HFs were exposed to total body irradiation with γ-rays at 4 to 12 Gy after intraperitoneal treatment with FGF18 or an FGF receptor inhibitor. A time course analysis was performed histologically and hair growth was observed 14 or 15 days after depilation. Skin specimens were analyzed by DNA microarrays and Western blotting.

Results: Telogen irradiation at 6 Gy resulted in transient cell growth arrest, leading to successful hair growth, whereas anagen irradiation failed to promote hair growth. Telogen irradiation did not induce apoptosis in HFs or reduce HF stem cells, whereas anagen irradiation induced apoptosis and reduced stem cell numbers. The Inhibition of FGF receptor signaling during the telogen phase promoted HF cell proliferation; however, hair failed to grow after irradiation. In contrast, recombinant FGF18 induced transient cell growth arrest after anagen irradiation with enhanced

Sources of support: This work was supported in part by the Cosmetology Research Foundation and Japan Society for the Promotion of Science Grants-in-Aid for Scientific Research (KAKENHI) Grant Number 24591856.

Conflicts of interest: None.

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http://dx.doi.org/10.1016/j.adro.2016.05.004

DNA repair, leading to the inhibition of apoptosis, maintenance of HF stem cells, and successful hair growth. Moreover, FGF18 reduced the expression levels of genes promoting G2/M transition as well as the protein expression levels of cyclin B1 and cdc2 in skin, and induced G2/M arrest in the keratinocyte cell line HaCaT.

Conclusions: These results suggest that FGF18 signaling mediates radioresistance in telogen HFs by arresting the cell cycle, and that FGF18 has potential as a radioprotector for radiation-induced alopecia.

Introduction

Radiation therapy is one of the most effective cancer treatments currently available; however, irradiation at high doses causes hair loss, which may lead to cosmetic and/or mental issues, thereby reducing the quality of life of cancer patients. We previously investigated murine hair follicle (HF) dystrophy induced by irradiation at relatively high doses that cause permanent alopecia using stage-specific histological parameters,[1,2] and found that this type of dystrophy is characterized by similar morphological changes to those of dystrophic catagen induced by chemotherapy.[3] However, temporary alopecia induced by relatively low doses of irradiation has not yet been examined in detail, despite its greater potential to be treated successfully.

Radiation generally causes more damage to rapidly dividing cells than nondividing cells.[4] The radiosensitivity of HFs also depends on the hair cycle stage, that is, telogen (resting phase) matrix cells are more radioresistant than proliferating anagen (growth phase) cells[5] because anagen HF cells are proliferating more rapidly than telogen ones. Moreover, a previous study reported that the expression of a DNA double-strand break (DSB) marker was weaker in telogen HFs than in anagen HFs, even without irradiation.[6] These findings suggest that high DNA repair activity levels may contribute to the radioresistance of telogen HFs; however, the molecular mechanisms underlying radioresistance during telogen remain unknown.

The hair cycle depends on the cyclic activation of HF stem cells, which determines the proliferation, differentiation, and apoptosis of a variety of hair-forming cells. HF stem cells are located in the bulge region of HFs and characterized by stem cell markers such as K15 or CD34.[7-9] Fibroblast growth factors (FGFs) play a key role in regulating the hair cycle.[10] The expression of FGF18 transcripts has been shown to increase during the telogen phase and decease during the anagen phase.[11] FGF18 is expressed in K6-positive bulge cells together with bone morphogenetic factor 6 during telogen[12] to maintain bulge stem cell quiescence.[9] In addition, FGF18 inhibits the proliferation of hair germ cells expressing a low level of FGF18 transcripts.[13] Accordingly, FGF18 maintains the telogen phase and determines its duration, while simultaneously inhibiting the anagen phase.[14] In contrast, several FGFs protect against radiation-induced damage.[15-19] Moreover, a treatment with FGF7 was previously shown to increase hair survival after x-ray irradiation.[20] However, the radioprotective effects of FGF18 have not yet been elucidated in detail.

We have investigated the involvement of FGF18 in the radioresistance of telogen HFs in BALB/c mice and the in vivo radioprotective effects of FGF18 on anagen HFs using symptoms, histological parameters, and a molecular analysis. Our results demonstrated that FGF18 mediated the radioresistance of HFs by arresting the cell cycle during the telogen phase and has a potential as a radioprotector against radiation-induced alopecia.

Methods and materials

Reagents

Recombinant human FGF18 was purchased from PeproTech (Rocky Hill, NJ) or produced as reported previously.[21] AZD4547,[22] a pan-FGF receptor (FGFR) inhibitor, was purchased from AdooQ Bioscience (Irvine, CA). Antibodies and other reagents are listed in Table E1 (available as supplementary material online only at www.practicalradonc.org).

Animals

Seven-week-old male BALB/c mice were depilated to induce anagen HFs and subjected to total body irradiation (TBI) with γ-rays, as described previously (Appendix E1).[3,19] FGF18 was administered intraperitoneally in 0.5 mL of saline containing 5% mouse serum or 5 μg/mL heparin to mice 24 hours before TBI. Dorsal skin specimens were sampled over time after depilation and TBI, and staining with hematoxylin and eosin was performed on paraffin-embedded sections. Hematoxylin and eosin–stained longitudinal HF sections were photographed, and more than 1000 HFs were screened in each

mouse to assess histological parameters by measuring HF numbers (density), HF lengths, and hair bulb diameters on the photographs (n = 2-5). All protocols complied with the Animal Care Guidelines of the National Institute of Radiological Sciences and were approved by the Animal Research Committee for Animal Experimentation of the National Institute of Radiological Sciences.

TUNEL assay

Apoptosis was evaluated in paraffin-embedded sections of skin by a terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) analysis as described previously (Appendix E1).[19]

BrdU assay

The 5-bromo-2′-deoxyuridine (BrdU) assay was performed as described previously (Appendix E1).[18] Mouse immunoglobulin G was blocked using a VECTOR M.O.M. immunodetection kit (Vector Laboratories, CA) according to the manufacturer's protocol.

Western blot assay

The expression of FGF18, a cell-cycle regulator, and DNA repair proteins in skin was analyzed by Western blotting as described in Appendix E1. The experimental conditions for each antibody are described in Table E1.

Microarray analysis

The messenger RNA expression profiles of irradiated skin were obtained by DNA microarray analysis 4 hours after the FGF18 pretreatment, as described in Appendix E1. Microarray data were submitted to the National Center for Biotechnology Information Gene Expression Omnibus (accession no.: GSE69501).

