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Original Article

The regulation of the metastasis phenotype of laryngeal carcinoma cells using miRNA-34a-3p to target AXL the PI3K pathway, and the epithelial-mesenchymal transition

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Abstract: Objective: To explore the role of miRNA-34a-3p in laryngeal carcinoma cells and its mechanisms. Methods: Specimens from thirty-three patients with laryngeal carcinoma were collected to analyze their miRNA-34a-3p expression levels and clinicopathological features. CCK-8 and colony formation assays were used to evaluate the effects of miRNA-34a-3p on laryngeal carcinoma cell growth, and a Transwell assay was used to determine the impact on cell migration and invasion. Bioinformatics predictions, a luciferase assay, and gene and protein level analyses were used to analyze the interactions between the miRNA and the target genes. Finally, the effect of miRNA-34a-3p in vivo were assessed using a xenograft model. Results: We found significantly decreased levels of miRNA-34a-3p in the laryngeal carcinoma patients with positive lymph node metastasis, low and moderate differentiation, and being in the T3+T4 periods and the III+IV clinical periods compared to the laryngeal carcinoma patients with negative lymph node metastasis, high differentiation, and being in the T1+T2 periods and the I+II clinical periods. The overexpression of miRNA-34a-3p downgraded cell multiplication, colony formation, and cell migration and invasion. In the laryngeal carcinoma cells with an overexpression of miRNA-34a-3p, the AXL levels decreased, the Akt phosphorylated, the Snail and vimentin levels increased, and the E-cadherin and N-cadherin expression levels were upgraded. In addition, the miRNA-34a-3p mimics were able to reduce the growth of the tumor cells in vivo. Conclusions: We proved that miRNA-34a-3p regulates the metastasis phenotype of laryngeal carcinoma cells by targeting AXL the PI3K pathway, and the epithelial-mesenchymal transition.

Keywords: miRNA-34a-3p, AXL, PI3K pathway, laryngeal cancer

Introduction

Laryngeal carcinoma, the most common type of squamous cell carcinoma on the head and neck, accounts for nearly 1%-5% of all malignant tumors [1, 2]. Although the diagnosis and treatment of cancer have improved [3], the incidence and mortality rate of laryngeal cancer have increased by about 1.86 per 100,000 and 1.01 per 100,000 respectively in China [4, 5]. Therefore, an elaboration of the mechanisms involved in the pathogenesis of laryngeal carcinoma will help provide effective therapeutic intervention.

MicroRNA (miRNA), a type of non-coding RNA around 22 nucleotides in length, has the ability to regulate genes by interacting with the 3' untranslated region (3'UTR) of genes [6]. It is aberrantly expressed in varied types of cancers, such as breast cancer, colon cancer, bladder cancer, and other tumors. The functions of miRNAs on cancer cell behavior regulation include cell proliferation, metastasis, angiogenesis, etc. However, the accuracy of the target gene and a possible change of the main pathways that miRNAs involve can be the key point to the pathophysiological function of miRNAs [7]. miRNA-34a-3p is an miRNA that has been studied recently. Domestic and foreign studies have shown that miRNA-34A-3P is closely related to the occurrence and development of various tumors and may become a potential bio-

logical indicator for the diagnosis and treatment of a variety of cancers [8, 9]. In our preliminary studies, we discovered that miRNA-34a-3p is downregulated in laryngeal carcinoma tissues and correlates with poor patient prognosis. However, the detailed regulation mechanisms of miRNA-34a-3p have yet to be fully probed.

In the present study, the aim was to investigate the function of miRNA-34a-3p in laryngeal carcinoma cells and explore its mechanisms, in order to provide new biological indexes for the clinical diagnosis and treatment of laryngeal carcinoma.

Materials and methods

The ethical approval of the research plan

The study was approved by the Institutional Review Board of the First Affiliated Hospital of Soochow University, Suzhou City, China. All the participants provided a written, informed consent. The study was conducted according to the Declaration of Helsinki. In addition, the animal experiments carried out in the study were approved by the Institutional Animal Care and Use Committee of Soochow University.

Subjects and specimens

A total of 33 cases of surgically resected laryngeal carcinoma specimens with complete clinical data were collected from the Surgery Department, the First Affiliated Hospital of Soochow University, from January 2014 to June 2016. The specimens were frozen using liquid nitrogen, and stored at -80°C for later analysis. The specimens came from 20 males and 13 females, and the patients had an average age of (51.58±9.12) years old. There were 14 cases with lymph node metastasis, and 6 cases in the T1 stage, 13 cases in the T2 stage, 10 cases in the T3 stage, and 4 cases in the T4 stage. According to their TNM stages, 4 cases were in stage I, 14 cases were in stage II, 9 cases were in stage III, and 6 cases were in stage IV. All the patients were diagnosed and classified by 2 experienced pathologists according to the guidelines established by the Union for International Cancer Control (UICC). The research plan was approved by the Ethics Committee of The First Affiliated Hospital of Soochow University. Formal informed consents in writing were provided by all the patients.

Inclusion and exclusion criteria

Inclusion criteria: patients pathologically diagnosed with laryngeal carcinoma who had not undergone radiotherapy or chemotherapy before their surgery, and patients (or their families) who provided a written, informed consent.

Exclusion criteria: patients with incomplete clinical data, and patients with other primary tumors.

