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Expression of HIF-1α in keloids and its correlation with inflammatory responses and apoptosis

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Abstract

The aim of this study was to investigate the expression of hypoxia-inducible factor-1α (HIF-1α) in keloids and its correlation with inflammatory responses and apoptosis. The keloid specimens resected in our hospital from November 2015 to February 2017 were selected as the pathological group, and the normal skin tissues from our hospital during the same period were selected as the control group. The expression of HIF-1α, inflammatory response cytokines, and apoptotic molecules in the tissues of two groups were detected. The messenger RNA (mRNA) expression of HIF-1α in the keloids in the pathological group was significantly higher than that in the control group, and the mRNA expression of interleukin (IL)-1β, IL-2, IL-6, and tumor necrosis factor (TNF)-α in the pathological group was significantly higher than those in the control group. The mRNA expression of Bax in the pathological group was significantly higher than that in the control group. The mRNA expression of Bcl-2, livin, and hPEBP4 in the pathological group was significantly lower than that in the control group. Pearson test showed that there was a positive correlation between the mRNA expression of HIF-1α and inflammatory cytokines including IL-1β, IL-2, IL-6, and TNF-α. There were also a positive correlation between the mRNA expression of HIF-1α and Bax and a negative correlation between the mRNA expression of HIF-1α and Bcl-2, livin, and hPEBP4. In conclusion, HIF-1α was highly expressed in keloids and closely related to inflammatory response cytokines and apoptosis molecules. Increased expression of HIF-1α in keloids may be an important factor in inflammatory responses and increased apoptosis in skin tissues.

Keywords

apoptosis, hypoxia-inducible factor-1α, inflammatory responses, keloids

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Introduction

Keloids are formed when hyperplasia occurs in connective tissues and exceeds the original damage area in the healing process of skin injury such as trauma, surgery, inflammation, or burns.[1] Keloids have persistent proliferation characteristics and are fibrous tissue tumors. In the field of plastic surgery, keloid is the most prevalent disease. Its existence is extremely unfavorable for psychological condition of patients and affects the patients' social activities. It needs timely treatment to restore skin smoothness and improve symptoms. And the treatment difficulty has become the research focus in the field of plastic surgery. At present, the clinical treatment of keloids is not ideal and is easy to relapse after resection.[2] Microenvironmental hypoxia is a common feature of many diseases and can cause the production of a series of adaptive responses to maintain oxygen

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homeostasis.[3] A large number of transcription factors are involved in hypoxia response, while hypoxia-inducible factor (HIF)-1 is considered to be an important transcription factor mediating hypoxia response.[4] HIF-1α plays an important role in immune and inflammatory responses.[5] In fact, HIF-1α can regulate the differentiation of immune cells, promote adhesion, affect cell metabolism, induce angiogenesis and regulate cell proliferation and apoptosis.[6] At present, there are few researches on the relationship between HIF-1α and keloids. In this study, we compared the difference of HIF-1α in keloid tissues and normal skin tissues and further explored the relationship between HIF-1α and skin tissue inflammation and skin cell apoptosis.

Materials and methods

Subjects

A total of 50 keloid specimens resected in our hospital from November 2015 to February 2017 were selected as the pathological group, and the general information of the patients was as follows: 31 males and 19 females, age from 37 to 65 years with a mean age of 54.6 ± 1.7 years. A total of 50 cases of the normal skin tissues from our hospital during the same period were selected as the control group, and the general information of the patients was as follows: 30 males and 20 females, age from 36 to 64 years with a mean age of 54.3 ± 1.5 years. All patients voluntarily participated in the study and signed informed consent. There were no significant differences between the two groups on sex, age, and other characteristics at baseline ($P > 0.05$).

RNA extraction methods

TRIzol lysates were added to the pathological keloid specimens and normal skin tissues and the tissue was fully ground. A volume of 200 μL of chloroform was added to the suspension and the suspension was kept unchanged after full shock. The suspension was centrifuged after liquid stratification at 12,000 r/min speed for 20 min. The supernatant was absorbed adding equal volume of isopropanol; a slight shock was applied and kept static for 20 min and then centrifuged at 12,000 r/min speed for another 20 min to get RNA precipitation. Add diethyl pyrocarbonate (DEPC) water after drying RNA for the following study. Some sample of RNA was dissolved in DEPC water and converted to complementary DNA (cDNA) by reverse transcription using cDNA synthesis kits.

Detection of messenger RNA expression

The resultant cDNA samples were used to perform reverse transcription polymerase chain reaction (RT-PCR) under the following conditions: denaturation at 95°C for 10 min, then 40 cycles at 95°C for 15 s, and 60°C for 1 min. The mouse *GAPDH* gene expression value was normalized as an endogenous control for *HIF-1α*, interleukin (*IL*)*-1β, IL-2, IL-6*, tumor necrosis factor (*TNF*)*-α, Bax, Bcl-2, livin* and *hPEBP4* gene expression.

