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Far-infrared radiation alleviates cisplatin-induced vascular damage and impaired circulation via activation of HIF-1α

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Abstract

Severe vascular damage and complications are often observed in cancer patients during treatment with chemotherapeutic drugs such as cisplatin. Thus, development of potential options to ameliorate the vascular side effects is urgently needed. In this study, the effects and the underlying mechanisms of far-infrared radiation (FIR) on cisplatin-induced vascular injury and endothelial cytotoxicity/dysfunction in mice and human umbilical vein endothelial cells (HUVECs) were investigated. An important finding is that the severe vascular stenosis and poor blood flow seen in cisplatin-treated mice were greatly mitigated by FIR irradiation (30 minutes/day) for 1-3 days. Moreover, FIR markedly increased the levels of phosphorylation of PI3K and Akt, and VEGF secretion, as well as the expression and the activity of hypoxia-inducible factor 1α (HIF-1α) in cisplatin-treated HUVECs in a promyelocytic leukemia zinc finger protein (PLZF)-dependent manner. However, FIR-stimulated endothelial angiogenesis and VEGF release were significantly diminished by transfection with HIF-1α siRNA. We also confirmed that HIF-1α, PI3K, and PLZF contribute to the inhibitory effect of FIR on cisplatin-induced apoptosis in HUVECs. Notably, FIR did not affect the anti-cancer activity and the HIF-1α/VEGF cascade in cisplatin-treated cancer cells under normoxic or hypoxic condition, indicating that the actions of FIR may specifically target endothelial cells. It is the first study to demonstrate that FIR effectively attenuates cisplatin-induced vascular damage and impaired angiogenesis through activation of HIF-1α–dependent processes via regulation of PLZF and PI3K/Akt. Taken together, cotreatment with the noninvasive and easily performed FIR has a therapeutic potential to prevent the pathogenesis of vascular complications in cancer patients during cisplatin treatment.

KEYWORDS
angiogenesis, cisplatin, far-infrared radiation, HIF-1α, vascular damage

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1 | INTRODUCTION

Currently, chemotherapeutic drugs are still widely used as anticancer drugs by inducing cellular apoptosis. Unfortunately, administration of chemotherapeutic drugs often causes vascular complications and cellular toxicity due to their unspecific effects.[1,2] Many clinical and experimental studies have indicated that the vascular complications caused by cisplatin, the most common chemotherapeutic drug, are characterized by thromboembolism, increased intima-media thickness of the carotid artery, and impaired angiogenesis.[3–6] Therefore, preventing vascular damage, impaired blood flow, and endothelial toxicity/dysfunction is an effective way to ameliorate these vascular complications caused by cisplatin.

Hypoxia-inducible transcription factor (HIF), a transcriptional factor, consists of an inducible oxygen-sensitive alpha subunit (HIF-1α) and a constitutive oxygen-insensitive beta subunit (ARNT/HIF-1β). In response to hypoxia or reactive oxygen species, the protein stability of HIF-1α is markedly increased by suppressing ubiquitin proteasome system–mediated HIF-1α degradation.[7] Then, the HIF-1α translocates into the nucleus and dimerizes with ARNT to initiate the transcription of downstream target genes involved in glucose metabolism, cell growth, and cellular survival.[8,9] Additionally, HIF-1α is a key transcriptional factor promoting angiogenesis by upregulating proangiogenic genes such as vascular endothelial growth factor (VEGF).[9] The VEGF and phosphatidylinositol-3 kinase (PI3K)/protein kinase B (Akt) pathways are thought to enhance various cell survival, including vascular endothelial cells.[10–12] Notably, the PI3K/Akt/mTOR signaling pathway is confirmed to increase HIF-1α expression and VEGF release and subsequent endothelial angiogenesis.[13] As angiogenesis is essential for revascularization, blood flow, wound healing, and tissue regeneration, maintaining angiogenic activity is a promising target to mitigate cardiovascular diseases.[14] Accordingly, we hypothesize that reinforcing the PI3K/Akt/HIF-1α/VEGF signaling pathway is able to attenuate cisplatin-induced endothelial toxicity and dysfunction.

Several studies have confirmed that far-infrared radiation (FIR) exhibits a protective effect against cardiovascular disorders and endothelial inflammation via heme oxygenase-1 induction.[15,16] These results suggest that FIR may be an alternative option to alleviate vascular injury and endothelial dysfunction. However, to our knowledge, whether FIR attenuates cisplatin-triggered vascular damage and stenosis and endothelial apoptosis has not yet been investigated. Therefore, in the present study the impact of FIR on the pathological effects of cisplatin on vessels and endothelial angiogenic activity was examined. Moreover, the molecular mechanisms, especially focusing on the role of the HIF-1α/VEGF axis, underlying the beneficial effects of FIR were investigated.

2 | MATERIALS AND METHODS

2.1 | Materials

The primary antibodies for caspase-3, Bcl-2, β-actin, HIF-1α, VEGF-A, Histone H3, PI3K, phospho-PI3K (Tyr458), Akt, phospho-Akt (Ser473), hydroxy-HIF-1α (Pro564), and p53 were purchased from Cell Signaling. The primary antibodies for promyelocytic leukemia zinc finger protein (PLZF) and von Willebrand factor (vWF) were purchased from Santa Cruz Biotechnology and Abcam, respectively. Primary Von Hippel Lindau (VHL) antibody was purchased from Genetex Inc. All chemicals of reagent grade were obtained from Sigma.

2.2 | Animals

Male 8-week-old C57BL/6J mice were obtained from Lasco Technology, and the animal experiment was approved by the Taipei Medical University Committee of Experimental Animal Care and Use (No. LAC-2014-0291). There were six groups (each group contained five mice): (1) the control group; (2) cisplatin (4 mg/kg, i.v.)-alone–treated group; (3) cisplatin + FIRx1 group: the cisplatin-treated mice were irradiated with FIR (30 minutes/day) on the 1st day (FIRx1); (4) cisplatin + FIRx2 group: the cisplatin-treated mice were irradiated with FIR for 2 days; (5) cisplatin + FIRx3 group: the cisplatin-treated mice were irradiated with FIR for 3 days; (6) cisplatin + 40℃ group: the cisplatin-treated mice were covered with an electric blanket set to 40℃ for 3 days (30 minutes/day). For FIR performance, the mice anesthetized by isoflurane were irradiated by FIR on their abdomen using the WS TY101 FIR emitter (WS Far Infrared Medical Technology Co., Ltd.) with an intensity of 0.13 mW/cm^2 (at the irradiation distance of 20 cm) for 30 minutes per day.

2.3 | Blood flow monitoring

The Laser Doppler Imager moorLDI system (Moor Instruments) was used to monitor the blood perfusion units (BPU) in the abdomen of the mice. Throughout the experiment, the BPU was recorded every 5 minutes. Data were expressed as a percentage of the baseline value, and individual readings were averaged at each time point.

2.4 | Immunohistochemistry (IHC) assay and vWF measurement

Peritoneal blood vessels were fixed with 10% formaldehyde and embedded in paraffin. Paraffin sections (5 μm thick) were cut for HE staining to evaluate the pathological changes. For IHC assay, the slides were stained with vWF antibody according to the manufacturer's instructions. The levels of vWF in mice blood plasma were measured with a mouse vWF ELISA kit (MyBioSource).

2.5 | Cell culture

Human umbilical vein endothelial cells purchased from the Bioresource Collection and Research Center were cultured in

90% medium 199 with 25 U/ml heparin and 30 μg/ml endothelial cell growth supplement (ECGS) adjusted to contain 1.5 g/L sodium bicarbonate with 10% fetal bovine serum (FBS). The prostate cancer cells (PC-3 and DU145) and lung cancer A549 cells were purchased from American Type Culture Collection (ATCC) and cultured in RPMI-1640 and Dulbecco's Modified Eagles Medium (DMEM) with 10% FBS, respectively. In the Cis + FIRx1 group, the cells were treated with cisplatin for 30 minutes, followed by FIR irradiation with WS TY101 FIR emitter for 30 minutes. In the Cis + FIRx2 group, the HUVECs or cancer cells were treated with cisplatin for 30 minutes, followed by exposure to FIR for 30 minutes twice with an interval of 2 hours between the two exposures. For some hypoxic tests, DU145 cells were incubated in a hypoxic chamber flushed with a gas mixture of 94% N_2, 5% CO_2, and 1% O_2.

