Mechanism of Astrin in head and neck squamous cell carcinoma

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ABSTRACT

Head and neck squamous cell carcinoma (HNSCC) is a malignant epidermal tumor that seriously threatens human life and health. The main factors affecting the death of patients are local recurrence and lymph node metastasis. Astrin antibody is the basic component of the mitotic spindle required for normal chromosome separation and later development. There are few domestic studies on the mechanism of Astrin in HNSCC. Based on this, this article is studying Astrin in HNSCC. The expression and function of Astrin, and analyze its correlation with clinical pathological parameters and prognosis of patients, and further explore the relevant mechanisms involved in the progression of Astrin in HNSCC. In this experiment, the real-time fluorescent quantitative polymerase chain reaction (PCR) method was used to detect the expression of the Astrin antibody in HNSCC cell lines A and B. Secondly, this article will focus on high metastatic HNSCC cells B. Divided into five groups (blank control group, overexpression positive group, overexpression negative control group, expression suppression positive group, expression suppression negative control group), using real-time fluorescent quantitative PCR technology to detect the expression of Astrin in each group, and then speculate the mechanism of Astrin in HNSCC. Experiments have shown that Astrin is expressed in A and B cells, but its expression in B is significantly higher than its expression in A, and the difference is statistically significant (P<0.001). This shows that the inhibition of Astrin expression has a significant anti-tumor effect and that Astrin plays an important role in the occurrence and development of tumors. It is expected to provide new ideas and reference basis for exploring new therapeutic strategies for targeted therapy of HNSCC.

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Introduction

Squamous cell carcinoma of the head and neck is the world’s sixth most common malignant tumor. Its survival rate has not improved significantly in the past few years, and lymph node metastasis is also one of the most important prognostic factors closely related to patients’ poor long-term survival rate. It is clear that miRNAs play an important regulatory role in cell life activities such as cell proliferation, differentiation and apoptosis. For head and neck tumors, surgery damages large defects and even affects organ function, and serious side effects caused by radiotherapy often affect patients’ quality of life. In the occurrence and development of HNSCC, many oncogenes and tumor suppressor genes play a key role in it, which is helpful for the early diagnosis and treatment strategy formulation and prognosis evaluation of patients with head and neck malignancies, especially HNSCC. It is expected to improve the survival rate of patients (1-3).

Research on the treatment of HNSCC in foreign countries is much earlier than that in China, and the discovery of the Astrin antibody is also earlier than in China, and the development and update of HNSCC treatment technology is fast. The treatment methods and methods of HNSCC have been greatly improved and developed. It is believed that the use of Astrin antibody treatment will become an important breakthrough in the near future. Zhang HD found that miR-17-5p and miR-20a negatively regulate the translation of cyclin D1, and inhibit cells from entering the S phase, thereby inhibiting the proliferation of breast cancer cells and acting as tumor suppressor genes (1). Thavarajah R evaluated the effect of miR-20a on the invasion and migration of oral squamous cell carcinoma cells through CCK-8, Transwell and scratch experiments and found that the expression of miR-20a was down-regulated; after up-regulating the expression of HPV-16E7, the expression of miR-20a increased (2). Azimi studied the role of miR-20a in oral squamous cell carcinoma and found that HPV16E7 inhibited the proliferation, invasion and metastasis of oral squamous cell carcinoma by up-regulating the expression of miR-20a (3).

The use of Astrin antibody to treat HNSCC originated in Western countries. Compared with Western countries, our country’s HNSCC treatment technology started late and its development is relatively slow. With the continuous development of science and technology and the maturity of biomaterials, the prevention of local recurrence and regional lymph node metastasis after our country’s treat-
mental of HNSCC has become increasingly mature. Warna-
kulasuriya S proposed that the mechanism of miR-20a is
mainly through up-regulating or down-regulating the
expression of miR-20a in tumor cells, and then observing
the changes in the biological behavior of tumor cells, and
then evaluating the relationship between miR-20a and tu-
more development (4). Liu Y cultured cell lines of head and
neck tumors in vitro, and used PCR to determine the infec-
tion status of the cells and then grouped them, and irradiat-
ted the positive and negative tumor cell lines in a test tube
(5). Lakshmanaperumal AS cultured cervical cancer cell
lines and head and neck squamous cell cancer cell lines in
vitro, and used the antiviral drug cidovir to reduce the
E6/E7 protein at the transcription level and found that it
induces cyclin-dependent kinase inhibition in tumor cell
lines accumulation of agents (6).

