Original Article

YAP1 expression in nasal polyps and its relationship with epithelial mesenchymal transition

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Received December 12, 2020; Accepted February 7, 2021; Epub June 15, 2021; Published June 30, 2021

Abstract: Objective: This study explored and analyzed the expression of YAP1 in nasal polyps and its correlation with the epithelial-mesenchymal transition (EMT). Method: 58 patients with chronic sinusitis and nasal polyps, who were hospitalized in the otorhinolaryngology department of our hospital from January 2019 to May 2020 were recruited as the study cohort and placed in a nasal-polyp group, and, at the same time, another 30 nasal septum deviation with inferior turbinate hypertrophy patients were placed in a control group. The expressions of the YAP1 gene in the nasal polyp and turbinate mucosa tissues (using the immunohistochemical method), the YAP1 mRNA, E-cadherin mRNA, and vimentin mRNA expressions (using the RT-PCR method), and the YAP1, E-cadherin, and vimentin protein expressions (using Western blot) were measured, and the correlations between YAP1 and the expressions of E-cadherin and vimentin were analyzed. Results: The immunohistochemistry results revealed that the positive rate of YAP1 expression in the nasal-polyps group was critically higher than the YAP1 expression in the control group (P<0.05). According to the RT-PCR results, the YAP1 mRNA and vimentin mRNA relative expression levels in the nasal-polyps group were significantly higher than they were in the control group (P<0.05), but the E-cadherin mRNA relative expression level in the nasal-polyp group was notably lower than it was in the control group (P<0.05). Our Western blot analysis showed that the protein expressions of YAP1 and vimentin protein in the nasal-polyps group were significantly higher than the corresponding protein expressions in the control group (P<0.05), but the E-cadherin expression in the nasal-polyp group was especially lower than it was in the control group (P<0.05). In addition, in the nasal polyp tissues, the relative expression between the YAP1 mRNA and the E-cadherin mRNA reflected a notably negative correlation (P<0.05), but the YAP1 mRNA and vimentin mRNA showed a positive correlation (P<0.05). Conclusion: High expressions of YAP1 and EMT occur in nasal polyp tissues, and YAP1 is likely to be involved in the regulation of EMT and might be one of the mechanisms in nasal polyps.

Keywords: YAP1, nasal polyps, Immunohistochemistry, epithelial mesenchymal transition

Introduction

Chronic rhinosinusitis with nasal polyps (CRSwNP) is a common chronic inflammatory disease seen in clinical otorhinolaryngology head and neck surgery with a prevalence of 1%-4%. CRSwNP has become an increasingly serious health problem globally [1]. At present, CRSwNP is primarily treated using comprehensive drug treatment, including nasal corticosteroids, and with endoscopic sinus surgery. However, studies have shown that the recurrence rate of the disease can be as high as 55.3% [2]. Therefore, it is particularly significant to explore the pathogenesis of the disease and to seek more ideal curative methods to improve the cure rate of the disease. Studies have found that the epithelial mesenchymal transition (EMT) is an important cause of chronic inflammatory tissue remodeling in multiple organs, especially interstitial fibrosis, and it participates in the pathophysiological process of CRSwNP [3, 4]. Yap1 is the most critical core transcriptional co-activator in the downstream Hippo pathway, and is located on human chromosome 11q22 [5, 6]. Current research of
tumor and kidney disease has found evidence that YAP1 participates in the regulation of EMT [7, 8], but the expression of YAP1 in nasal polyps and its correlation with EMT have not yet been reported. This study explored and analyzed YAP1 gene expression in nasal polyps and its correlation with EMT.

Materials and methods

Research subjects

58 patients with chronic sinusitis and nasal polyps, who were hospitalized in the otorhinolaryngology department of our hospital from January 2019 to May 2020 were recruited as the study cohort and placed in the nasal-polyp group, and, at the same time, another 30 nasal septum deviation with inferior turbinate hyper trophy patients were recruited to serve in the control group. This study was approved by the hospital’s ethics committee.

Inclusion and exclusion criteria

Inclusion criteria: (1) Patients who agreed to undergo a partial resection of the inferior turbinate, (2) Patients whose specimens were verified postoperatively by two or more pathologists, and (3) Patients who signed the informed consent forms voluntarily.

Exclusion criteria: (1) Patients with a history of nasal polyps, allergic rhinitis, or asthma, (2) Patients with allergies or other systemic diseases.