2.6 | Cell viability assay and apoptosis detection by flow cytometry

Human umbilical vein endothelial cells (10^4 cells/well) or cancer cells (10^5 cells/well) were cultured in a 96-well microtiter plate in a final volume of 200 μl/well of culture medium. Then, cell viability was measured as previously described.[17] An apoptosis detection kit (BD Biosciences) was used to perform FITC-Annexin V–specific binding and PI staining and analyzed by flow cytometry.

2.7 | Nuclear protein purification and Western blot analysis

Cells were suspended in cold buffer A (containing 10 mM KCl, 0.1 mM EDTA, 1 mM DTT, and 1 mM PMSF) for 15 minutes and lysed by addition of 10% NP-40, followed by centrifugation at 5000 *g* to obtain nuclear pellets. The nuclear pellets were resuspended in cold buffer B (containing 20 mM HEPES, 1 mM EDTA, 1 mM DTT, 1 mM PMSF, and 0.4 mM NaCl), followed by vigorous agitation and centrifugation. The protein expression of target protein was determined by Western blot analysis as previously described.[13]

2.8 | HIF-1α transcriptional activity assay

The transcriptional activity of HIF-1α was determined by using HIF-1α transcription factor assay kit (Abcam) according to the manufacturer's instructions.

2.9 | Quantitative real-time PCR assay

Total RNA was isolated from HUVECs by using TRIzol reagent (Life Technologies). For quantitative real-time PCR assay, the mixture

of total RNA, primers, and Power SYBR Green PCR Master Mix (Applied Biosystems) was incubated at 50℃ for 10 minutes and 95℃ for 5 minutes, followed by 45 cycles of 95℃ for 10 seconds and 72℃ for 30 seconds. Each reaction was performed in duplicate; the threshold (Ct) value of each mRNA was subtracted from that of β-actin mRNA, and the average was taken. The primer sequences used in the PCRs were the following: HIF-1α (forward) 5'-TGAGGAAATGAGAGAAATGCTTACA-3'; HIF-1α (reverse) 5'-ACACTGAGGTTGGTTACTGTTGGT-3'; β-actin (forward) 5'-TCTGGCACCACACCTTCTACAA-3'; β-actin (reverse) 5'-GTACATGGCTGGGGTGTTGAAG-3'.

2.10 | Short interfering (si)RNA transfection

The siRNAs (assay ID s15200 for PLZF, assay ID s6539 for HIF-1α) and control siRNAs were purchased from Thermo Fisher Scientific (Grand Island). Cells were grown to 70% confluence, and siRNAs (10 nM) were transfected using the lipofectamine RNAiMAX reagent (Thermo Fisher Scientific).

2.11 | Vascular endothelial growth factor measurement and caspase-3 activity assay

The amounts of VEGF in the medium were determined by a VEGF Human ELISA kit (Thermo Fisher Scientific). The caspase-3 activity in HUVECs was detected with a caspase-3 activity assay kit (Cell Signaling) according to the manufacturer's instructions.

2.12 | Co-immunoprecipitation assay

The HUVECs were lysed at 4°C in lysis buffer (50 mM Tris, pH 7.5; 1% Nonidet P-40; 0.5% sodium deoxycholate; 150 mM NaCl; and protease inhibitor cocktail [Thermo Fisher Scientific]). The immunocomplexes were precipitated by protein A agarose (Roche Molecular Biochemicals) with VHL or p53 antibodies. Then, the eluted proteins of hydroxyl-HIF-1α and HIF-1α were detected by Western blotting.

2.13 | Endothelial cell tube formation assay

Matrigel (12.5 mg/ml, BD Biosciences) at 4°C was pipetted onto a 96-well plate and allowed to solidify for 10 minutes. Human umbilical vein endothelial cells (1×10^4 cells/well) were seeded on the surface of Matrigel and incubated for 30 minutes. After adhesion of the cells, the medium was removed and replaced by fresh medium supplemented with VEGF (50 ng/ml) or cisplatin (30 μM) for 18 hours. The tube formation in various groups was photographed with an Olympus IX 70 invert microscope (Olympus America,

FIGURE 1 Effects of far-infrared radiation (FIR) on blood flow and vascular stenosis in cisplatin-treated mice. A, The blood flow in the abdomen was determined by using Laser Doppler Imager moorLDI system at indicated times. The histological changes of mesenteric blood vessels were examined with (B) low and (C) high magnification rate. D, The middle colic artery diameter of mice was measured. Values are presented as mean ± SD ($n = 5$). *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ versus cisplatin-alone–treated mice. Cis, cisplatin
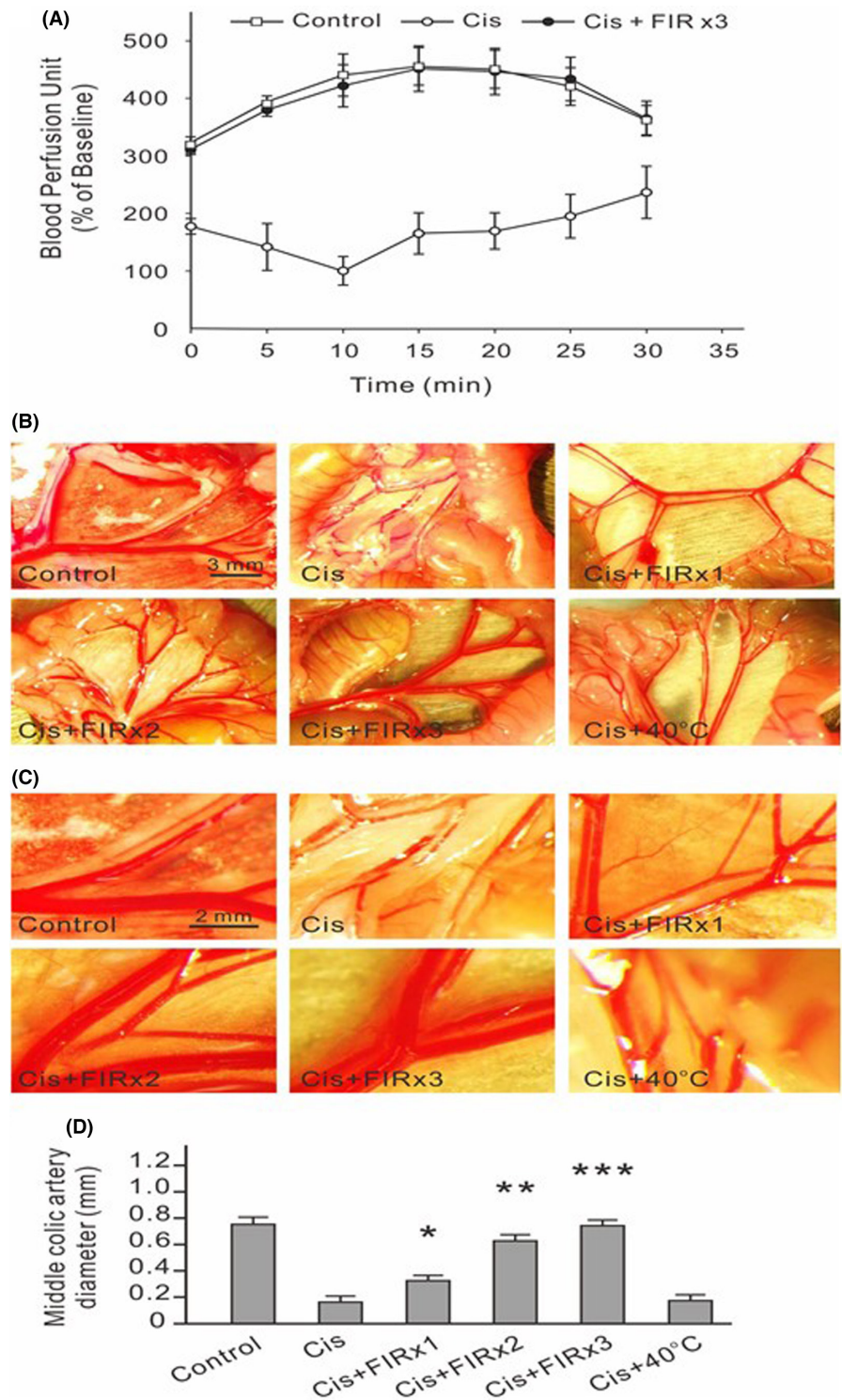
Inc.). The vessel length and branches were quantified by using an angiogenesis-measuring software (KURABO).[13]