Statistical analysis

SPSS 20 software was used to analyze the data. Comparisons on measurement data between the two groups were conducted using t test, and correlation analysis between the two groups was performed using Pearson test. $P < 0.05$ indicates a significant difference.

Results

Comparisons on messenger RNA expression of HIF-1α between the two groups

The messenger RNA (mRNA) expression of HIF-1α in keloid tissues in the pathological group was 2.73 ± .35, while mRNA expression of HIF-1α in keloid tissues in the control group was 1.01 ± 0.26. The mRNA expression of HIF-1α in keloid tissues in the pathological group was significantly higher than that in the control group, as shown in Figure 1.

Comparisons on mRNA expression of inflammatory cytokines between the two groups

The mRNA expression of IL-1β, IL-2, IL-6, and TNF-α in keloids in the pathological group was significantly higher than that in the control group, as shown in Figure 2.

Comparisons of mRNA expression of apoptosis molecules between the two groups

The mRNA expression of apoptosis molecule Bax in the pathological group was significantly higher than that in the control group. The mRNA expression of anti-apoptotic molecules, including Bcl-2,

livin, and hPEBP4, in the pathological group was significantly lower than those in the control group, as shown in Figure 3.

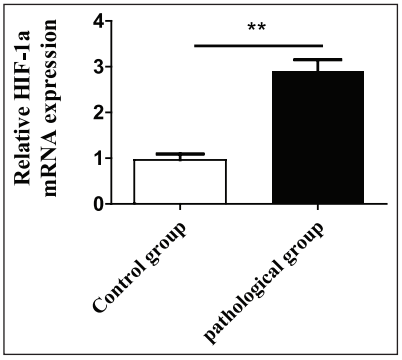

Figure 1. Comparisons of mRNA expression of HIF-1α between the two groups (**$P < 0.01$).

Correlation analysis

Pearson test showed that there was a positive correlation between the mRNA expression of HIF-1α and inflammatory cytokines, including IL-1β, IL-2, IL-6, and TNF-α. There was also a positive correlation between the mRNA expression of HIF-1α and Bax and a negative correlation between the mRNA expression of HIF-1α, Bcl-2, livin, and hPEBP4 ($P < 0.05$), as shown in Table 1.

Discussion

Keloid is a special type of scar, which has the characteristics of continuous growth. Although it is benign hyperplasia of fibrous tissue, it has aggressive behavior of malignant tumor, and it is easy to relapse after resection.[7] At present, the pathogenesis