Real-time quantitative PCR for miRNA-34a-3p

The cancer tissues were collected and ground into powder before total tissue RNA extraction was performed with TRIzol reagent (Thermo Fisher). A one-step process PrimeScript miRNA cDNA synthetic Kit that was purchased from Takara Co. was used for the miRNA cDNA synthesis. A one-step SYBR® PrimeScript™ RT-PCR kit (Takara, Dalian of China) and an ABI 7500 Real-Time PCR (Applied Biosystems, Foster City, California, U.S.) were employed for the RT-PCR. The glyceraldehyde 3-phosphate dehydrogenase (GAPDH) and U6 expression levels were respectively adopted as internal controls for the AXL and miRNA expressions. The $2^{-\Delta\Delta CT}$ measure was taken for the gene expression assay [14]. The oligonucleotide primers specific for AXL, miRNA-34a-3p, U6, and GAPDH are set out in **Table 1**.

The cell culture

Human-derived laryngeal cancer cell lines we used were AMC-HN8, Tu177, and TU686 and purchased from the American Type Culture Collection (ATCC, Manassas, VA, U.S.). The cells, which contained 10% fetal bovine serum (FBS; Thermo Fisher), streptomycin (100 μg/mL) and penicillin (100 U/mL), were maintained in Dulbecco's modified Eagle medium (DMEM, Thermo Fisher, CA, USA). The preserving condition of the cells was a humidified atmosphere of 5% CO_2 at 37°C.

Cell transfection assays

In order to upregulate the miRNA-34a-3p expression, the laryngeal cancer cells were conducted by transfections with the miRNA-34a-3p mimic and a mimic control obtained from RiboBio Co. (Genepharm, China) respectively. TU686 cells (2×10^4 cells/hole) were

Table 1. Primers for real-time PCR

Gene name	Forward primer (5'-3')	Reverse primer (5'-3')
AXL	GAGAACATTAGTGCTACGCGGAA	CCTTAGCCCTATGTCCATTAGCA
GAPDH	TCGACAGTCAGCCGCATCTT	GGCGCCCAATACGACCAAAT
miRNA-34a-3p	GCGGCCAATCAGCAAGTATACT	GTGCAGGTCCGAGGT
U6	AGAGAGATTACATGGCCCCT	CTAATGTCACGCACGATTCT

plated in 6-hole plates, and the cell transfection assays were carried out using Lipofectamine 3000 reagent (Thermo Fisher) in line with the manufacturer's instructions. The miRNA-34a-3p mimic and mimic control were transfected at a concentration of 20 nM.

Cell counting kit-8 (CCK-8) experiment

The proliferation of the TU686 laryngeal carcinoma cells after the transfection of the miRNA-34a-3p mimic and mimic control was assessed using CCK-8 assays (Beyotime, Nantong, China). TU686 cells (3×10^3 cells/hole) were plated in 96-well cell culture plates for 24, 48, and 72 hours. The cell proliferation was assessed after the transfection, and 10 μL of CCK-8 solution was injected into every hole. The cells, then, were kept for another 2 h in 5% CO_2 at 37°C, and the absorbance was measured at a wavelength of 450 nm through a microplate reader (Bio-Rad).

Colony formation assay

The TU686 laryngeal cancer cells (800 cells) infected with the miRNA-34a-3p mimic or the mimic control lentivirus were seeded in 6-hole plates in complete medium and preserved at 37°C under a humidified atmosphere with 5% CO_2 for a week until stained with crystal violet. The colony survival rate was checked using a lighted microscope with a definition of >50 cells. The steps were repeated three times to acquire an even number of colony formations.

Migration and invasion assays

A modified Transwell migration assay with 8 μm pore filters inserted in 24-hole plates was provided for the cell migration evaluation (Becton, Dickinson and Company, San Jose, CA, U.S.). 24 h after the transfection, the cells were placed in a serum-free medium at a level of 3×10^5 cells and moved to the upper chamber. We then filled the lower chamber with DMEM and supplemented it with a chemoattractant using 20% FBS. After that, we incubated the cells on a Transwell plate for 24 h, and erased the residue cells on upper chamber using a cotton swab. We then cut the Transwell membrane that was closest to the lower chamber, and stained it with crystal violet for a microscopic examination. The last step was to count the cells in the photographs. The cell invasion assay was similar to the migration assay, except that the 8 μm pore filters inserted were coated with Matrigel before the cell seeding.

Bioinformatics analysis

The miRDB database (http://mirdb.org/miRDB/ [10]) and TargetScan, the prediction of regulatory targets software (http://www.targetscan.org), were employed for miRNA-34a-3p.

Luciferase report gene assays

The binding sites between AXL, miRNA-34a-3p and mutated AXL were predicted and are shown in **Figure 2A**. The co-transfections with pmirGLO, pmir-GLO-AXL-wt or pmir-GLO-AXL-mut, and the miRNA-34a-3p mimic or the mimic control were performed in the TU686 laryngeal cancer cells following the manufacturer's instructions for Lipofectamine 3000 (Thermo Fisher). After 48 h of transfection, a luciferase reporter gene assay system (Promega, E1910) was employed to identify the relative luciferase activities of the TU686 cells.

