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Research Article

MiRNA-106a-5p Promotes Laryngeal Carcinoma Proliferation and Migration Through *PI3K/AKT/m-TOR* Pathway by *AKTIP*

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Background: Laryngeal cancer (LC) remains one of the most common tumors of the respiratory tract, the exact pathogenesis remains unclear. *MiRNA-106a-5p* is aberrantly expressed in a variety of cancers and plays a pro- or anti-cancer role, but is indistinct in LC.

Objectives: Showing the role of *miRNA-106a-5p* in the development of LC.

Materials and Methods: Quantitative reverse transcription-polymerase chain reaction was used for *miR-106a-5p* measurement in clinical samples and LC cell lines (AMC-HN8 and TU212), first. The expression of *miR-106a-5p* was inhibited by inhibitor, then followed clonogenic and flow cytometric assays for cell proliferation; wood healing, and Transwell assays for cell migration. Dual luciferase reporter assay was performed for interaction verification, and the activation of the signal pathway was detected by western blots.

Results: *MiR-106a-5p* was significantly over-expressed in LC tissues and cell lines. The proliferation ability of the LC cells was significantly reduced after *miR-106a-5p* inhibition, and most LC cells were stagnated in the G1 phase. The migration and invasion ability of the LC cells was decreased after the *miR-106a-5p* knockdown. Further, we found that *miR-106-5a* is bound with 3'-UTR of AKT interacting protein (*AKTIP*) mRNA specifically, and then activate *PI3K/AKT/m-TOR* pathway in LC cells.

Conclusions: A new mechanism was uncovered that miR-106a-5p promotes LC development via *AKTIP/PI3K/AKT/m-TOR* axis, which guides clinical management and drug discovery.

Keywords: AKTIP, Laryngocarcinoma, *MiRNA-106a-5p*, *PI3K/AKT/m-TOR*

1. Background

Laryngeal cancer (LC) is found in the upper respiratory tract of the human body and is the most common type of head and neck cancer globe wide. LC mainly develops in males, but with exposure to such as smoking, alcohol, and air deterioration, LC is becoming more frequent and de-sexualized (1, 2). The current treatment protocols for LC include surgical resection, radiotherapy, and chemotherapy, etc. However, due to the high recurrence after surgery and damage caused by radiotherapy and chemotherapy to the weak body, the clinical treatment outcome is not satisfactory (3, 4). To eliminate the pain of patients, there is an urgent requirement to find specific biological targets in the development of LC,

to provide a theoretical basis for the development of specific drugs and the optimization of clinical treatment strategies.

MicroRNAs (miRNAs) are small non-coding RNAs of approximately 22 bases that are involved in regulating various life activities such as apoptosis, cell division, and cell proliferation (5, 6). By binding to the 3′UTR of target messenger RNA (mRNA), miRNAs repress/promote specific mRNA translation and participate in post-translation regulation (7). MiRNAs are involved in cancer initiation, drug escaping, and cancer metastasis as well (8, 9). For example, *miR-107* inhibits the development of hepatocellular carcinoma by increasing drug sensitivity (10); high expression of *miR-125b* in pancreatic cancer accelerates cancer progression and metastasis (11), and *miR-139* promotes prostate cancer progression by acting on *RIG-1* (12). Currently, the disordered expression of multiple miRNAs like *miR-376a* (13), *miR-892a* (14), *miR-29a-3p* (15), *miR-145-5p* (16) and others are involved in the development of LC. Also, *miR-106a-5p* is involved in the development of a variety of cancers and has anti- or promote roles in different cancers. In hepatocellular carcinoma (HCC), the *FER1L4-miR-106a-5p* regulatory axis was found to promote drug sensitivity in HCC cells (17); mesenchymal stem cell-derived *miR-106a-5p* promoted cancer growth via exosomes delivery to breast cancer cells (18), and *miR-106a-5p* expression promoted proliferation and invasion of ovarian cancer cells (19). However, the specific role of *miR-106a-5p* in LC remains unclear.

We found that the level of *miR-106a-5p* was considerably elevated in LC tissues compared to adjacent tissues. However, the exact mechanism remains unclear. We conducted a preliminary work using commercial cell lines in vitro. This study is an initial validation of *miR-106a-5p,* and provides a theory to reveal the mechanism of LC carcinogenesis and progression.

2. Objectives

To demonstrate the significance and specific mechanisms of *miR-106a-5p* in the development and metastasis of LC through clinical sample testing and cell biology approaches.

3. Materials and Methods

3.1. Clinical Specimens

The LC tissues and paired normal adjacent tissues (>0.5 cm from the safe border of the tumor) were collected from 30 patients who underwent surgery from Jan 2019 to Oct 2020. All patients had not received any treatments before sampling (details presenting in **supplement Table 1**). All specimens were placed into liquid nitrogen quickly. The study was approved by the ethics committee of First Affiliated Hospital of Jinzhou Medical University (No. 202288), informed consent was obtained from each patient included in the study and the study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki.

3.2. Cell Lines and Culture

Human LC cell line AMC-HN8, TU212 (AC338377, HTX2130) was purchased from the Shanghai Cell Bank, Chinese Academy of Sciences. The cells were cultured in DMEM medium (Gibco, USA) + 10% fetal bovine serum (FBS, Gibco, USA) and incubated at 37 °C and 5% CO_2.

3.3. Cell Transfection

Logarithmically grown cancer cells were digested with 0.5% trypsin and inoculated into 6-well plates (2-3×105 cell), then *miR-106a-5p* sponge virus (Hanheng, Shanghai; 5’-CUACCUGCACUGUAAGCACUUUU-3’)or *miR-106a-5p* mimic (Hanheng, Shanghai, 5’-UCUA CUCUUUCUAGGAGGUUGUGA-3’) and liposome transfection reagent (Polyplus, France) were used in serum-free medium. The stable infections were screened by using puromycin and then detected by RT-PCR.

3.4. Quantitative Reverse Transcription-Polymerase Chain Reaction (qRT-PCR)

Tissues or cells were washed with PBS and then lysized (1 mL Buffer RLM). RNA was then extracted using an RNA extraction kit (TaKaRa, Japan) and *miR-106a-5p* was synthesized using the Takara One Step PrimeScript miRNA cDNA Synthesis Kit (TaKaRa, Japan). Real-time PCR (ABI) was applied to amplify *miR-106a-5p*, *U6* was used as the internal reference, and the 2-ΔΔCT values were used to represent the fold difference of *miR-106a-5p* in the experimental group relative to each group.