2.14 | Aortic ring angiogenesis assay

According to previously described methods,[18] the thoracic aortas were removed from 6- to 9-week-old BALB/c mice after cervical dislocation and cut into 1-mm-thick rings. Each ring was placed on a prechilled 96-well plate coated with 40 μl Matrigel and incubated at 37°C for 20 minutes. Then, additional 40 μl Matrigel was added and incubated at 37°C for 20 minutes. The embedded rings were incubated with 150 μl MCDB 131 medium (ThermoFisher) in the presence or absence of 20 ng/ml VEGF, with replacement of the medium every 2 days. In the cisplatin-treated rings, cisplatin (5 μM) was added for 2 days, and cisplatin was withdrawn for subsequent incubation. In the VEGF + cisplatin + FIR group, cisplatin (5 μM) was added for 30 minutes, followed by exposure to FIRx2/day for 2 days. Then, the medium without cisplatin was replaced, and the treatment of the rings with FIRx2 was continued for 3 days. On day 5,
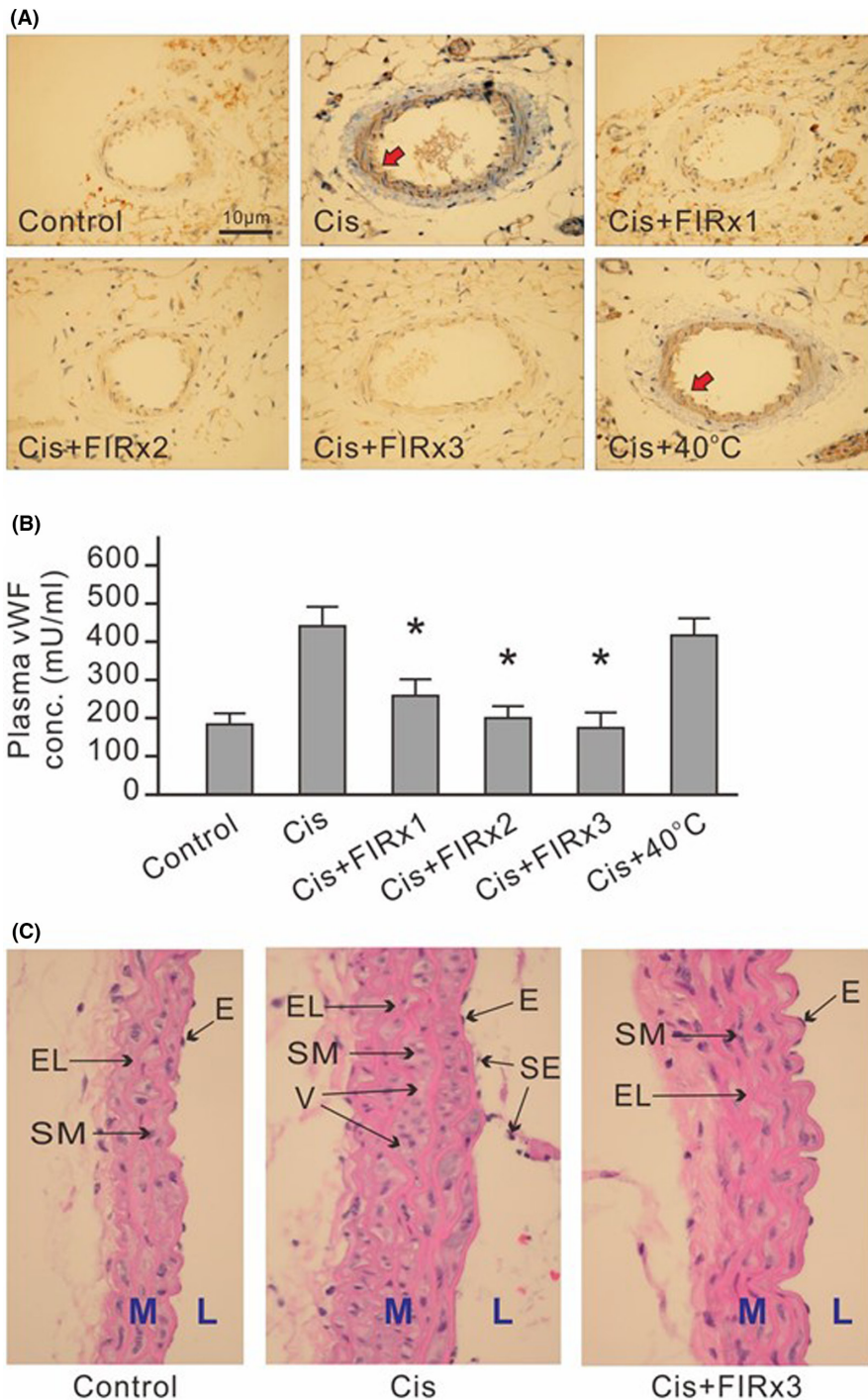
(A)

(B)

(C)

FIGURE 2 Effects of far-infrared radiation (FIR) on arterial morphology changes in cisplatin-treated mice. A, The expression of von Willebrand Factor (vWF) (brown color) in peritoneal blood vessels was evaluated by immunohistochemistry (IHC). The arrows indicate the vascular endothelium. B, The plasma levels of vWF were determined. C, The representative stains of superior mesenteric artery. E, endothelial cells; EL, elastic lamina; L, lumen; M, media; SE, sloughing endothelial cells; SM, smooth muscle cells; V, vacuoles. Values are presented as mean $\pm$ SD ($n = 5$). *$p < 0.05$ versus cisplatin-alone–treated mice

the angiogenesis was evaluated by the capillary sprouts from aortic rings by using an inverted microscope equipped with a digital camera (SAGE VISION).

2.15 | Statistical analysis

The experimental data were expressed as the mean $\pm$ SD of at least four independent experiments. Statistical differences between two groups were determined using Student's t test. The differences were considered significant if the P-value was less than 0.05.

3 | RESULTS

3.1 | FIR improves blood flow in cisplatin-treated mice

A marked reduction of blood flow in the abdomen that occurred in cisplatin-treated mice was completely restored after FIR treatment for 3 days (Figure 1A). Similarly, the severe vascular stenosis reflected by a significant decrease in the middle colic artery diameter in cisplatin-treated mice was remarkably attenuated by FIR in an exposure time–dependent manner (Figure 1B, C). It is known that FIR can steadily