Main test reagents and instruments

Rabbit anti-human YAP1 polyclonal antibody (Abcam, UK), Rabbit anti-human E-cadherin polyclonal antibody (Abcam, UK), Rabbit anti-human vimentin polyclonal antibody (Abcam, UK), HRP-labeled anti-rabbit antibody (Fuzhou Maixin Biotechnology Development Co., Fujian), DAB chromogenic kits (Fujian Maixin Biotechnology Development Co.,), High-efficiency RNA extraction kits (Beijing Youshengte Biotechnology Co., Ltd.), cDNA preparation reverse transcription kits (Beijing Youshengte Biotechnology Co., Ltd.) and immunohistochemical staining (SABC method) kits (Wuhan Boster Biological Technology Co., Ltd.).

YAP1 expressions measured using immunohistochemistry

After they were removed, the specimens were immediately fixed in a formaldehyde solution and embedded in paraffin for preparation. We sectioned the paraffin specimens consecutively with a thickness of 5 μM, and performed routine HE staining. We routinely dewaxed the sections, rinsed them with distilled water three times, performed antigen repair for 10 minutes using citric acid, and let them cool naturally. We performed immunohistochemical staining according the kit’s instruction manual and DAB dyeing. We then lightly counterstained the sections with hematoxylin, dehydrated them, made them transparent, mounted them with a neutral gum, and then we observed them under a microscope. We adopted PBS as negative control instead of the first antibody, and set the existing positive sections as the positive control group.

Results determination: The staining expressions of YAP1 after the immunohistochemistry were primarily located in cytoplasms and nuclei, and the positive cells were in yellow/brown granules. We used the semi-quantitative method to judge the results: (1) Scoring the strength of the cell staining: 0 points indicated non-staining. The light yellow cells were scored 1 point. The brown cells were scored 2 points, and the sepia cells were scored 3 points. (2) We randomly chose five high vision (400) to calculate the percentage of the positive staining cells to the total number of cells was calculated: 0 points, <10%, 1 point, 10%-25%, 2 points, 25%-50%, 3 points, 50%-75%, 4 points, >75%. We then multiplied the above two scores as the final score: 0-4 points for negative, and 5-12 points for positive.

Measuring the YAP1 mRNA, E-cadherin mRNA, and vimentin mRNA expressions using RT-PCR

We took two sets of paraffin specimens, extracted their total RNA using kits, and determined the purity and concentration. We prepared the cDNA according to cDNA preparation reverse transcription kit’s instructions. The PCR reaction system included: 2× SYBR Green Real-Time PCR Master Mix 10 μl, Primer Mix 1 μl, ddH2O 7 μl, template cDNA 2 μl, and a total...
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Table 1. Comparison of the clinical data between the two groups

<table>
<thead>
<tr>
<th>Group</th>
<th>Number of cases</th>
<th>Gender</th>
<th>Age (years, ( \bar{x} \pm s ))</th>
<th>BMI (kg/m(^2), ( \bar{x} \pm s ))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nasal-polyps group</td>
<td>58</td>
<td>Male</td>
<td>42.84±9.37</td>
<td>21.47±3.94</td>
</tr>
<tr>
<td>Control group</td>
<td>30</td>
<td>Female</td>
<td>41.70±8.95</td>
<td>21.22±3.50</td>
</tr>
<tr>
<td>t/(X^2)</td>
<td>-</td>
<td></td>
<td>0.083</td>
<td>0.549</td>
</tr>
<tr>
<td>P</td>
<td>-</td>
<td></td>
<td>0.774</td>
<td>0.584</td>
</tr>
</tbody>
</table>

Table 2. YAP1 expressions in the tissues of the two groups

<table>
<thead>
<tr>
<th>Group</th>
<th>n positive cases</th>
<th>Positive rate (%)</th>
<th>(X^2)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>nasal-polyp group</td>
<td>58</td>
<td>41</td>
<td>70.69</td>
<td>20.417</td>
</tr>
<tr>
<td>control group</td>
<td>30</td>
<td>6</td>
<td>20.00</td>
<td></td>
</tr>
</tbody>
</table>

volume of 20 μl. The reaction conditions of PCR: to complete 40 rounds at 95°C of 10 min, 95°C of 10 s and 60°C for 1 min. We took GAPDH as an internal reference. The forward and reverse primers of YAP1 were 5'-TAGCCCTGCGATCCAGTTA-3' and 5'-TCATGCTTACGTCACGTGTCTG; E-cadherin: 5'-CGCATTGCCACATACA-3' and 5'-CGTACGCTTTCTCA-3'; vimentin: 5'-CCGCTTGCCAACACTAC-3' and 5'-AGGCGATCCACTCAGAC-3'; GAPDH: 5'-TGGTGCCTTGGTGTCGTCAC-3' and 5'-ATGGCATGGACGACTGTCAT. The relative expression in each targeted gene was calculated using the 2\(^{-\Delta\Delta\text{Ct}}\).