In this paper, by detecting the expression of Astrin in
HNSCC cells and clinical specimens, analyzing the rela-
tion between its expression level and relevant clinicopha-
tological parameters and patient survival rate, reveal-
ing the expression and clinical significance of Astrin in
the occurrence and development of HNSCC, lay the foun-
dation for further study of Astrin’s functions and biologi-
cal effects in vivo and in vitro. At the same time, further,
evaluate the imaging data of patients with related HNSCC
and unrelated HNSCC after Astrin antibody treatment,
calculate the degree of tumor regression of the two before
and after treatment, and further perform tumor regression
degree classification to compare the two in the differen-
tivity of Astrin antibody therapy provides new ideas and
reference for future clinical treatment options for
HNSCC.

As the main treatment for patients with HNSCC, ra-
diotherapy has achieved great success in the treatment
of early nasopharyngeal carcinoma. However, conven-
tional fractional radiotherapy is not satisfactory in terms
of local control rate and survival rate of patients with
advanced HNSCC, so radiotherapy many doctors in the
world have changed the traditional conventional treatment
methods and use unconventional segmentation treatment.
Hyperfractionated radiotherapy increases the number of
radiotherapy divisions under the premise that the total
radiotherapy dose remains unchanged and shortens the
radiotherapy time, thereby improving the control rate of
local lesions (7-8). The dose-dependence of radiotherapy
in normal tissues is greater than that of early responding
tissues such as tumors. Increasing the number of radio-
therapy divisions while reducing the division dose can give
tumor cells a relatively high dose and reduce normal tissue
radiation damage.

Radiotherapy is one of the main treatment methods for
HNSCC. The fundamental purpose of precise radiothe-
rapy is to increase the dose in the target area while giving
the greatest protection to surrounding tissues and organs.
Applying techniques such as cone beam tomography and
CT simulation three-dimensional planning can help imple-
ment precise positioning during radiotherapy to better take
advantage of the highly conformable dose of intensity-
modulated radiation therapy. Early diagnosis of malignant
tumors, precise positioning during radiotherapy and pro-
gnostic analysis of patients all rely on advanced imaging
equipment. With the development of technology, imaging
technology will play an increasingly important role in tar-
geted radiotherapy (9-10).

The main purpose of image preprocessing is to suppress
the influence of image speckles on ultrasound images,
then evaluating the relationship between miR-20a and tu-
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The main purpose of image preprocessing is to suppress
the influence of image speckles on ultrasound images,
ability of highly metastatic HNSCC cells are analyzed in six aspects.

**Experimental Method**

**Astrin expression in HNSCC**

Take out an A and a B cell from the liquid nitrogen irrigation. After the liquid nitrogen evaporates, place them in a 37-degree constant temperature water bath for rapid dissolution. Add the lysed cells to a 15ml centrifuge tube containing 9ml of the medium, centrifuge at 800rpm for 3min, discard the supernatant, add 2ml of complete medium to the centrifuge tube, use a pipette to blow evenly into a single cell suspension, and then inoculate it into the culture flask. Place it in an incubator at 37°C and 5% CO2. The A and B cells in the culture flask were collected separately, and all RNA was extracted after centrifugation, and the RNA concentration was determined by PCR.

**Cell transfection and PCR detection after transfection**

Choose high-metastasis B cells in good condition for laying 6-well plates, divide the experiment into five groups, and mark the groups on the 6-well plate. After plating, place the plate in a 37°C, 5% CO2 incubator for 24h. After 24h, perform no treatment, overexpression positive transfection, overexpression negative transfection, expression suppression positive transfection, expression suppression negative transfection operation, and then continue to culture for 48h, harvest cells from time to time, and do PCR to detect Astrin after transfection express the situation.

