Cancer Management and Research

Dovepress

open access to scientific and medical research

Open Access Full Text Article

ORIGINAL RESEARCH

LncRNA-IUR Sponges miR-24 to Upregulate P53 in Laryngeal Squamous Cell Carcinoma

This article was published in the following Dove Press journal:
Cancer Management and Research

Cen Wei,[1,*] Huaqing Wei,[2,*] Xun Wu,[3] Guangyao Nong,[1] Chenglin Wu[4] Jinli Lee,[5] Ning Meng,[3] Dahai Yu,[6] Jiping Su,[7] Mengzhu Guo,[6] Jiangyuan Qin,[8] Xuemin Fan[3]

[1]ENT & HN Surgery Department, Wuming Hospital of Guangxi Medical University, Nanning 530199, People's Republic of China; [2]Department of Cardiology, Wuming Hospital of Guangxi Medical University, Nanning 530199, People's Republic of China; [3]Department of Maxillofacial Surgery, Guangxi Medical University College of Stomatology, Nanning 530021, People's Republic of China; [4]Department of ENT, Sixth Affiliated Hospital of Guangxi Medical University, Nanning 537000, People's Republic of China; [5]Department of Gastroenterology, 923 Hospital of the Joint Logistics Support Force of the Chinese People's Liberation Army, Nanning 530021, People's Republic of China; [6]Department of Stomatology, The First Affiliated Hospital of Guangxi Medical University, Nanning 530021, People's Republic of China; [7]ENT & HN Surgery Department, The First Affiliated Hospital of Guangxi Medical University, Nanning 530199, People's Republic of China; [8]Department of Otolaryngology, Guangxi General Hospital of Chinses People's Armed Police Force, Nanning 530000, People's Republic of China

*These authors contributed equally to this work

Correspondence: Xun Wu
Department of Maxillofacial Surgery, Guangxi Medical University College of Stomatology, No. 10 Shuangyong Road, Nanning City, Guangxi Province 530021, People's Republic of China
Email kc4704@163.com

Guangyao Nong
ENT & HN Surgery Department, Wuming Hospital of Guangxi Medical University, No. 26 Yongning Road Wuming District, Nanning City, Guangxi Province 530199, People's Republic of China
Tel +86 771-6023602
Fax +86 771-6213919
Email lmqvqaymlhn43@163.com

Objective: The functions of lncRNA-IUR in laryngeal squamous cell carcinoma (LSCC) were investigated in this study.

Methods: RT-qPCR and paired *t*-test were used to measure and compare expression levels of IUR, miR-24 and p53 in LSCC and non-tumor tissues. Human LSCC cell line UM-SCC -17A was used and transfected by pcDNA3.1 vector to overexpress IUR and miR-24. The transwell assay and wound healing assay illustrated the effect of overexpression of IUR or miR-24 in the cell invasion and migration of LSCC. Subcutaneous tumor model in nude mice was carried out to demonstrate the mechanism between IUR and miR-24 in regulating tumor growth.

Results: We found that IUR was downregulated in LSCC. Low expression levels of IUR were correlated with the poor survival of LSCC patients. Overexpression experiments showed that overexpression of IUR led to increased, while overexpression of miR-24 led to decreased expression levels of p53 in LSCC cells. And bioinformatics analysis showed that IUR may sponge miR-24. Cell proliferation assay showed that overexpression of IUR and p53 led to decreased proliferation rate of LSCC cells, while overexpression of miR-24 led to increased proliferation rate of LSCC cells. We also illustrated that overexpression of IUR promoted cell migration and invasion while miR-24 had opposite effects. In addition, subcutaneous tumor model in nude mice showed that overexpression of miR-24 attenuated the effects of overexpression of IUR on the expression of p53 and cancer cell proliferation.

Conclusion: IUR sponges miR-24 to upregulate p53 in LSCC, thereby inhibiting cancer cell proliferation.

