JOURNAL OF PLASTIC SURGERY AND HAND SURGERY
2020, VOL. 54, NO. 5, 290–296
https://doi.org/10.1080/2000656X.2020.1771719

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ARTICLE

Resveratrol inhibits proliferation and promotes apoptosis of keloid fibroblasts by targeting HIF-1α

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ABSTRACT
A keloid is characterized by red, tickling, hard, and irregular raised tissues, and it tends to outgrow its origin. It frequently occurs in young adults and appears to be refractory to prevailing therapies. Resveratrol is a new drug that has anti-proliferative effect. In this study, keloid-derived fibroblasts were cultured under hypoxia environment and was treated by resveratrol. CCK-8 assay and Annexin V-FITC were used to evaluate cell activity and apoptosis level. Western blot and RT-qPCR were also used to assess the expression of HIF-α, Collagen I and Collagen III. Besides, siRNA was also used to explore the mechanisms of resveratrol's effect. In this study, hypoxia promotes proliferation and inhibits apoptosis of keloid fibroblasts. These findings highlight the potential obstacle in treating keloids. Furthermore, we demonstrated that resveratrol could reverse the effect of hypoxia on keloids through down-regulation of HIF-1α. Moreover, collagen synthesis in keloid fibroblasts was also inhibited by resveratrol, which corresponded with HIF-1α suppression. These results provide evidence for resveratrol's treatment effect against keloids through inhibiting cell proliferation and promoting cell apoptosis, while, HIF-1α may play the key role in this process.

ARTICLE HISTORY
Received 14 February 2020
Revised 18 April 2020
Accepted 15 May 2020

KEYWORDS
keloid; resveratrol; HIF-1α; collagen synthesis; hypoxia

Introduction

A keloid is a benign dermal tumor that is characterized by red, tickling, hard, and irregular raised tissues, and it occurs at a high frequency in young adults [1,2]. It tends to extend beyond the wound boundaries and is refractory to regular treatment [3]. There have been intractable cases showing recurrence of a keloid, which if not eradicated might lead to necrosis, suppuration, and recurrent hemorrhage. Histological studies have revealed that a keloid contains overabundant fibroblasts undergoing mitotic division, and excessive collagen deposition and myxoid stroma may be observed within the keloid tissue [4]. Keloids has partial similar characteristics and molecular mechanisms with tumor, and tumor growth is often driven by abnormal overexpression of oncogenes, resulting in uncontrolled growth of cells [5–7]. Indeed, therapies developed for the treatment of a keloid, such as interferon therapy, intralesional administration of 5-fluorouracil and tamoxifen, excision, and radiation therapy [8,9], are based on cancer therapy. Currently, no single therapeutic modality has been found to achieve the best outcome.

Low oxygen level has been frequently reported in solid tumors, including prostate cancer, melanoma, liver cancer, breast cancer, and ovarian cancer [10–14]. Hypoxic stress in the tumor microenvironment can be caused by rapid growth of tumor tissue, which consumes oxygen more rapidly than normal tissue, and perfusion defects resulting from abnormal tumor blood vessel structure and function [15]. A keloid resembles a tumor in many aspects, such as uncontrolled proliferation, tendency to outgrow the origin, and resistance to therapies. Hypoxia also exists within keloid tissues; however, how much it contributes to the malignant properties of keloids is unclear.

Resveratrol is a natural polyphenolic compound, and it has been reported to have various beneficial effects, such as cardioprotective, anti-cancer, anti-inflammatory, and anti-oxidative functions. The protective function of resveratrol in hypoxic pulmonary hypertension is one of the most studied aspects. Csiszar et al. [16] first demonstrated that resveratrol prevents monocrotaline-induced pulmonary hypertension in rats, and Chen et al. [17] further proved that the anti-proliferative effect of resveratrol on human pulmonary artery smooth muscle cells involves a decreased expression of hypoxia-induced arginase II expression and Akt-dependent signaling. In scar researches, Ikeda's study explored the effect of Resveratrol towards keloid derived fibroblasts and indicated its anti-fibrogenesis and pro-apoptosis [18], Zeng also concluded that Resveratrol could reduce collagen expression by inhibiting proliferation and producing apoptosis in human hypertrophic scar fibroblasts [19]. In view of these finding, we hypothesized that resveratrol could alleviate the effect of hypoxia in keloid tissues.

In this study, we focused on the treatment effect of resveratrol against keloid fibroblasts under hypoxia environment. In addition, the mechanisms of resveratrol would also be explored, such as the function of key target HIF-1α.

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Table 1. Profile of samples for primary culture.

No.	Sex	Age	Site	Keloid duration (years)
1	Female	42	Shoulder	10
2	Male	45	Back	11
3	Male	38	Chest	3
4	Female	35	Shoulder	4
5	Male	24	Ear	2

Materials and methods

Source of keloid fibroblasts

Keloid tissues were obtained from five Chinese patients who underwent surgery at the Plastic Surgery Department of our hospital. Detailed information on these samples is provided in Table 1. Written informed consent was obtained from all of the patients, and the study was approved by the Ethics Committee of our hospital. None of the patients had received any therapy prior to sampling or had any complicating disease that affects wound healing. All of the keloid tissue specimens were examined and confirmed pathologically, and full-thickness biopsy specimens were obtained.

Cell culture

Tissues were dissected and minced into pieces measuring 1 mm in diameter, and they were then cultured in DMEM supplemented with 5% FBS in 5% CO_2 and 37 °C humidified atmosphere. At day 12, the cells proliferating out of the explanted tissues were passaged, and the central colonies in 3–6 passages were used for subsequent experiments.

