Correlations of SOX9 expression with serum IGF1 and inflammatory cytokines IL-1α and IL-6 in skin lesions of patients with acne


Department of Dermatology, Jiangsu Province Hospital of Traditional Chinese Medicine, Nanjing, China

Abstract. – OBJECTIVE: To study the correlations of sex determining region Y-box 9 (SOX9) expression with serum type-1 insulin-like growth factor (IGF-1), interleukin-1α (IL-1α), and interleukin-6 (IL-6) in skin lesion tissues of patients with acne.

PATIENTS AND METHODS: Six patients with acne who were treated for the first time in our outpatient clinic from June 2017 to July 2017 were selected as observation group, and 6 normal subjects were selected as control group. The expression of SOX9 was detected by immunohistochemistry. The protein expressions of IGF-1, IL-1α, and IL-6 were detected by enzyme-linked immunosorbent assay (ELISA). SOX9 was detected by quantitative polymerase chain reaction (qPCR).

RESULTS: Compared with that in control group, the expression of SOX9 in observation group was significantly increased ($p < 0.05$). Compared with those in control group, the expressions of IGF-1, IL-1α, and IL-6 in observation group were significantly increased ($p < 0.05$). Compared with that in control group, the mRNA expression of SOX9 in observation group was significantly increased ($p < 0.05$). SOX9 was positively correlated with IGF-1, IL-1α and IL-6.

CONCLUSIONS: The expressions of SOX9, IGF-1, IL-1α, and IL-6 in skin lesion tissues of patients with acne are increased, and SOX9 is positively correlated with IGF-1, IL-1α, and IL-6 and can be used as a target for the treatment of acne inflammation.

Key Words: Acne, SOX9, IGF-1, IL-1α, IL-6, Correlation analysis.

Introduction

As a clinically common chronic skin disease, acne usually occurs in adolescent males and females. It easily damages facial skin, leaving facial skin injury marks and permanent scars, which seriously affect the mental health of patients. The pathogenesis of acne is complex and not yet fully understood. However, at present, most scholars believe that the pathogenesis of acne is closely related to abnormal keratosis of hair follicle sebaceous gland, excessive secretion of androgens and subsequent inflammation. Excessive secretion of androgens causes hypertrophy of sebaceous glands, excessive secretion of sebum and hyperkeratosis of hair follicle sebaceous duct openings, resulting in narrowing or even occlusion of sebaceous gland openings. The sebum deposition in the hair follicles finally leads to inflammation. Therefore, inflammation is one of the important factors in the pathogenesis of acne. The inflammatory cytokines interleukin-1α (IL-1α) and interleukin-6 (IL-6) are the main cytokines involved in inflammation. Insulin like growth factor (IGF-1), which can promote androgen secretion and its signal receptor transduction, is considered to be a mediator of acne and plays a key role in the pathogenesis of acne. In addition, it can regulate the proliferation and lipid formation of sebaceous gland cells. At the same time, as a downstream substrate of IGF1, transcription factor sex determining region Y-box 9 (SOX9) is considered to play an important role in the morphogenesis of hair follicles and sebaceous glands, which can promote epidermal proliferation and inhibit its differentiation.

The purpose of this work was to clarify the correlations of SOX9 expression with IGF-1, IL-1α and IL-6 in skin lesion tissues of acne, and provide more specific targets for the treatment of acne in clinic.

Patients and Methods

Patients

Six patients with acne vulgar treated for the first time in the outpatient clinic from June 2017...
to July 2017 were collected. Their skin lesion tissues were taken as observation group. Six normal subjects were collected, whose facial skin tissues were taken as control group. Those who had received oral or topical drug treatment prior to this treatment were excluded from the study. The degree of skin lesions included in this work was in accordance with the Pillsbury improved grading method (Table I). This research was approved by the Ethics Committee of Jiangsu Province Hospital of Traditional Chinese Medicine. Signed written informed consents were obtained from all participants before the study.