Cell-cycle analysis

The cell cycle of the human keratinocyte cell line HaCaT was analyzed by propidium iodide staining and flow cytometry as described in Appendix E1.

Statistical analysis

All values represent the mean ± standard deviation of results obtained from 10 samples in each mouse (n = 2-5), and the values in each group were compared using analysis of variance and Fisher's protected least significant difference (*$P < .05$; **$P < .01$; ***$P < .001$).

Results

Irradiation during telogen suppresses the development of HFs, leading to hair regeneration

BALB/c mice were subjected to TBI at anagen or telogen to assess the radiosensitivity of HFs by hair growth and histological parameters of HF dystrophy (Fig 1A). Missing data depend on the radiation doses and days after irradiation because mice were exposed to TBI at lethal doses (Fig 1). After exposure to irradiation at 4 Gy, hair regenerated on the back 14 days after depilation. However, anagen irradiation at more than 6 Gy markedly delayed hair regeneration 14 days after depilation (Fig 1B). In contrast, hair regeneration was observed on the back 14 days after telogen irradiation at either 4 or 6 Gy (Fig 1B). Histopathologically, whole HFs were markedly degenerated after anagen irradiation at more than 6 Gy; this was accompanied by hair bulb shrinkage (Fig 1C). On the other hand, follicle length was shortened after telogen irradiation. However, significant morphological dystrophy was not detected in HFs after telogen irradiation at 4, 6, and 8 Gy (Fig 1C).

HF lengths were significantly shorter in anagen- and telogen-irradiated mice that in the nonirradiated control, and these reductions occurred in a dose-dependent manner (Fig 1D). HF lengths returned to the control length 9 days after telogen irradiation at 4 Gy, but continued shortening for up to 14 days after irradiation at 6 Gy (Fig 1D). The hair bulb, which is the thick proximal end of HF, increases in size until anagen IIIc and is a primary target of pharmacological manipulations.[23] Irradiation has also been shown to influence the diameter of the hair bulb; thus, it may be a good indicator of radiation damage.[3] In the present study, the diameter of the hair bulb decreased 3 days after anagen irradiation (Fig 1E). It then started to increase and surpassed the diameter of the nonirradiated control 6 days after anagen irradiation at 4 Gy. However, the diameter of the hair bulb did not increase after anagen irradiation at more than 6 Gy and remained smaller than that of the nonirradiated control (Fig 1E). On the other hand, telogen irradiation induced the growth of hair bulb even at 6 Gy, and was slightly larger than that of the nonirradiated control 12 days after irradiation (Fig 1E).

Irradiation during telogen arrests cell proliferation in HFs

BALB/c mice were subjected to anagen or telogen irradiation to assess the proliferation and apoptosis of hair bulb cells as well as the number of CD34+ HF stems in HFs (Fig 2A). During anagen, HF keratinocytes strongly proliferated in the hair bulb and continued proliferating for 3 days after anagen irradiation at 4, 6, and 8 Gy (Fig 2B). In contrast, very few cells proliferated in HF during telogen.