**Establish model evaluation index system**

The evaluation index is a specific evaluation item determined according to some evaluation goals, which can reflect some basic characteristics of the evaluation object. The index is specific and measurable, and it is the observation point of the goal. Definite conclusions can be drawn through actual observation of objects. Generally speaking, the evaluation index system includes three levels of evaluation indexes: they are the relationship between gradual decomposition and refinement. Among them, the first-level evaluation indicators and the second-level evaluation indicators are relatively abstract and cannot be used as a direct basis for evaluation. The third-level evaluation indicators should be specific, measurable and behavior-oriented and can be used as a direct basis for teaching evaluation.

**Statistical processing**

Statistical analysis was performed with SPSS 13.0 statistical software. The significance test of difference was performed by one-way analysis of variance, the difference between the two groups was tested by LSD-t, and the PCR results of Astrin expression in cells were performed by group t-test. P<0.05 is considered significant and statistically significant.

**Results and Discussion**

**Evaluation index system based on index reliability testing**

Here we carry out a reliability analysis on each index of each analysis object, and the results are shown in Table 1.

It can be seen from Figure 1 that the effect of Astrin on the proliferation of highly metastatic HNSCC cells, the effect of Astrin on the apoptotic ability of highly metastatic HNSCC cells, the effect of Astrin on the migration ability of highly metastatic HNSCC cells, and the effect of Astrin on the migration ability of highly metastatic HNSCC cells. The influence of the invasion ability of metastatic HNSCC cells the data obtained by various indicators have a very good effect on the impact of this experiment (α>0.8). The differential expression of Astrin in HNSCC cells and Astrin in the highly metastatic head and neck the data obtained from the differential expression of various indicators in squamous cell carcinoma cells has an acceptable impact on this experiment (α>0.7), which illustrates the six selected in this article when studying the mechanism of Astrin in HNSCC. The indicators are reasonable, which provides a basis for subsequent experiments.

**Based on inspection data**

**Differential expression of Astrin in HNSCC**

The PCR method was used to detect the expression of Astrin in low metastatic HNSCC A and high metastatic HNSCC B. The results are shown in Table 2.

From Figure 2 we can see that Astrin is expressed in both high metastatic HNSCC B and HNSCC A, but its ex-

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**Table 1. Data sheet of evaluation index system for index reliability testing.**

<table>
<thead>
<tr>
<th></th>
<th>Very Clear</th>
<th>Clear</th>
<th>General</th>
<th>Not Clear</th>
<th>Chaotic</th>
<th>Alpha</th>
</tr>
</thead>
<tbody>
<tr>
<td>Differential Expression</td>
<td>3.81</td>
<td>4.44</td>
<td>4.37</td>
<td>0.51</td>
<td>0.46</td>
<td>0.7754</td>
</tr>
<tr>
<td>High Metastatic Differential Expression</td>
<td>3.68</td>
<td>3.68</td>
<td>4.54</td>
<td>0.57</td>
<td>0.35</td>
<td>0.7319</td>
</tr>
<tr>
<td>Cell Proliferation</td>
<td>3.51</td>
<td>3.32</td>
<td>4.18</td>
<td>0.31</td>
<td>0.35</td>
<td>0.8328</td>
</tr>
<tr>
<td>Apoptosis</td>
<td>3.55</td>
<td>3.57</td>
<td>4.38</td>
<td>0.67</td>
<td>0.41</td>
<td>0.8361</td>
</tr>
<tr>
<td>Cell Migration</td>
<td>3.76</td>
<td>3.75</td>
<td>4.61</td>
<td>0.40</td>
<td>0.32</td>
<td>0.8576</td>
</tr>
<tr>
<td>Cell Invasion</td>
<td>3.83</td>
<td>4.45</td>
<td>4.77</td>
<td>0.63</td>
<td>0.31</td>
<td>0.8194</td>
</tr>
</tbody>
</table>
pression in high metastatic HNSCC B is higher than that of HNSCC B. Of squamous cell carcinoma cells, the difference was statistically significant (P<0.05). At the same time, because A came from the primary tumor of HNSCC patients, B came from the same HNSCC patients’ lymph node metastases. This also proves that B is a cell line with high metastatic ability, while A is a cell line with low metastatic ability.

