Abstract. – OBJECTIVE: The purpose of this study was to uncover the correlations of expression levels of microRNA-429 (miRNA-429) and SOX2 with clinical parameters and prognosis of nasopharyngeal carcinoma.

PATIENTS AND METHODS: Nasopharyngeal carcinoma patients (n=95) and nasopharyngitis patients (n=95) in the same period were enrolled. The relative levels of miRNA-429 and SOX2 in nasopharyngeal tissues collected from these patients were detected by reverse transcriptase-polymerase chain reaction (RT-PCR). Then, the potential correlation between miRNA-429 and SOX2 was analyzed by Pearson correlation test. Next, the influences of miRNA-429 and SOX2 levels on clinical parameters of nasopharyngeal carcinoma patients were assessed. At last, the factors influencing the prognosis of nasopharyngeal carcinoma were determined using the Cox regression model.

RESULTS: It was found that downregulated miRNA-429 and upregulated SOX2 were observed in nasopharyngeal tissues collected from nasopharyngeal carcinoma patients. MiRNA-429 level was negatively correlated with that of SOX2 in nasopharyngeal carcinoma tissues. In addition, miRNA-429 and SOX2 levels were related to age, tumor differentiation, T stage, N stage, and clinical grade of nasopharyngeal carcinoma patients. Moreover, worse prognosis was seen in nasopharyngeal carcinoma patients expressing low level of miRNA-429 or high level of SOX2. Furthermore, Cox regression analysis showed that T3-T4 stage, moderate to high differentiation, and high level of SOX2 were risk factors, while high level of miRNA-429 was the protective factor for nasopharyngeal carcinoma.

CONCLUSIONS: Downregulation of miRNA-429 and upregulation of SOX2 are unfavorable to the prognosis of nasopharyngeal carcinoma.

Key Words: Nasopharyngeal carcinoma, MiRNA-429, SOX2, Clinical parameters, Prognosis.

Introduction

Nasopharyngeal carcinoma (NPC) is one of the most common malignancies in Southern China and Southeast Asia. About 60-85% of nasopharyngeal carcinoma patients develop tumor metastases at the initial diagnosis. Therefore, tumor metastasis is the leading cause of treatment failure in nasopharyngeal carcinoma. With the continuous advancement of diagnosis and treatment technology, comprehensive treatments based on surgery and postoperative radiotherapy effectively control the primary tumors. Nevertheless, sufficient treatments on metastases are limited. The 5-year survival of nasopharyngeal carcinoma has not been greatly improved. It is urgent to find out biomarkers for effectively predicting the prognosis of nasopharyngeal carcinoma.

MicroRNAs (miRNAs) are highly conserved, non-coding, single-stranded RNAs with approximately 19-25 nt in length. They primarily target on mRNA 3’-untranslated region (3’-UTR) and thereby block the translation or degrade the target mRNAs. Chen et al. detected 35 differentially expressed miRNAs in 13 nasopharyngeal carcinoma tissues and 9 normal nasopharyngeal tissues. Among them, 11 miRNAs are upregulated, including miR-17-92 clusters, miR-155, miR-138, miR-25, etc., and a total of 24 miRNAs are downregulated. MiRNA-429-encoded gene locates on human chromosome 1p36.33, which is closely linked to tumorigenesis. In most types of tumors, miRNA-429 serves as a tumor-suppressor gene. It is reported that miRNA-429 suppresses nasopharyngeal carcinoma cells to proliferate and metastasize by downregulating TLN1.

SOX2 is a key transcription factor responsible for maintaining stem cell pluripotency. As an important biomarker for cancer stem cells, SOX2 is
closely related to multiple aspects of cancer cell activities\textsuperscript{2,13}, and it is generally upregulated in cancer, and linked to the disease progression\textsuperscript{14-17}. Wu et al\textsuperscript{18} suggested that the upregulation of SOX2 promotes the growth of nasopharyngeal carcinoma cells.

A previous study has demonstrated the potential interaction between miRNA-429 and SOX2, thereafter influencing the occurrence and progression of cancer\textsuperscript{19}. The progression of glioblastoma multiforme is alleviated by miRNA-429 by targeting SOX2\textsuperscript{20}. In this paper, the influences of miRNA-429 and SOX2 on prognosis of nasopharyngeal carcinoma were mainly explored.

Patients and Methods

Baseline Characteristics

A total of 95 nasopharyngeal carcinoma patients who were treated with radical surgery in Affiliated Hospital of Guizhou Medical University from July 2016 to December 2018 were enrolled, including 57 males and 38 females with the age of 33-75 years old (mean age: 54.6±9.2 years old). Their clinical data were collected. Inclusion criteria: patients initially diagnosed with nasopharyngeal carcinoma by biopsy, those whose tumor staging and pathology were confirmed, those receiving no pre-operative treatment of chemotherapy or radiotherapy, and those providing complete clinical data and follow-up data. Exclusion criteria: Patients not initially diagnosed with nasopharyngeal carcinoma was, those with distant metastases at the first diagnosis, those with autoimmune diseases, diabetes, blood diseases, etc., those with other malignancies, or those with unavailable complete clinical data and follow-up data.