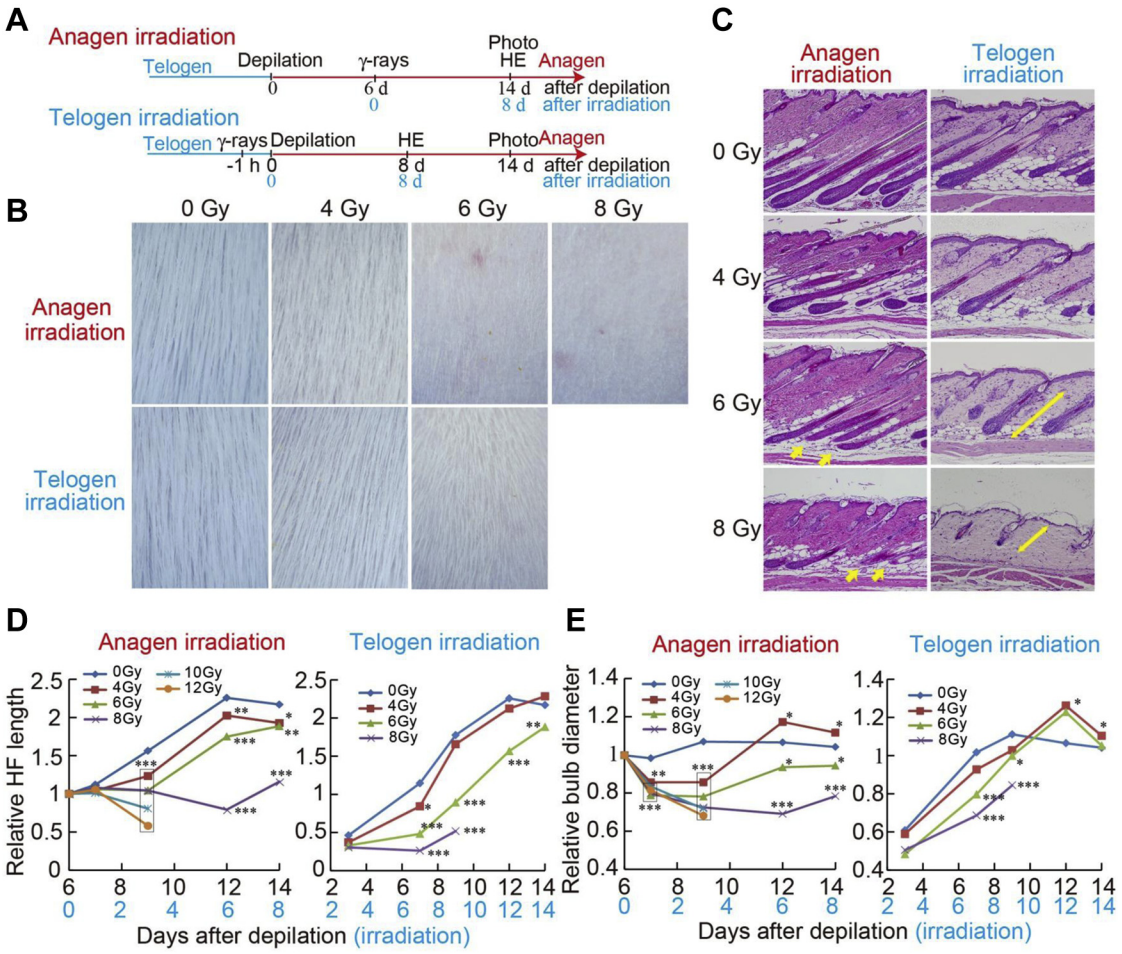

Figure 1 Telogen hair follicles are radioresistant. (A) Experiment protocols of radiation-induced hair damage depending on the hair-cycle stage. Anagen hair follicles (HFs) were irradiated 6 days after depilation (anagen irradiation), whereas telogen HFs were irradiated and then depilated 1 hour after irradiation (telogen irradiation). (B) BALB/c mice were subjected to total body irradiation (TBI) during anagen or telogen with γ-rays at the indicated doses. Representative appearance of skin 14 days after depilation. (C) Representative hematoxylin and eosin–stained sections of dorsal skin 8 days after TBI during anagen or telogen. HF dystrophy (yellow arrows) and shortened HFs (double-pointed arrows) were observed after anagen and telogen irradiation, respectively. (D) Lengths of HF and (E) bulb diameters were determined in HE-stained sections of dorsal skin after TBI. Each value is shown as a relative value from the nonirradiated control 6 days after depilation. $*P < .05$; $**P < .01$; $***P < .001$ significantly different from the nonirradiated control.

Telogen irradiation further reduced cell proliferation, resulting in complete arrest 3 days after telogen irradiation at 6 Gy with postirradiation depilation (Fig 2B). Although radiation-induced apoptosis was not detectable in the bulge under the current experimental conditions, anagen irradiation induced apoptosis in the hair bulb in a dose-dependent manner.[3] However, in contrast to anagen irradiation, telogen irradiation did not induce apoptosis in HFs (Fig 2C). The number of HF stem cells progressively decreased in the bulge over time during the anagen phase (Fig E1). In addition, anagen irradiation further reduced the number of CD34+ and K15+ stem cells in HFs in a dose-dependent manner (Fig 2D; Fig E2A-B). However, telogen irradiation did not significantly reduce the number of HF stem cells, regardless of postirradiation depilation (Fig 2D; Fig E2A-B). LGR5+ cells are actively proliferating and multipotent stem cells that are located in the lower outer sheath of anagen HFs.[24] Irradiation markedly reduced LGR5+ stem cell numbers during anagen (Fig E2C). In contrast, LGR5+ cells in the bulge and secondary germ of telogen HFs were maintained after irradiation (Fig E2C); therefore, cyclic activation of HF stem cells during growth phase may contribute to the greater radiosensitivity of stem cells.

FGF signaling is critical for the radiosensitivity of HFs

FGF18 is strongly expressed in telogen HFs; however, its expression was markedly weaker in in HFs after the induction of anagen by depilation and remained at low levels up to 15 days after depilation (Fig 3A; Fig E3A). The expression of FGF18 also correlated with that of the telogen marker Krt24 (Fig E3AB).[25] FGF18 regulates the