Western-blotting

The TU686 cells obtained after the miRNA-34a-3p mimic and mimic control transfection were dissolved in a RIPA buffer, followed by high speed centrifugation at 12000 g at 4°C for 15 min for the protein quantification analysis using a bicinchoninic acid assay. The cellular proteins were separated first through sodium dodecyl sulfate-polyacrylamide gel electrophoresis and then moved to polyvinylidene difluoride membranes. After the blocking-up, the membranes were then cultured with anti-total-

or -phospho-Akt, E-cadherin, N-cadherin, vimentin, Snail and AXL monoclonal primary antibodies (Technology of the Cell Signaling Pathway, Cambridge, MA). GAPDH (Santa Cruz Biotechnology Inc, Santa Cruz, California) was utilized as the loading control. Appropriate secondary antibodies that bind horseradish peroxidase were applied for the quantification of the labeled proteins. The protein mass spectrometry was developed using SuperSignal Ultra Chemiluminescent Substrate (Pierce, Rockford City, IL) on x-ray film (Japan's Kodak, Tokyo City).

Nude mice model of xenograft tumors

We obtained 6-week old athymic nude mice from the Shanghai SLAC Laboratory Animal Center. The 2×10^6 TU686 cell infected miRNA-34a-3p mimic or the mimic control lentivirus that levitated in 50 μl PBS were hypodermically injected into the mice's dorsal thigh area. We weighed the mice every two days and measured the lengths and widths of their tumors for assessment. At the fourth week after the treatment, all the mice were killed and their tumors were excised for weight calculation and photographing. For the histological analysis, the organs of the treated groups and the control group were held in 4% formalin and then conducted with paraffin-cut for the HE staining. The treated slices were recorded using digital microscope equipment (Leica QWin).

Statistical analysis

The descriptive statistics for the patient group were reported as the mean ± standard deviation (SD) or the median plus range value. The data were presented by category as numbers and percentages. The calculation of the differences between the relative expressions of miRNA-34a-3p according to the stratification of the clinical data of the patients were performed with the Student's t distribution. $P<0.05$ indicated statistical significance. The statistical analysis was carried out using SPSS 19.0 (SPSS Inc., Chicago, IL).

Results

The relationship between miRNA-34a-3p and the clinicopathological features of the laryngeal carcinoma patients

In order to identify the function of miRNA-34a-3p in laryngeal carcinoma patients, we collected cancer tissues, evaluated the expression of miRNA-34a-3p, and explored the connection between the expression level of miRNA-34a-3p and the clinicopathological features of the laryngeal carcinoma cases. As indicated in **Table 2** and **Figure 1**, a significantly decreased level of miRNA-34a-3p in laryngeal carcinoma cases with positive lymph node metastasis, low and moderate differentiation, being in the T3+T4 period and the III+IV clinical period compared to negative lymph node metastasis, high differentiation, being in the T1+T2 period and the I+II clinical period, respectively. These results indicated that miRNA-34a-3p might be involved in the metastatic features of laryngeal carcinoma.

The overexpression of miRNA-34a-3p decreased cell reproduction, colony formation, and cell migration and invasion

With the decrease level of miRNA-34a-3p in laryngeal carcinoma, we further investigated whether the malignant phenotype of the laryngeal carcinoma could be reversed using the miRNA-34a-3p mimic. We checked the miRNA-34a-3p expression in the r4 laryngeal carcinoma cell lines AMC-HN8, Tu177, TU686, and Hep-2. As the lowest level was found in the TU686 cells, it was selected for the following analysis. The results showed that the overexpression of miRNA-34a-3p could significantly decrease the cell proliferation (0.41±0.04 vs. 0.53±0.05 at 24 h, 0.64±0.06 vs. 0.96±0.08 at 48 h and 0.99±0.08 vs. 1.26±0.10 at 48 h), colony formation (150±10 vs. 250±15 clones), cell migration (108±12 vs. 220±15 cells), and invasion (45±5 vs. 188±12 cells) (**Figure 1**). These results indicate that the overexpression of miRNA-34a-3p can reverse the malignant features of laryngeal carcinoma cells.

miRNA-34a-3p targets AXL in laryngeal carcinoma cells

According to previous description, miRNA exerts its effects by targeting mRNA, thereby affecting gene translation. In order to explore the possible mechanisms, we first used the bioinformatics tools to identify the target gene, and confirmed AXL as the target gene of miRNA-34a-3p (**Figure 2A**). Luciferase activity was decreased in the WT reporting plasmid, but not in the MUT plasmid, suggesting a substantial interaction between miRNA-34a-3p and AXL

