EXPRESSION AND CORRELATION ANALYSIS OF KEY MOLECULES OF MTOR SIGNALLING PATHWAY-PI3K, AKT AND MTOR IN PATIENTS WITH PATHOLOGICAL SCAR

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ABSTRACT

Objective: *To investigate the expression and correlation analysis of phosphatidylinositol 3-kinase (pi3k), protein kinase B (akt) and mammalian target of rapamycin (mtor) in the mtor signalling pathway in patients with pathological scars.*

Methods: *Thirty-six patients with pathological scars diagnosed in our hospital from April 2014 to January 2017 were divided into a hypertrophic scar group (n=18) and a keloid group (n=18) according to their pathological types. The normal skin tissue of 18 cases of the hypertrophic scar group was selected as a control group. RT-PCR and Western Blot was used to detect the expression of pi3k, akt, mtor, mRNA and protein in normal tissue, hypertrophic scar tissue and keloid tissue. Moreover, the correlation between mRNA of pi3k, akt and mtor in pathological scar was analysed.*

Results: *The results of RT-PCR showed that the expression of pi3k mRNA in hypertrophic scar tissue and keloid tissue was not significantly different from normal skin tissue (P>0.05). The expression of akt and mtor mRNA in hypertrophic scar tissue and keloid tissue was significantly lower than normal skin tissue (P<0.05). Moreover, the expression of akt and mtor mRNA in hypertrophic scar tissue was significantly higher than keloid tissue (P>0.05). Western Blot results showed that akt and mtor protein bands in hypertrophic scar tissue and keloid tissue were significantly lower than those in normal skin tissue, but pi3k protein bands in hypertrophic scar tissue and keloid tissue were not significantly decreased. The expression levels of akt and mtor protein in hypertrophic scar tissue and keloid tissue was significantly lower than in normal skin tissue, and the difference was statistically significant (P<0.05). However, the expression levels of pi3k protein in hypertrophic scar and keloid tissue was not significantly different from normal skin tissue (P>0.05). The expression levels of akt and mtor protein in hypertrophic scar tissue was significantly higher than keloid tissue. The results of further correlation analysis showed that there was a positive correlation between mRNA expression of pi3k and akt, akt and mtor, and pi3k and mtor in pathological scar tissue.*

Conclusion: *The expression of mRNA and protein of pi3k, akt and mtor was low in pathological scar tissue. There was a positive correlation between mRNA expression of pi3k and akt, akt and mtor, and pi3k and mtor in pathological scar tissue.*

Keywords: *Pathological scar, PI3K, AKT, mTOR.*

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Introduction

The pathological scar is a skin fibrosis disease caused by excessive production and deposition of a connective tissue matrix such as collagen, including keloid and hypertrophic scars, which is formed by excessive healing of skin trauma. Pathological scars not only affect the appearance of the skin, but also cause serious dysfunction[(1)]. In recent years, the correlation analysis of human pathological scars has gradually increased at home and abroad. It has been demonstrated that the key molecules of mammalian target of rapamycin (mtor) signalling pathway such as protein kinase B (akt), phosphatidylinositol-3-kinase (pi3k) and mtor are abnormally expressed in breast cancer[(2)], gastric cancer[(3)] and colorectal adenoma cancer[(4)], but there are few studies on the expression and correlation analysis of pi3k, akt and mtor in human pathological scars. In this study, the expression of pi3k, akt and mtor in pathological scar tissue and normal skin tissue, and the mRNA and protein expression of pi3k, akt and mtor in fibroblasts of pathological scar were detected, and the expression difference of pi3k, akt and mtor in human pathological scar was analysed.

Materials and methods

Clinical data

Thirty-six patients with pathological scars diagnosed in our hospital from April 2014 to January 2017 were divided into a hypertrophic scar group (n=18) and a keloid group (n=18) according to their pathological types. The normal skin tissue of 18 cases in the hypertrophic scar group was selected as a control group.

Selection criteria:

• No malignant lesions confirmed by pathology;

• Patients with a course of 6-12 months;

• Patients without immune disease;

• The symptom of a pathological scar is prominent.

Exclusion criteria:

• Patients had recently received other treatments for scars;

• Poor compliance, unable to receive regular follow-up;

• Complicated with neoplasms and other serious diseases.