FIGURE 3 Effects of far-infrared radiation (FIR) on cisplatin-induced apoptosis in human umbilical vein endothelial cells (HUVECs). A, After the cells were treated with cisplatin or vehicle (DMSO) for 24 h, cell viability was determined. Values are presented as mean ± SD ($n = 4$). *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ versus the control group. B, The representative flow cytometric plots of cell apoptosis and (C) the relative apoptotic rate were determined. D, The protein levels of Bcl-2 and cleaved caspase-3 in HUVECs treated with cisplatin (30 μM), cisplatin (30 μM) + FIRx1, or cisplatin (30 μM) + FIRx2 were determined. Values are presented as mean ± SD ($n = 5$). *$p < 0.05$, **$p < 0.01$ versus cisplatin (30 μM)-alone–treated group
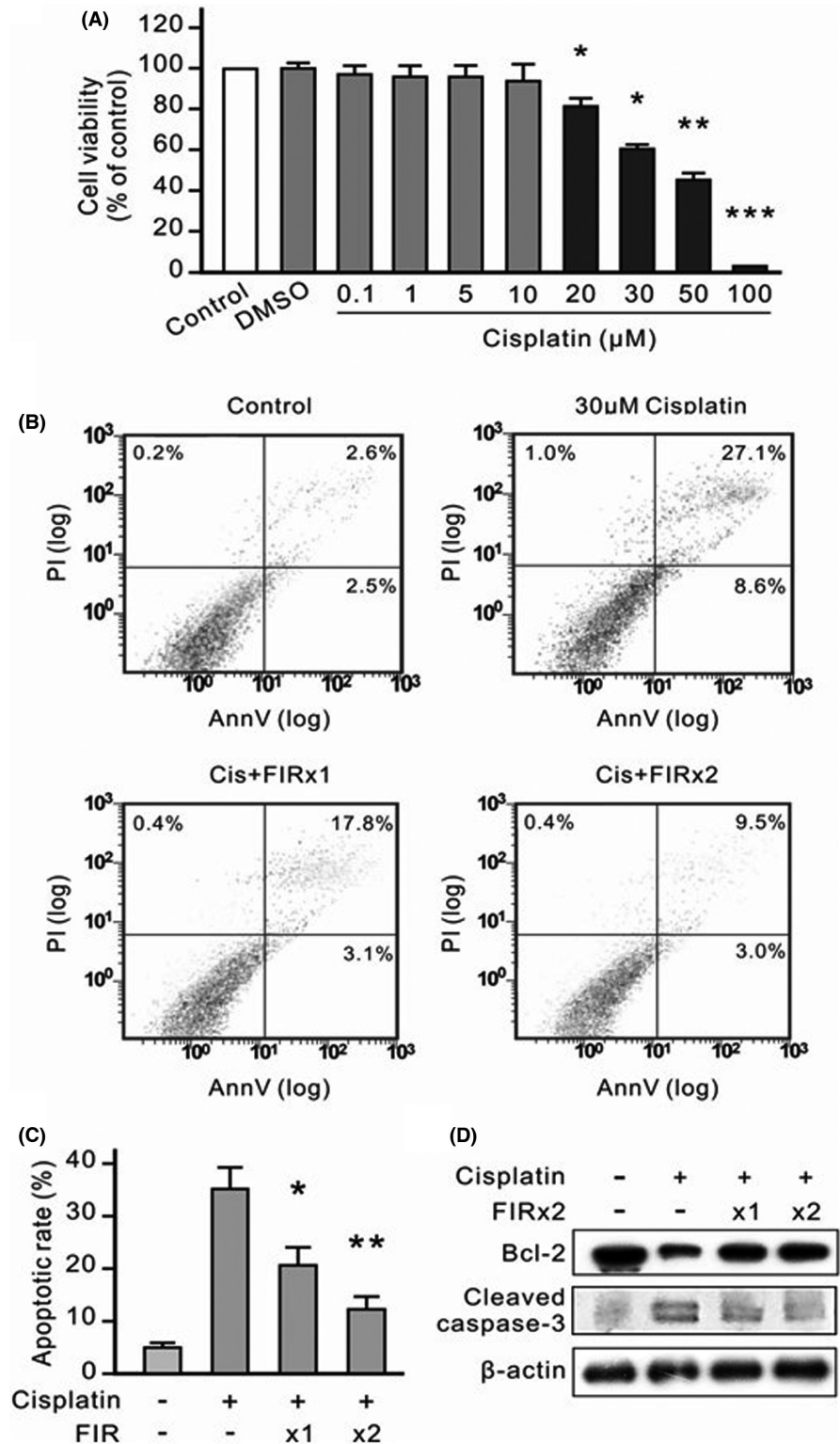
increase the skin temperature to about 40°C after FIR irradiation for 30-60 minutes. To clarify whether the effects of FIR are due to its nonthermal effects, the increase in the body temperature of mice that received FIR was less than 1°C under our set condition, and the 40°C-alone–treated mice acted as heated control group. As only heating with a 40°C-warm blanket failed to exert the above beneficial effects (Figure 1C, D), the protective effects of FIR in the cisplatin-treated mice may largely be attributed to the nonthermal effects of FIR.

3.2 | FIR attenuates cisplatin-induced vascular injury

Significantly elevated levels of vWF, a marker of vascular endothelial damage and vascular disorders,[19] in artery endothelium and plasma in cisplatin-treated mice were greatly reduced by FIR irradiation, which was not observed in the warm-compress-alone–treated mice (Figure 2A, B). Consistently, the severe histological
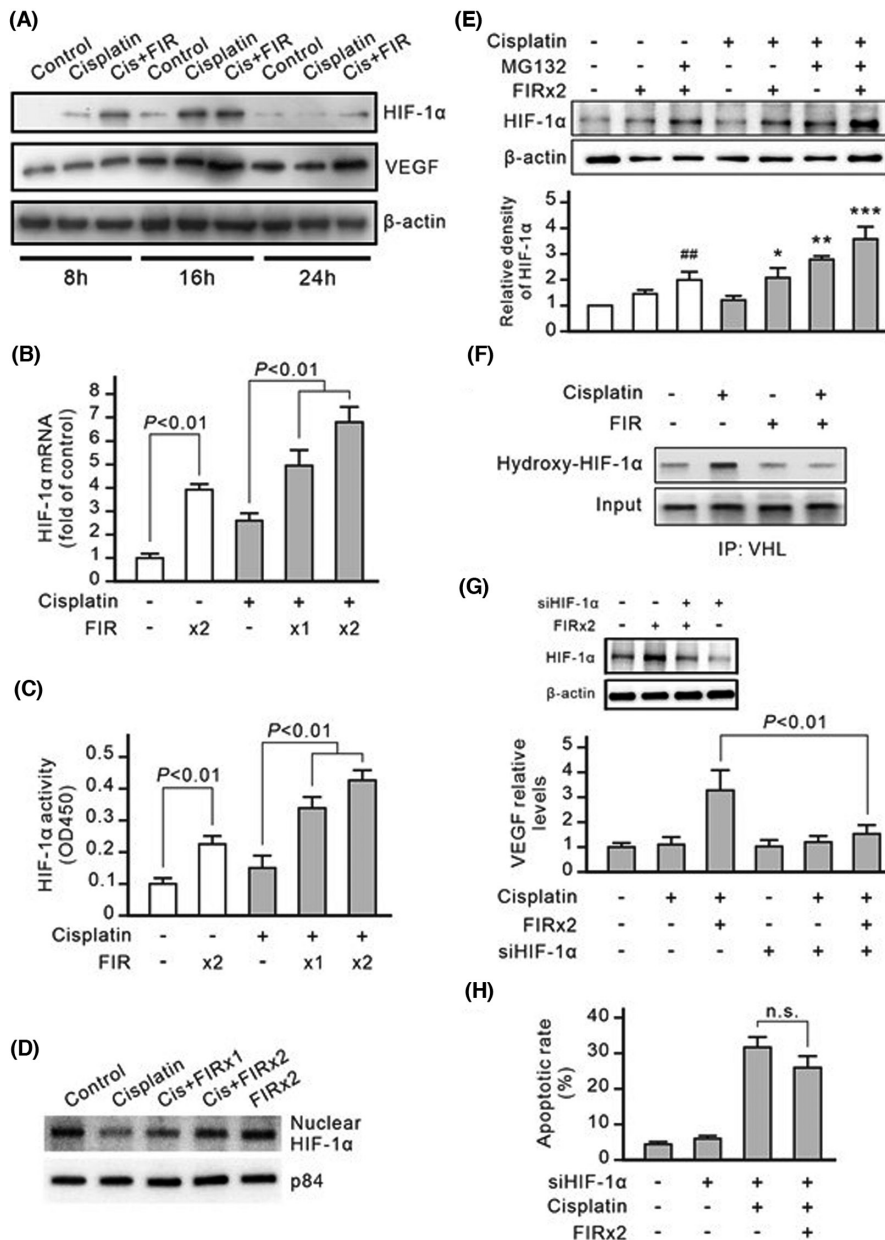
FIGURE 4 Effects of far-infrared radiation (FIR) on hypoxia-inducible factor 1α (HIF-1α)/VEGF cascade and apoptosis in human umbilical vein endothelial cells (HUVECs). A, The protein expression of HIF-1α and VEGF at indicated times was determined. After cells were treated with different treatments for 6 h, (B) the mRNA level, (C) the transcriptional activity, and (D) the nuclear level of HIF-1α were determined. E, After cells were treated with different treatments for 8 h, the relative HIF-1α levels were determined in the presence or absence of MG132 (10 μM). $^{##}p < 0.01$ versus control group; $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ versus the cisplatin group. F, The cell protein was immunoprecipitated with Von Hippel Lindau (VHL) antibody, and the level of hydroxylated HIF-1α was determined by Western blot. The cells or cells transfected with HIF-1α siRNA were treated with different combined treatments for 24 h. G, The relative amounts of VEGF in the medium and (H) the apoptotic rate were measured

changes in the superior mesenteric artery vessels in cisplatin-treated mice were greatly improved by FIR (Figure 2C).