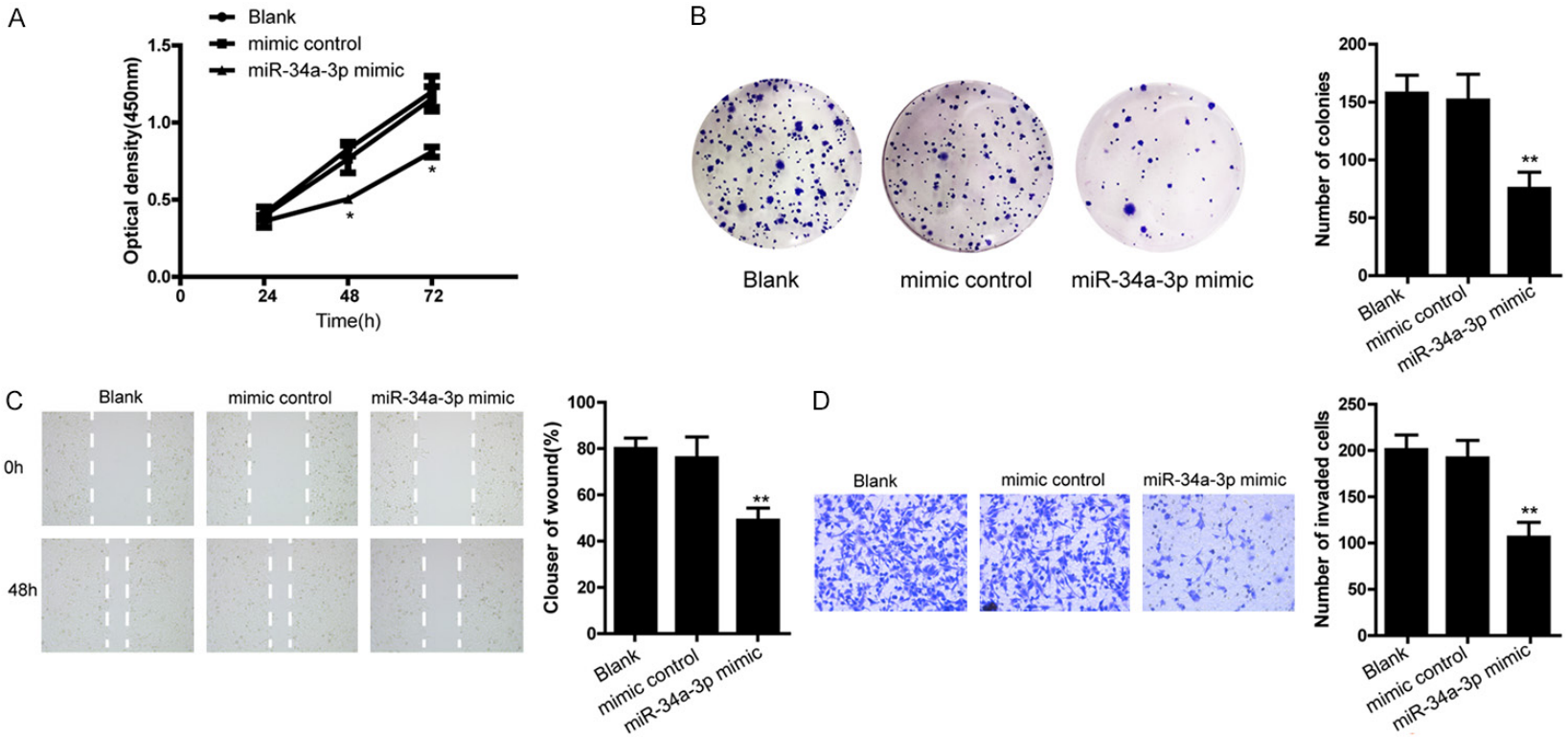

Figure 1. The overexpression of miRNA-34a-3p decreases cell reproduction, colony formation, and cell migration and invasion. miRNA-34a-3p overexpression decreases cell proliferation (A), colony formation (B), cell migration (C) and invasion (D). *P<0.05 compared to the mimic control or the blank.