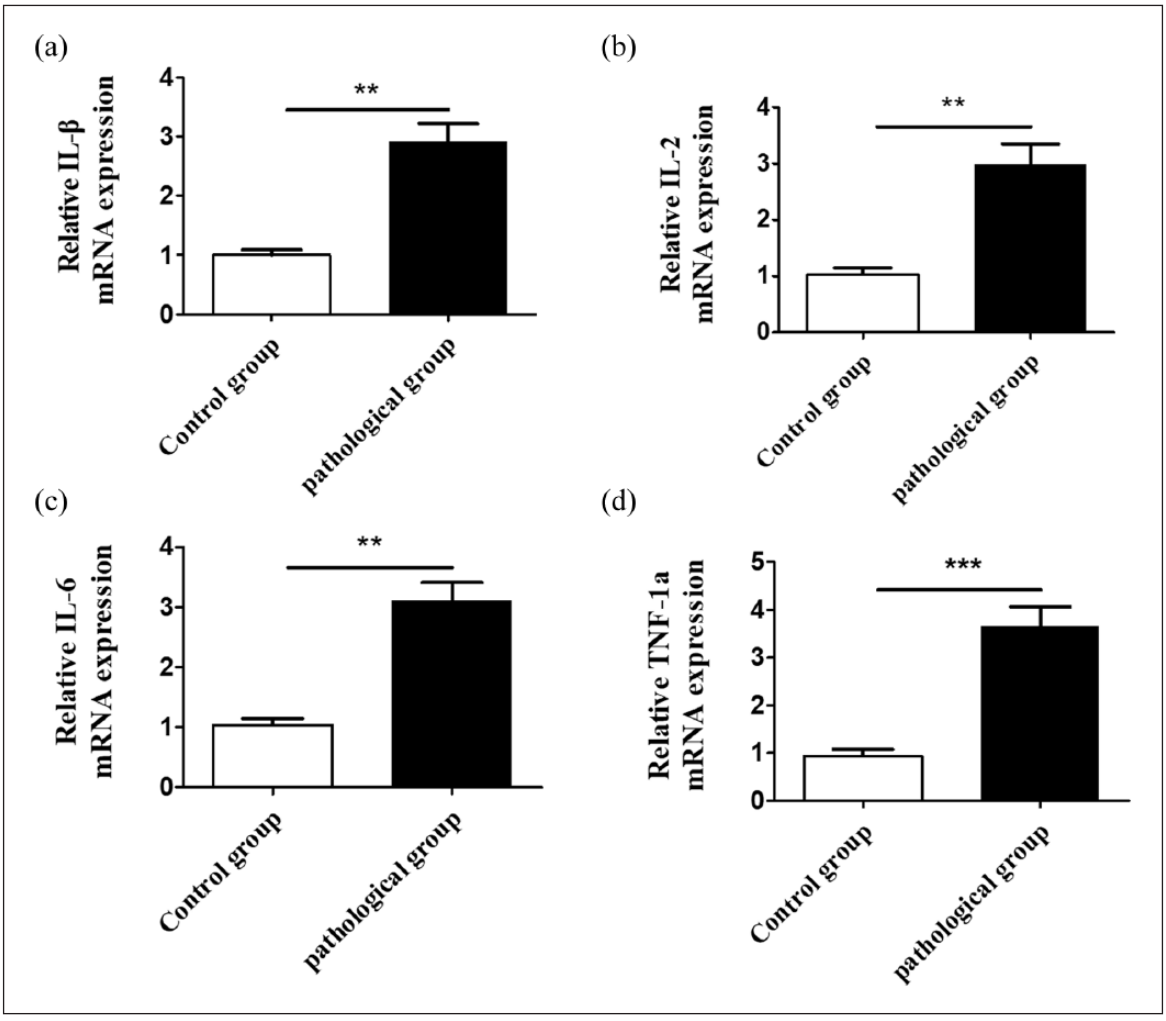

Figure 2. qT-PCR was performed to examine the mRNA expression of inflammatory cytokines (a) IL-β, (b) IL-2, (c) IL-6, and (d) TNF-1α between control groups and pathological groups (**$P < 0.01$, ***$P < 0.001$).