## 3.3 | FIR inhibits cisplatin-induced apoptosis in HUVECs

The apoptotic pathway is reported to trigger cisplatin-induced renal tubular cell death.[20] Similarly, cisplatin dose-dependently caused endothelial toxicity evidenced by increased apoptotic rate, evaluated by flow cytometry (Figure 3A–C). Importantly, FIR exerts an antiapoptotic activity accompanied by downregulation of proapoptotic protein cleaved caspases-3 and upregulation of antiapoptotic protein Bcl-2 in cisplatin-treated HUVECs (Figure 3D). As expected, cisplatin-induced DAPI-staining

fragmented nuclei and TUNEL staining in HUVECs were remarkably inhibited by FIR (Figure S1).

3.4 | FIR enhances the induction and activation of HIF-1α

A novel finding of this study is that FIR irradiation significantly increased the protein and mRNA levels, transcriptional activity, and nuclear accumulation of HIF-1α in HUVECs compared with those of the control or cisplatin-alone–treated groups (Figure 4A–D). When MG132, a proteasome inhibitor, was simultaneously added to block HIF-1α degradation, the protein level of HIF-1α in cisplatin + FIR–treated cells was still higher than that in cisplatin-treated cells (Figure 4E). The interaction of hydroxylated HIF-1α and VHL

FIGURE 5 Effects of far-infrared radiation (FIR) on the angiogenesis in human umbilical vein endothelial cells (HUVECs) and aortic rings. The cells or cells transfected with hypoxia-inducible factor 1α (HIF-1α) siRNA were treated with VEGF (50 ng/ml), cisplatin (30 μM), or cisplatin + FIRx2 for 18 h. A, Then, the images of capillary-like tube formation were photographed and evaluated by (B) total tube length and total branching points. C, The representative images of the sprouting angiogenesis from aortic rings in various groups are presented. Values are presented as mean ± SD ($n = 5$)
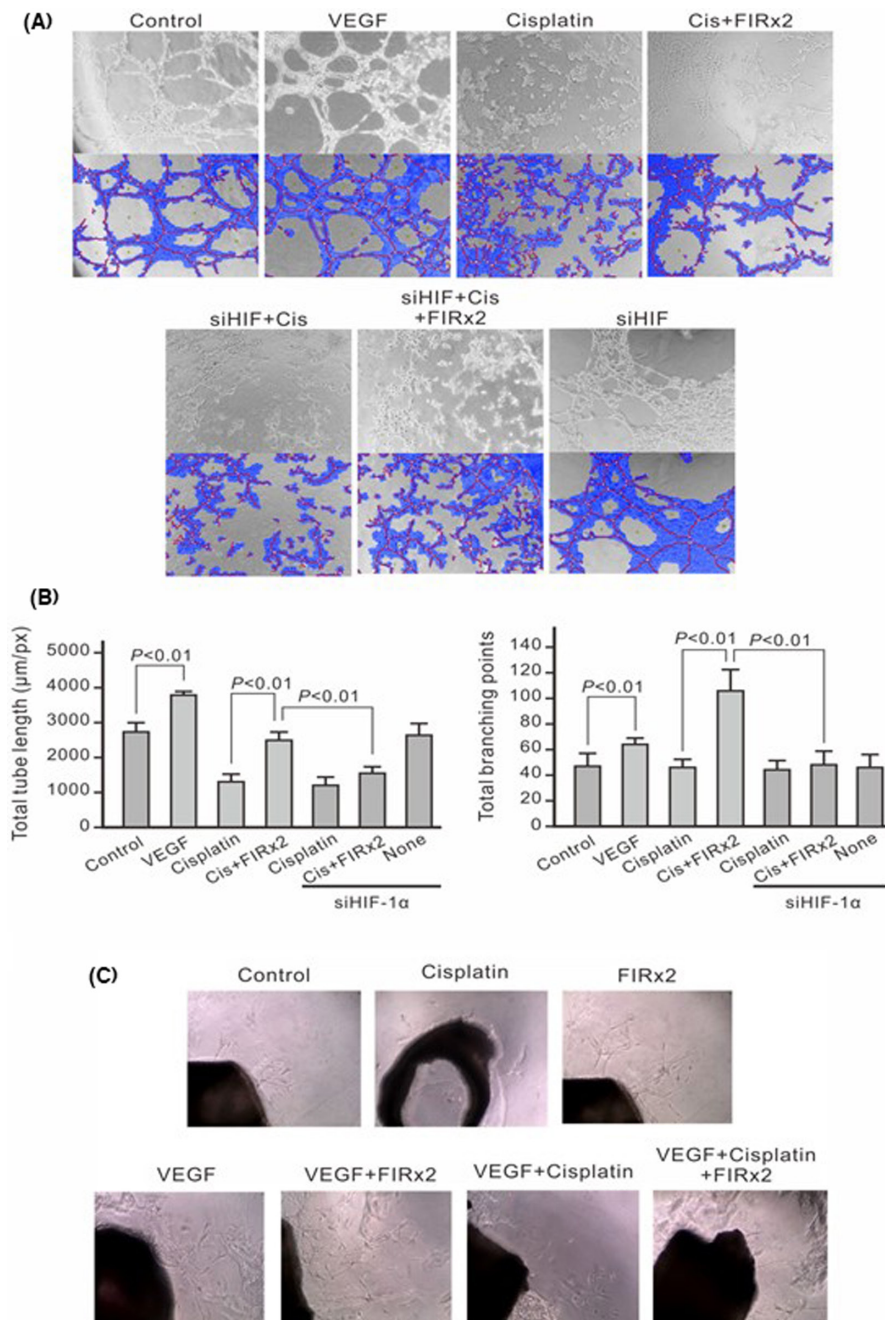
ultimately promotes proteasomal degradation of HIF-1α.[21] Our data showed that FIR markedly inhibited the binding of hydroxylated HIF-1α and VHL compared with the cisplatin-alone–treated group (Figure 4F). Therefore, FIR-mediated increase in HIF-1α protein level may result from enhancing HIF-1α synthesis and inhibiting HIF-1α protein degradation. The HIF-1α/VEGF axis is considered to maintain endothelial survival and functions.[22] Far-infrared radiation–mediated VEGF release and suppression of apoptosis in cisplatin-treated HUVECs were completely abolished by transfection with HIF-1α siRNA (Figure 4G, H), suggesting that the beneficial effects of FIR may largely be attributed to HIF-1α–dependent processes.