Construction of a hypoxic model

The air was customized to contain 1% O_2, 5% CO_2, and 94% N_2, and it was stored in a sterile CO_2 air tank. Before hypoxia treatment, the incubator (SANYO, Japan) was equipped with customized air overnight at stable air pressure. When the CO_2 content reached a level of 5%, the O_2 content was 1%.

CCK-8 cell proliferation assay

The Cell Counting Kit-8 (CCK-8; Sigma, St. Louis, Missouri, USA) was used to quantify cell proliferation. Cells were seeded in 96-well plates until the cells adhered to the plate wall. Cells were divided into normal control and hypoxia challenged groups, and each group contained five plates. Cell counts per 25 cm^2 were quantified. Cells were seeded at a density of 1000 cells/100 μl, 3000 cells/100 μl, and 5000 cells/100 μl, with five replicates each. Cells in both the experimental group and the control group were incubated under respective conditions for 5 days. Every 24 h, the CCK-8 reagent (10 μl/well) was added to the well for 2 h before the absorbance was measured at 450 nm by the ELISA plate reader (Thermo, Waltham, MA, USA), which was then processed with Gen5.

Annexin V-FITC apoptosis detection

FITC Annexin V/Dead Cell Apoptosis kit (Thermo, Waltham, Massachusetts, USA) was used to assess the apoptosis rate of keloid fibroblasts. Then, 100 μl cells were suspended in 1 × Annexin-binding buffer and incubated with 5 μl fluorescein isothiocyanate (FITC) annexin V and 5 μl PI for 15 min at room temperature in darkness. After adding 400 μl to each replicate, the viability and apoptosis were analyzed by flow cytometry at 530 nm and more than 575 nm, respectively (BD Biosciences, NJ, USA).

Caspase-3 activity assay

Caspase-3 activity assay kit (Beyotime, Shanghai, China) was used to determine caspase activity in keloid fibroblasts. Then, 50 μl chilled cell lysis buffer was added to the cells that were collected by centrifugation at 600*g* for 5 min. After 10 min of lysis, the lysate was centrifuged at 16,000*g* for 10 min. The supernatant was transferred to a pre-cooled centrifuge tube, and it was dispensed to a 96-well plate in which a 50 μl sample, 40 μl reaction buffer, and 10 μl Ac-DEVD-pNA (2 mM) substrate were mixed and incubated for 90 min at 37 °C. Until the color change was obvious, absorbance was measured at a wavelength of 405 nm with ELISA reader. In parallel, small amounts of samples were used to determine the protein concentration with the Bradford method, which helps to normalize the absorbance values.

Western blot

Keloid fibroblasts were lysed, and total proteins were extracted using RIPA. Aliquots of the extracted proteins were then subjected to 10% sodium dodecylsulfate–polyacrylamide gel electrophoresis (SDS–PAGE) and then transferred to nitrocellulose membranes. Furthermore, 5% skimmed milk was used to block the background, and 0.1% Tween 20 was resolved in PBS (pH 7.4), which was then incubated with the primary antibodies for 60 min at 37 °C. The following antibodies were used: anti-HIF-1α (1:250, Abcam), anticollagen I (1:1000, Abcam), anti-collagen III (1:1000, Abcam), and β-actin (1:1000). Horseradish peroxidase (HRP) conjugated secondary antibody (diluted in 0.01 M PBS) was added, followed by washing five times with 0.01M PBS. Enhanced chemiluminescence solution was used to visualize the antigens, and Image J was employed to assess the signal intensities.

Quantitative RT-PCR

Total RNA was extracted using Trizol. cDNA synthesis was performed by Revert Aid M-MulV Reverse Transcriptase, which served as the template for PCR with SYBR mix (Roche Applied Science, Penzberg, Upper Bavaria, Germany) as per the manufacturer's instructions. Each sample contained three replicates. The primer sequences were as follows (Invitrogen, USA): HIF1A forward: 5′-CGTTCCTTCGA TCAGTTGTC-3′, and antisense: 5′-TCAGTGGTGGC AGTG GTAGT-3′; COL1A1 forward: 5′-TACAGCGTCACTGTCGATGGC-3′, and reverse: 5′-TCAATCACTGTCTTGCCCCAG-3′; COL3A1 forward: 5′-AATTTGGTGTGGACGTTGGC-3′, and reverse: 5′-TTGTCGG TCACTTGCAC TGG-3′; and β-actin forward: 5′-GAGACCTTCAACACC CCAGCC-3′, and reverse: 5′-AATGTCACGCACGATTTCCC-3′.

siRNA transfection and construction of HIF-1α

Cells were transfected with HIF-1α siRNAs and scrambled siRNAs (QIAGEN) using the Lipofectamine^{TM} 2000 Transfection Reagent (Invitrogen, USA), and they were grown to 70–90% confluence. Two days after incubation at 37 °C, cells were harvested for the subsequent assay. For HIF-1α overexpression constructs, pAdTrack-CMV and pAdEasy-1 were transfected into HEK-293 cells to produce a recombinant adenovirus, which was then used to transfect keloid fibroblasts with Lipofectamine^{TM} 2000 when the cells had grown to 60% confluence.