In the meantime, 95 patients with chronic inflammation of the nasopharynx were enrolled as controls, including 66 males and 29 females with 34-69 years old (mean age: 55.7±8.3 years old). This study was approved by Ethics Committee of Affiliated Hospital of Guizhou Medical University. Signed written informed consents were obtained from all participants before the study.

Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR)

Nasopharyngeal tissues were lysed for isolating total RNA using TRIzol method (Invitrogen, Carlsbad, CA, USA). RNA was reversely transcribed into complementary deoxyribose nucleic acid (cDNA) using PrimeScript RT Reagent (TaKaRa, Otsu, Shiga, Japan), and applied for RT-PCR. Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) and U6 were used as the internal reference. miRNA-429: forward: 5'-GGGGGTATACTGTCTGGT-3' and reverse: 5'-TGCGTGTCGTTGAGTC-3', U6: forward: 5'-GCTTCCGCACATATAAAAAAT-3' and reverse: 5'-CGCTTTACGATTTGCTGTGCT-3', SOX2: forward: 5'-TGAGCAGAGACATGCGTGTT-3', GAPDH: forward: 5'-TGAAGGGCAGGAGCGTCAT-3' and reverse: 5'-TGGAGCATGGTGCAGTC-3'.

Follow-Up

Every patient was followed up through outpatient visit or telephone call for at least 5 years. The follow-up was conducted once every three months in the first year and once every six months since after. The last follow-up was conducted in 2018. The death of patient was recorded as the termination of follow-up.

Statistical Analysis

Statistical Product and Service Solutions (SPSS) 22.0 (IBM Corp., Armonk, NY, USA) was used for data analyses. Data were expressed as mean ± standard deviation. The differences between the two groups were analyzed by the t-test. Kaplan-Meier method was introduced for survival analysis, followed by Log-rank test for comparison between curves. Then, the correlation between miRNA-429 and SOX2 was evaluated by Pearson correlation test. At last, potential factors influencing prognosis of nasopharyngeal carcinoma were analyzed by Cox regression model. \( p<0.05 \) was considered as statistically significant.

Results

Downregulated miRNA-429 and Upregulated SOX2 in Nasopharyngeal Carcinoma Tissues

Relative levels of miRNA-429 and SOX2 in nasopharyngeal tissues collected from 95 nasopharyngeal carcinoma patients and 95 nasopharyngitis patients were detected by RT-PCR. It was found that compared with normal nasopharyngeal tissues, miRNA-429 was downregulated and SOX2 was upregulated in tumor tissues (Figure 1A, 1B).
Correlation Between MiRNA-429 and SOX2

Pearson correlation test revealed a negative correlation between miRNA-429 and SOX2 in nasopharyngeal carcinoma tissues \( r=-0.9164, \ p<0.001 \), Figure 2).

Relationship Between Expression Levels of MiRNA-429 and SOX2 with Clinical Parameters of Nasopharyngeal Carcinoma Patients

To validate potential influences of miRNA-429 and SOX2 on the progression of nasopharyngeal carcinoma, patients were assigned into high-level group \( (n=48) \) and low-level group \( (n=47) \) based on the median level of miRNA-429 \( (2.20\pm1.07) \). According to the results, miRNA-429 level was negatively correlated with age, tumor differentiation (moderate to high differentiation), T\(_3\)-T\(_4\) stage, N\(_2\)-N\(_3\) stage, and clinical grade III-IV of nasopharyngeal carcinoma patients. Likewise, the patients were assigned into high-level group \( (n=50) \) and low-level group \( (n=45) \) based on the median level of SOX2 \( (8.29\pm3.39) \). SOX2 level was positively related to the abovementioned parameters (Table I).

Influences of MiRNA-429 and SOX2 on Prognosis of Nasopharyngeal Carcinoma Patients

Patients were followed up for 5 years for collecting prognostic information. By depicting Kaplan-Meier curves, overall survival was found to be better in nasopharyngeal carcinoma patients expressing high level of miRNA-429 \( (HR=7.517, \ p=0.0061, \ \text{Figure 3A}) \). Conversely, overall survival was worse in nasopharyngeal carcinoma patients expressing high level of SOX2 \( (HR=8.828, \ p=0.0030, \ \text{Figure 3B}) \). It is suggested that miRNA-429 is favorable, while SOX2 is unfavorable to the prognosis of nasopharyngeal carcinoma.