3.5. Dual Luciferase Reporting Assay

AMC-HN8 (TU212) cells were incubated in 24-well

plates for 24 hours. The pGL3-*AKT*IP-3'-UTR wt and pGL3-*AKT*IP-3'-UTR mutant reporter plasmids were constructed in advance. AMC-HN8 and TU212 cells were mixed with *miR-106a-5p* mimics or *miR-106a-5p* inhibitors with 0.1 μg reporter plasmids and transiently transfected using Lipofectamine 2000 according to the manufacturer's instructions. 48 h later, the dual luciferase reporter gene assay system (Promega, USA) was used to detect luciferase activity and recorded using a 96-microplate reader (Promega, USA).

3.6. Western Blot

The treated cells were fully lysed to extract proteins and prepare suspensions, 10% SDS-PAGE were prepared, electrophoresed at 40V for 4-5h, then transferred to nitrocellulose membranes and blocked, antibody incubation was performed, and finally visualized and image analysis was carried out. *PI3K*/p-*PI3K* (Abcam, ab154598, UK), *AKT*/p-*AKT* (Abcam, ab38449, UK), *mTOR/p-mTOR* (Abcam, ab134903, UK), *Bcl-2* (Invitrogen, PA5-11379, USA), *Bax* (Invitrogen, 14-6997-82, USA), *GAPDH* (Invitrogen, 39-8600, USA).

3.7. Colony Formation Assay

The treated AMC-HN8 and TU212 LC cells were prepared into single-cell suspensions. The cell suspensions of each group were inoculated in 6-well culture plates (400 cells/well) and incubated at 5% CO_2 and 37 °C for 48h. The cells were washed twice with PBS, fixed with 4% paraformaldehyde liquid for 20min then discarded, and an appropriate amount of Jimson's staining solution was added to them and stained for 30min, washed, and dried at room temperature. Microscopically observe the number of cell clones.

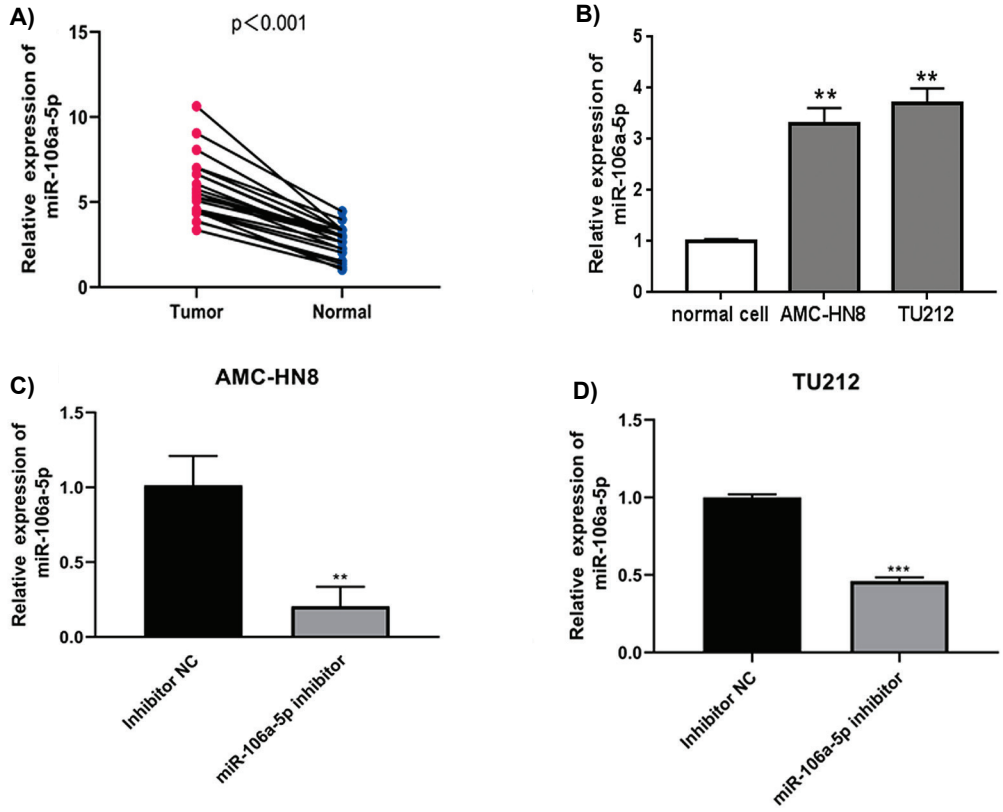

Figure 1. *MiR-106a-5p* is overexpressed in LC tissues and cell lines. A) Expression of *miR-106a-5p* in tissues (n=30). **B)** Expression of *miR-106a-5p* in cell lines, AMC-HN8 and TU212 (n=3). **(C, D)** Inhibition of miR-106a-5p by inhibitor, mimic as negative control. Mean ± SEM, ANOVA, **, p<0.01; ***, p<0.001.