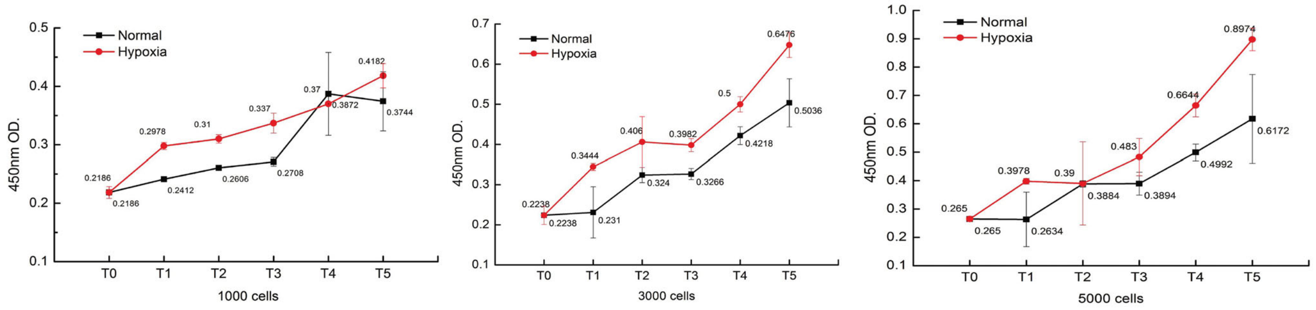

Figure 1. Growth curves of keloid fibroblasts under hypoxia and normoxia. At three different cell seeding densities (1000 cells/100 µl, 3000 cells/100 µl, and 5000 cells/100 µl), the difference between growth rate of keloid fibroblasts under hypoxia and normoxia were compared. *y*-axis suggest OD value of the culture, and *x*-axis represent duration under set conditions. Keloid fibroblasts have a higher proliferation level under hypoxia environment even similar proliferation level were showed at some time point when compared with normoxia environment (normal).

Statistical analysis

Data are reported as mean ± standard deviation (SD). SPSS statistics 24.0 software (SPSS, Inc., Chicago, IL, USA) was used. Student's *t* test was used to test the significance of differences, and $p < 0.05$ was considered significant.

Results

Hypoxia induces proliferation and inhibits apoptosis of keloid fibroblasts

The growth rate in the hypoxia group was significantly higher than that in the control group (Figure 1). At 1000 cells/µl, the cell number in the control group outnumbered that in the hypoxia group at day 4, and after that, it decreased, while the cell number in the hypoxia group kept increasing. At 3000 cells/µl, the hypoxia group showed higher cell number and an increased OD450 value, which was the same as that at 5000 cells/µl.

Resveratrol attenuates the effect of hypoxia on keloid fibroblasts

On supplementation with different concentrations of resveratrol and incubation under hypoxia for 5 days, the proliferation of keloid fibroblasts was reduced (Figure 2(a)), which seemed to display a dose-dependent pattern (3000 cells/100 µl). After 2 days of treatment, the cell viability displayed remarkable divergence. At 80 µM, the OD450 value indicating the cell number showed a significant difference. Under a microscope, we observed that excessive number of resveratrol-treated cells were shrunk and deformed. Annexin-V flow cytometry was performed to assess cell apoptosis of keloid fibroblasts treated with 80 µM resveratrol under hypoxia. Results showed that the apoptosis rates of resveratrol-treated cells were significantly higher than those of control cells after 48 h (Figure 2(b)). No significant difference in necrosis was observed between keloid and normal fibroblasts (Figure 2(b)). To further examine the difference in apoptosis, we compared the caspase-3 activity in keloid fibroblasts treated with resveratrol and control under hypoxia.

Suppression of HIF-1α accounts for attenuation of proliferation and induction of apoptosis in hypoxia-challenged keloid fibroblasts

Western blotting showed that HIF-1α expression in hypoxia-challenged cells was higher than that in control cells, and this increase was mitigated by resveratrol treatment (Figure 3(a)). Moreover, HIF-1α deficient keloid fibroblasts showed stagnant growth and an increased proportion of cells underwent apoptosis (Figure 3(b,c)).

Overexpression of HIF-1α reverses the inhibition of proliferation and induction of apoptosis by resveratrol

Compared with untreated keloid fibroblasts, resveratrol treatment of cells overexpressing HIF-1α did not cause a significant change in the cell number after 48 h (Figure 4(a,b)). The active caspase-3 in resveratrol-treated cells was also rescued by overexpression of HIF-1α, and both levels were significantly increased in untreated HIF-1α overexpressing cells (Figure 4(c)).

HIF-1α promotes collagen synthesis and resveratrol inhibits collagen synthesis

The protein levels of collagen I and collagen III were substantially reduced in resveratrol-treated cells, and HIF-1α overexpression seemed to alleviate this effect (Figure 5).

Discussion

Tumors proliferate rapidly by consuming the excess oxygen, and they produce a hypoxic microenvironment. The hypoxic microenvironment in turn confers a proliferative advantage on tumor cells. A keloid, generally considered to be a benign hyperplastic dermal tumor, thrives in a hypoxic microenvironment and exhibits similar characteristics as those of a tumor, including uncontrolled proliferation, migration and invasion, and escape from apoptosis. During wound repair, the injured area is in a hypoxic state, which induces fibroblast proliferation and angiogenesis. Therefore, we suspected that hypoxia contributes to the tumor-like characteristics of keloids. In the cell viability assay, the hypoxia-challenged cells showed more rapid growth than cells under normal conditions. Other researches also indicated the cell growth changes under hypoxia environment, which could increase periostin expression and affect cell proliferation, apoptosis, migration and invasion. In addition, cells could also possess myofibroblast-like phenotype under hypoxia environment. These observations were in accordance with multiple lines of evidence showing the effects of low oxygen environment on cell growth in various cell types [20–23]. Furthermore, a decreased apoptosis rate was also observed in hypoxia-treated keloid fibroblasts. Taken together, we deemed that the existence of hypoxia in the wound area may confer keloid fibroblasts with a growth advantage and resistance to apoptosis, and it contributes to resistance of keloids to treatment; however, the underlying molecular mechanism remains to be determined.