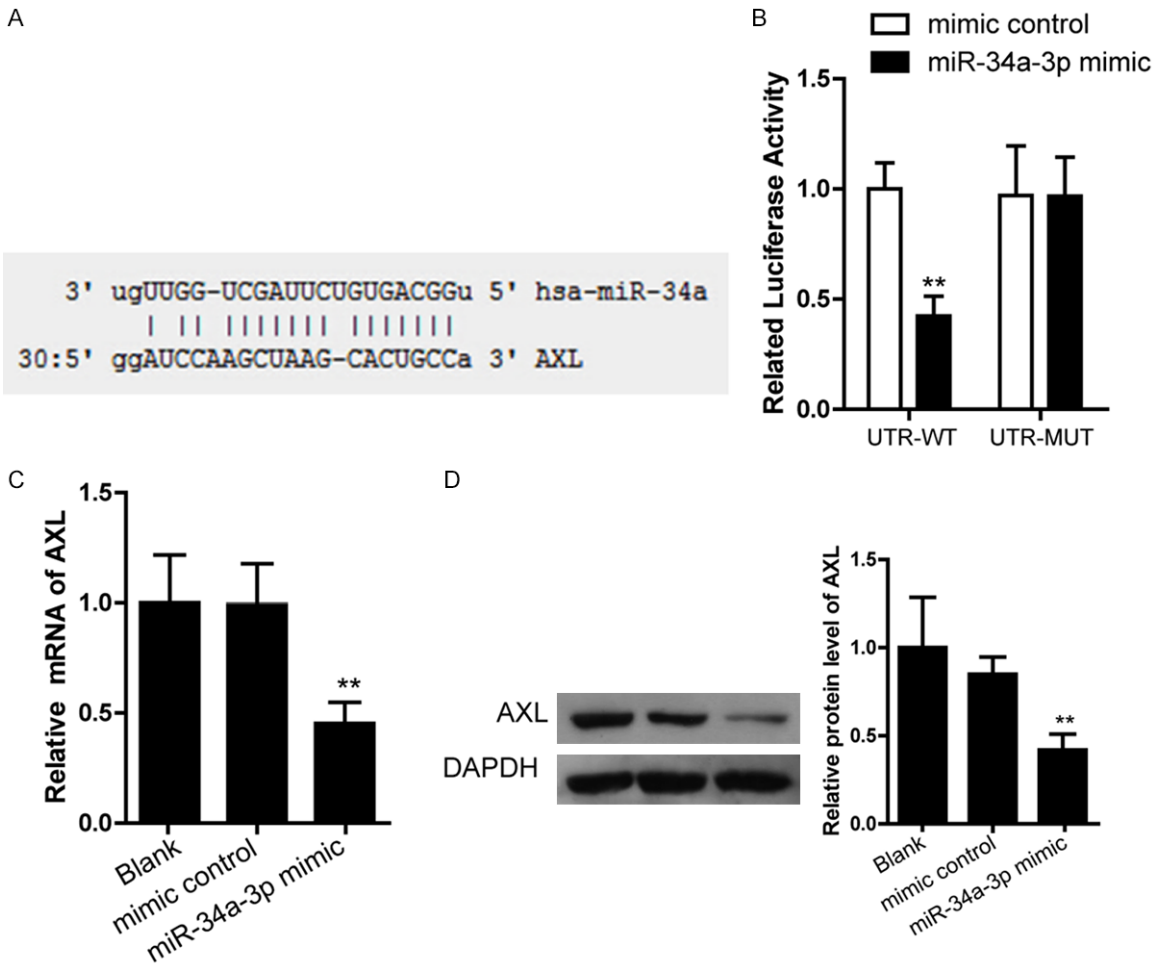

Figure 2. miRNA-34a-3p targets AXL in laryngeal carcinoma cells. A. Schematic figures about the binding sites between miRNA-34a-3p and AXL. B. Luciferase activity analysis of the TU686 cells co-transfected with the luciferase reporter plasmid containing either wildtype (WT) or mutant (MUT) AXL 3'-UTR, and either the miRNA-34a-3p mimics or the mimic control. C. Quantitative PCR analysis of the expression level of AXL. D. Western-blotting analysis the protein level of AXL in laryngeal carcinoma cells.

(**Figure 2B**). Besides, further gene and protein level verified a decrease in the AXL mRNA and protein after the overexpression of miRNA-34a-3p (**Figure 2C**, **2D**). These results indicated that miRNA-34a-3p targets AXL to regulate the behavior of laryngeal carcinoma cells.

The overexpression of miRNA-34a-3p regulates PI3K/Akt pathway and epithelial-mesenchymal transition (EMT) in laryngeal carcinoma cells

Multiple signaling pathways, including the PI3K/Akt pathway and EMT, are known to perform a major role in the metastatic behavior of laryngeal carcinoma cells. Therefore, we examined the related protein levels. The results reflected dramatically decreased Akt phosphorylation, Snail, and vimentin levels and uplifted E-cadherin and N-cadherin levels were found in the miRNA-34a-3p overexpressed laryngeal carcinoma cells. Therefore, this revealed that miRNA-34a-3p changed the malignant phenotype of laryngeal carcinoma cells via the PI3K/Akt pathway and EMT. As shown as **Figure 3**.

The overexpression of miRNA-34a-3p decreased laryngeal carcinoma cell growth in vivo

After analyzing the effects of miRNA-34a-3p in vitro, we further evaluated the role of miRNA-34a-3p in vivo using a xenograft transplantation model. The results showed that significant-

Table 2. The relationship between miRNA-34a-3p and the clinicopathological features of the laryngeal carcinoma patients

Clinicopathological features	Quantity	miRNA-34a-3p	t	*P*
Gender				
Male	20	0.362±0.047	1.886	0.069
Female	13	0.389±0.026		
Age (years old)				
≥60	15	0.372±0.045	0.760	0.453
<60	18	0.359±0.052		
Lymph node metastasis				
Yes	14	0.278±0.047	8.001	0.000
No	19	0.431±0.059		
Differentiation Grade				
Well-differentiated	17	0.217±0.022	27.931	0.000
poorly and moderately differentiated	16	0.548±0.040		
T Staging				
T1+T2	19	0.497±0.031	36.264	0.000
T3+T4	14	0.197±0.016		
TNM Staging				
I+II	18	0.512±0.042	24.162	0.000
III+IV	15	0.228±0.019		

ly decreased tumor volumes could be found at weeks 2, 3, and 4, which was also verified by tumor images and H&E staining figures. These results indicated that miRNA-34a-3p overexpression can decrease laryngeal carcinoma cell growth in vivo.

Discussion

MicroRNA plays a very important role in varied physical and pathological processes such as the growth of cells, differentiation and tumor formation [11]. Currently, aberrant miRNA expressions have been detected in a variety of tumors and are closely related to tumor growth [12], metastasis [13], angiogenesis [14] and many other malignant biological characteristics, such as oncogenes or tumor suppressors. Therefore, it is of great significance to study the mechanism of action of microRNAs on tumor cells to inform cancer treatment.

miRNA-34a-3p, with a size of 22 bases, is a member of the miR-34 family [15]. Under physiological conditions, miRNA-34a-3p is mainly distributed in brain tissue, and it is located at human chromosome 1p36.23 [16, 17]. According to previous reports, the transcription of miRNA-34a-3p is initiated via the binding of the classical tumor suppressor gene p53 and the first exon of the miRNA-34a-3p located gene [15]. Studies have confirmed that significantly lower expression levels of miRNA-34a-3p in breast tumors [18], bladder tumors [19], esophageal tumors [20], and other tumor tissues compared to the corresponding normal tissues. The overexpression of miRNA-34a-3p in human lung cancer A549 cells [21] and human chronic myeloid leukemia K562 cells [22] can significantly reduce various protein molecules involved in cell proliferation and apoptosis inhibition, such as CDK2, CDK4, CCND1, and Bcl-2, thereby achieving tumor suppression effects. However, in laryngeal carcinoma, few studies focusing on the role of miRNA-34a-3p have been published. Our previous small sample studies found that miRNA-34a-3p is down-regulated in laryngeal carcinoma and is relevant to an inferior clinical prognosis, but its function in the development of laryngeal carcinoma is undetermined.