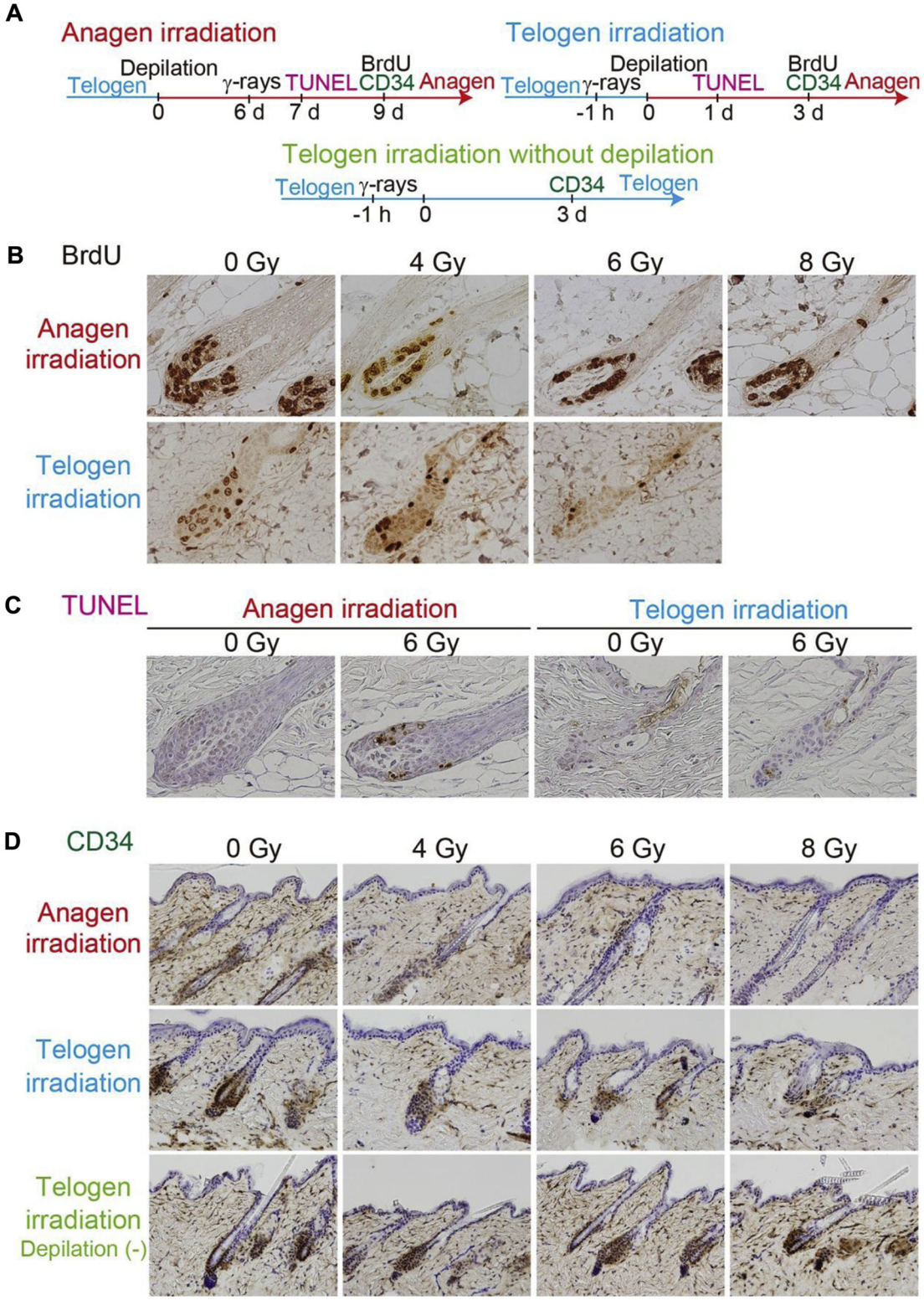

Figure 2 Proliferation of hair follicle (HF) cells is arrested after telogen irradiation. (A) Protocols of experiments following anagen or telogen irradiation. Telogen irradiation was performed without depilation for CD34 staining. (B) The cell proliferation of HFs was examined by the incorporation of 5-bromo-2′-deoxyuridine (BrdU) 3 days after irradiation at the indicated doses. (C) Terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) assays were performed using paraffin-embedded sections 24 hours after irradiation. (D) Staining with an anti-CD34 antibody was performed on frozen sections 3 days after anagen or telogen irradiation at the indicated doses to evaluate reductions in HF stem cell numbers. In addition, experiments were performed without the induction of anagen by depilation after irradiation at telogen.

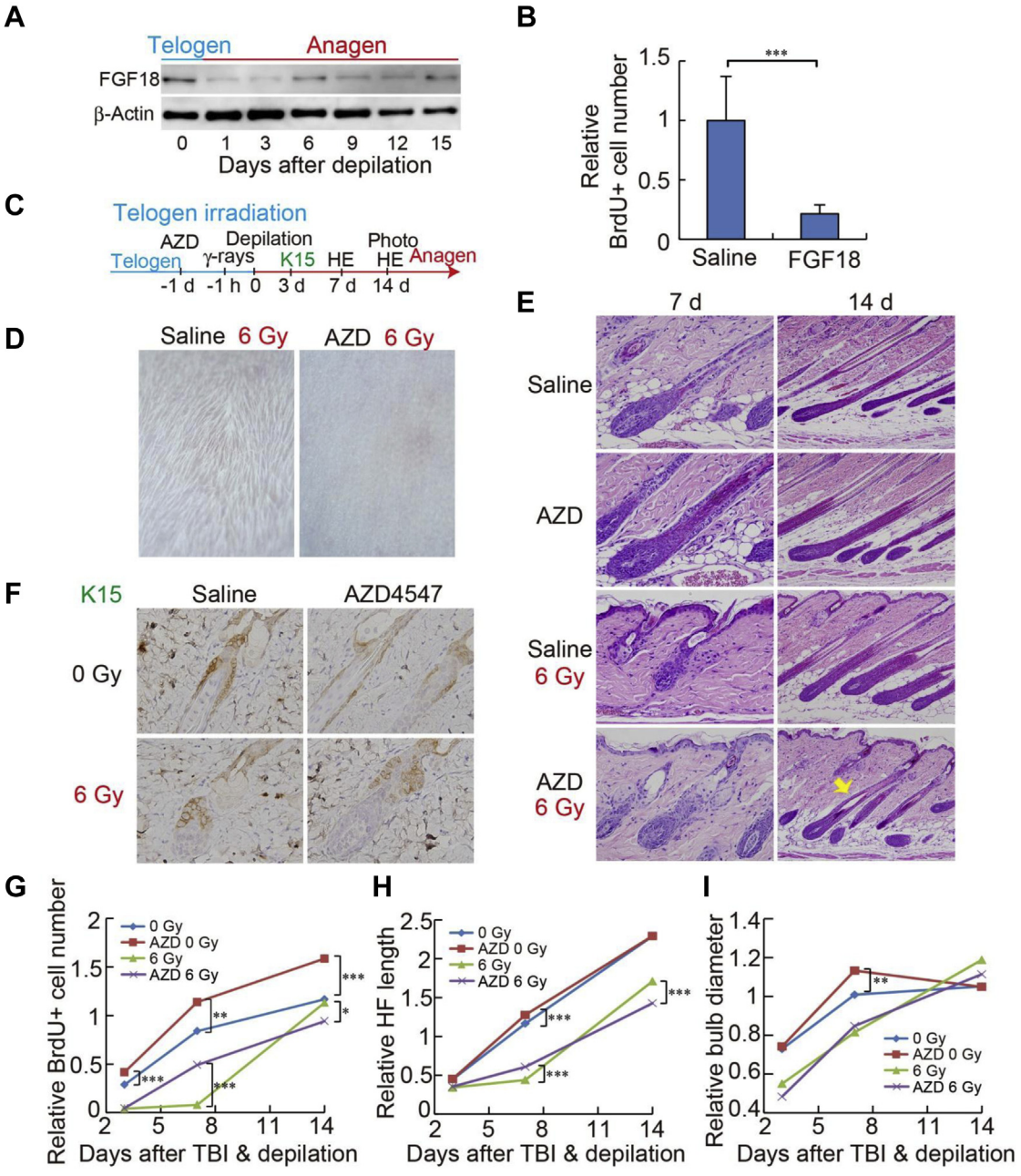

Figure 3 Inhibition of fibroblast growth factor receptor (FGFR) signaling increases the radiosensitivity of telogen hair follicles (HFs). (A) The protein expression level of fibroblast growth factor 18 (FGF18) over time in skin was examined by Western blot analysis after depilation. (B) The relative number of 5-bromo-2′-deoxyuridine (BrdU+) cells in HFs was examined 24 hours after depilation. One hundred micrograms of FGF18 was administered intraperitoneally 1 hour before depilation. (C) Protocol of experiments using the FGFR inhibitor AZD4547. Five hundred microliters of 0.5 mM AZD4547 was administered intraperitoneally 24 hours before telogen irradiation. (D) Representative appearance of skin 14 days after irradiation at 6 Gy. (E) Representative hematoxylin and eosin (HE)-stained sections of dorsal skin 7 and 14 days after irradiation. HF dystrophy was observed 14 days after telogen irradiation with the AZD4547 pretreatment (yellow arrow). (F) Staining with an anti-K15 antibody was performed using paraffin-embedded sections 3 days after irradiation. (G) Cell proliferation in HFs, (H) relative lengths of HFs, and (I) relative bulb diameters were examined over time after TBI. $^{*}P < .05$; $^{**}P < .01$; $^{***}P < .001$.

hair cycle by maintaining the telogen phase and inhibiting the anagen phase,[14] and compensation by FGF18 inhibited depilation-induced cell proliferation in HFs (Fig 3B). Therefore, the involvement of FGF in radiation-induced HF damage was examined by administrating an FGFR inhibitor to mice during telogen (Fig 3C). The FGFR inhibitor (AZD4547) suppressed hair growth after telogen irradiation (Fig 3D). In contrast, the FGFR inhibitor