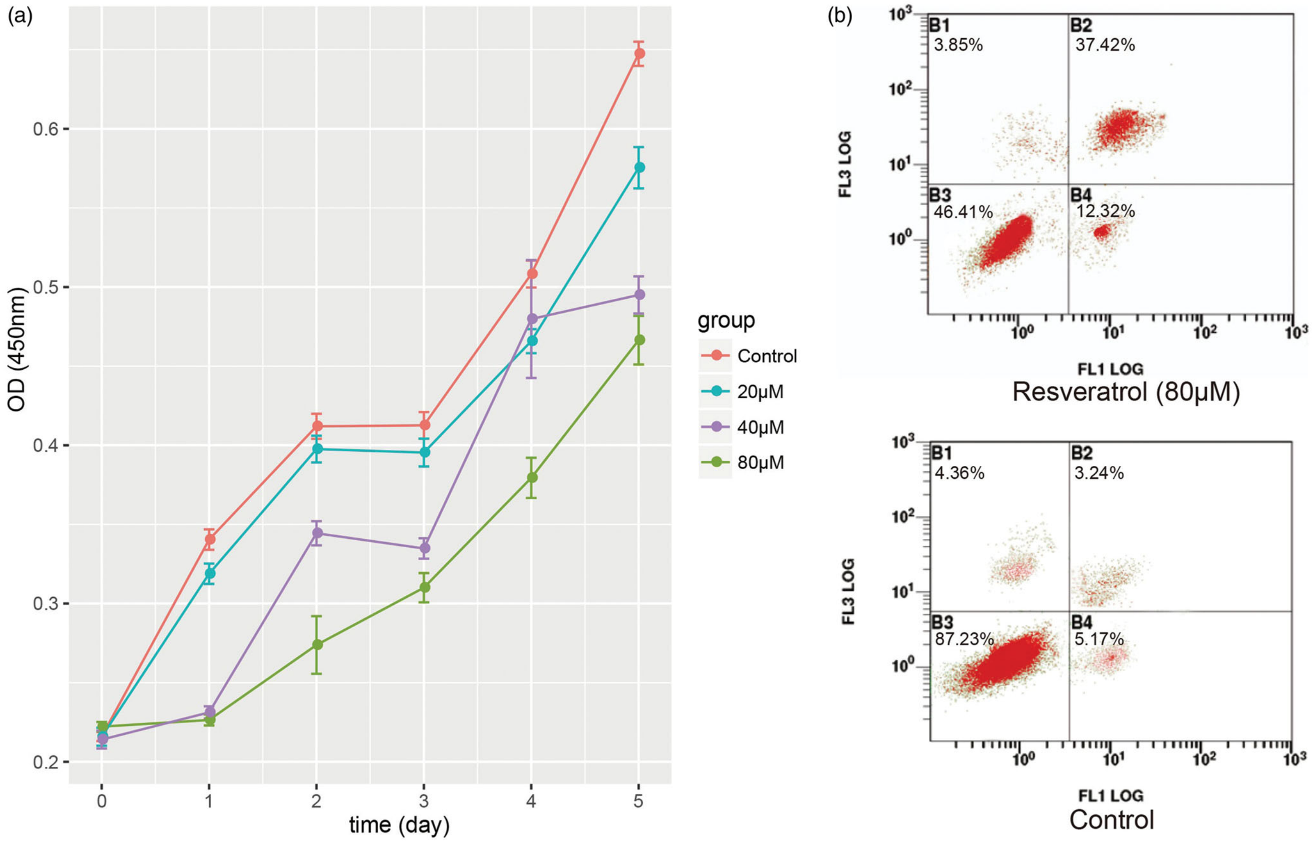

Figure 2. Resveratrol attenuates effect of hypoxia on keloid fibroblasts. (a). The growth curves of keloid fibroblasts cultured with different concentration of resveratrol under hypoxia for 5 days, and the OD values were taken every 24 h. (b). Annexin V apoptosis flow cytometry analysis of keloid fibroblasts treated with 80 μM resveratrol for 2 days. Upper left quadrant represents necrosis, upper right represents late apoptosis, and lower right represents early apoptosis (control: keloid fibroblasts under hypoxia environment with 0 μM resveratrol).