In the hypertrophic scar group, 10 cases were male, 8 cases were female, aged 5 to 53 years old, with the median age of 29 years old. Among the 18 patients with hypertrophic scars, 6 cases had hypertrophic scars in extremities, 7 cases had hypertrophic scars on the head and face and 5 cases had hypertrophic scars on the neck and shoulder. In the keloid group, 9 cases were male, 9 cases were female, aged 6 to 56 years old, with the median age of 31 years old. Among the 18 patients with keloids, 5 cases had keloids in extremities, 6 cases had keloids on the head and face and 7 cases had keloids on the neck and shoulder. There was no significant difference in age and sex between the two groups ($P>0.05$). This study was approved by the ethics committee of our hospital. All patients and their families were informed and signed an informed consent form.

Main reagent

The main reagents are rabbit anti-human mtor monoclonal antibody (Wuhan ABclonal Biotechnology Co., Ltd.); Rabbit anti-pi3k monoclonal antibody (Shanghai Tian Yuan Biotechnology Co., Ltd.); Rabbit anti-human akt monoclonal antibody (Shanghai Haochen Biotechnology Co., Ltd.); Reverse transcription kit superscript III (Beijing Yaanda Biotechnology Co., Ltd.); SDS-PAGE gel preparation kit (Shanghai Weiao Biotechnology Co., Ltd.); Goat anti-rabbit IgG second antibody (Shanghai Yiji industrial co., Ltd.); PBS phosphate buffer (Nanjing Senbeijia Biotechnology Co., Ltd.); Total RNA extraction kit (Beijing Tiangen Biochemistry Technology Co., Ltd.); Reverse transcription kit (Shanghai Yubo Biotechnology Co., Ltd.); PCR polymerase (Beijing Suolaibao Technology Co., Ltd.); pi3k primer (Shanghai Ruiqi Biotechnology Co., Ltd.); akt primer (Dalian takara Biological Engineering Co., Ltd.); mtor primer (Shanghai Guantai Biotechnology Co., Ltd.).

RT-PCR detection

RT-PCR was used to detect the expression of pi3k, akt and mtor mRNA in normal tissue, hypertrophic scar tissue and keloid tissue. According to the operation steps of the TrizolReagent total RNA extraction kit, total RNA was extracted from various tissues, the complementary DNA was synthesized, and the mRNA expression of reverse transcription products of pi3k, akt and mtor was detected by RT-PCR. The relative expression of mRNA (RQ) was expressed by the ratio of the grey value of each target gene and the internal reference gene β-action. The expression levels $RQ=2^{-\triangle\triangle Ct}$.

Western Blot assay

Western Blot was used to detect the expression of pi3k, akt and mtor protein in normal tissue, hypertrophic scar tissue and keloid tissue. The three kinds of tissues were washed with a PBS buffer, and the cell lysis solution was added and left standing for 15 minutes, then centrifuged, and the supernatant was extracted.

The protein in the supernatant was quantified by the Lowry method and 50 μg protein was sampled for electrophoresis. The first antibody was added and incubated overnight at 4 °C, then the second antibody was added and incubated at 25 °C for 1 h. After washing the film, the Odyssey infrared fluorescence imager scans the film and imaging. The image is analysed by the Image Pro-Plus image analysis system, and the grey value of each band is analysed by the Quantity-one gel imaging analysis system.

Observation indices

These indices are the expression levels of pi3k, akt and mtor mRNA in normal tissues, hypertrophic scar tissues and keloid tissues; the expression levels of pi3k, akt and mtor protein in normal tissue, hypertrophic scar tissue and keloid tissue; and the correlation of mRNA expression in pi3k, akt and mtor.

Statistical method

All the data of this study are statistically analysed by SPSS 23.0 software. The measurement data were expressed as mean±standard deviation ($\bar{x}\pm s$), and the single factor analysis of variance (ANOVA) was used for comparison. LSD-t test was used for pairwise comparison, and person correlation was used for correlation analysis. P<0.05 indicated that the difference was statistically significant.

Results

The mRNA expression of pi3k, akt and mtor

The mRNA expression of pi3k in hypertrophic scar tissue and keloid tissue was not significantly different from normal skin tissue (P>0.05). The expression of akt and mtor mRNA in hypertrophic scar tissue and keloid tissue was significantly lower than normal skin tissue, and the difference was statistically significant (P<0.05). The expression of akt and mtor mRNA in hypertrophic scar tissue was significantly higher than keloid tissue (P<0.05). The results are shown in table 1.