Differential expression of Astrin in highly metastatic HNSCC

Through transient transfection technology, the overexpression vector, inhibitor and blank vector were respectively transfected into B cells. The experiment was divided into five groups, namely blank control group, overexpression positive group, overexpression negative control group, and expression suppression positive group, expression suppression negative control group, the results are shown in Table 3.

From Figure 3, we can see that the expression of Astrin in the expression suppression positive group was significantly lower than that in the expression suppression negative control group and the blank control group. The results were statistically significant and the difference was significant (P<0.05); the expression suppression negative control group and the blank control group Compared with the control group, there was no difference in the expression of Astrin, showing no difference (P>0.05). It shows that the overexpression plasmid vector we constructed can increase the expression of Astrin; the constructed Astrin inhibitor can inhibit the expression of Astrin, and the transfection is effective.

Effect of Astrin on the proliferation of highly metastatic HNSCC

PCR method was used to detect the effect on the proliferation of HNSCC B. The experiment was divided into five groups, namely blank control group, overexpression positive group, overexpression negative control group, expression suppression positive group, and expression suppression negative control group, each group was measured at 0, 24, 48, 72, 96 hours after transfection Table 4 shows the OD value of the cells.

From Figure 4, we can see that the OD values of each group are basically the same at 0h. On the whole, the differences in cell proliferation in each group were obvious, and the differences in cell proliferation at each time point were also statistically significant. The cell proliferation

### Table 2. Astrin differential expression data table in HNSCC.

<table>
<thead>
<tr>
<th>Time</th>
<th>A</th>
<th>B</th>
<th>t</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>1h</td>
<td>1.85</td>
<td>2.77</td>
<td>0.54</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>2h</td>
<td>2.33</td>
<td>3.97</td>
<td>0.49</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>4h</td>
<td>2.86</td>
<td>3.77</td>
<td>0.71</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>12h</td>
<td>3.39</td>
<td>4.55</td>
<td>0.47</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>24h</td>
<td>3.97</td>
<td>4.79</td>
<td>0.45</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>48h</td>
<td>4.38</td>
<td>5.33</td>
<td>0.74</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

### Table 3. Astrin differential expression data table in highly metastatic HNSCC.

<table>
<thead>
<tr>
<th>Blank Control Group</th>
<th>Overexpression Positive Group</th>
<th>Overexpression Negative Control Group</th>
<th>Expression Suppression Positive Group</th>
<th>Expression Suppression Negative Control Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>1h</td>
<td>3.80</td>
<td>4.51</td>
<td>3.71</td>
<td>2.70</td>
</tr>
<tr>
<td>2h</td>
<td>3.65</td>
<td>4.76</td>
<td>3.78</td>
<td>3.04</td>
</tr>
<tr>
<td>4h</td>
<td>4.10</td>
<td>4.63</td>
<td>4.11</td>
<td>3.24</td>
</tr>
<tr>
<td>12h</td>
<td>4.02</td>
<td>4.56</td>
<td>4.15</td>
<td>3.05</td>
</tr>
<tr>
<td>24h</td>
<td>4.13</td>
<td>4.76</td>
<td>4.01</td>
<td>3.49</td>
</tr>
<tr>
<td>48h</td>
<td>4.10</td>
<td>4.67</td>
<td>3.95</td>
<td>3.44</td>
</tr>
<tr>
<td>P</td>
<td>0.976</td>
<td>0.001</td>
<td>0.854</td>
<td>0.005</td>
</tr>
</tbody>
</table>
ability of the overexpression positive group was significantly higher than that of the overexpression negative control group and the blank control group (P<0.05); the cell proliferation ability of the inhibition positive group was significantly lower than that of the expression inhibition negative control group and the blank control group (P<0.05), and the difference was statistically significant. This shows that B cell proliferation ability is enhanced after overexpression of Astrin, and B cell proliferation ability is reduced after inhibiting Astrin expression.