Univariate and Multivariate Analyses on Factors Influencing Prognosis of Nasopharyngeal Carcinoma

To further clarify the factors affecting survival in nasopharyngeal carcinoma patients, the Cox regression model was applied. Univariate Cox regression analysis identified that age, tumor differentiation, T stage, N stage, clinical grade, expression levels of miRNA-429 and SOX2 all could affect survival in nasopharyngeal carcinoma. Nevertheless, multivariate Cox regression analysis showed that moderate to high differen-

Figure 1. Downregulated miRNA-429 and upregulated SOX2 in nasopharyngeal carcinoma tissues. Relative levels of miRNA-429 (A) and SOX2 (B) in nasopharyngeal tissues collected from 95 nasopharyngeal carcinoma patients and 95 nasopharyngitis patients.

Figure 2. Correlation between miRNA-429 and SOX2 in nasopharyngeal carcinoma tissues.
MiRNA-429 and SOX2 in nasopharyngeal carcinoma

Table I. Relationship between expression levels of miR-429 and SOX2 with clinical parameters of nasopharyngeal carcinoma patients.

<table>
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<tr>
<th>Variable</th>
<th>N</th>
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<th>SOX2</th>
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<td></td>
<td></td>
<td>High level</td>
<td>Low level</td>
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<tr>
<td></td>
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<td>(n = 50)</td>
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<tr>
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Figure 3. Influences of miRNA-429 (A) and SOX2 (B) on prognosis of nasopharyngeal carcinoma patients.

tiation, T_{3}-T_{4} stage and SOX2 were risk factors, while miRNA-429 was a protective factor for the prognosis of nasopharyngeal carcinoma (Table II).

Discussion

Nasopharyngeal carcinoma has nodular, ulcerative, and submucosal infiltration morphology. Its pathological types mainly include squamous cell carcinoma, adenocarcinoma, and undifferentiated carcinoma\textsuperscript{21}. Due to the surgery difficulties and limitations, chemotherapy and radiotherapy are preferred for nasopharyngeal carcinoma even if the therapeutic efficacy is limited\textsuperscript{22}. Therefore, identification of effective diagnostic biomarkers and targeted therapies for nasopharyngeal carcinoma is critical to improve clinical outcomes.

MiRNA-429 is critical in tumor progression\textsuperscript{23}. By targeting Bcl-2 and SP1, miRNA-429 suppresses invasion and stimulates apoptosis in esophageal carcinoma\textsuperscript{24}, and it inhibits breast cancer cell growth by negatively regulating invasion and metastasis inducers\textsuperscript{25}. Here, miRNA-429 was downregulated in nasopharyngeal carcinoma tissues. Moreover, its level was closely linked to age, tumor differentiation, T stage, N stage, and clinical grade of nasopharyngeal carcinoma patients. It is suggested that miRNA-429 was involved in the progression of nasopharyngeal carcinoma.

The SOX gene family is a class of genes encoding transcription factors with HMG domains. The HMG sequences are highly conserved, and SOX family members are related
SOX2 is able to maintain cellular polymorphisms and to induce polymorphisms\textsuperscript{26}. Sholl et al\textsuperscript{27} showed that SOX2 is expressed in 50% of patients with stage I lung adenocarcinoma. SOX2 is associated with age, gender, and prognosis, rather than tumor stage and smoking history of lung adenocarcinoma patients. Poor prognosis is observed in patients with positive expression of SOX2. In addition, through recruitment of KLF4, SOX2 could regulate the proliferative ability of nasopharyngeal carcinoma via the PI3K/AKT pathway\textsuperscript{28}. In glioma, miRNA-429 inhibits tumor proliferation and metastasis by targeting SOX2\textsuperscript{14}. Consistently, the findings of this study illustrated that SOX2 was the direct target of miRNA-429, and it was upregulated in nasopharyngeal carcinoma tissues. Meanwhile, SOX2 was related to age, tumor differentiation, T stage, N stage, and clinical grade of nasopharyngeal carcinoma patients. In addition, survival analysis revealed that worse prognosis was seen in nasopharyngeal carcinoma patients expressing low level of miRNA-429 or high level of SOX2. Furthermore, T\textsubscript{1}-T\textsubscript{4} stage, moderate to high differentiation, and high level of SOX2 were risk factors, while high level of miRNA-429 was the protective factor for nasopharyngeal carcinoma. Collectively, it is believed that miRNA-429 and SOX2 may be utilized as biomarkers for predicting the prognosis of nasopharyngeal carcinoma patients. This laid the foundation for clinical targeted diagnosis and treatment, and provided new research directions and ideas.

### Conclusions

Downregulation of miRNA-429 and upregulation of SOX2 are unfavorable to the prognosis of nasopharyngeal carcinoma.

### Conflict of Interest

The Authors declare that they have no conflict of interests.

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MiRNA-429 and SOX2 in nasopharyngeal carcinoma

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