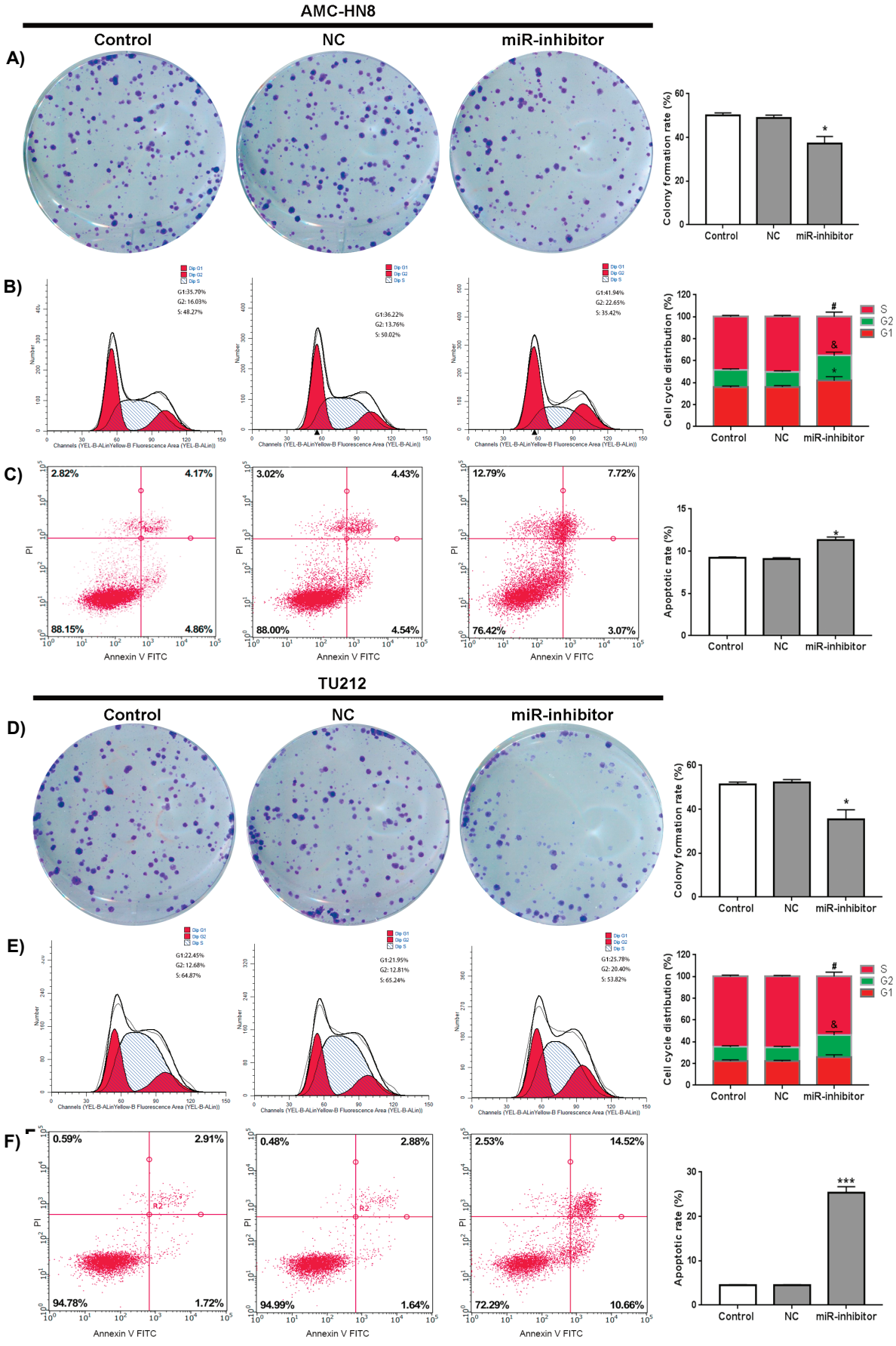

Figure 2. Legend continued on next page.

3.8. Cytometry Analysis

Cell cycle: Cells were harvested and washed with PBS. 5mL or more of pre-cooled 70-80% ethanol was added drop by drop, mixed the cells overnight at 4 °C (>18 hours). The cells were Centrifuged at 1000-1500 rpm for 10 minutes and discard the supernatant. Cell I/RNase staining: Cells are resuspended in 0.5 mL PI/RNase (Invitrogen, USA) staining solution and incubated for 15 minutes at room temperature and avoiding light. The samples were stored at 4°C and protected from light before analysis and tested by flow cytometry within 1 hour.

Apoptotic: The cells were collected after digestion by EDTA-free trypsin (Life, USA, trypsin digestion time should not be too long) and then washed twice with PBS (2000 rpm, 5 min), 1-5×10^5 cells were prepared; 500 μL of Binding Buffer was added to resuspend the cells; 5 μL of Annexin V-FITC (Life, USA,) was added and mixed, then 5 μL of PI (Life, USA,) was added and mixed; the reaction was carried out for 5-15 min at 25 °C and protected from light; the cells were observed and detected by flow cytometry within 1 hour.

3.9. Wound Healing Assay

The LC cells were planted into 6-well plates at concentration of 2×10^6, cells were cultured with DMEM medium containing 10% FBS. When the cells spread across the bottom of wells, a 200ul pipettor was taken and scratched across the diameter of each well of the 6-well plate, washed 3 times with PBS, and recorded under the microscope. Serum-free medium added and keep culture. 8h or 24h after, the scratch distance was observed and measured. Calculate the migration rate respectively. Image results were taken with the microscope at 10x.

3.10. Transwell Assay

LC cells were digested with trypsin, and then the single-cell suspension was prepared. 2 × 10^4 LC cells were added to the upper compartment, and the lower compartment added culture medium containing 10% FBS and HGF (20 ug.mL^{-1}) (GlpBio, USA). Under the incubation condition of 37 °C and 5% CO_2 ,the compartment was put into the incubator for 24 h. The LC cells of each group were fixed with 1 mL 4% formaldehyde for 10 min then washed, then stained with 1 mL 0.1% crystal violet solution for 30 min, and then washed with PBS. Count under a microscope after drying (20x).

3.11. Statistical Analysis

All statistical analyses were carried out using SPSS (13.0). Statistical analyses were performed using the One-way ANOVA. Data were showed with mean + SEM. P-values less than 0.05 were considered to be statistically different.

4. Results

4.1. MiR-106a-5p is Over-Expressed in LC Tissues and Cell Lines

To investigate the role of *miR-106a-5p* in the development of LC, we first examined the expression of *miR-106a-5p* using clinical tissues (in paired cancer and adjacent tissues). RNA was extracted from the tissues and cDNA was obtained by reverse transcribed. Using qRT-PCR experiments we found that *miR-106a-5p* expression was significantly higher in LC tissues (5.71±1.65 foldchange) than in adjacent cancer tissues (2.66±0.87 foldchange), $p<0.001$ (**Fig. 1A**). We also examined LC cell lines, AMC-HN8 and TU212, for the expression of *miR-106a-5p*. The results showed that the expression of *miR-106a-5p* was notably higher in LC cell lines compared to normal cells (**Fig. 1B**), consistent with the clinical tissue test findings. To investigate whether the high expression of *miR-106a-5p* promotes the development of LC, *miR-106a-5p* was knockdown by *miR-106a-5p* inhibitor transfection in AMC-HN8 and TU212 cells. PCR results showed that intracellular *miR-106a-5p* was accurately targeted and reduced the *miR-106a-5p* expression (**Fig. 1C, D**).

Figure 2. The proliferative ability of *miR-106a-5p* in cancer cells. The *miR-106a-5p* inhibitor was transfected into cancer cells, empty vector as NC, then the cell proliferation **(A, D)**, cell cycle **(B, E)** and apoptotic **(C, F)** were analyzed by colony formation and flow cytometry analysis. Mean ± SEM, n=3, ANOVA, *, $p<0.05$.