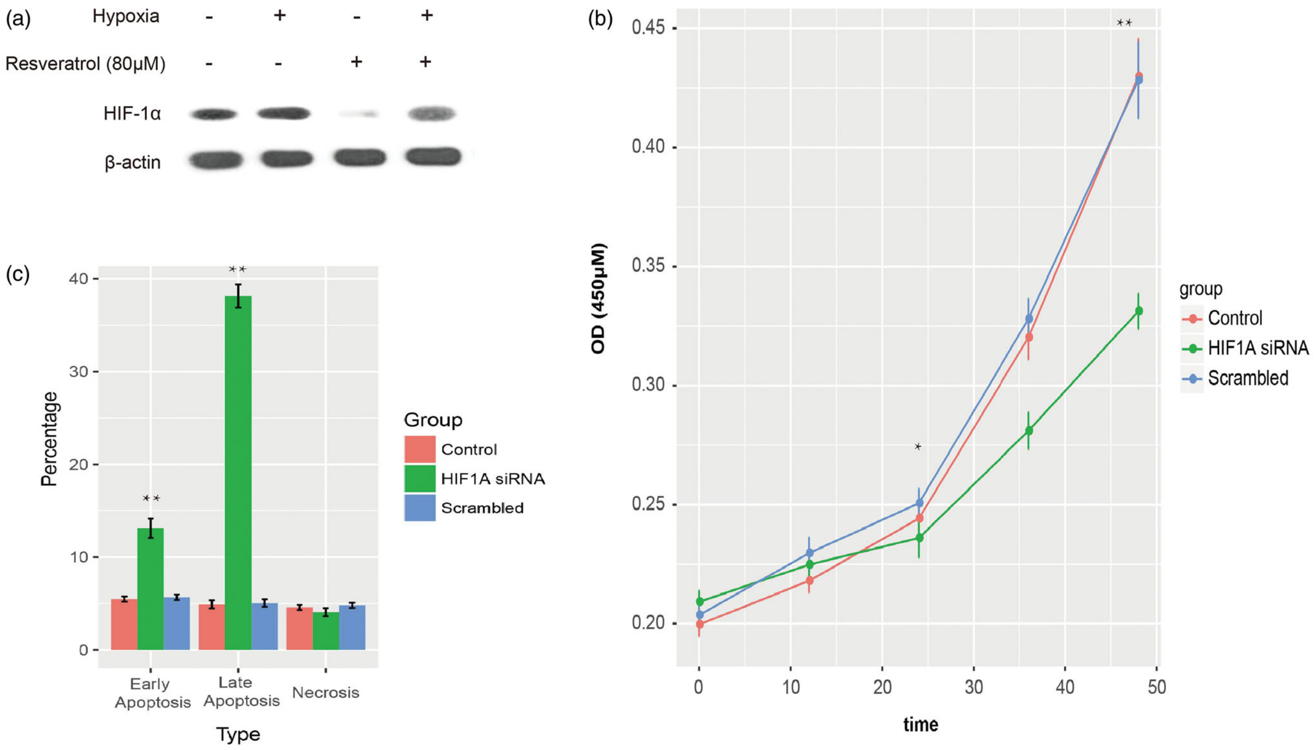

Figure 3. Suppression of HIF-1α accounts for attenuation on proliferation and induction of apoptosis in hypoxia-challenged keloid fibroblasts. (a) Western blot of HIF-1α of keloid fibroblasts treated/untreated with resveratrol under hypoxia/normoxia. (b) Cell growth curves of HIF-1α deficient keloid fibroblasts. HIF-KD: HIF-1α deficient, $*p < 0.05$, $**p < 0.01$. (c) Apoptosis rates of HIF-1α deficient keloid fibroblasts. HIF-KD: HIF-1α deficient, $*p < 0.05$, $**p < 0.01$. (c) *y* axis represents percentage of cells in early apoptosis, late apoptosis, or necrosis (control: keloid fibroblasts under hypoxia environment).