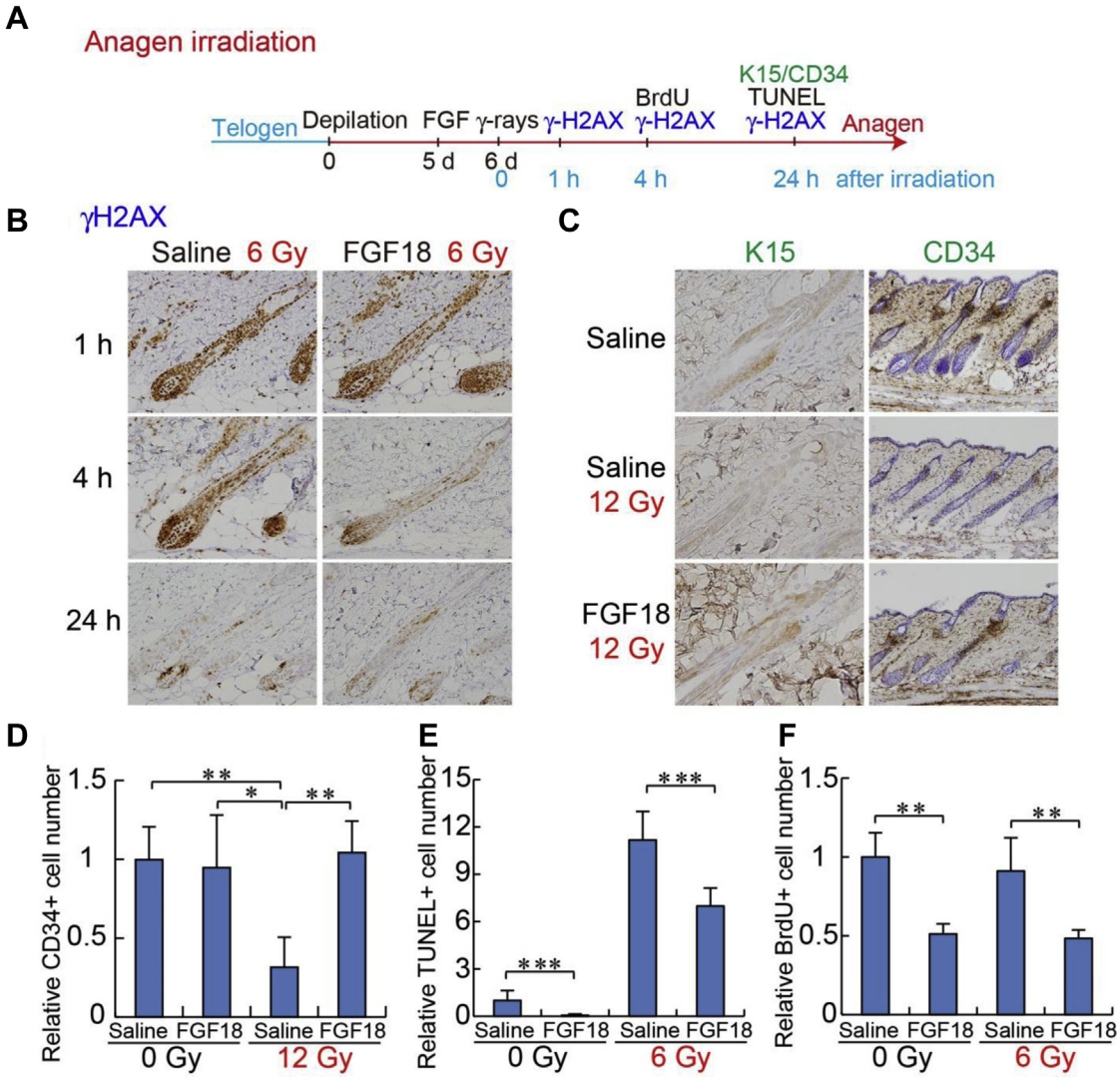

Figure 4 Exogenous FGF18 protects hair follicle (HF) keratinocytes and stem cells from radiation-induced damage. (A) Protocol of experiments after the administration of recombinant fibroblast growth factor 18 (FGF18). One hundred micrograms of FGF18 was administered intraperitoneally 24 hours before anagen irradiation. (B) Staining with an anti-γH2AX antibody was performed using paraffin-embedded sections 1, 4, and 24 hours after anagen irradiation to evaluate the repair of DNA double-strand breaks in hair bulb cells. (C) Staining with anti-K15 and anti-CD34 antibodies was performed 24 hours after irradiation at 12 Gy to detect HF stem cells. (D) The number of HF CD34+ cells was assessed in the HF bulge and secondary hair germ 24 hours after irradiation at 12 Gy. Each value is shown as a relative value from the number of cells in the nonirradiated saline control. (E) Apoptosis was determined by terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) assays 24 hours after anagen irradiation. (F) Cell proliferation in HFs was examined by the incorporation of 5-bromo-2′-deoxyuridine (BrdU) 4 hours after irradiation at 6 Gy. $^{*}P < .05$; $^{**}P < .01$; $^{***}P < .001$.

promoted HF development during the initial anagen phase, but induced morphological dystrophy in HFs 14 days after irradiation (Fig 3E). In addition, the FGFR inhibitor slightly decreased the number of K15+ stem cells in HFs without irradiation because the inhibition of FGFR during telogen induced the anagen phase instead of depilation (Fig 3F). The FGFR inhibitor increased the incorporation of BrdU into HF cells and slightly enhanced the elongation of HFs 7 days after irradiation, which were shorter 14 days postirradiation than the irradiated control (Fig 3G-H). Similar to the HF length, the bulb diameter also showed a similar change in size in response to the FGFR inhibitor and irradiation: however, these changes were not significantly different form the irradiated control (Fig 3I).