The above results tentatively showed that *miR-106a-5p* expression was significantly increased in LC tissues and cells, but the exact role of *miR-106a-5p* needs further investigation.

4.2. MiR-106a-5p Promote LC Cells Proliferation In Vitro

We first studied the effect of *miR-106a-5p* on the proliferation of LC cells. By clonogenesis assay we found that after *miR-106a-5p* was inhibited, the number of clonogenesis of LC cells was significantly decreased compared to control and NC groups (**Fig. 2A, D**), indicating that the growth of LC cells was inhibited. Clonogenesis rates of AMC-HN8 cells were: 49.02±0.63 % (control), 48.83±1.53 % (NC), 37.17±0.76 % (miR-inhibitor), respectively. The clonogenic rates of TU212 cells were: 50.96±2.56 % (control), 52.33±2.36 % (NC), and 35.50±2.65 % (miR-inhibitor), respectively. In addition, we further analyzed the cell cycle distribution and apoptosis of cancer cells after *miR-106a-5p* inhibition using cell flow cytometry assays.

The results show that AMC-HN8 and TU212 cells transfected with *miR-106a-5p* inhibitor presenting a significant decrease in S-phase cells and a significant increase in G1-phase cells (**Fig. 2B, E**), indicating that the transition from G1 to S-phase of tumor cells was blocked after *miR-106a-5p* inhibition. The apoptosis assay revealed that the apoptosis rate of cancer cells after *miR-106a-5p* inhibition was significantly higher than that of the control and NC groups (**Fig. 2C, F**). The apoptosis rates of AMC-HN8 cells were: 9.19±0.21 % (control), 9.08±0.14 % (NC), 10.76±0.6 % (miR-inhibitor); the apoptosis rates of TU212 cells were: 4.4±0.29 % (control), 4.5±0.12 % (NC) and 25.35±0.17 % (miR-inhibitor), respectively. These results indicated that *miR-106a-5p* inhibition could prevent cancer cells from progressing from the G1 phase to the S phase, which in turn led to blocked cell proliferation and increased apoptosis, suggesting that *miR-106a-5p* could promote the growth and development of LC.

4.3. MiR-106a-5p Facilitate LC Cells Migration and Invasion

Next, we investigated the role of *miR-106a-5p* in the migration and invasion of LC cells by wound healing and Transwell assays. In AMC-HN8 cells, we found that the migration rate of cancer cells was significantly reduced by transfection with *miR-106a-5p* inhibitor (**Fig. 3A**), and the cell migration rates in control, NC, and miR-inhibitor groups were 64.10±5.94 %, 71.43±8.45 %, and 38.96±5.87 %, respectively. The results of the Transwell assay showed that the invasive ability of the cells was significantly decreased after *miR-106a-5p* inhibition comparing to control and NC (**Fig. 3B**). We have observed the same results in TU212 cells, where cells transfected with miR-inhibitor had significantly lower migratory and invasive abilities than both control and NC groups (**Fig. 4A, B**). These results suggest that *miR-106a-5p* plays a role in promoting cell metastasis and invasion in LC cells.

4.4 MiR-106a-5p Activate PI3K/AKT/mTOR Pathway Through AKTIP

Further, the specific mechanism of miR-106-5p in LC was studied. By *TargetScan*, we found that the *AKT* interacting protein (*AKT*IP) was one of the specific targets of miR-106-5p. *In vitro*, the expression of *AKT*IP in LC cells was dramatically decreased after miRNA inhibitor addition in culture medium (**Fig. 5A**). To verify the specificity of this interaction, sequence mutations were modified in the miRNA binding region (**Fig. 5B**, GCACUUU) and a dual luciferase reporting assay was performed (**Fig. 5C**). The figures show that the luciferase activity was decreased in the wt group rather than the mut group demonstrating that the specific interaction between miR-106-5p and *AKT*IP mRNA. *AKT*IP (encode by FT1/FTS) plays a role in cell apoptotic and activates the protein kinase/*AKT* pathway by enhancing the phosphorylation state. As shown in **Figure. 5D**, the phosphorylated *PI3K*/*AKT*/mTOR pathway was decreased when miR-106-5p was knockdown, and meanwhile, the *Bcl-2* was declined and *Bax* was up-regulated, indicating that the cell was apoptotic.

5. Discussion

It has been widely documented that miRNA play an important role in cancer progression (10). *MiR-106a-5p*, a member of the *miR-17* family, is abnormally expressed in several tumors and plays a pro- or anti-cancer role (20). However, the contribution of *miR-106a-5p* in LC has not been described. In the present study, we found that *miR-106a-5p* was generally overexpressed in LC clinical tissues and commercial cell lines, as well as that there was a significant reduction in both cell proliferation and migration capacity and an increase in apoptosis after inhibition of *miR-106a-5p* expression in LC cells.