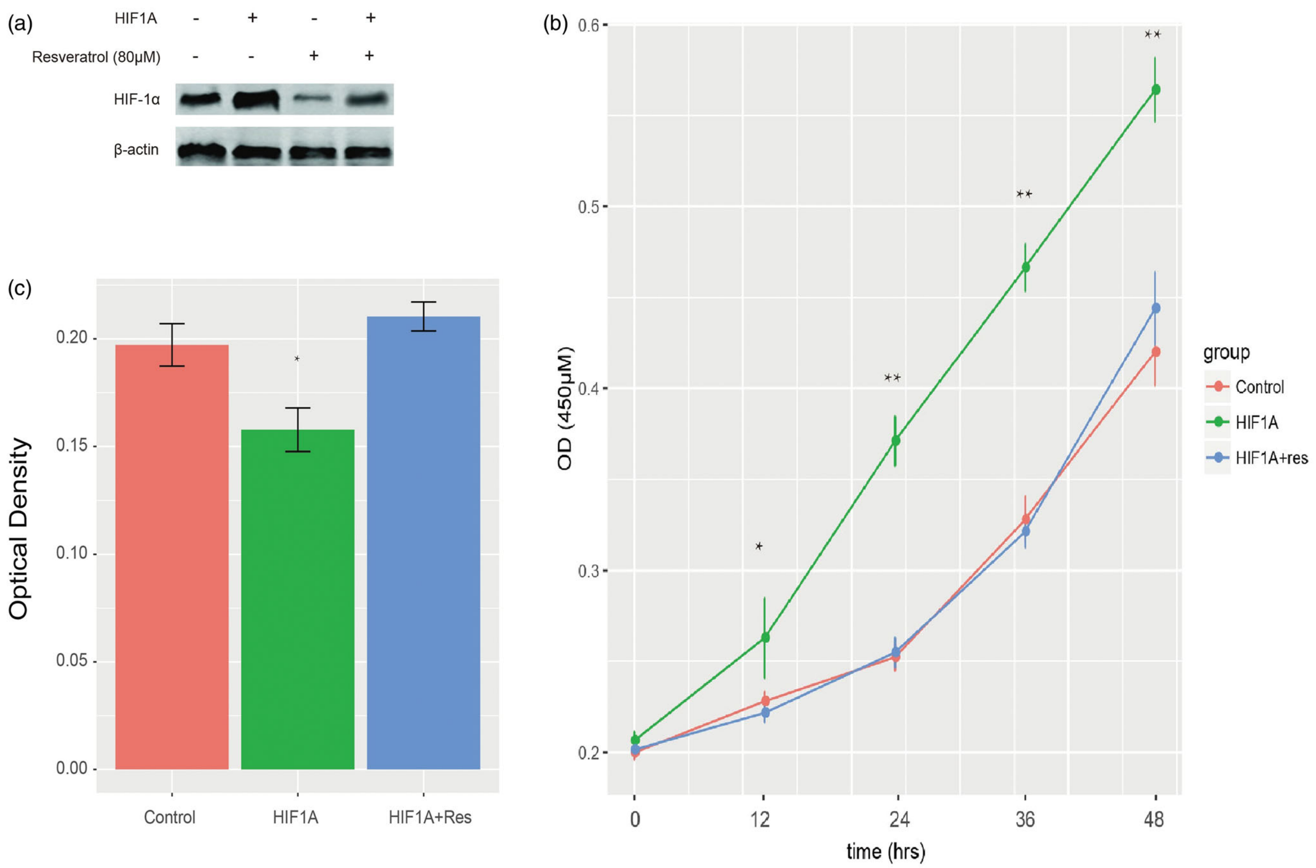

Figure 4. Overexpression of HIF-1α reverses the inhibition of proliferation and induction of apoptosis by resveratrol. (a). Western blot of HIF-1α in HIF1A overexpressing keloid fibroblasts. (b). Growth curves of keloid fibroblasts overexpressing HIF1A treated/untreated with resveratrol for 2 days. *y* axis represents OD values under 450 nm and *x* axis represents time points when the OD values were taken. Error bars indicate standard deviation. $^{*}p < 0.05$, $^{**}p < 0.01$. (c). Caspase-3 activity of HIF1A overexpressing keloid fibroblasts treated/untreated with resveratrol. $^{*}p < 0.05$. (control: keloid fibroblasts under hypoxia environment without resveratrol).

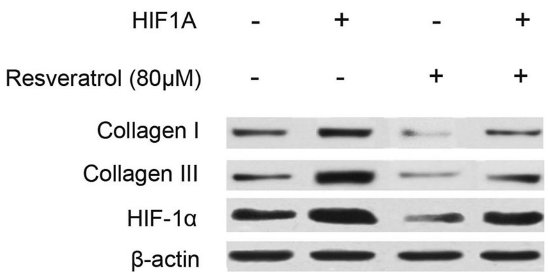

Figure 5. Western blot image of each group. Under hypoxia environment, HIF-1α showed higher expression which could increase the expression of collagen I and collagen III. Besides, resveratrol could significantly decrease collagen expression.

Resveratrol is a natural polyphenol compound found abundantly in grapes and berries [24]. Over the past decade, resveratrol has received much attention in anti-cancer therapy [25,26], and it has been integrated into food supplements. In addition to its anti-proliferative and pro-apoptotic properties in carcinogenesis, resveratrol is also well-known for its ability to reduce energy expenditure *in vivo* [27]. Recently, the inhibitory effect of resveratrol on cellular glucose metabolism has been highlighted in several studies [28]. Anthony et al. [29] reported that resveratrol inhibited phosphatidylinositol 3-kinase (PI-3K) signaling and glucose metabolism, accompanied by cell-cycle arrest, in germinal center B-cell-like LY1 and LY8 human diffuse large B-cell lymphomas (DLBCLs). Consistently, Mara et al. [30] revealed that resveratrol treatment decreased the ability of HepG2 cells to utilize glucose and amino acids, as well as it slowed down the cell cycle in the S phase but without inducing apoptosis. Furthermore, they also demonstrated that Sirt1, an energy status sensitive protein that harnesses NAD + as a co-enzyme for its acetylase activity, increased in the presence of resveratrol. Sirt1 is a deacetylase that is sensitive to NAD+/NADH. Reduced level of NAD + during hypoxia downregulated SIRT1, which inhibited deacetylation and led to activation of HIF-1α [31]. Conversely, when glycolysis was blocked, SIRT1 may be upregulated, thus resulting in excessive deacetylated HIF-1α, which impairs its activity. These pieces of evidence point to the inhibitory effect of resveratrol on hypoxia-induced biological processes. Indeed, in the present study, we observed that hypoxia-induced proliferation and inhibition of apoptosis in keloid fibroblasts was attenuated by resveratrol treatment, and these findings indicate the therapeutic value of resveratrol in treating keloids.

HIF-1α is a subunit of HIF-1, which is a transcriptional regulator of cellular and physiological processes in response to hypoxia stress. The expression of HIF-1α is tightly regulated by hypoxia stress, while HIF-1β is constitutively expressed. Accumulation of HIF-1α promotes its binding with HIF-1β to form active dimers, which were identified as transcriptional factors of more than 100 genes implicated in cellular proliferation, apoptosis, and angiogenesis [32,33]. Overexpression of HIF-1α has been found to be associated with many human cancers and to promote their metastasis. Moreover, the expression level of HIF-1α showed a correlation with the aggressive tumor phenotype, such as a more advanced tumor grade. Therefore, HIF-1α is a potential marker for evaluating the prognosis, mortality risk, or therapeutic efficacy [34]. In keloids, abnormal expression of HIF-1α has also been frequently reported. Zhang et al. [35] found that HIF-1α accumulation was augmented in a co-culture of keloid fibroblasts and human mast cells, and it involved the activation of ERK1/2 and an increase in VEGF. Ma et al. [36] demonstrated that HIF-1α was highly expressed in hypoxia-exposed keratinocytes and the epithelial layer of keloid tissue. They further found that vimentin and fibronectin, markers of epithelial-to-mesenchymal transition, were upregulated during hypoxia, which was concurrent with reduced expression of E-cadherin and zonula occludens-1 (ZO-1). This reduced expression of E-cadherin and ZO-1 can be reversed by silencing of HIF-1α. In the present study, overexpression of HIF-1α was found in hypoxia-challenged keloid fibroblasts, thus suggesting its potential role in promoting cell growth and suppressing apoptosis of keloid fibroblasts. Resveratrol treatment downregulated HIF-1α, and this effect was attenuated by overexpressing HIF-1α. This suggests that HIF-1α may be a mediator of the effect of resveratrol in keloid fibroblasts. On the other hand, glycolysis can be blocked by resveratrol, and Sirt1 would be upregulated accordingly. Deacetylation of HIF-1α by excess of Sirt1 could further inhibit the activity of HIF-1α. Therefore, resveratrol may be a potential therapeutic agent for inhibiting and suppressing HIF-1α.