Exogenous FGF18 protects HF keratinocytes and stem cells from radiation-induced damage

The involvement of FGF18 in the protection of HF keratinocytes and stem cells during exposure to radiation was examined by assessing DNA DSBs, stem cells, apoptosis, and proliferation (Fig 4A). The expression of γH2AX, a DSB marker,[26] was enhanced HFs 1 hour after irradiation at 6 Gy (Fig 4B); this was followed by DNA

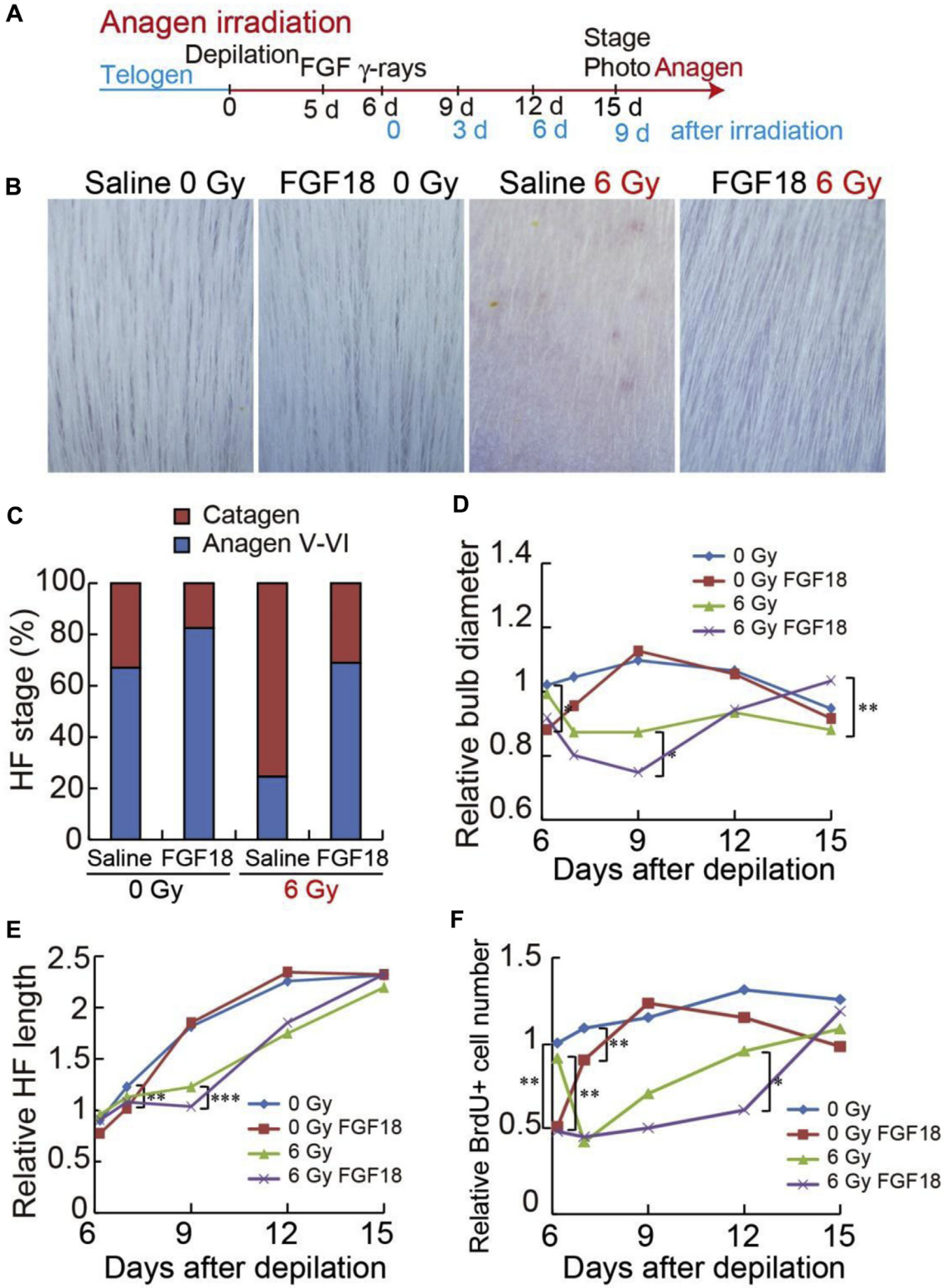

Figure 5 Fibroblast growth factor 18 (FGF18) promotes hair regeneration after irradiation. (A) Protocol of experiments after the administration of recombinant FGF18. One hundred micrograms of FGF18 was administered intraperitoneally 24 hours before anagen irradiation at 6 Gy. (B) Representative appearance of skin 9 days after irradiation. (C) The proportion of hair follicles (HFs) at each hair-cycle stage was measured by hematoxylin and eosin staining of dorsal skin 15 days after total body irradiation. (D) Changes in hair bulb diameters and (E) HF lengths over time were measured after irradiation. (F) Cell proliferation in HFs was examined by the incorporation of terminal deoxynucleotidyl transferase dUTP nick end labeling over time after irradiation. Each value is shown as a relative value from the nonirradiated control 6 days after depilation. $*P < .05$; $**P < .01$; $***P < .001$ significantly different from the nonirradiated control.