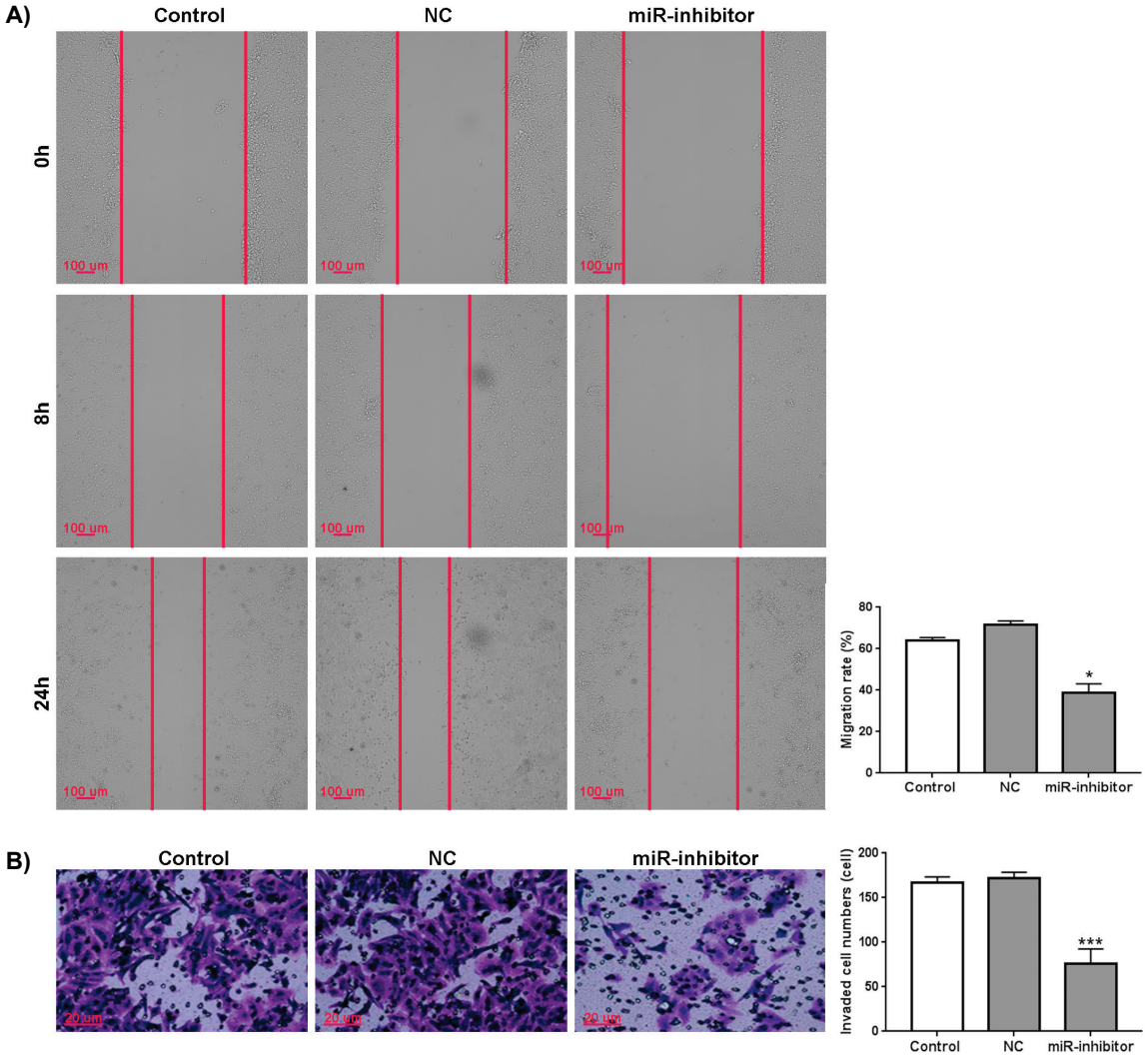

Figure 3. *MiR-106a-5p* facilitates AMC-HN8 cells migration and invasion. AMC-HN8 cells were transfected with *miR-106a-5p* inhibitor, empty vector as NC, then cell's migration (0h, 8h and 24h) **A)** and invasion capacity **B)** were measured with wound healing and Transwell assays. Mean ± SEM, n=3, ANOVA, *, $p<0.05$; ***, $p<0.001$.

Mechanismly, *AKT*IP was down-regulated and *PI3K*/*AKT*/mTOR pathway was inactivated by *miR-106a-5p* inhibition, demonstrating that the miR-106-5p-*AKT*IP-*PI3K*/*AKT*/mTOR axis mediates LC development.

Many miRNAs are involved in LC modulation. Recently, it was reported that *miR-892a* was found to be elevated in LC tissues and that inhibition of its expression significantly reduced cell proliferation (14). CeRNA (competing endogenous RNAs) are thought to be a new mechanism by which miRNAs function. *LncNEAT1* (Long noncoding RNA NEAT1), a novel oncogene in LC, competitively binding to *miR-29a-3p*, thereby inhibits the regulation of downstream targets by miRNAs (15). Previous study shows that *miR-106a-5p* increases the sensitivity of head and neck cancers to radiotherapy by directly acting on *RUNX3*, thus exhibiting an anti-cancer efficacy (21). Furthermore, in a recent study in nasopharyngeal carcinoma, the anti-cancer potency of *miR-106a-5p* was also found. The *LncRNA SMAD5-AS1* was act as a sponge on *miR-106a-5p* to promote the epithelial-mesenchymal transition (EMT) of cancer cells and

accelerate cancer metastasis, but abrogated by over-expression of *miR-106a-5p* (22). In another study it was similarly demonstrated that *miR-106a-5p* was at a low level in kidney cancer tissues and cells, however, their experiments revealed that *miR-106a-5p* exhibited anti-oncogenic effects through the regulation of *VEGFA* (23). However, what we found in LC was quite the contrary. We found that *miR-106a-5p* was significantly more abundant in LC tissues than adjacent tissues in clinical specimens. We then constructed LC cell lines that inhibited *miR-106a-5p* expression in AMC-HN8 and TU212 cells, and found that the clonogenic ability of LC cells was significantly decreased after *miR-106a-5p* inhibition. Flow cytometric analysis showed that most of the cells stopped at the G1 phase, and apoptosis increased when cell proliferation decreased. The migration and invasion ability of LC cells were then examined by wound healing assay and Transwell assay. The results showed that the migration rate and invasion ability of AMC-HN8 and TU212 cells were reduced with different degrees after *miR-106a-5p* inhibition. Therefore, our results demonstrated that *miR-106a-5p*