In the present study, we found that significantly decreased levels of miRNA-34a-3p in laryngeal carcinoma cases with positive vs. negative lymph node metastasis, low and moderate vs. high differentiation, and being in the T3+T4 vs. the T1+T2 stages and in the III+IV and I+II clinical stage comparisons. The overexpression of miRNA-34a-3p reduces cell proliferation, colo-

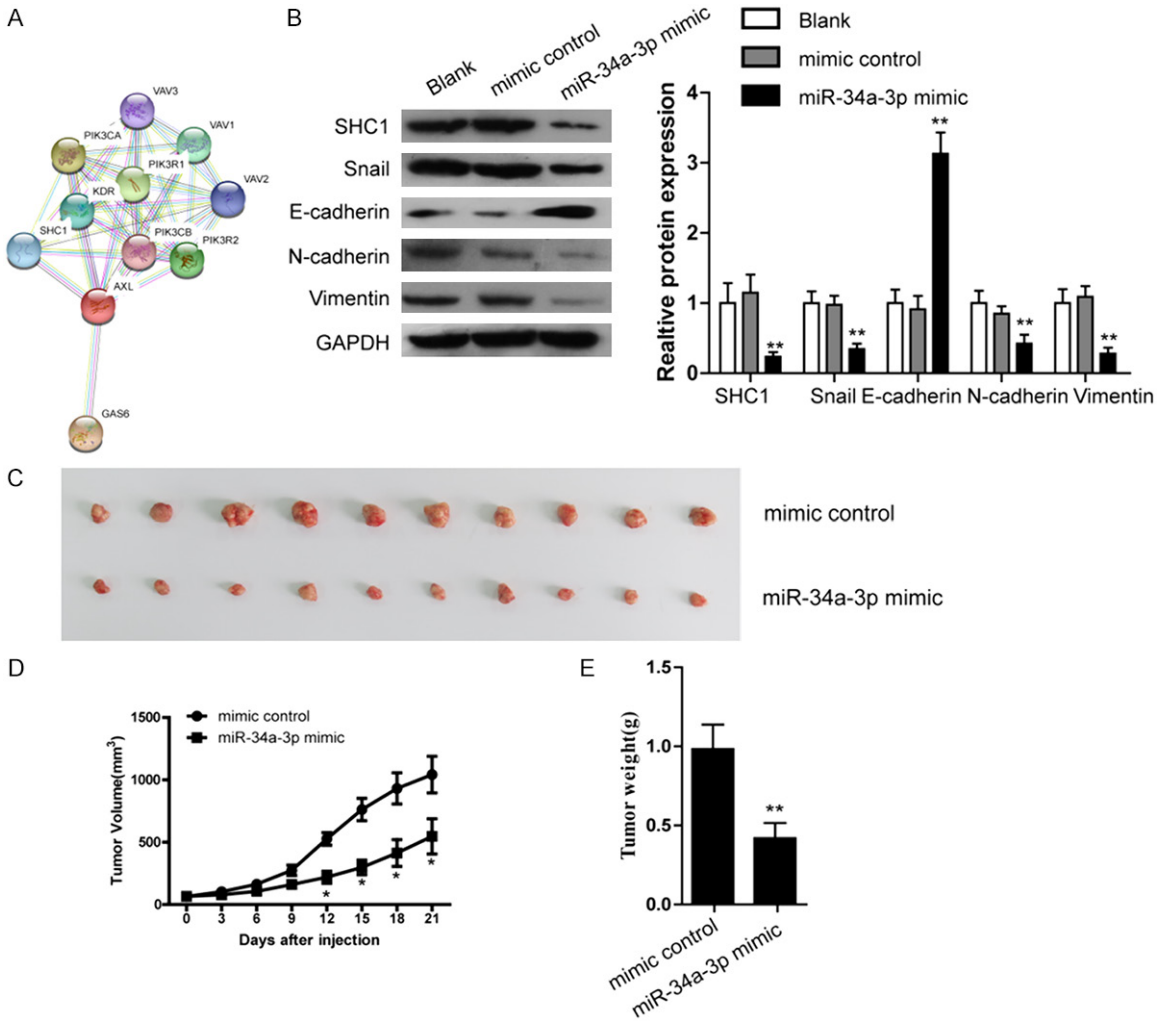

Figure 3. The overexpression of miRNA-34a-3p regulates the PI3K/Akt pathway and the epithelial-mesenchymal transition in laryngeal carcinoma cells. The decreased of Akt phosphorylation, Snail and vimentin levels and the increased of E-cadherin and N-cadherin levels.

ny formation, and cell migration and invasion. Decreased AXL levels, Akt phosphorylation, Snail and vimentin and uplifted levels of E-cadherin and N-cadherin were found in laryngeal carcinoma cells with miRNA-34a-3p overexpression. In addition, the miRNA-34a-3p mimic was able to decrease tumor cell growth in vivo.