3.5 | FIR increases the angiogenesis in HUVECs and mice aortic rings

As expected, addition of VEGF significantly increased the capillary tube–like formation of HUVECs. In contrast, cisplatin markedly inhibited endothelial angiogenesis (Figure 5A), which was greatly attenuated by FIR treatment evidenced by higher total tube length and branch points (Figure 5B). However, the proangiogenic activity of FIR was also diminished by HIF-1α siRNA, indicating that HIF-1α–dependent responses are involved in the actions of FIR. Moreover, the angiogenic sprout formation from mice aortic rings was enhanced by FIR. Consistently, decreased
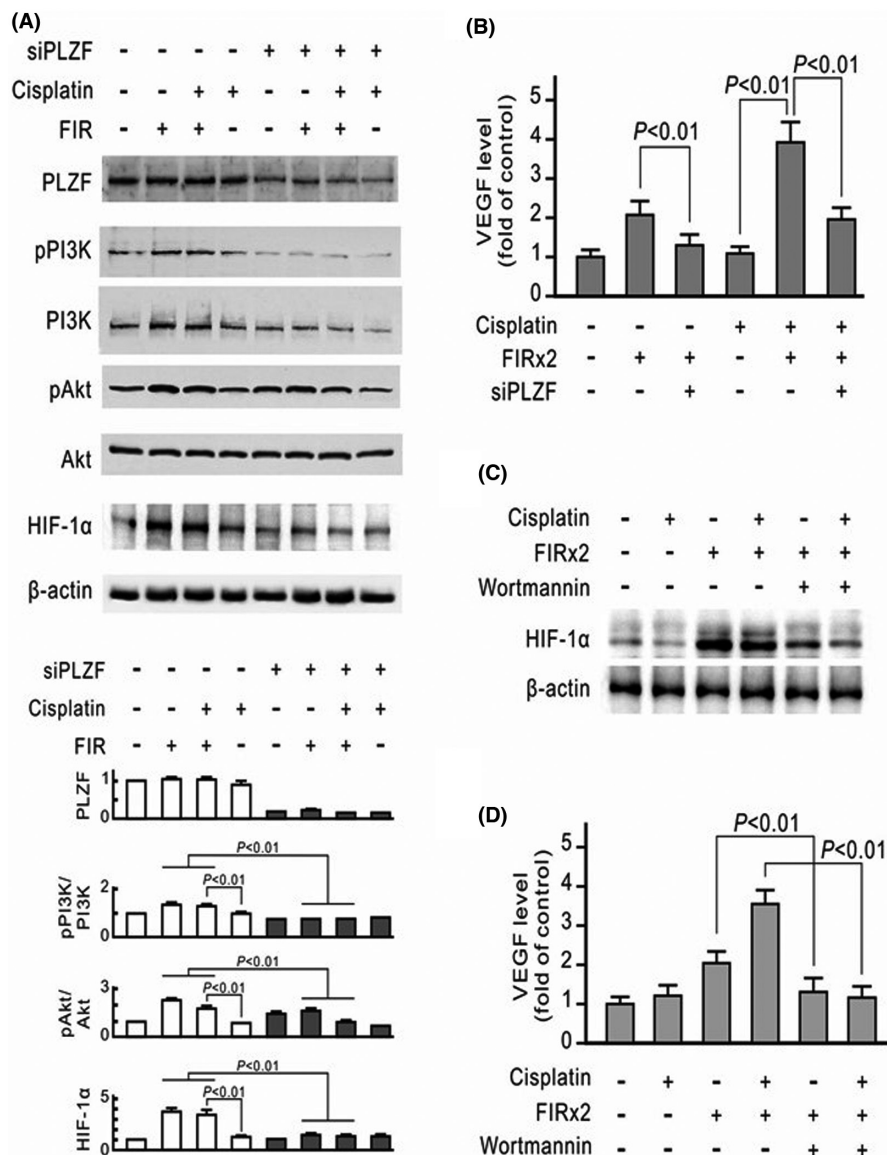
FIGURE 6 The role of promyelocytic leukemia zinc finger protein (PLZF) and phosphatidylinositol-3 kinase (PI3K) on the far-infrared radiation (FIR)-mediated hypoxia-inducible factor 1α (HIF-1α)/VEGF cascade in cisplatin-treated human umbilical vein endothelial cells (HUVECs). A, The relative levels of target genes at specific times were determined (2 h for PLZF, phospho-PI3K, and PI3K; 4 h for phospho-Akt and Akt; 8 h for HIF-1α) in HUVECs transfected with PLZF siRNA or scrambled siRNA. B, The relative levels of VEGF in the medium were measured in cells treated with different treatments for 24 h. C, The HUVECs of various groups in the presence or absence of Wortmannin (1 μM) were incubated for 8 h to determine the HIF-1α expression. D, The cells treated for 24 h were used to measure the relative amounts of VEGF in the medium. Values are presented as mean ± SD ($n = 4$)

angiogenic sprouts caused by cisplatin in the presence or absence of VEGF were improved by FIR irradiation (Figure 5C). These findings strongly support that FIR has a proangiogenic activity even under cisplatin treatment.

3.6 | Promyelocytic leukemia zinc finger protein and PI3K regulate FIR-mediated HIF-1α expression and apoptosis

Compared with untreated or cisplatin-treated HUVECs, FIR significantly upregulated phospho-PI3K, PI3K, phospho-Akt, and HIF-1α, as well as VEGF secretion, which were almost completely suppressed by transfection with PLZF siRNA (Figure 6A, B). Thus, PLZF is an important transcriptional factor in the activation of the Akt/HIF-1α/VEGF pathway by FIR. Addition of wortmannin, a PI3K inhibitor, also remarkably abolished FIR-induced HIF-1α expression and VEGF secretion in HUVECs in the

presence or absence of cisplatin (Figure 6C, D). Notably, HIF-1α exerts a proapoptotic activity via binding to p53.[23] The increased interaction of HIF-1α and p53, as well as caspase-3 activity, in cisplatin-treated HUVECs was significantly inhibited by FIR, whereas it was strongly reversed by transfection with PLZF siRNA (Figure 7A, B). Similarly, the antiapoptotic activity of FIR in cisplatin-treated HUVECs was suppressed by wortmannin (Figure 7C, D). Collectively, FIR-regulated HIF-1α induction, VEGF generation, and the antiapoptotic activity in cisplatin-treated HUVECs are mediated by PLZF and PI3K/Akt.

3.7 | FIR does not affect the cell death and HIF-1α VEGF cascade in cisplatin-treated cancer cells

Cell death and expression of HIF-1α and VEGF in cisplatin-treated cancer cells were not altered by FIR (Figure 8A, B). Similar effects of FIR on HIF-1α and VEGF expression in hypoxic DU145 cells in

the presence or absence of cisplatin were observed (Figure 8C). As expected, the VEGF production in normoxic or hypoxic cisplatin-treated cancer cells was not changed by FIR irradiation (Figure 8D). Accordingly, the effects of FIR may be specific for endothelial cells without affecting the anticancer activity of cisplatin on cancer cells.

4 | DISCUSSION

There are many severe cardiovascular complications during cisplatin therapy, thereby greatly limiting its therapeutic efficacy and clinical application.[4] Therefore, development of safer and effective treatment or supplements to mitigate the vascular side effects of cisplatin is urgently needed. A marked decrease in blood flow in the abdomen and severe vascular injury evidenced by a significant increase in the level of vWF in cisplatin-treated mice were greatly improved by FIR irradiation. Far-infrared radiation also activates the HIF-1α/VEGF axis and its regulated angiogenesis and antia-poptotic activity in cisplatin-treated HUVECs. Thus, it is proposed that the protective effects of FIR against cisplatin-triggered vascular damage and endothelial toxicity may be mediated by activation of HIF-1α/VEGF-dependent processes. However, only warm-compress treatment did not alleviate the pathological symptoms caused by cisplatin, indicating that the protective effects of FIR