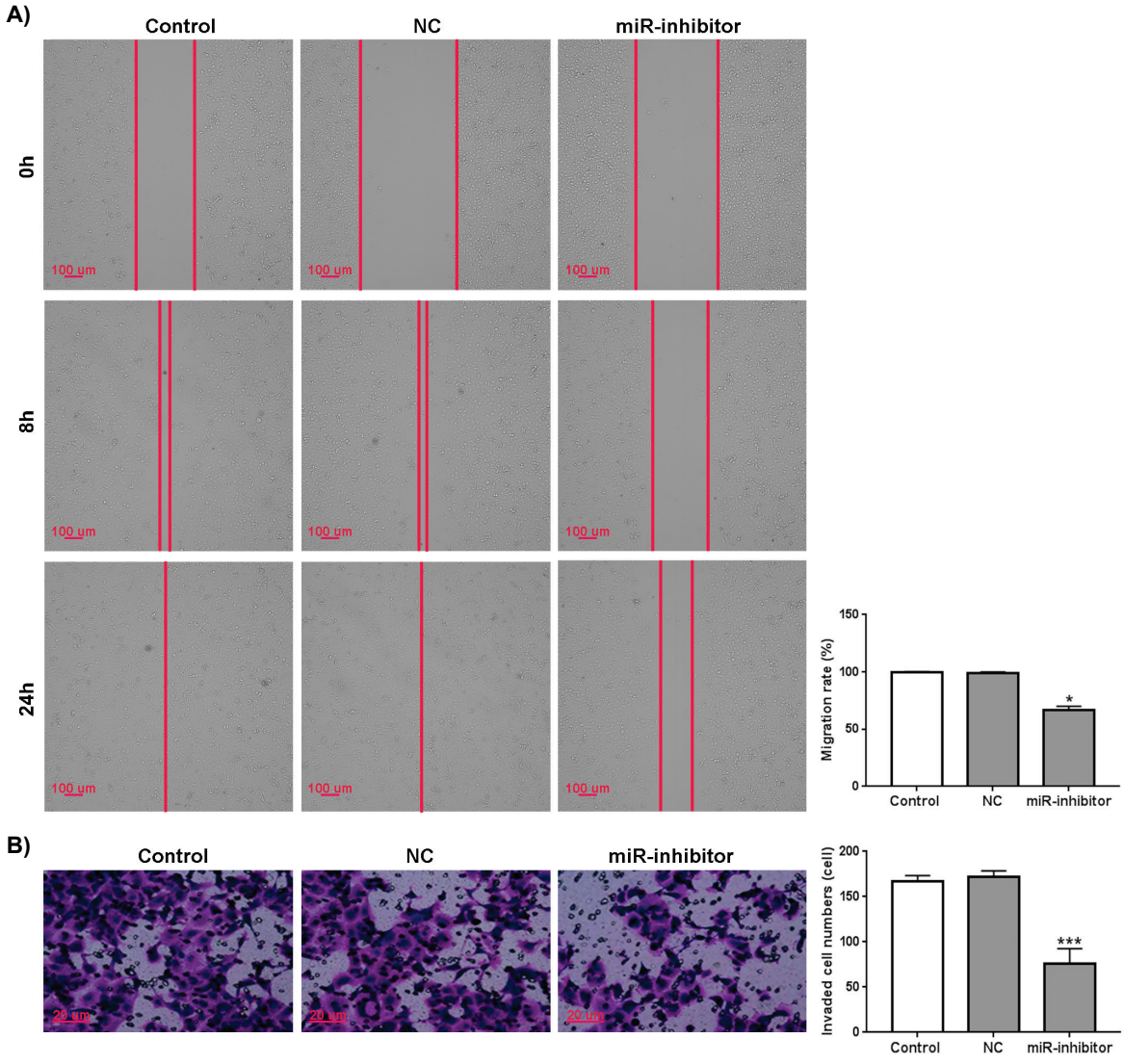

Figure 4. *MiR-106a-5p* facilitates TU212 cells migration and invasion. TU212 cells were transfected with *miR-106a-5p* inhibitor, empty vector as NC, then cell's migration (0h, 8h and 24h) **A)** and invasion capacity **B)** were measured with wound healing and Transwell assays. Mean ± SEM, n=3, ANOVA, *, $p<0.05$; ***, $p<0.001$.

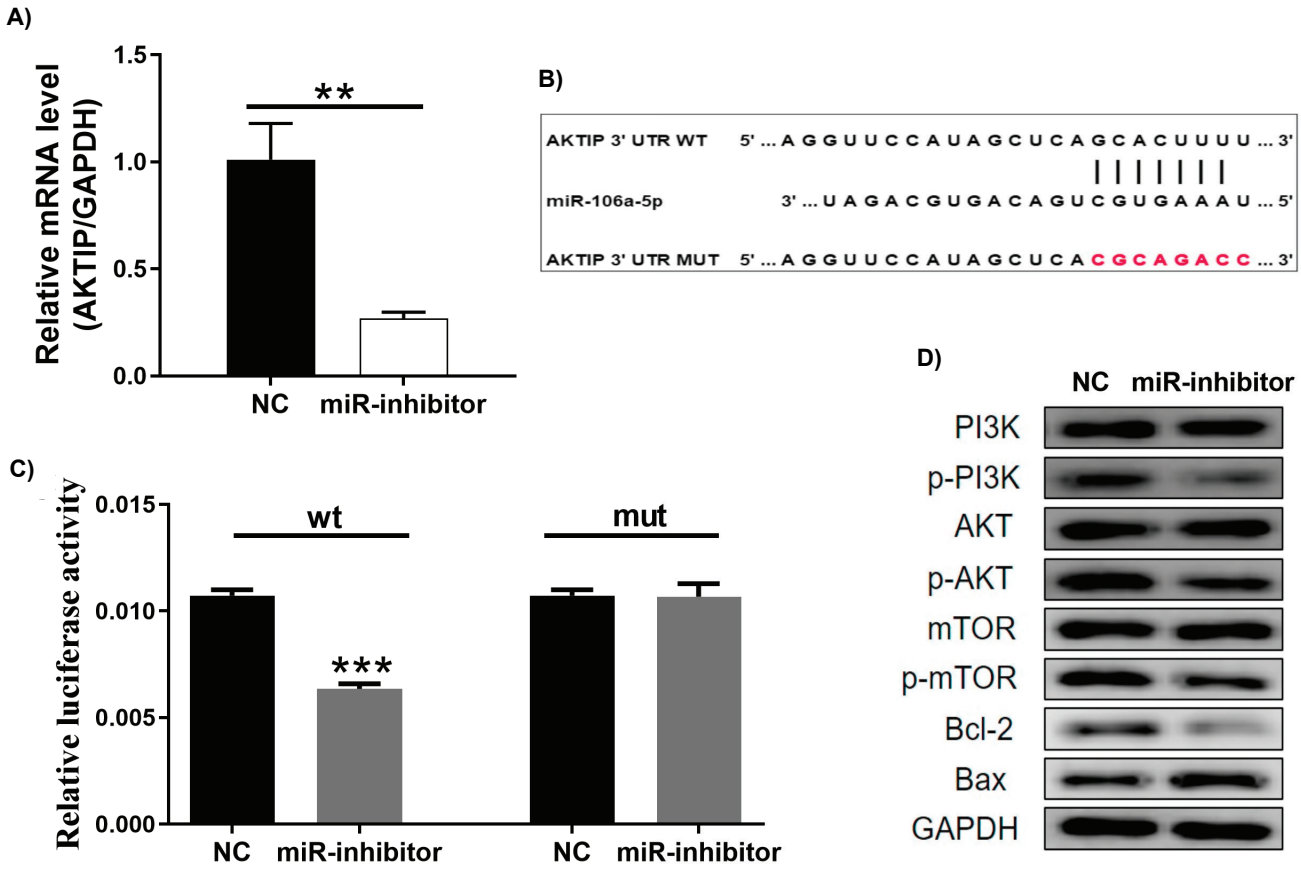

Figure 5. ***MiR-106a-5p*** **activate** ***PI3K/AKT/mTOR*** **pathway through** ***AKTIP*****. A)** Relative *AKTIP* expression. **B)** Binding region of *miR-106a-5p* and *AKTIP.* **C)** Dual luciferase reporter assay. **D)** Detection of *PI3K/AKT/m-TOR* pathway and cell proliferation. Mean ± SEM, n=3, ANOVA, **, p<0.01; ***, p<0.001.