Measuring the YAP1, E-cadherin, and vimentin expressions using Western blot

We took the two sets of paraffin specimens, extracted the total protein using PIPA, and measured its concentration using the bicinchoninic acid (BCA) method. We prepared 10% SDS-PAGE gel, and adjusted the protein content of each sample to the same level according to its protein concentration. The protein loading amount was about 10 μg, the concentration gel of 60 V, and the separation gel of 120 V, and the measurement lasted for about 1.5 h. We used 80 mA transfer membrane for semi-dry transferring in 30 min. After the completion of the transmembrane, we sealed the membrane in a shaker with a blocking solution at room temperature for 1 h. After finishing the closure, the membrane was rinsed with TBSST three times, and then a primary antibody (YAP1, 1:1500; E-cadherin, 1:2000; vimentin: 1:1200) was added and incubated overnight at 4°C. We then incubated the second antibody (1:4000) at room temperature. We prepared a HRP substrate working solution, placed the contact sur-

face of the membrane and glue face up in a clean container, the solution and incubated it at room temperature for 5 min. Finally, we drained the remaining liquid, wrapped it with preservative film to avoid bubbles and wrinkles, and put it into a cold CCD imaging system for shooting. The internal reference was taken by β-actin.

Statistical analysis

The data processing and analysis were conducted with SPSS 25.0. The measurement data was represented by (\( \bar{x} \pm s \)), the two groups of measurement data were compared using t-tests, the enumeration data were expressed as percentages, and the two sets of enumeration data were compared using \( X^2 \) tests. A difference of \( P<0.05 \) was treated as statistically significant.

Results

Clinical data

The comparative differences in the clinical data between the two sets of were insignificant (\( P>0.05 \)), as shown in Table 1.

The YAP1 expressions measured using immunohistochemistry

The YAP1 expressions in the two groups were quantified using the immunohistochemical method. In the nasal-polyp group, 41 patients had positive YAP1 expressions in their nasal polyp tissue, so the positive rate was 70.69%. In the control group, six patients had positive YAP1 expressions in their turbinate mucosa tissue, so the positive rate was 20.00%. The positive rate of YAP1 expression in nasal-polyp group was critically higher than it was in the control group (\( X^2=20.417, P<0.0001 \)), as shown in Table 2 and Figure 1.

The YAP1 mRNA, E-cadherin mRNA, and vimentin mRNA expression measured using RT-PCR

The YAP1 mRNA, E-cadherin mRNA, and vimentin mRNA relative expressions in the two sets of subjects were determined using RT-PCR. According to the results, the relative expression
levels of YAP1 mRNA and vimentin mRNA in the nasal-polyps group were remarkably higher than the corresponding expressions in the control-group \((P<0.05)\), but the relative expression level of E-cadherin mRNA in the nasal-polyp group was notably lower than it was in the control-group \((P<0.05)\), as illustrated in Table 3 and Figure 2.

Correlation analysis

In the nasal polyp tissues, the relative expressions between the YAP1 mRNA and the E-cadherin mRNA presented a notably negative correlation \((P<0.05)\), but the YAP1 mRNA and the vimentin mRNA showed a positive correlation \((P<0.05)\), as illustrated in Figure 4.

Discussion

The current mechanism of nasal polyps has yet been fully elucidated. A large number of studies on the pathogenesis of nasal polyps has focused on epithelial cells, body immunity, and pathogens, considering that the damage to the epithelial barrier is the cause of the pathogenic invasion. In the case of immune imbalance, damage to the epithelial barrier promotes the occurrence of chronic inflammation and tissue remodeling of the nasal mucosa, which is then histologically characterized by interstitial edema, a thickening of the basement membrane, and an infiltration of inflammatory cells [9, 10]. EMT is the chronic inflammatory tissue remodeling of multiple organs, and it participates in the pathophysiological process of CRSwNP [11]. As the first cellular defense line of respiratory tract immunity, epithelial cells both work as the natural physical barrier, and as the origin of the inflammatory response [12]. Under certain circumstances, the epithelial cells turn into mesenchymal phenotype cells.
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Table 4. The expressions of YAP1, E-cadherin, and vimentin in the two groups