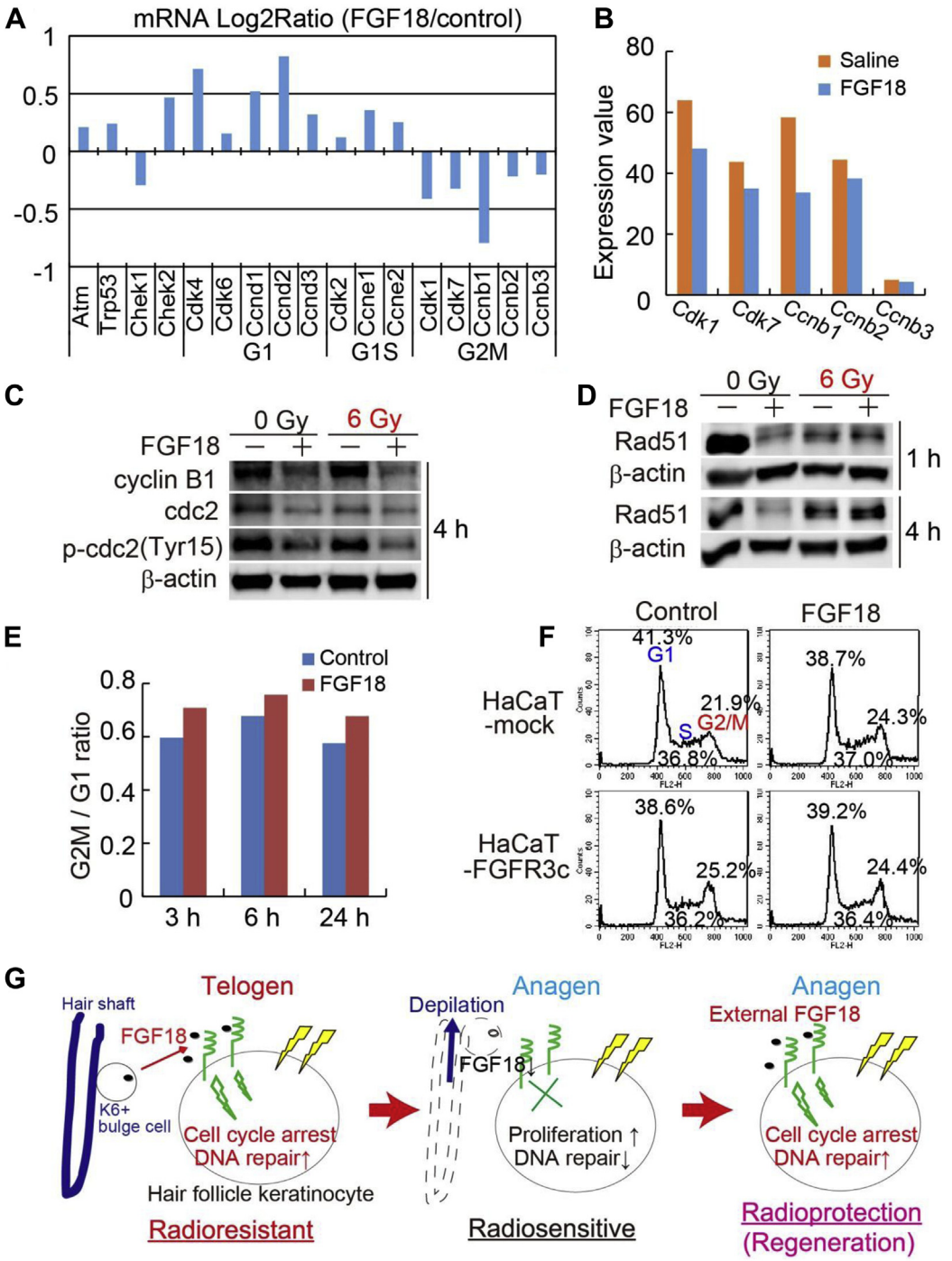

Figure 6 Fibroblast growth factor 18 (FGF18) enhances G2/M arrest in keratinocytes. (A-D) One hundred micrograms of FGF18 was administered intraperitoneally 24 hours before anagen irradiation at 6 Gy. (A) Total RNA was isolated from skin 4 hours after total body irradiation (TBI). The expression levels of each gene in FGF18-treated mice relative to those in saline-treated mice were measured by DNA microarrays. (B) The expression levels of genes promoting G2/M transition were measured by DNA microarrays. (CD) Cell lysates were prepared from dorsal skin specimens isolated 1 and/or 4 hours after TBI and subjected to a Western blot analysis using anti-cyclin B1, anti-cdc2, anti-phospho-cdc2 (Tyr15), and anti-Rad51 antibodies. (E) The cell-cycle distribution of HaCaT cells was examined 3, 6, and 24 hours after the treatment with 100 ng/mL FGF18 by propidium iodide (PI) staining using flow cytometry. The ratio of G2/M to G1 cell numbers was calculated. (F) The cell-cycle distributions of HaCaT-mock and HaCaT-FGFR3c transfectants were examined by PI staining using flow cytometry 24 hours after the treatment with 100 ng/mL FGF18. (G) FGF18 is expressed in K6-positive bulge cells[12] to maintain bulge stem cell quiescence[9]. FGF18 signaling mediates the relationship between radioresistance and the hair-cycle stage. FGF18 plays a key role in both the resting state of hair follicles (HFs) and cell-cycle arrest of HF cells for DNA repair. In addition, the recombinant FGF18 protein promotes the regeneration of HFs after irradiation by enhancing the repair of HF damage through the slowing of hair cycling. mRNA, messenger RNA.

damage repair, leading to the disappearance of most γH2AX foci 24 hours postexposure. A pretreatment with recombinant FGF18 reduced γH2AX foci in the HFs 4 hours after irradiation (Fig 4B). However, FGF18 did not affect γH2AX foci 1 hour after TBI, suggesting that it is associated with DSB repair, but not the induction of DSBs. The application of FGF18 also inhibited reduction in K15+ and CD34+ stem cell numbers after irradiation (Fig 4CD) and decreased radiation-induced apoptosis in the hair bulb (Fig 4E); thus, FGF18 may protect stem and progenitor cells in anagen HFs against radiation damage. On the other hand, the number of BrdU-labeled cells in FGF18-treated mice was decreased to 50% of that in control mice (Fig 4F). These results suggest that the administration of FGF18 may arrest the growth of HFs during anagen, resulting in increases in DNA repair and cell survival in the hair bulb and HF stem cells.

FGF18 promotes hair regeneration after radiation damage

The effects of FGF18 on hair regeneration after irradiation were examined in anagen HFs (Fig 5A). Although anagen irradiation at 6 Gy delayed hair regeneration, exogenous FGF18 induced successful hair growth even after anagen irradiation at 6 Gy (Fig 5B). Irradiation during anagen increased anagen-catagen transition and increased the number of catagen HFs 15 days after depilation; however, the FGF18 treatment suppressed the radiation-induced anagen-catagen transition of HFs (Fig 5C). The FGF18 treatment decreased the hair bulb diameter more than the saline treatment 3 days after irradiation; this was followed by full recovery (Fig 5D). Moreover, the HF length was reduced by the FGF18 treatment 3 days after irradiation; this was followed by an increase in size to more than that of the control without the FGF18 treatment (Fig 5E). Irradiation transiently reduced the number of proliferating cells in hair bulbs to approximately 50% that in the nonirradiated control (Fig 5F). The FGF18 treatment reduced the number of proliferating cells after anagen irradiation more significantly than in the irradiated control, which maintained a lower level of proliferation for a longer duration than the irradiated control. Accordingly, the time course of HF development during the FGF18 treatment was similar to the pattern of the successful regrowth of HFs, including the early suppression and late overgrowth stages (Fig 1E).