Excessive deposition of collagen is a typical characteristic of keloid tissues. Resveratrol was found to markedly reduce the levels of fibrotic markers, including fibronectin, collagen I, and collagen IV, and to decrease ECM deposition via regulation of autophagy in a SIRT1-dependent manner [37]. Karin et al. [38] showed that deletion of HIF-1α disrupted collagen modification, cell migration, and metastasis in murine sarcoma models. In the present study, we chose collagen I and collagen III to represent the level of collagen, and they constitute 80–90% and 10–15% of total collagen present in the skin, respectively [39,40]. We demonstrated that HIF-1α deficiency impaired the expressions of pro-collagen type I and type III, and the mRNA levels of COL1A1 and COL3A1 were also downregulated, thus indicating that resveratrol not only exerts an inhibitory effect on collagen synthesis via SIRT1 functionality but also hampers the expressions of COL1A1 and COL3A1 through downregulation of HIF-1α. Taken together, a complementary mechanism underlying the regulation of collagen synthesis by resveratrol was revealed in our study.

In our results, the proliferation of keloid fibroblasts was enhanced under hypoxic conditions. Resveratrol has been reported to be an anti-cancer adjuvant. A similar effect of resveratrol on keloid fibroblasts was also observed under hypoxia environment. The results of HIF-1α deficient and overexpression in keloid fibroblasts suggesting that HIF-1α might be involved in the mechanism underlying the pharmaceutical effect of resveratrol in keloid fibroblasts.

In conclusion, our study showed that hypoxia promotes proliferation and inhibits apoptosis of keloid fibroblasts, and these findings highlight the potential obstacle in treating keloids. Furthermore, we demonstrated that resveratrol could reverse the effect of hypoxia on keloids through downregulation of HIF-1α, a stress–responsive protein that was then found to disrupt collagen synthesis in keloid tissues. These results provide complementary evidence for multiple molecular signaling pathways controlled by resveratrol and suggest a pathway implicating that resveratrol regulates the expression of pro-collagen via HIF-1α.

Disclosure Statement

No potential conflict of interest was reported by the author(s).

Funding

This work was supported by National Natural Science Foundation of China [81801926].

References

[1] Werner S, Krieg T, Smola H. Keratinocyte–fibroblast interactions in wound healing. J Invest Dermatol. 2007;127(5):998–1008.

[2] Botwood N, Lewanski C, Lowdell C. The risks of treating keloids with radiotherapy. Br J Radiol. 1999;72(864):1222–1224.

[3] Tuan T, Nichter L. The molecular basis of keloid and hypertrophic scar formation. Mol Med Today. 1998;4(1):19–24.

[4] Andrews J, Marttala J, Macarak E, et al. Keloids: the paradigm of skin fibrosis—pathomechanisms and treatment. Matrix Biol. 2016;51:37–46.

[5] Shang Y, Yu D, Hao L. Liposome-adenoviral hTERT-siRNA knockdown in fibroblasts from keloids reduce telomere length and fibroblast growth. Cell Biochem Biophys. 2015;72(2):405–410.

[6] Sidgwick G, Bayat A. Extracellular matrix molecules implicated in hypertrophic and keloid scarring. J Eur Acad Dermatol Venereol. 2012;26(2):141–152.

[7] Aoki M, Miyake K, Ogawa R, et al. siRNA knockdown of tissue inhibitor of metalloproteinase-1 in keloid fibroblasts leads to degradation of collagen type I. J Invest Dermatol. 2014;134(3):818–826.

[8] Gragnani A, Warde M, Furtado F, et al. Topical tamoxifen therapy in hypertrophic scars or keloids in burns. Arch Dermatol Res. 2010;302(1):1–4.

[9] Nanda S, Reddy B. Intralesional 5-fluorouracil as a treatment modality of keloids. Dermatol Surg. 2004;30(1):54–56.

[10] Sooriakumaran P, Kaba R. Angiogenesis and the tumour hypoxia response in prostate cancer: a review. Int J Surg. 2005;3(1):61–67.

[11] Selvendiran K, Bratasz A, Kuppusamy M, et al. Hypoxia induces chemoresistance in ovarian cancer cells by activation of signal transducer and activator of transcription 3. Int J Cancer. 2009;125(9):2198–2204.

[12] Jensen R. Brain tumor hypoxia: tumorigenesis, angiogenesis, imaging, pseudoprogression, and as a therapeutic target. J Neurooncol. 2009;92(3):317–335.

[13] Higgins L, Withers H, Garbens A, et al. Hypoxia and the metabolic phenotype of prostate cancer cells. Biochim Biophys Acta. 2009;1787(12):1433–1443.

[14] Vaupel P, Briest S, Höckel M. Hypoxia in breast cancer: pathogenesis, characterization and biological/therapeutic implications. Wien Med Wochenschr. 2002;152(13–14):334–342.

[15] Majmundar A, Wong W, Simon M. Hypoxia-inducible factors and the response to hypoxic stress. Mol Cell. 2010;40(2):294–309.

[16] Csiszar A, Labinskyy N, Olson S, et al. Resveratrol prevents monocrotaline-induced pulmonary hypertension in rats. Hypertension. 2009;54(3):668–675.