Keywords: laryngeal squamous cell carcinoma, IUR, miR-24, p53

Introduction

Laryngeal cancer is the most common type of head and neck malignancy and the second most common type of respiratory system cancer.[1] Laryngeal cancer accounts for 1–5% of all cancer cases across different regions of the world.[2,3] In China, laryngeal cancer affects 1.55 out of 100,000 people with about 8-time higher incidence observed in males than in females.[2–4] Laryngeal squamous cell carcinoma (LSCC) is the most common subtype of laryngeal cancer. With the development of novel therapeutic strategies, such as surgical resection of the primary tumors, and the development of different chemotherapies, the treatment outcomes of LSCC have been significantly improved during the past decades.[5] However, postoperative recurrence is common and long-term survival is still poor.[6]

With the increased understanding of the molecular pathogenesis of LSCC, more and more genetic factors have been identified to play critical roles in the

development and pathogenesis of this disease.[7,8] The identified genetic players provided novel insights into the development of targeted therapies.[9] Tumor protein p53 is a well-characterized tumor suppressor that plays critical roles in cancer biology by regulating the expression of multiple downstream genes to prevent the occurrence of mutations of the genome.[10] In effect, some miRNAs, such as miR-24, can target p53 and promote cancer development.[11] Recent studies of lncRNA cDNA microarray identified IUR as a tumor suppressor in leukemia,[12] while its roles in other human cancers are unknown. Many studies have shown that miRNAs and long (>200 nt) non coding RNAs (lncRNAs) are important players in LSCC. In this study, we found that IUR can potentially form base pairing with miR-24. This study was carried out to explore the interactions between miR-24 and IUR in LSCC.

Materials and Methods

Tissue Sample Collection

A total of 60 fresh LSCC and paired non-tumor tissues were collected from 60 patients with LSCC (38 males and 22 females, 44 to 68 years old, mean age 56.1 ± 7.0 years old). These patients were admitted to Guangxi Medical University College of Stomatology, Wuming Hospital of Guangxi Medical University between March 2012 and March 2014. This study was approved by the Ethics Committee of the aforementioned hospital. All the patients were newly diagnosed LSCC patients and no recurrent LSCC patients were enrolled. Patients with initiated therapies before admission and patients who were suffering from multiple clinical disorders were excluded. All tissue samples were immediately frozen in liquid nitrogen and stored in a liquid nitrogen sink before the following experiments. All patients were informed of the design of this project and they all signed the informed consent. LSCC staging system was used to stage the 60 patients. The results revealed 12, 15, 18 and 15 cases at stages I–IV, respectively.

Treatment and Follow-Up

The 60 patients were staged according to AJCC staging system. The 60 patients included 10, 18, 17 and 15 cases at clinical stage I, II, III and IV, respectively. Treatment approaches, such as surgical resection, chemotherapy, radiation therapy and targeted therapy were determined based on clinical stages and patients' health conditions. All patients were followed up for 5 years or until their deaths. Follow-up was performed in a monthly manner through telephone.

LSCC Cells and Transient Transfections

Human LSCC cell line UM-SCC-17A (MilliporeSigma, USA) was used. Cell culture medium was composed of 10% FBS and 90% DMEM and cell culture conditions were 95% humidity, 5% CO_2 and 37°C. Vectors expressing IUR and p53 were constructed using pcDNA3.1 vector (Invitrogen) as backbone. Negative control (NC) miRNA and miR-24 mimic were purchased from Sigma-Aldrich. UM-SCC-17A cells were harvested at 75–85% confluence and counted, followed by transfecting 10 nM vector or 50 nM miRNA into 10^6 cells using lipofectamine 3000 (Sigma-Aldrich). Untransfected cells were used as the control (C) cells. Cells transfected with empty vector or NC miRNA were used as NC cells. All following experiments were performed using cells harvested at 48 h post-transfection.

Dual Luciferase Reporter Assay

IUR vector was constructed using pGL4.23 luciferase vector (Promega) as backbone. A total of 10^6 cells were transfected with IUR expression vector + NC miRNA or IUR expression vector + miR-24 mimic. Cells were harvested at 48 h post-transfection and Dual-Luciferase® Reporter Assay System (Promega Corporation) was used to measure luciferase activity.