Phosphatidylinositol-3-kinase (PI3K) is a clan of lipid kinases that phosphorylates 3'-OH in the inositol ring. Classical PI3Ks are made up of catalytic subunit p110 and regulatory subunit p85. Akt is a 57KD serine/threonine protein kinase, which consists of a N-terminal pleckstrin homology (PH) region, an intermediate kinase region, and a carboxy-terminal regulatory region. The binding site between Akt and inositol 3-phosphate is the PH region that contains one hundred amino acid residues. Because AKT is structurally similar to kinase A and C, it is also called protein kinase B (PKB) [23]; in tumors, PI3K/Akt can promote tumor cell EMT through various pathways, thereby promoting the occurrence of tumor invasion and metastasis, so the PI3K/Akt/Snail signaling pathway is one of the most important pathways [24]. Snail is a kind of DNA-binding protein that contains a zinc finger structure, shuttles between the cytoplasm and the nucleus, recognizes and binds to the E-box region upstream of the E-cadherin promoter, restrains the expression of the E-cadherin gene, and boosts EMT. Snail positioning in the nucleus also affects the transcrip-

tion of itself and β-catenin, thereby upregulating the expression of vimentin, fibronectin, matrix metalloproteinase 19, and N-cadherin. The regulation of Snail by the PI3K/AKT signal is a complex process. On the one hand, PI3K/Akt signaling can inhibit the degradation of Snail; on the other hand, PI3K/Akt can directly up-regulate the expression level of Snail in cells. Thus, the inhibition of the PI3K/Akt/Snail signaling pathway is effective in inhibiting the invasion and metastasis abilities of tumor cells.

AXL is a constituent of the receptor tyrosine kinase clan. The 3'-UTR of AXL mRNA has a specific binding sequence of miRNA-34a-3p and is one of the downstream targets of miRNA-34a-3p. The upstream signaling molecule of PI3K/Akt can significantly promote the phosphorylation of PI3K/Akt in a variety of tumors, thereby activating a series of downstream signal transduction pathways that promote cell growth and invasion [25], and AXL is one of the effector molecules. Our preliminary study using small samples also found that miRNA-34a-3p is down-regulated in laryngeal carcinoma tissues and is associated with poor clinical pathological features. Similar to the related study results [26], miRNA-34a-3p may play a tumor suppressor gene role in the occurrence and development of laryngeal carcinoma, and its mechanism of action may be through inhibiting the PI3K/Akt/Snail signaling pathway, thereby effectively inhibiting tumor cell invasion and metastasis.

However, due to the relatively small number of samples included, there may be some deviation in the conclusion. In future studies, the sample size will be expanded so that more reliable research results can be obtained.

In conclusion, we demonstrated that miRNA-34a-3p regulates the metastasis phenotype of laryngeal carcinoma cells by targeting AXL, the PI3K pathway, and the epithelial-mesenchymal transition.

Disclosure of conflict of interest

None.

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References

[1] Gao W, Wu Y, He X, Zhang C, Zhu M, Chen B, Liu Q, Qu X, Li W, Wen S and Wang B. Microrna-204-5p inhibits invasion and metastasis of laryngeal squamous cell carcinoma by suppressing forkhead box c1. J Cancer 2017; 8: 2356-2368.

[2] Yuan Z, Xiu C, Song K, Pei R, Miao S, Mao X, Sun J and Jia S. Long non-coding RNA AFAP1-AS1/miR-320a/RBPJ axis regulates laryngeal carcinoma cell stemness and chemoresistance. J Cell Mol Med 2018; 22: 4253-4262.

[3] Adeel M, Faisal M, Rashid A, Usman S, Khaleeq U, Abbas T, Rehman A, Malik K, Hussain R and Jamshed A. An overview of laryngeal cancer treatment at a tertiary care oncological center in a developing country. Cureus 2018; 10: e2730.

[4] Liu Y, Zhao Q, Ding G, Zhu Y, Li W and Chen W. Incidence and mortality of laryngeal cancer in China, 2008-2012. Chin J Cancer Res 2018; 30: 299-306.

[5] Takeuchi T, Kawasaki H, Luce A, Cossu AM, Misso G, Scrima M, Bocchetti M, Ricciardiello F, Caraglia M and Zappavigna S. Insight toward the microRNA profiling of laryngeal cancers: biological role and clinical impact. Int J Mol Sci 2020; 21: 3693.

[6] Yang T, Li S, Liu J, Yin D, Yang X, Tang Q and Wang S. Long non-coding RNA KRT16P2/miR-1294/EGFR axis regulates laryngeal squamous cell carcinoma cell aggressiveness. Am J Transl Res 2020; 12: 2939-2955.

[7] Ye HF, Jin QZ, Wang XQ and Li Y. MicroRNA-802 inhibits cell proliferation and induces apoptosis in human laryngeal cancer by targeting cAMP-regulated phosphoprotein 19. Cancer Manag Res 2020; 12: 419-430.