[17] Chen B, Xue J, Meng X, et al. Resveratrol prevents hypoxia-induced arginase II expression and proliferation of human pulmonary artery smooth muscle cells via Akt-dependent

signaling. Am J Physiol Lung Cell Mol Physiol. 2014;307(4): L317–L325.

[18] Ikeda K, Torigoe T, Matsumoto Y, et al. Resveratrol inhibits fibrogenesis and induces apoptosis in keloid fibroblasts. Wound Repair Regen. 2013;21(4):616–623.

[19] Zeng G, Zhong F, Li J, et al. Resveratrol mediated reduction of collagen by inhibiting proliferation and producing apoptosis in human hypertrophic scar fibroblasts. Biosci Biotechnol Biochem. 2013;77(12):2389–2396.

[20] Hung SP, Ho JH, Shih YR, et al. Hypoxia promotes proliferation and osteogenic differentiation potentials of human mesenchymal stem cells. J Orthop Res. 2012;30(2):260–266.

[21] Zhang Z, Nie F, Kang C, et al. Increased perostin expression affects the proliferation, collagen synthesis, migration and invasion of keloid fibroblasts under hypoxic conditions. Int J Mol Med. 2014;34(1):253–261.

[22] Mehta M, Branford OA, Rolfe KJ. The evidence for natural therapeutics as potential anti-scarring agents in burn-related scarring. Burns Trauma. 2016;4:15–27.

[23] Zhang B, Guan H, Liu JQ, et al. Hypoxia drives the transition of human dermal fibroblasts to a myofibroblast-like phenotype via the TGFβ1/Smad3 pathway. Int J Mol Med. 2017;39(1):153–159.

[24] Athar M, Back JH, Tang X, et al. Resveratrol: a review of preclinical studies for human cancer prevention. Toxicol Appl Pharmacol. 2007;224(3):274–283.

[25] Xu W, Zhao Y, Zhang B, et al. Resveratrol attenuates hyperoxia-induced oxidative stress, inflammation and fibrosis and suppresses Wnt/β-catenin signalling in lungs of neonatal rats. Clin Exp Pharmacol Physiol. 2015;42(10): 1075–1083.

[26] Athar M, Back JH, Kopelovich L, et al. Multiple molecular targets of resveratrol: anti-carcinogenic mechanisms. Arch Biochem Biophys. 2009;486(2):95–102.

[27] Timmers S, Konings E, Bilet L, et al. Calorie restriction-like effects of 30 days of resveratrol supplementation on energy metabolism and metabolic profile in obese humans. Cell Metab. 2011;14(5):612–622.

[28] Crandall JP, Oram V, Trandafirescu G, et al. Pilot study of resveratrol in older adults with impaired glucose tolerance. J Gerontol A Biol Sci Med Sci. 2012;67(12):1307–1312.

[29] Faber AC, Dufort FJ, Blair D, et al. Inhibition of phosphatidylinositol 3-kinase-mediated glucose metabolism coincides with resveratrol-induced cell cycle arrest in human diffuse large B-cell lymphomas. Biochem Pharmacol. 2006;72(10): 1246–1256.

[30] Massimi M, Tomassini A, Sciubba F, et al. Effects of resveratrol on HepG2 cells as revealed by (1)H-NMR based metabolic profiling. Biochim Biophys Acta. 2012;1820(1):1–8.

[31] Lim JH, Lee YM, Chun YS, et al. Sirtuin 1 modulates cellular responses to hypoxia by deacetylating hypoxia-inducible factor 1 alpha. Mol Cell. 2010;38(6):864–878.

[32] Mancini M, Gariboldi MB, Taiana E, et al. Co-targeting the IGF system and HIF-1 inhibits migration and invasion by (triple-negative) breast cancer cells. Br J Cancer. 2014; 110(12):2865–2873.

[33] Conde E, Alegre L, Blanco-Sánchez I, et al. Hypoxia inducible factor 1-alpha (HIF-1 alpha) is induced during reperfusion after renal ischemia and is critical for proximal tubule cell survival. PLos One. 2012;7(3):e33258.

[34] Zhang Q, Tang X, Lu QY, et al. Resveratrol inhibits hypoxia-induced accumulation of hypoxia-inducible factor-1alpha and VEGF expression in human tongue squamous cell carcinoma and hepatoma cells. Mol Cancer Ther. 2005;4(10): 1465–1474.

[35] Zhang Q, Oh CK, Messadi DV, et al. Hypoxia-induced HIF-1 alpha accumulation is augmented in a co-culture of keloid fibroblasts and human mast cells: involvement of ERK1/2 and PI-3K/Akt. Exp Cell Res. 2006;312(2):145–155.

[36] Ma X, Chen J, Xu B, et al. Keloid-derived keratinocytes acquire a fibroblast-like appearance and an enhanced invasive capacity in a hypoxic microenvironment in vitro. Int J Mol Med. 2015;35(5):1246–1256.

[37] Agarwal R, Agarwal P. Targeting extracellular matrix remodeling in disease: could resveratrol be a potential candidate? Exp Biol Med (Maywood)). 2017;242(4):374–383.

[38] Eisinger-Mathason T, Zhang M, Qiu Q, et al. Hypoxia-dependent modification of collagen networks promotes sarcoma metastasis. Cancer Discov. 2013;3:1990–1205.

[39] Nasseri YY, Krott E, Groningen KMV, Berho M, et al. Abnormalities in collagen composition may contribute to the pathogenesis of hemorrhoids: morphometric analysis. Tech Coloproctol. 2015;19(2):83–87.

[40] Cicchi R, Vogler N, Kapsokalyvas D, et al. From molecular structure to tissue architecture: collagen organization probed by SHG microscopy. J Biophoton. 2013;6(2): 129–142.