**Reagents**

Primary antibody: anti-SOX9 antibody (Abcam, Cambridge, MA, USA). Immunohistochemistry kit (Maxim, Fuzhou, China), IGF-1 enzyme-linked immunosorbent assay (ELISA) kit, IL-1α ELISA kit, IL-6 ELISA kit, association of consulting engineers of Québec (AceQ) quantitative polymerase chain reaction (qPCR) Synergy Brands (SYBR) Green Master Mix kit (Vazyme, Nanjing, China), HiScript II Q RT Radioisotope Tracer (RT) SuperMix for qPCR [+] genomic deoxyribonucleic acid (gDNA) wiper kit (Vazyme, Nanjing, China), optical microscope (Leica DMI 4000B/DFC425C, Wetzfar, Germany), fluorescence qPCR instrument (ABI 7500, Waltham, MA, USA) and Image-lab analysis system (BioRad, Hercules, CA, USA).

**Methods**

**Sampling**

Venous blood was collected immediately after the participants were included in the study. The supernatant was collected after the blood was centrifuged. ELISA kits were used to detect the expression levels of inflammatory cytokines (IGF-1, IL-1α, and IL-6) in venous blood according to the instructions of the kits. Skin tissues were obtained by puncture. Some of tissues were stored at -20°C for stand-by application, and the others were fixed in 4% paraformaldehyde solution for 48 h, embedded and made into paraffin sections.

**Immunohistochemistry**

5 μm-thick paraffin tissue sections were routinely dewaxed, immersed into water, then added with citric acid buffer and heated in a microwave oven for antigen repair. After being rinsed with phosphate-buffered saline (PBS) solution, the sections were incubated with endogenous peroxidase blocker for 10 min. After being rinsed with PBS solution, the sections were added with goat serum for blocking for 20 min, then the serum blocking solution was shaken off, and anti-SOX9 primary antibody (1:200) was added for incubation at 4°C overnight. After being rinsed with PBS solution, the sections were incubated with secondary antibody for 10 min. Next, they were incubated with streptomyces biotin peroxidase solution for 10 min. Diaminobenzidine (DAB) was used for color development. Hematoxylin was used for counterstaining, neutral balsam was used for covering and a microscope was used for observation and shooting.

**ELISA Detection**

(1) Loading: each well was added with 100 μL standard or serum to be detected, and the reaction plate was fully mixed and placed for reaction at 37°C for 40 min. (2) Plate washing: the reaction plate was thoroughly washed with washing solution for 4-6 times and blotted dry on the filter paper. (3) Each well was added with 50 μL distilled water and 50 μL biotinylated antibody in the kit (except blank group), and the reaction plate was fully mixed and placed for reaction at 37°C for 20 min. (4) Plate washing: the reaction plate was thoroughly washed with washing solution for 4-6

---

**Table I.** Pillsbury improved grading method for acne.

<table>
<thead>
<tr>
<th>Grade</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Pimples mainly, a small number of papules and pustules, and the total number of skin lesions is less than 30</td>
</tr>
<tr>
<td>II</td>
<td>Pimples, a moderate number of pimpls and pustules, and the total number of skin lesions is 31-50</td>
</tr>
<tr>
<td>III</td>
<td>A large number of papules and pustules, and the total number of skin lesions is 50 to 100, with less than 3 nodules</td>
</tr>
<tr>
<td>IV</td>
<td>Nodular/cystic acne or polymorphic acne, and the total number of skin lesions is more than 100, with more than 3 nodules/cysts</td>
</tr>
</tbody>
</table>
times and blotted dry on the filter paper. (5) Each well was added with 100 μL enzyme conjugate in the kit, and the reaction plate was placed for reaction at 37°C for 10 min. (6) Plate washing: the reaction plate was thoroughly washed with washing solution for 4-6 times and blotted dry on the filter paper. (7) Each well was added with 100 μL substrate working solution in the kit, and the reaction plate was placed in a dark environment for reaction at 37°C for 15 min. (8) 100 μL stop solution in the kit were added into each well and mixed well. (9) Optical density was measured at 450 nm using a microplate reader.