FIGURE 7 The role of promyelocytic leukemia zinc finger protein (PLZF) and phosphatidylinositol-3 kinase (PI3K) on far-infrared radiation (FIR)-mediated antiapoptotic activity in human umbilical vein endothelial cells (HUVECs). A, The interaction of hypoxia-inducible factor 1α (HIF-1α) and p53 was examined as described in "Methods". HUVECs or HUVECs transfected with PZLF siRNA were treated with cisplatin (30 μM), FIRx2, or cisplatin + FIRx2 for 24 h. B, The relative caspase-3 activity was measured. C, D, The cells of various groups were incubated with or without wortmannin (Wort, 1 μM) for 24 h, and the relative apoptotic rate was examined. Values are presented as mean ± SD (n = 5). E, Proposed molecular mechanisms regulating the angiogenesis and apoptosis by FIR in cisplatin-treated HUVECs
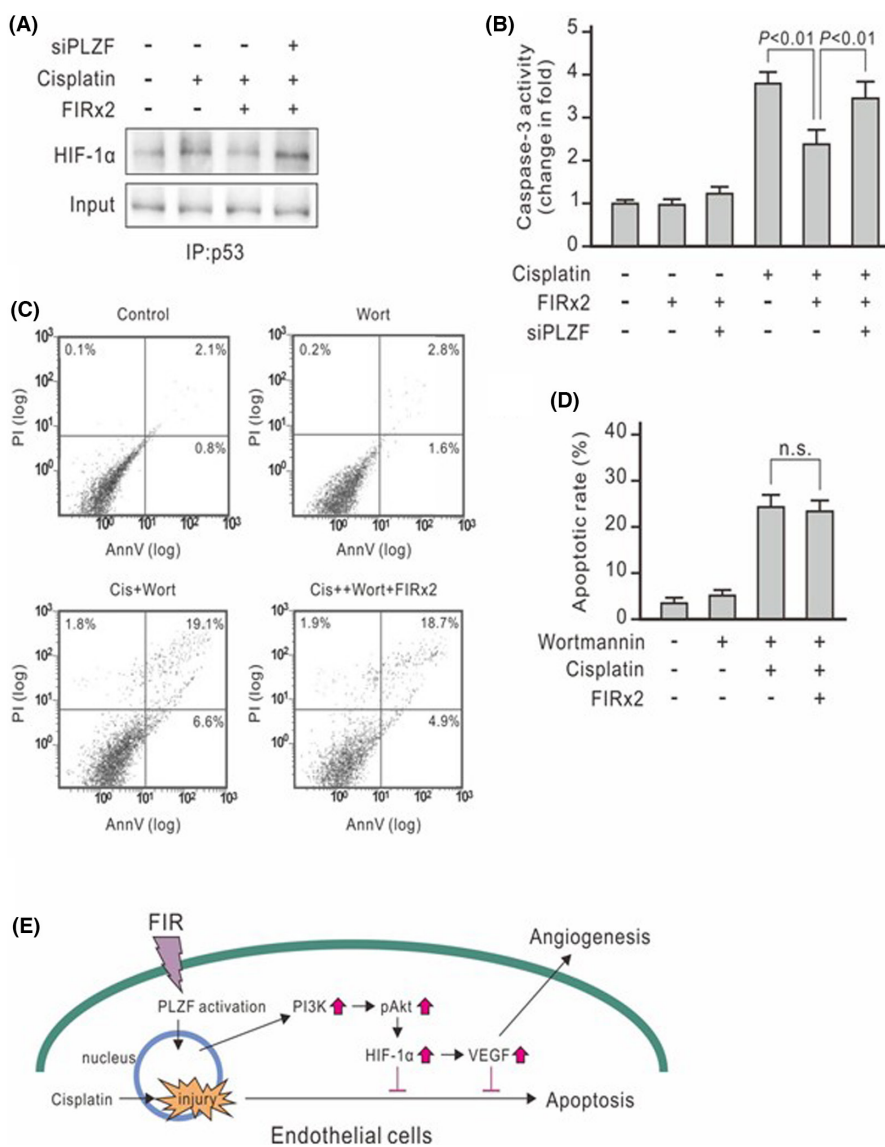

FIGURE 8 Effects of far-infrared radiation (FIR) on cell viability and the hypoxia-inducible factor 1α (HIF-1α)/VEGF cascade in cisplatin-treated cancer cells. Cancer cells were pretreated with or without cisplatin (15 or 30 μM) for 30 min, followed by exposure to FIRx2, and further incubated for 24 h. A, Cell viability, (B) expression of HIF-1α and VEGF, and (D) VEGF production in the medium were determined. After DU145 cells were incubated for 6 h under hypoxic condition (1% O_2), the levels of HIF-1α and VEGF (C) and VEGF production in the medium (D) were determined. Values are presented as mean ± SD (n = 5). *$p < 0.05$, ***$p < 0.001$, #$p < 0.05$ versus respective control group
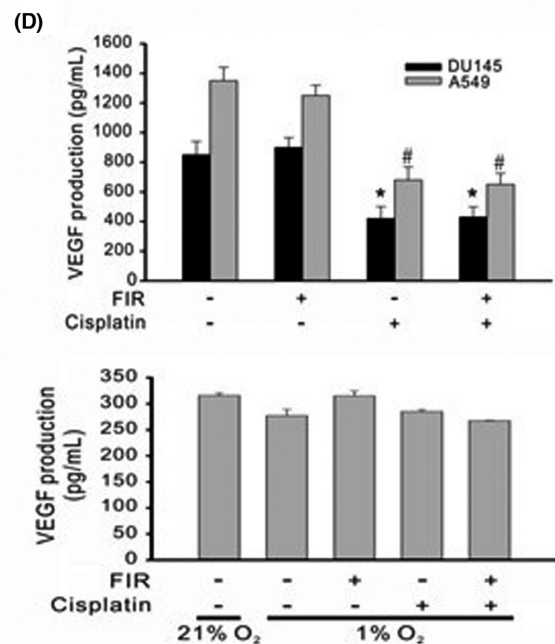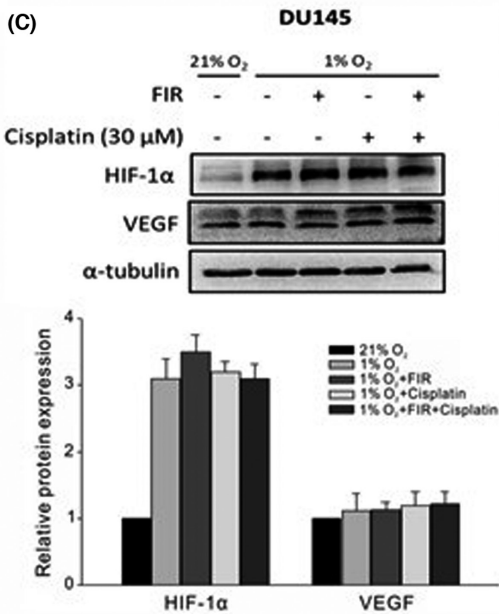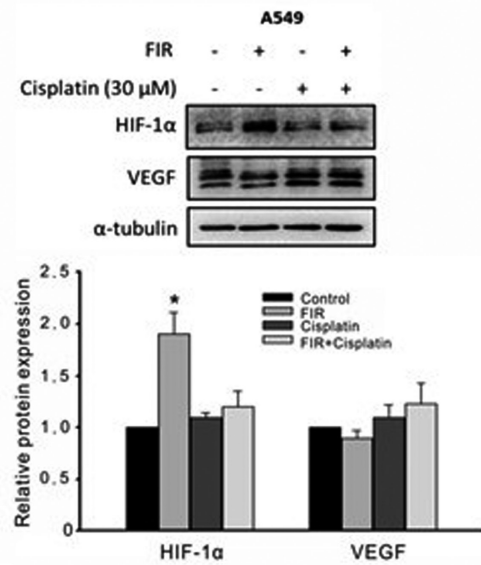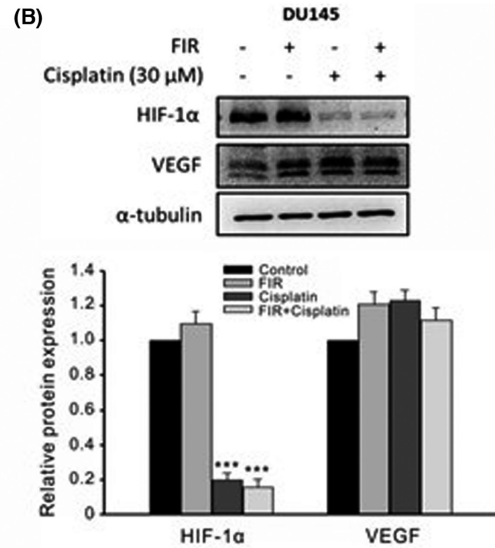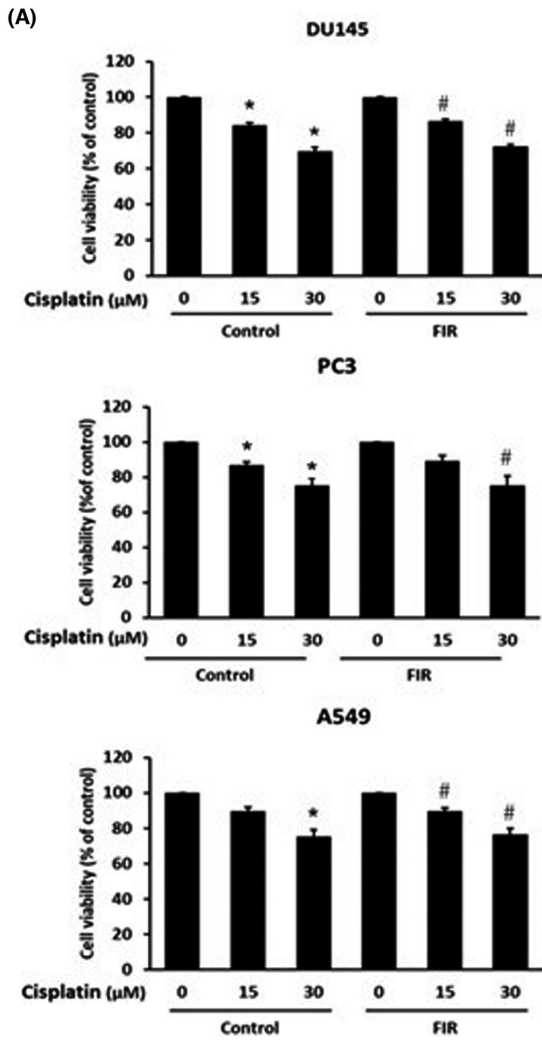
against cisplatin-triggered endothelial toxicity and dysfunction are not due to thermal effects.