promote the development of LC. Most recently, and consistent with our results, Li (*J Cell Mol Med. 2021 Oct;25(19):9183-9198*) found that serum levels of exosomal *miR-106a-5p* were higher in chemotherapy-resistant and final-cycle nasopharyngeal carcinoma patients than in non-resistant and first-cycle patients, and that exosomal *miR-106a-5p* enhanced the proliferation of nasopharyngeal carcinoma cells. As we know, the mechanism of miRNAs action is to target specific genes and regulate downstream genes expression and to signal pathway responses. Based on *Targetscan* databases, *AKT*IP was found and selected for mechanism study. *AKT*IP associated with telomerase complex and mediate cell growth and programmed death (24). Here, we have proved that *AKT*IP was down-regulated by *miR-106a-5p* inhibition, and their interaction have been verified by dual luciferase reporting assay. In previous reports, *AKT*IP has been proved promotes cell's proliferation and cell connection through *PI3K*/*AKT*/mTOR signaling pathway (25). To determine whether the promotion of LC by miR-106a-5p was mediated by the *PI3K*/*AKT*/*mTOR* pathway, we examined the activation status of this pathway using WB assays after *miR-106a-5p* inhibition and found that the levels of p-*PI3K*, p-*AKT*, and *p-mTOR* were significantly reduced, and the expression of *Bcl-2*, a protein associated with cell proliferation, was also reduced; In contrast, the expression of the apoptotic protein *Bax* was increased. These results confirm our suspicions about the mechanism of *miR-106a-5p* in LC development.

The pro-cancer role of *PI3K*/*AKT*/*mTOR* signal pathway

was fully studied as one of the major cellular signaling pathways. The *PI3K*/*AKT*/*mTOR* pathway regulates cell growth, metabolism and motility. Genetic members of this pathway have been extensively studied and found to be frequently activated in human cancers (26). Recently, Wang *et al.*, recently, have found that *miR-214* regulates the activation of *PI3K*/*AKT*/*mTOR* pathway in cervical cancer (27). In another hand, m (6). A modification also mediated the enhanced phosphorylation in *PI3K*/*AKT*/*mTOR* pathway (28). Inhibition of the *PI3K*/*AKT*/*mTOR* pathway has been shown to benefit human tumors regression and has been validated in numerous clinical trials (29). Meanwhile, a number of molecular inhibitors specifically targeting this pathway, such as Idelalisib, Arqule, Everolimus, etc. were approved by the Food and Drug Administration after demonstrating excellent performance and safety in clinical trials (30). In this study, we identified an important role of *miR-106a-5p-AKT*IP-*PI3K*/*AKT*/*mTOR* pathway axis in the development of LC for the first time, providing a theoretical basis for personalized clinical treatment and the development of new molecular inhibitors in LC.

6. Conclusion

In this study, we found that the level of *miR-106a-5p* was elevated in LC tissue than normal, further experiments were conducted using cell lines, and we demonstrated that the expression of *miR-106a-5p* was significantly raised in LC cell lines. Moreover, we found that miR-106-5p mediated the activation of *PI3K*/*AKT*/*mTOR* pathway by interacting with *AKT*IP, thus promoting the growth and invasion of LC. This study is a first step in the study of *miR-106a-5p* in LC carcinogenesis and development. The specific mechanism of *miR-106a-5p* in LC needs to be further explored.

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Conflicts of Interest

All authors have completed the *ICMJE* uniform disclosure form. The authors have no conflicts of interest to declare.

Ethical Statement

The study was approved by the ethics committee of First Affiliated Hospital of Jinzhou Medical University (No. 202288); informed consent was obtained from each patient included in the study and the study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki.

Reference

1. Miller KD, Goding Sauer A, Ortiz AP, Fedewa SA, Pinheiro PS, Tortolero-Luna G, *et al*. Cancer Statistics for Hispanics/Latinos, 2018. CA: *Cancer J Clin*. 2018;**68**(6):425-445.doi: 10.3322/caac.21494
2. Lou ZC, Li X. Leukoplakia or LPR: The Misdiagnosis of Laryngeal Tuberculosis. *Ear Nose Throat j*. 2019:14556131 9891264.doi:10.1177/0145561319891264
3. Huang WT. (Female laryngocarcinoma--clinical analysis of 267 cases). Zhonghua zhong liu za zhi (Chinese journal of oncology). 1990;**12**(3):219-221
4. Zhang SY, Lu ZM, Luo XN, Chen LS, Ge PJ, Song XH, *et al*. Retrospective analysis of prognostic factors in 205 patients with laryngeal squamous cell carcinoma who underwent surgical treatment. *PloS One*. 2013;**8**(4):e60157. doi:10.1371/journal.pone.0060157
5. Lampri ES, Chondrogiannis G, Ioachim E, Varouktsi A, Mitselou A, Galani A, *et al*. Biomarkers of head and neck cancer, tools or a gordian knot? *Int J Clin Exp Med*. 2015; **8**(7):10340-10357
6. Lu TX, Rothenberg ME. MicroRNA. *J Allergy Clin Immunol*. 2018;**141**(4):1202-1207. doi:10.1016/j.jaci. 2017. 08.034
7. Bayraktar R, Van Roosbroeck K, Calin GA. Cell-to-cell communication: microRNAs as hormones. *Mol Oncol*. 2017; **11**(12):1673-1686. doi: 10.1002/1878-0261.1244
8. Mziaut H, Henniger G, Ganss K, Hempel S, Wolk S, McChord J, *et al*. MiR-132 controls pancreatic beta cell proliferation and survival through Pten/Akt/Foxo3 signaling. *Mol Metabol*. 2020;**31**:150-162. doi:10.1016/j.molmet.2019.11.012
9. Ma L. MicroRNA and Metastasis. *Adv Cancer Res*. 2016; **132**:165-207. doi:10.1016/bs.acr.2016.07.004
10. Bach DH, Hong JY, Park HJ, Lee SK. The role of exosomes and miRNAs in drug-resistance of cancer cells. *Int J Cancer*. 2017;**141**(2):220-230. doi:10.1002/ijc.30669
11. Chen HA, Li CC, Lin YJ, Wang TF, Chen MC, Su YH, *et al*. Hsa-miR-107 regulates chemosensitivity and inhibits tumor growth in hepatocellular carcinoma cells. *Aging*. 2021;**13**. doi: 10.18632/aging.202908
12. Wang P, Zheng D, Qi H, Gao Q. Thioredoxin-interacting protein is a favored target of miR-125b, promoting metastasis

and progression of pancreatic cancer via the HIF1alpha pathway. *J Biochem Mol Toxicol* 2021:e22782. doi:10.1002/jbt.22782