**qPCR Detection**

The total ribonucleic acid (RNA) was extracted from skin tissues preserved at -20°C for stand-by application via RNA extraction kit. The total RNA extracted was reversely transcribed into complementary deoxyribonucleic acid (cDNA) using reverse transcription kit. The reaction system was 20 μL. The reaction conditions are as follows: reaction at 51°C for 2 min, initial denaturation at 96°C for 10 min, denaturation at 96°C for 10 s, annealing at 60°C for 30 s, 40 cycles. Glyceraldehyde-3-phosphate dehydrogenase (GADPH) was used as the internal reference to calculate the relative expression of Sox messenger ribonucleic acid (mRNA). The primer sequences are shown in Table II.

**Observation Indicators**

The expression of SOX9 in skin tissues was detected by immunohistochemistry. The protein expressions of SOX9, IGF-1, IL-1α, and IL-6 in serum were detected by ELISA. The mRNA expression of SOX9 in skin tissues was detected by qPCR.

**Statistical Analysis**

Statistical Product and Service Solutions (SPSS) 20.0 software (IBM, Armonk, NY, USA) were used for statistical analysis. Enumeration data were expressed as mean ± standard deviation, t-test was used for those conforming to normal distribution and homogeneity of variance, corrected t-test was used for those conforming to normal distribution but heterogeneity of variance, and nonparametric test was used for those not conforming to normal distribution and homogeneity test of variance. Rank-sum test was used for ranked data. The χ²-test was used for enumeration data. Person correlation analysis was used for correlation analysis. p < 0.05 suggested that the difference was statistically significant.

**Results**

**The Expression of SOX9 was Detected by Immunohistochemistry**

As shown in Figure 1, the positive expression of SOX9 was tan. The positive expression of SOX9 in control group was significantly less than that in observation group, and the difference was statistically significant (p < 0.05). The results of statistical analysis of the positive expressions are shown in Figure 2. The positive expression of SOX9 in observation group was significantly more than that in control group, and the difference was statistically significant (p < 0.05). The results suggest that the expression of SOX9 is high in skin lesion tissues of acne.

**Detection of Protein Expressions of IGF-1, IL-1α and IL-6 by ELISA**

The expressions of IGF-1, IL-1α and IL-6 in control group were significantly less than those in observation group, and the differences were statistically significant (p < 0.05). The results suggest that the protein expressions of IGF-1, IL-1α and IL-6 are increased in skin lesion tissues of acne (Figure 3).

**Detection of SOX9 mRNA Expression by qPCR**

The mRNA expression of SOX9 was less in control group, while that in observation group

<table>
<thead>
<tr>
<th>Name</th>
<th>Primer sequences</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sox9</td>
<td>Forward primer: 5’GCTCTACTCCACCTTCACC3’</td>
</tr>
<tr>
<td></td>
<td>Reverse primer: 5’CTCTGTCACCATTGCTCTT 3’</td>
</tr>
<tr>
<td>GADPH</td>
<td>Forward primer: 5’ACGGCAAGTTCAACCGCACAG 3’</td>
</tr>
<tr>
<td></td>
<td>Reverse primer: 5’GAAGACGCACGTAGACTCCACGAC 3’</td>
</tr>
</tbody>
</table>
was significantly increased, and difference was statistically significant ($p < 0.05$). The results suggest that the mRNA expression of SOX9 is increased and the transcriptional ability is enhanced in skin lesion tissues of acne (Figure 4).

**Correlation Analyses of SOX9 with IGF-1, IL-1α and IL-6**

By calculation, the $r$-value of correlation analysis between SOX9 and IGF-1 was 0.828, indicating a positive correlation between SOX9 and IGF-1 (Figure 5). The $r$-value of correlation analysis between SOX9 and IL-1α score was 0.879, indicating a positive correlation between SOX9 and IL-1α (Figure 6). The $r$-value of correlation analysis between SOX9 and IL-6 scores was 0.877, indicating a positive correlation between SOX9 and IL-6 (Figure 7).