[8] Tan W, Liu B, Qu S, Liang G, Luo W and Gong C. Micrornas and cancer: key paradigms in molecular therapy. Oncol Lett 2018; 15: 2735-2742.

[9] Reddy KB. Microrna (mirna) in cancer. Cancer Cell Int 2015; 15: 38-40.

[10] Hu A, Huang JJ, Xu WH, Jin XJ, Li JP, Tang YJ, Huang XF, Cui HJ, Sun GB, Li RL and Duan JL. MiR-21/miR-375 ratio is an independent prognostic factor in patients with laryngeal squamous cell carcinoma. Am J Cancer Res 2015; 5: 1775-1785.

[11] Li JZ, Gao W, Lei WB, Zhao J, Chan JY, Wei WI, Ho WK and Wong TS. MicroRNA 744-3p promotes MMP-9-mediated metastasis by simultaneously suppressing PDCD4 and PTEN in laryngeal squamous cell carcinoma. Oncotarget 2016; 7: 58218-58233.

[12] Li Y, Liu J, Hu W, Zhang Y, Sang J, Li H, Ma T, Bo Y, Bai T, Guo H, Lu Y, Xue X, Niu M, Ge S, Wen

S, Wang B, Gao W and Wu Y. miR-424-5p promotes proliferation, migration and invasion of laryngeal squamous cell carcinoma. Onco Targets Ther 2019; 12: 10441-10453.
[13] Oliveto S, Mancino M, Manfrini N and Biffo S. Role of micrornas in translation regulation and cancer. World J Biol Chem 2017; 8: 45-56.
[14] Li P, Lin XJ, Yang Y, Yang AK, Di JM, Jiang QW, Huang JR, Yuan ML, Xing ZH, Wei MN, Li Y, Yuan XH, Shi Z, Liu H and Ye J. Reciprocal regulation of miR-1205 and E2F1 modulates progression of laryngeal squamous cell carcinoma. Cell Death Dis 2019; 10: 916.
[15] Zhao X, Zhang W and Ji W. MYO5A inhibition by miR-145 acts as a predictive marker of occult neck lymph node metastasis in human laryngeal squamous cell carcinoma. Onco Targets Ther 2018; 11: 3619-3635.
[16] Wu Y, Yu J, Ma Y, Wang F and Liu H. miR-148a and miR-375 may serve as predictive biomarkers for early diagnosis of laryngeal carcinoma. Oncol Lett 2016; 12: 871-878.
[17] Slabakova E, Culig Z, Remsik J and Soucek K. Alternative mechanisms of miRNA-34a-3p regulation in cancer. Cell Death Dis 2017; 8: e3100.
[18] Zhu X and Zhu R. Curcumin suppresses the progression of laryngeal squamous cell carcinoma through the upregulation of miR-145 and inhibition of the PI3K/Akt/mTOR pathway. Onco Targets Ther 2018; 11: 3521-3531.
[19] Gao W, Zhang C, Li W, Li H, Sang J, Zhao Q, Bo Y, Luo H, Zheng X, Lu Y, Shi Y, Yang D, Zhang R, Li Z, Cui J, Zhang Y, Niu M, Li J, Wu Z, Guo H, Xiang C, Wang J, Hou J, Zhang L, Thorne RF, Cui Y, Wu Y, Wen S and Wang B. Promoter methylation-regulated miR-145-5p inhibits laryngeal squamous cell carcinoma progression by targeting FSCN1. Mol Ther 2019; 27: 365-379.
[20] Cui XB, Peng H, Li RR, Mu JQ, Yang L, Li N, Liu CX, Hu JM, Li SG, Wei Y, Laibo Y, Zhou H, Li F and Chen YZ. Microrna-34a functions as a tumor suppressor by directly targeting oncogenic plce1 in kazakh esophageal squamous cell carcinoma. Oncotarget 2017; 8: 92454-92469.
[21] Yao XD, Li P and Wang JS. MicroRNA differential expression spectrum and microRNA-125a-5p inhibition of laryngeal cancer cell proliferation. Exp Ther Med 2017; 14: 1699-1705.
[22] Cui X, Xiao D, Cui Y and Wang X. Exosomes-derived long non-coding RNA HOTAIR reduces laryngeal cancer radiosensitivity by regulating microRNA-454-3p/E2F2 axis. Onco Targets Ther 2019; 12: 10827-10839.
[23] Cui W, Meng W, Zhao L, Cao H, Chi W and Wang B. TGF-β-induced long non-coding RNA MIR155HG promotes the progression and EMT of laryngeal squamous cell carcinoma by regulating the miR-155-5p/SOX10 axis. Int J Oncol 2019; 54: 2005-2018.
[24] Zhong JT and Zhou SH. Warburg effect, hexokinase-II, and radioresistance of laryngeal carcinoma. Oncotarget 2017; 8: 14133-14146.
[25] Ricciardiello F, Capasso R, Kawasaki H, Abate T, Oliva F, Lombardi A, Misso G, Ingrosso D, Leone CA, Iengo M and Caraglia M. A miRNA signature suggestive of nodal metastases from laryngeal carcinoma. Acta Otorhinolaryngol Ital 2017; 37: 467-474.
[26] Jiang C, Zhou L, Wang H, Zhang Q and Xu Y. Axl is a potential cancer prognostic marker for the migration and invasion of nasopharyngeal carcinoma. Adv Clin Exp Med 2016; 25: 531-537.