FGF18 induces G2/M arrest in keratinocytes to mediate the radioresistance of HFs

The FGF18 treatment promoted the repair of DNA damage by reducing DSBs in the HFs (Fig 4B). DSBs are the most frequently induced and harmful type of DNA damage incurred by irradiation and are repaired by nonhomologous end joining or homologous recombination through the assembly of DNA repair molecules.[27] To screen for molecules involved in FGF18-induced radioresistance, the messenger RNA expression profiles of irradiated skin were obtained using a DNA microarray analysis 24 hours after the FGF18 pretreatment. The expression levels of genes that promote G2/M transition, *Cdk7*, *Cdk1* (cdc2), and *Ccnb1* (cyclin B1), were lower than those in the control, whereas those of genes that promote G1 phase progression, *Cdk4, Ccnd2* (cyclin D2), and *Ccnd1* (cyclin D1), were higher (Fig 6A-B and Fig E4A). In addition, the pretreatment with FGF18 reduced the protein expression of cyclin B1 and cdc2 in skin (Fig 6C). Although the dephosphorylation of cdc2 (Tyr 15), a marker of the activation of cdc2, was observed after the FGF18 treatment, it may have been due to compensation for the reduction in cdc2 protein levels. These results suggest that FGF18 promotes G2/M arrest in skin after irradiation, because G2/M transition is tightly regulated by the cdc2/cyclin B complex.[28] In contrast, the FGF18 treatment did not increase the expression of DNA repair genes in skin (Fig E4B). The protein expression of Rad51, which plays an essential role in DNA repair by homologous recombination,[27] did not increase in skin or HFs after the FGF18 treatment (Fig 6D; Fig E5).

FGF18 induced cell-cycle arrest at G2/M in HaCaT keratinocytes 3, 6, and 24 hours after the addition of FGF18 (Fig 6E). Furthermore, transfection of the human *FGFR3c* gene into HaCaT cells increased the G2/M population without the addition of FGF18 (Fig 6F) because FGFR3c is a receptor of FGF18.[29] The G2/M arrest induced in HaCaT keratinocytes by FGF18 was consistent with the reduced expression of cdc2 and cyclin B1 in skin after anagen irradiation with the FGF18 pretreatment (Fig 6A-C).

Discussion

DNA damage arrests cell-cycle progression, which provides cells with time to repair the damage induced.[30] In the present study, successful hair growth after anagen irradiation was characterized by the morphological pattern of HF changes, namely early suppression and late growth stages (Fig 1E). A similar pattern of HF changes was observed after telogen irradiation at 6 Gy, which resulted in hair regrowth (Fig 1E). In contrast, FGF18 was strongly expressed during telogen (Fig 3A; Fig E3A),[11] and has been shown to play an essential role in the maintaining the telogen phase.[14] Moreover, the inhibition of FGFR induced a reversal in the morphological pattern of hair regrowth, comprising early growth and late suppression stages (Fig 3). These results suggest that FGF18 signaling is involved in the regeneration of HFs after irradiation through the suppression of HF development.

Recombinant FGF18 effectively enhanced the primary recovery of HFs after irradiation because FGF18 mediated the cessation of HF development, inhibition of apoptosis in HF keratinocytes, and maintenance of HF stem cells, thereby promoting the repair of HF damage (Fig 4, 5). The rationale for these results was FGF18 enhancing G2/M arrest in keratinocytes to provide more time for DNA repair in these cells (Fig 6). Although FGF18-induced G2/M arrest has not been demonstrated in cells, FGF2 has been shown to induce G2/M arrest in Ras-driven mouse malignant cell lines.[31] FGF1 also reduces cyclin B1/CDK1 activity to induce transient G2 arrest in rat chondrosarcoma chondrocytes. However, the mechanisms underlying FGF1-induced reductions in cyclin B1/CDK1 have not yet been clarified, but have already been investigated in an attempt to identify the signaling pathways responsible using several inhibitors including an ERK1/2 inhibitor.[32] Thus, further studies are needed to identify novel signaling molecules to clarify the involvement of FGF/FGFR signaling pathways in cell-cycle regulation. Although FGF1 is expressed in HFs during telogen[11] and has markedly broader FGFR specificity than FGF18,[29] recombinant FGF1 was found to be less effective for promoting HF regeneration after radiation damage than FGF18 (data not shown). In contrast, FGF18 has been shown to react with limited members of FGFRs including FGFR3c[29] and chondrocytes express FGFR3[33]; therefore, FGFR3 signaling may play a key role in the induction of G2/M arrest by FGF18.

Although telogen HFs have a morphologically quiescent appearance, telogen is an energy-efficient default state for a new regeneration cycle.[34] At the level of transcriptional regulation, the expression of key components of G2/M progression: *Cdk1, Cdca2, Nek2, Cenpf, Cenpn*, and *SMC2*, was decreased during the telogen phase, and this may have been because some HF cells are arrested in G2 to enable fast anagen activation.[25] During late telogen, hair germs contain a significantly higher percentage of G2/M phase cells than the bulge.[13] FGF18 is strongly expressed during telogen; therefore, it may exert cytostatic effects on HF keratinocytes by inducing G2/M arrest to provide time for the indirect promotion of DNA repair, leading to the development of radioresistance in telogen HFs. FGF18 maintains bulge stem cell quiescence[9] and inhibits the proliferation of hair germ cells that express a low level of FGF18 transcripts.[13] Although irradiation reduced not only the numbers of K15+ and CD34+ stem cells, but also that of LGR5+ cycling stem cells during anagen (Fig 2D; Fig E2), exogenous FGF18 maintained HF stem cell numbers after anagen irradiation (Fig 4C-D); therefore, FGF18 may exert cytostatic effects on not only keratinocytes, but also HF stem cells.

Thus, FGF18 signaling mediates the relationship between radioresistance and the hair-cycle stage because it plays a key role in both the resting state of HFs and cell-cycle arrest of HF cells for DNA repair. Although the expression of FGF18 decreased in HFs during anagen, that of FGFR3 gradually increased during the anagen phase, and this increase was enhanced by irradiation (Fig E3C-D). Therefore, FGF18 may be more reactive to FGFR3-expressing keratinocytes during anagen after irradiation, and, as such, the recombinant FGF18 protein could promote regeneration of anagen HFs by enhancing the repair of HF damage through the slowing of hair cycling (Fig 6G).

Accordingly, our results revealed that FGF18 mediates the radioresistance of telogen HFs. FGF18 has potential as a radioprotector, and its effects may provide a new approach for the treatment and prevention of radiation-induced alopecia.

Supplementary data

Supplementary material for this article (http://dx.doi.org/10.1016/j.adro.2016.05.004) can be found at www.practicalradonc.org.

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