**Effect of Astrin on the apoptotic ability of highly metastatic HNSCC**

The PCR method was used to detect the effect on the apoptosis of HNSCC cells B. The experiment was divided into five groups, namely blank control group, overexpression positive group, overexpression negative control group, expression suppression positive group, and expression suppression negative control group, each group was measured at 0, 24, 48, 72, 96 hours after transfection the number of apoptotic cells and the results are shown in Tables 5 and 6.

From Figure 5, we can see that the expression of Astrin is up-regulated, and the early apoptosis of HNSCC cells B is inhibited; when the expression of Astrin is reduced, the early apoptosis of HNSCC cells B is increased. Comparing the experimental group with the control group, P<0.05, which is statistically significant, and the difference is significant; while the negative control group and the blank control group, P>0.05, which is not statistically significant, indicating that there is no difference between the groups.

**Effect of Astrin on the migration ability of highly metastatic HNSCC**

The effect of Astrin on the migration ability of head
and neck squamous cell carcinoma cell B was studied by PCR method. The number of cells passing through the cell membrane reflects the level of cell migration ability. After transfection, the number of cells passing through the cell was calculated, and the result is shown in Figure 6.

From Figure 6, we can see that the overexpression positive group, overexpression negative control group, and blank control group are compared in pairs, and statistical analysis is performed. The result shows that overexpression negative control group=blank control group<overexpression positive group, that is, overexpression. After Astrin, the number of cells passing through the chamber was significantly more than that of the negative control and the blank control group, and there was no significant difference between the negative control group and the blank control group (P>0.05). It shows that overexpression of Astrin can up-regulate the migration ability of B cells (P<0.05); the same reason can show that inhibiting Astrin expression can down-regulate the migration ability of B cells (P<0.05).

**Effect of Astrin on the invasion ability of highly metastatic HNSCC**

The effect of Astrin on the invasion ability of head and neck squamous cell carcinoma B was studied by PCR method. The cells passing through the Matrigel glue reflect the level of cell invasion. The results are shown in Figure 7.

From Figure 7 we can see that the overexpression positive group, the overexpression negative control group and the blank control group are compared. The result is that the overexpression negative control group=blank control group<the overexpression positive group, that is, the overexpression vector is transfected. The number of B cells passing through Matrigel gel was significantly more than that of the other two groups of cells. It shows that overexpression of Astrin can up-regulate the invasion ability of B cells (P<0.05); the same reason can show that inhibiting Astrin expression can down-regulate the invasion ability of B cells (P<0.05). In this regard, there are already similar reports (23-25).

The expression of Astrin in the high-metastatic HNSCC cell B was higher than that in the low-metastatic HNSCC cell A. Astrin can promote the proliferation, migration and invasion of HNSCC cells, and inhibit the apoptosis of HNSCC cells. The expression of Astrin in the overexpression positive group was significantly higher than that in the overexpression negative control group and the blank control group, and the results were statistically significant and the difference was significant (P<0.05); the expression of Astrin in the expression suppression positive group was significantly lower than the expression suppression negative control group compared with the blank control group, the results are statistically significant and the difference is significant (P<0.05), indicating that the overexpression plasmid vector we constructed can increase the expression of Astrin; the constructed Astrin inhibitor can inhibit the expression of Astrin, and the transfection is effective.

Astrin is abnormally highly expressed in HNSCC and has important clinical significance. Therefore, this paper uses PCR in vitro to study the biological functions of Astrin in tumorigenesis and development. Astrin can significantly inhibit the proliferation, migration and invasion of HNSCC cells and enhance the sensitivity to chemotherapeutic drugs. Inhibition of Astrin expression has a significant anti-tumor effect, indicating that Astrin plays an important role in the development of tumors and is expected to be a target for exploration of new treatment strategies for HNSCC providing new ideas and reference basis.

Astrin is highly expressed in patients with HNSCC, showing its tumor-promoting effect. In order to better study the biological functions of Astrin, this article studied its effect on the proliferation, invasion, metastasis and monoclonal formation ability of HNSCC cell lines A and B in vitro. In this paper, PCR and linear regression analysis proved that miRNAs and Astrin in HNSCC tissues are obviously negatively correlated, and the expression level of Astrin in HNSCC cells transfected with miRNAs was significantly reduced.

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**Interest conflict**
The authors declare that they have no conflict of interest.

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