The protein level of HIF-1α is controlled by its synthesis and degradation or a combination of both. Under normoxic condition, the two specific critical proline residues of HIF-1α are hydroxylated by prolyl hydroxylases (PHDs). Hydroxylated HIF-1α is required for binding to VHL, thereby promoting HIF-1α proteasomal degradation.[21] As FIR significantly inhibited cisplatin-evoked increased interaction of hydroxylated HIF-1α and VHL in HUVECs, suppressing PHD/VHL-induced HIF-1α degradation may be a mechanism to enhance HIF-1α protein stability. In the presence of MG132, a specific proteasome inhibitor, the HIF-1α protein level in FIR + cisplatin–treated cells was higher than that in cisplatin-treated cells. Furthermore, the mRNA level of HIF-1α in cisplatin-treated HUVECs was greatly elevated by FIR. Collectively, FIR-induced HIF-1α protein accumulation in cisplatin-treated HUVECs may be associated with accelerating HIF-1α synthesis and attenuating HIF-1α degradation.

In response to mild cellular stress, activation of HIF-1α/VEGF-regulated responses is thought to be an adaptive strategy to maintain endothelial cell proliferation and survival.[24,25] Accordingly, we proposed that the antiapoptotic activity of FIR in cisplatin-treated HUVECs may be mediated by HIF-1α activation evidenced by the result that the inhibitory effect of FIR on apoptosis was markedly abolished by blocking HIF-1α activity. Interestingly, HIF-1α has both antiapoptotic and proapoptotic activities that depend on different experimental conditions and its posttranslational modification.[26] Under severe hypoxic or pathological condition, the dephosphorylated HIF-1α prefers binding to tumor suppressor p53 leading to p53-induced apoptosis.[23] Thus, the interaction of HIF-1α and p53 is regarded as a crucial switch to apoptosis. A significant increase in the interaction of HIF-1α and p53 observed in cisplatin-treated HUVECs was almost completely inhibited by FIR, suggesting that cisplatin-regulated HIF-1α may largely switch to p53-dependent apoptosis rather than exerting the physiological functions of HIF-1α, including promoting endothelial survival and angiogenesis. Therefore, it is likely that attenuating cisplatin-induced endothelial cell death by FIR may be related to suppressing HIF-1α/p53-dependent apoptosis.

It is noteworthy that cisplatin treatment may increase the risk of thrombotic complications by triggering platelet activation.[27,28] Consistent with the improvement of the poor blood flow by FIR in cisplatin-treated mice, the impaired angiogenic sprout formation from mice aortic rings caused by cisplatin was greatly restored by FIR irradiation. Similarly, FIR significantly prevented cisplatin-evoked decreased capillary tube–like formation in HUVECs, while the event was strongly diminished by transfection with HIF-1α siRNA, indicating the involvement of HIF-1α in the proangiogenic activity of FIR. Previous studies have confirmed that FIR is able to alleviate ischemia/reperfusion injury and the poor blood flow in ischemic hindlimb of diabetic mice by enhancing endothelial progenitor cell functions and activating eNOS/NO cascade.[29-31] Accordingly, FIR has a therapeutic potential for ischemia-associated cardiovascular diseases by augmenting HIF-1α–dependent new vessel formation and vasodilation.

Then, the molecular mechanisms underlying FIR-mediated induction of HIF-1α and VEGF were investigated. FIR irradiation significantly increased the phosphorylation of PI3K and Akt in cisplatin-treated HUVECs. However, addition of wortmannin remarkably diminished FIR-mediated elevation of HIF-1α expression and VEGF secretion and inhibition of apoptosis in cisplatin-treated HUVECs, suggesting that activation of the PI3K/Akt axis contributes to the effects of FIR. Promyelocytic leukemia zinc finger protein belongs to the family of Krüppel-like zinc finger proteins. It is a transcriptional repressor implicated in many physiological functions.[32] As the transcriptional activity of PLZF can be activated by FIR in HUVECs,[17] it is possible that PLZF may involve the induction of HIF-1α by FIR. To date, whether PLZF modulates HIF-1α expression remains unknown and is worthy to explore. In the present study, we found that the changes of the PI3K/Akt/HIF-1α cascade, VEGF release, caspase-3 activity, and the interaction of HIF-1α and p53 by FIR in cisplatin-treated HUVECs were all significantly reversed by PLZF siRNA. These results confirm that PLZF and its regulated PI3K/Akt cascade are key upstream mediators responsible for the FIR-regulated HIF-1α/VEGF axis and the resistance to apoptosis in cisplatin-treated HUVECs.

Notably, activation of the HIF-1α/VEGF pathway also promotes cancer progression.[33] Considering the clinical application, the effects of FIR on cisplatin-treated cancer cells were examined. Our results revealed that FIR did not affect cancer cell cytotoxicity, the expression of HIF-1α and VEGF, and VEGF formation in cisplatin-treated cancer cells under normoxic or hypoxic condition. Therefore, the actions of FIR may specifically target endothelial cells without abating the anticancer activity of cisplatin, suggesting that FIR can be used for combination therapy with anticancer drugs. In addition, FIR could significantly inhibit cell proliferation and colony formation in various cancer cells without a significant effect on cancer cell viability.[34,35] Clinical study further confirmed that FIR did not increase the recurrence and metastasis of breast cancer and related tumor marker level and adverse effects in breast cancer patients.[36] Experimental and clinical evidence strongly supports that FIR is an effective and oncologically safe treatment for cancers. However, the effects of FIR on cancer may be diverse depending on different cancer types, gene expression, the protocols of FIR treatment, and various combinations of other therapies. To examine the anticancer activity of FIR, more animal and clinical studies are needed.

In summary, we demonstrated for the first time that FIR effectively prevents cisplatin-induced vascular injury and stenosis, endothelial toxicity, and impaired angiogenesis in mice and HUVECs via activation of the PLZF/PI3K/Akt/HIF-1α/VEGF signaling pathway (Figure 7E). Taken together, cotreatment with the noninvasive and easily performed FIR may have a high clinical application potential in cancer patients during cisplatin therapy.

DISCLOSURE

The authors declare no conflict of interest.

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