**Discussion**

Acne mainly occurs in the epidermal tissues and hair follicle sebaceous gland units. Its pathogenesis is mainly related to four factors, namely, hyperkeratosis of sebaceous duct and hair follicles, microbial invasion, inflammation...
Correlations of SOX9 expression with serum IGF1 and IL-1α and IL-6 in patients with acne

and exuberant sebum secretion. At present, it is believed that acne is an inflammatory disease. The inflammatory response is involved from the onset of acne and existed in the whole process of the disease, eventually leading to the occurrence of inflammatory lesions. In the inflammation of acne, IL-1α and IL-6, as the main inflammatory cytokines, are the main participants in the response. IL-1α is a member of the inflammatory cytokine IL-1 family and is the main form of IL-1 in the skin. The main role of IL-1α is to cause hyperkeratosis of hair follicles and sebaceous ducts, mediating the occurrence and development of acne. A research has found that IL-1α is highly expressed in sebaceous gland cells and hair follicles in patients with acne. As a strong inducer of adhesion molecules, IL-1α can promote the aggregation of various adhesion molecules in vascular endothelial cells. It binds to leukocytes to show the corresponding ligands, which is favorable for leukocyte adhesion to the vascular endothelium and mediating inflammatory exudation. IL-6, an agonist and catalyst for inflammatory response, can mediate and exacerbate inflammation. The IGF family includes IGF-1 and IGF-2, and the content of IGF-1 in the body is higher than that of IGF-2. IGF-1, as a ligand, binds to the IGF-1 receptors on the sebaceous gland cell membranes and plays an important role in regulating sebum secretion. It is currently known that IGF-1, as an upstream...
cell signal, activates the downstream phosphatidylinositol 3-kinase (PI3K)/serine-threonine kinase (MAPK)/extracellular signal-regulated kinases (ERK) signaling pathways, causing severe sebum secretion. SOX gene family is a highly circulating transcription factor. In the family, SOX9 plays an important role in promoting the proliferation of hair follicle stem cells and in maintaining the development of sebaceous gland cells. In addition, it has been further confirmed that SOX9 gene is closely related to the proliferation and differentiation of hair follicle stem cells, the maintenance of stem cell characteristics and the occurrence of skin tumors through Sonic hedgehog (Shh) pathway activation.

We found that the expression levels of SOX9, IGF-1, IL-1α, and IL-6 in skin lesion tissues of acne were significantly higher than those in normal skin tissues, and the expression level of SOX9 was positively correlated with the expression levels of IGF-1, IL-1α, and IL-6. The results suggest that there is an inflammatory response in the loss lesion tissues after the occurrence of acne, and the high expressions of SOX9 and IGF-1 are closely related to the inflammatory response. The reason may be that IGF-1 is highly expressed. As an effective upstream cytokine of the signaling pathway, IGF-1 will increase the transcription and translation expression levels of transcription factor SOX9, and the highly expressed SOX9 further activates downstream PI3K/AKT, MAPK/ERK and other signaling pathways. These signaling pathways are closely related with inflammation, leading to elevated expression levels of inflammatory cytokines such as IL-1α and IL-6. Therefore, SOX9 is positively correlated with IGF-1, IL-1α, and IL-6.

Conclusions

SOX9, IGF-1, IL-1α, and IL-6, as important key substances in the inflammatory response after acne, can be used as targets for the treatment of acne inflammation. Intervention with these substances can provide a new idea for the clinical treatment of acne.

Conflict of Interest

The Authors declare that they have no conflict of interests.

References

11) Bohm M, Lugger TA. The pilosebaceous unit is part of the skin immune system. Dermatology 1998; 196: 75-79.
Correlations of SOX9 expression with serum IGF1 and IL-1α and IL-6 in patients with acne