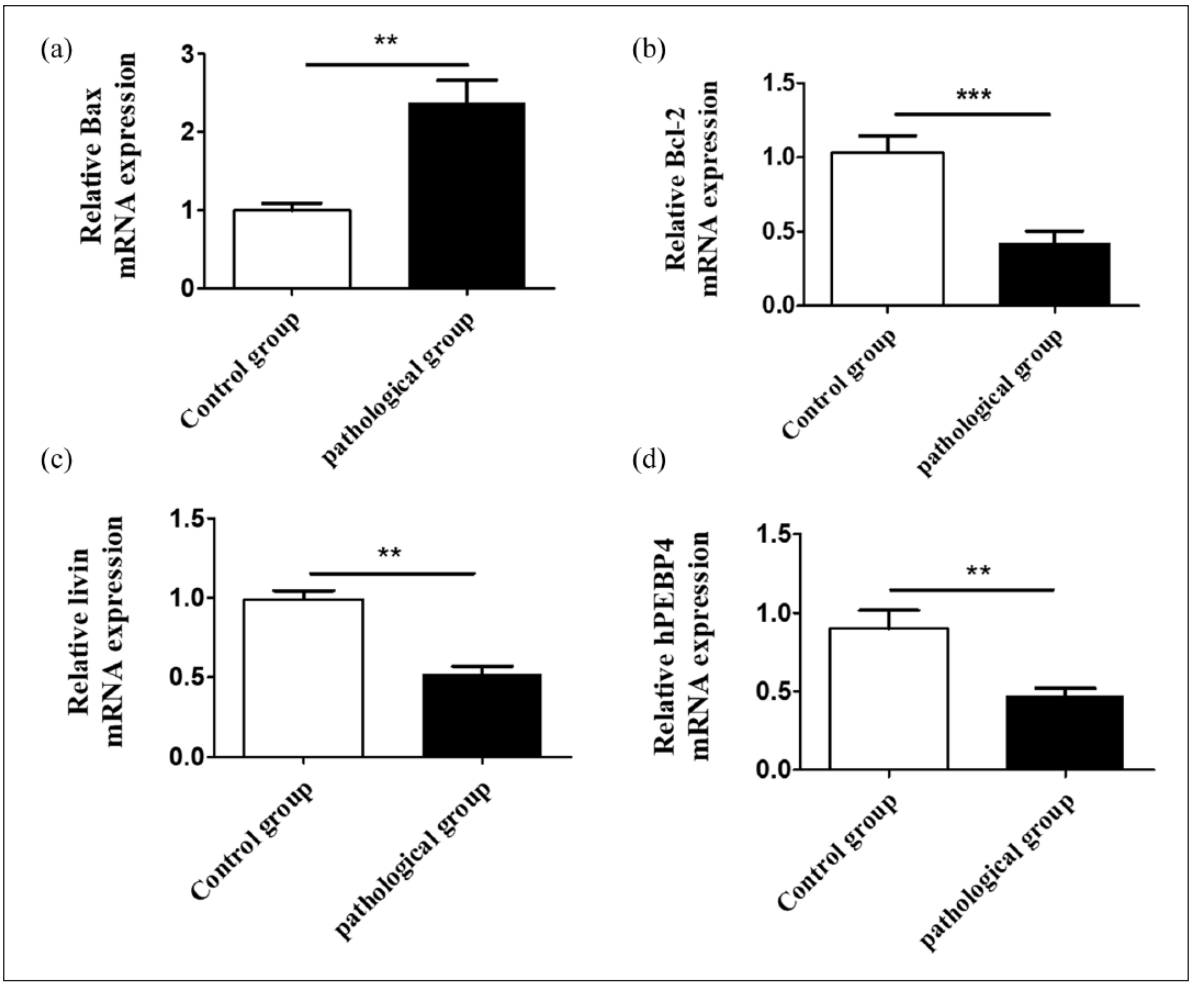

Figure 3. qT-PCR was performed to examine the mRNA expression of (a) Bax, (b) Bcl-2, (c) livin, and (d) Hpebp4 between control groups and pathological groups (**$P < 0.01$, ***$P < 0.001$).

Table 1. Correlation between mRNA expression of HIF-1α and inflammatory cytokines and apoptosis molecules.

HIF-1α mRNA	IL-1β mRNA	IL-2 mRNA	IL-6 mRNA	TNF-α mRNA	Bax mRNA	Bcl-2 mRNA	Livin mRNA	hPEBP4 mRNA
r	0.627	0.653	0.584	0.609	0.581	–0.125	–0.172	–0.166
P	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05

HIF: hypoxia-inducible factor; IL: interleukin; mRNA: messenger RNA.

of keloids has not yet been fully elucidated. In fact, keloid formation can cause inflammatory responses, cell proliferation, and apoptosis.[8] HIF-1α is the effector molecule of cells under hypoxia, and continuous hypoxia can increase the expression of HIF-1α.[9] By analyzing the difference of HIF-1α expression in keloid specimens and normal skin tissues, we found that the mRNA expression of HIF-1α in keloid tissues was significantly higher. This indicated that the increased expression of HIF-1α was related to the formation of keloids, and its effect on affecting the downstream inflammatory responses and cell apoptosis maybe the possible pathway in keloids.

The activation of inflammatory responses and the secretion of inflammatory cytokines involved in keloid formation can significantly promote the proliferation of fibroblasts.[10] After the formation of keloids, the function of mononuclear macrophage is activated, resulting in secretion of IL-1β, IL-2, and IL-6 and inducing neutrophil aggregating in local lesions. It can further promote the synthesis

of inflammatory mediators such as TNF-α and increase inflammatory responses. By analyzing the differences of inflammatory cytokines in keloids and normal skin tissues, we found that the mRNA expression of IL-1β, IL-2, IL-6, and TNF-α in keloids was significantly higher, which indicated that the increased expression of pro-inflammatory factors is related to the formation of keloids. Correlation analysis showed that there was a positive correlation between the mRNA expression of HIF-1α and the expression of IL-1β, IL-2, IL-6, and TNF-α, which meant that the increased expression of HIF-1α in keloids could affect the expression of inflammatory cytokines and promote inflammation responses.

The proliferation of fibroblasts in keloids is also closely related to the abnormality of apoptosis program.[11] *Bax* is a typical apoptosis gene that can promote tumor cell apoptosis, while *Bcl-2, livin*, and *hPEBP4* are genes that can inhibit apoptosis.[12] By comparing the difference in gene expression of apoptosis and anti-apoptotic genes, we found that the mRNA expression of the apoptosis molecule Bax in pathological group was significantly higher. However, the mRNA expression of anti-apoptotic molecules including Bcl-2, livin, and hPEBP4 in the pathological group was significantly lower. The results indicated that the upregulated expression of apoptosis molecule and downregulated expression of anti-apoptotic molecules were related to the formation of keloids. Pearson test showed that there was a positive correlation between the mRNA expression of HIF-1α and inflammatory cytokines including IL-1β, IL-2, IL-6, and TNF-α. There was also a positive correlation between the mRNA expression of HIF-1α and Bax and a negative correlation between the mRNA expression of HIF-1α, Bcl-2, livin, and hPEBP4, which meant that the increased expression of HIF-1α could affect the expression of cell apoptosis molecules and inhibit cell apoptosis.

In summary, HIF-1α was highly expressed in keloids and closely related to inflammatory response cytokines and apoptosis molecules. Increased expression of HIF-1α in keloids may be an important factor in inflammatory responses and increased apoptosis in skin tissues.

Declaration of conflicting interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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