Groups	pi3k	akt	mtor
Normal skin tissue (n=18)	7.258±0.108	6.856±0.095	8.790±0.139
Hypertrophic scar tissue (n=18)	7.308±0.112	5.142±0.112*	7.032±0.095*
Keloid tissue (n=18)	7.231±0.083	3.759±0.109*#	4.877±0.121*#
F	2.648	3885.711	4825.068
P	0.080	<0.001	<0.001

Table. 1: The mRNA expression of pi3k, akt and mtor in hypertrophic scar tissue, keloid tissue and normal skin tissue. *Compared with normal skin tissue, *P<0.05; Compared with hypertrophic scar tissue, *P<0.05.*

The protein expression of pi3k, akt and mtor

Akt and mtor protein bands in hypertrophic scar tissue and keloid tissue were significantly decreased compared with normal skin tissue. However, the decrease of pi3k protein band in hypertrophic scar and keloid tissue is not obvious (as shown in figure 1). The expression levels of mtor and akt protein in hypertrophic scar tissue and keloid tissue were significantly lower than normal skin tissue (P<0.05), but the expression of pi3k protein in hypertrophic scar and keloid tissue was not significantly different from normal skin tissue (P>0.05). The expression levels of akt and mtor protein in hypertrophic scar tissue is significantly higher than keloid tissue, as shown in table 2.

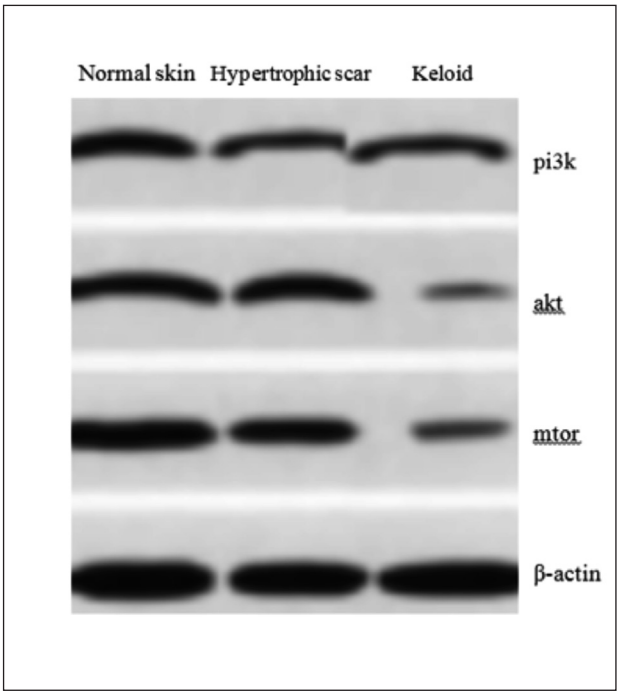

Figure 1: pi3k, akt and mtor protein electrophoretogram.

Groups	pi3k	akt	mtor
Normal skin tissue (n=18)	1.788±0.078	1.856±0.105	2.142±0.099
Hypertrophic scar tissue (n=18)	1.713±0.082	1.342±0.092*	1.533±0.098*
Keloid tissue (n=18)	1.677±0.093	0.785±0.081*#	0.921±0.079*#
F	3.007	594.76	784.78
P	0.058	<0.001	<0.001

Table. 1: The protein expression of pi3k, akt and mtor in hypertrophic scar tissue, keloid tissue and normal skin tissue. *Compared with normal skin tissue, *P<0.05; Compared with hypertrophic scar tissue, *P<0.05.*

Correlation of mRNA expression of pi3k, akt and mtor

There was a positive correlation between mRNA expression of pi3k and akt (r=0.819, P<0.001), akt and mtor (r=0.832, P<0.001), and pi3k and mtor (r=0.906, P<0.001) in pathological scar tissue. The results are shown in figure 2.