13. Nam RK, Benatar T, Amemiya Y, Seth A. MiR-139 Induces an Interferon-beta Response in Prostate Cancer Cells by Binding to RIG-1. *Cancer genomics & proteomics*. 2021;**18**(3):197-206. doi:10.21873/cgp.20252
14. Feng B, Chen K, Zhang W, Zheng Q, He Y. circPGAM1 enhances autophagy signaling during laryngocarcinoma drug resistance by regulating miR-376a. *Biochem Biophys Res Commun*. 2021;**534**:966-972. doi:10.1016/j.bbrc.2020.10.063
15. Dong J, Wang J, Shan C, Zhang H, Xu O. MicroRNA-892a regulates laryngocarcinoma cell proliferation via Dicer. *Exp Biol Med*. 2020;**245**(14):1222-1232.doi:10.1177/1535370220934036
16. Liu T, Wang W, Xu YC, Li ZW, Zhou J. Long noncoding RNA NEAT1 functions as an oncogene in human laryngocarcinoma by targeting miR-29a-3p. *Eur Rev Med Pharmacol Sci*. 2019;**23**(14):6234-6241. doi:10.26355/eurrev_201907_18442
17. Zhuang S, Liu F, Wu P. Upregulation of long noncoding RNA TUG1 contributes to the development of laryngocarcinoma by targeting miR-145-5p/ROCK1 axis. *J cell biochem*. 2019; **120**(8):13392-13402. doi:10.1002/jcb.28614
18. Wang X, Chen Y, Dong K, Ma Y, Jin Q, Yin S, *et al*. Effects of FER1L4-miR-106a-5p/miR-372-5p-E2F1 regulatory axis on drug resistance in liver cancer chemotherapy. *Mol Ther Nucleic Acids*. 2021;**24**:449-461. doi:10.1016/j.omtn.2021.02.006
19. Xing L, Tang X, Wu K, Huang X, Yi Y, Huan J. LncRNA HAND2-AS1 suppressed the growth of triple negative breast cancer via reducing secretion of MSCs derived exosomal miR-106a-5p. *Aging*. 2020;**13**(1):424-436. doi:10.18632/aging.202148
20. Chao H, Zhang M, Hou H, Zhang Z, Li N. HOTAIRM1 suppresses cell proliferation and invasion in ovarian cancer through facilitating ARHGAP24 expression by sponging miR-106a-5p. *Life Sci*. 2020;**243**:117296. doi:10.1016/j.lfs.2020.117296
21. Pan YJ, Wei LL, Wu XJ, Huo FC, Mou J, Pei DS. MiR-106a-5p inhibits the cell migration and invasion of renal cell carcinoma through targeting PAK5. *Cell Death Dis*. 2017**;8**(10):e3155. doi:10.1038/cddis.2017.561
22. Zhang C, Chen H, Deng Z, Long D, Xu L, Liu Z. DGCR8/miR-106 Axis Enhances Radiosensitivity of Head and Neck Squamous Cell Carcinomas by Downregulating RUNX3. *Front Med*. 2020;**7**:582097. doi:10.3389/fmed.2020.582097
23. Ma J, Wang W, Azhati B, Wang Y, Tusong H. miR-106a-5p Functions as a Tumor Suppressor by Targeting VEGFA in Renal Cell Carcinoma. *Dis Markers*. 2020;**2020**:8837941. doi:10.1155/2020/8837941
24. Burla R, Carcuro M, Raffa GD, Galati A, Raimondo D, Rizzo A, *et al*. AKTIP/Ft1, a New Shelterin-Interacting Factor Required for Telomere Maintenance. *PLos Gen*. 2015;**11**(6):e1005167. doi:10.1371/journal.pgen.1005167
25. Zhang E, Gao B, Yang L, Wu X, Wang Z. Notoginsenoside Ft1 Promotes Fibroblast Proliferation via PI3K/Akt/mTOR Signaling Pathway and Benefits Wound Healing in Genetically Diabetic Mice. *J Pharmacol Exp Ther*. 2016;**356**(2):324-332. doi:10.1124/jpet.115.229369
26. LoRusso PM. Inhibition of the PI3K/AKT/mTOR Pathway in Solid Tumors. *J Clin Oncol*. 2016;**34**(31):3803-3815. doi:10.1200/JCO.2014.59.0018
27. Wang F, Tan WH, Liu W, Jin YX, Dong DD, Zhao XJ, *et al*. Effects of miR-214 on cervical cancer cell proliferation, apoptosis and invasion via modulating PI3K/AKT/mTOR signal pathway. *Eur Rev Med Pharmacol Sci*. 2020;**24**(14):7573. doi:10.26355/eurrev_202007_22242
28. Zhao Q, Zhao Y, Hu W, Zhang Y, Wu X, Lu J, *et al*. m(6)A RNA modification modulates PI3K/Akt/mTOR signal pathway in Gastrointestinal Cancer. *THER*. 2020;**10**(21):9528-9543. doi:10.7150/thno.42971
29. Dienstmann R, Rodon J, Serra V, Tabernero J. Picking the point of inhibition: a comparative review of PI3K/AKT/mTOR pathway inhibitors. *Mol Cancer Ther*. 2014;**13**(5):1021-1031.doi:10.1158/1535-7163.MCT-13-0639
30. Alzahrani AS. PI3K/Akt/mTOR inhibitors in cancer: At the bench and bedside. *Semin Cancer Biol*. 2019;**59**:125-132. doi:10.1016/j.semcancer.2019.07.009