<table>
<thead>
<tr>
<th>Group</th>
<th>Number of cases</th>
<th>YAP1</th>
<th>E-cadherin</th>
<th>vimentin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nasal-polyps group</td>
<td>58</td>
<td>1.208±0.271</td>
<td>0.875±0.115</td>
<td>0.902±0.284</td>
</tr>
<tr>
<td>Control group</td>
<td>30</td>
<td>0.984±0.197</td>
<td>1.462±0.317</td>
<td>0.364±0.093</td>
</tr>
<tr>
<td>t</td>
<td></td>
<td>4.008</td>
<td>12.639</td>
<td>10.076</td>
</tr>
<tr>
<td>P</td>
<td></td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
</tr>
</tbody>
</table>

Figure 3. The expressions of YAP1, E-cadherin, and vimentin in the two sets of subjects. Note: (A) A Grey-analysis chart of the protein expression, compared with the control group, *P<0.05; (B) Western blot protein expression. a: The nasal-polyps group. b: The control group.

YAP1 is the most critical core transcriptional co-activator that exists downstream of the Hippo pathway. The Hippo signaling pathway, which has attracted much attention in recent years, is an important signaling pathway that regulates organ growth and tissue size. It is highly conserved in mammals, and it plays a crucial role in measuring cell proliferation and differentiation [15, 16]. The Hippo signaling pathway was initially discovered in Drosophila. It affects cell proliferation and apoptosis by controlling the growth, differentiation, and regeneration of different tissues [17, 18]. As a transcriptional co-activator, YAP can negatively regulate the Hippo signaling pathway, thereby regulating cell growth, proliferation, and apoptosis. YAP1 has currently been found to be involved in the regulation of EMT [19, 20]. The high levels of snail and N-cadherin, and the low level of E-cadherin can be detected on the surfaces of highly-expressed YAP cells, indicating that YAP may be an effective inducer of EMT. In cholangiocarcinoma, prostate cancer and breast cancer, the overexpression of YAP overcomes the contact restraint of the cells and EMT, thereby promoting tumor metastasis [21-23]. This research explored and analyzed YAP1 expression in nasal polyps and its correlation with EMT.

The immunohistochemistry results showed that the positive rate of YAP1 expression in the nasal-polyps group was critically superior to the positive rate of YAP1 expression in the control group. This is similar to the results of other scholars [24], and YAP1 expression may be regulated in the occurrence of nasal polyps. The RT-PCR and Western Blot results were consistent, indicating that the relative expression levels of YAP1 and vimentin in the nasal-polyps group were remarkably superior to the levels in the control-group, but the relative expression level of E-cadherin in the nasal-polyp group was...
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Figure 4. Correlation analysis. A: The YAP1 mRNA and E-cadherin mRNA correlation analysis ($r=0.598$, $P=0.000$); B: The YAP1 mRNA and vimentin mRNA correlation analysis ($r=0.522$, $P=0.000$).

notably lower than it was in the control-group. Vimentin and E-cadherin are molecular markers of EMT. Researchers have suggested that high expressions of vimentin and the deficiency of E-cadherin in tumor cells can boost the occurrence of EMT and tumor metastasis [25, 26]. The results of this study are consistent with our previous hypotheses, namely that high expressions of YAP1 and the activation of EMT occur in nasal polyps, further confirming that EMT may be one of the mechanisms responsible for nasal polyps. In addition, in nasal polyp tissues, the relative expression between YAP1 mRNA and E-cadherin mRNA reflects a notably negative correlation, while YAP1 mRNA and vimentin mRNA show a positive correlation. This is consistent with the findings of other scholars [27, 28], namely that YAP1 may be a regulatory protein of EMT, and its high expression can promote the occurrence of EMT, and there may be high expressions of YAP1 and the activation of EMT in nasal polyp disease, and there is a significant correlation between them.

However, our study cohort was small, and there is a lack of an in-depth analysis of the regulatory pathway between YAP1 and EMT. Therefore, it is necessary to further expand the sample size and clarify the regulatory mechanism between YAP1 and EMT.

To conclude, high expressions of YAP1 and EMT both exist in nasal polyp tissues, and YAP1 is likely to be involved in the regulation of EMT, and these factors may be two of the mechanisms involved in nasal polyps.

Acknowledgements

This work was supported by the Natural Science Foundation of Hainan Province, China (No. 818MS130) and the Health and Family Planning Science Foundation of Hainan Province, China (No. 19A200014).

Disclosure of conflict of interest

None.

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References

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VEGF release by nasal polyp fibroblasts. Allergol Int 2016; 65: 414-419.