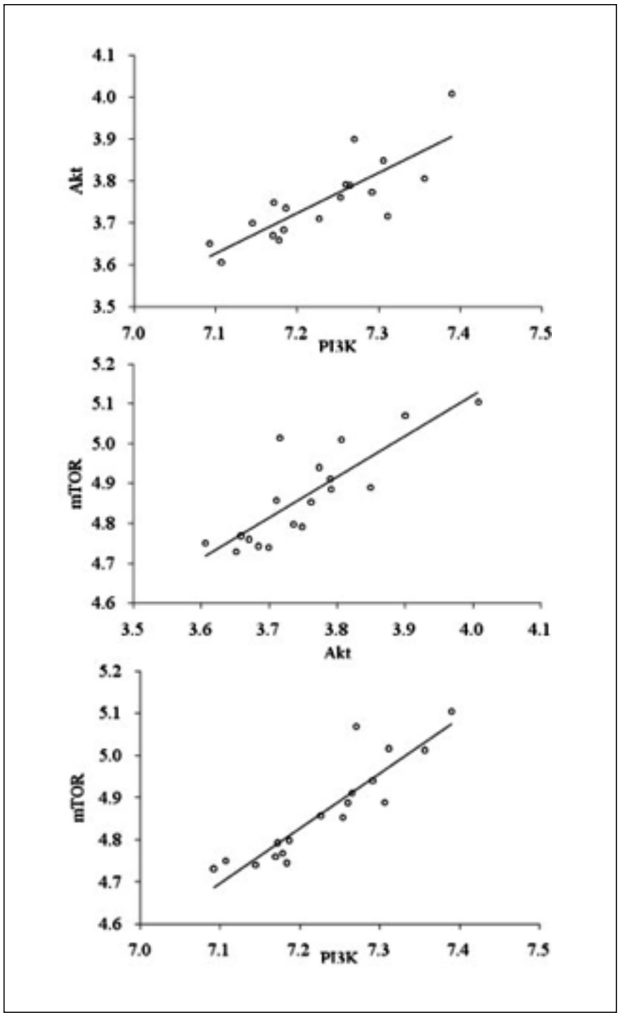

Figure 2: Correlation of mRNA expression of pi3k, akt and mtor in pathological scar tissue.

Discussion

The pathological scar belongs to a class of dermal fibrosis disease, in which hyperplastic pathological scar tissue is higher than the surrounding normal skin, mainly manifesting as pain, itching, scar contracture and other symptoms[7]. It is mainly caused by the excessive deposits of collagen, and collagen production and the deposits in scar tissue increases the strength of the wound[8-9]. Drug therapy, radiotherapy and surgical treatment were mainly used to treat the pathological scar, but the curative effect was not satisfactory and there were some limitations. Therefore, it is of great significance to study the correlation factors in pathological scars.

Pi3k consists of a regulatory subunit (p85) and a catalytic subunit (p110), which mediates signal transduction pathways to regulate cell proliferation, differentiation and apoptosis[10-11]. Qiaohui et al. [12] found that the pi3k signalling pathway is associated with the chemotherapeutic resistance of gastric cancer and plays an important role in the occurrence and development of gastric cancer. Akt is an important downstream molecule of pi3k, including akt1, akt2 and akt3, which play a very important role in regulating cell growth, proliferation, survival and glucose metabolism. Shengfang et al.[13] found that the expression of akt protein in breast cancer is related to the occurrence and development of the disease and prognosis. Mtor is a kind of threonine kinase, while the C-terminal of mtor protein has kinase activity and can receive many signals such as growth factor, nutrition, energy, etc. It is a key regulator of cell growth and proliferation. Xiaoli et al.[14] found that the gene polymorphism of mtor signalling pathway was closely related to the occurrence, invasion and metastasis of gastric cancer. The results of RT-PCR showed that the expression of pi3k mRNA in hypertrophic scar tissue and keloid tissue was not significantly different from normal skin tissue, but akt and mtor mRNA expression in hypertrophic scar tissue and keloid tissue was significantly lower than normal skin tissue, and the difference was statistically significant. The expression of akt and mtor mRNA in hypertrophic scar tissue was significantly higher than keloid tissue. Western Blot results showed that akt and mtor protein bands in hypertrophic scar tissue and keloid tissue were significantly lower than normal skin tissue, but pi3k protein bands in hypertrophic scar tissue and keloid tissue were not significantly decreased. The expression of akt and mtor protein in hypertrophic scar tissue and keloid tissue was significantly lower than normal skin tissue. However, the expression of pi3k protein in hypertrophic scar tissue and keloid tissue was not significantly different from normal skin tissue. The expression of akt and mtor protein in hypertrophic scar tissue was significantly higher than keloid tissue. Further correlation analysis indicated that there was a positive correlation between mRNA expression of pi3k and akt, akt and mtor, and pi3k and mtor in pathological scar tissue.

In conclusion, the expression of mRNA and protein of pi3k, akt and mtor was low in pathological scar tissue. Moreover, there was a positive correlation between mRNA expression of pi3k and akt, akt and mtor, and pi3k and mtor in pathological scar tissue. However, due to the small number of people included, further research is needed.

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