RNA Extractions and RT-qPCR

HigherPurity™ Total RNA Extraction Kit (Canvax Biotech) was used to extract total RNAs from tissue samples and UM-SCC-17A cells. All RNA samples were digested with LookOut® DNA Erase (Sigma-Aldrich). RNA concentrations were measured by NanoDrop 2000c Spectrophotometer (Thermo Scientific). Tetro Reverse Transcriptase (Bioline) was used to reverse transcribe total RNAs into cDNA, followed by preparation of qPCR mixtures using TaqMan probes and performed following the instructions from TaqMan™ MicroRNA Assay (Thermo Fisher Scientific). GAPDH was used as the endogenous control to measure the expression levels of IUR and p53 mRNA.

High Pure miRNA Isolation Kit (Sigma-Aldrich) was used to extract miRNAs from aforementioned tissue samples and cells. Measurement of the expression levels of mature miR-24 was detected using Custom TaqMan™ Small RNA Assay (Thermo Fisher Scientific). The endogenous control of miR-24 was U6. Fold changes of gene

expression were calculated using $2^{-\Delta\Delta CT}$ method. All PCR reactions were repeated 3 times.

Cell Proliferation Assay

CCK-8 assay kit was used to measure the proliferation rates of UM-SCC-17A cells harvested at 48 h post-transfection. Each well of a 96-well cell culture plate was filled with 10^4 UM-SCC-17A cells in 0.1 mL cell suspension. Cells were cultivated under aforementioned conditions and CCK-8 solution was added into each well to reach a final concentration of 10% at 4 h before the end of cell culture. Cells were harvested every 24 h until 96h. OD values were measured at 450 nm.

Wound Healing Assay

After 48 h of transfection, cells were inoculated in a 12-well plate and cultured for 12 h. Cells were gently scratched with a pipette head across the center of the well, and then washed with PBS for 3 times. After 24 h of incubation, the cell wound healing process was observed with an inverted fluorescence microscope (Nikon, Japan).

Transwell Assay

In the upper chamber of a transwell plate (8 μ pore size, Corning Incorporated, Corning, NY, USA), 200 μL diluted Matrigel (BD Biosciences) was added and dried overnight. Cells (2×10^4) were inoculated in the upper chamber of the transwell. In the lower chamber of the transwell, 300 μL DMEM medium containing 10% fetal bovine serum was added as a chemokine. After 24 h of incubation at 37°C with 5% CO_2, tune-penetrating cells on transwell were stained with 0.1% crystal violet (Beyotime). The invasion ability was evaluated by the number of cells migrating to the lower layer of the microporous membrane, and 10 fields were randomly selected from each group to take photographs using a microscope (Leica, DM4000B). The migrated cells were counted using ImageJ Software.

Xenograft Tumor Formation

UM-SCC-17A cells stably infected with the miR-24 or IUR or both were harvested and washed by phosphate-buffer saline. Then, 5×10^6 cells were subcutaneously injected into the right flank of BALB/c nude mice (5 per group). Normal UM-SCC-17A cells were used as the control cells. The width and length of tumors were measured every 7 d. Tumor volumes were calculated by the formula: $V = \text{width}^2 \times \text{length}/2$. On day 28 after implantation, mice were sacrificed and the tumor weights were assessed. The animal study was approved by the Animal Ethical Committee of Guangxi Medical University College of Stomatology, Wuming Hospital of Guangxi Medical University and the experimental processes were carried out in accordance with the guidelines for Animal Experiments of the National Cancer Center.

Western Blot Assay

RIPA solution (Beyotime) was used to extract total proteins from UM-SCC-17A cells harvested at 48 h post-transfection and total proteins from subcutaneous tumors. BCA assay kit (Beyotime) was used to measure protein concentrations. All protein samples were denatured by incubating the samples in boiling water for 15 min. After that, electrophoresis was performed using 12% SDS-PAGE gel to separate protein molecules. After that, proteins were transferred to PVDF membranes, followed by blocking in PBS containing 5% non-fat milk for 2 h at room temperature. After that, membranes were incubated with rabbit primary antibodies of GAPDH (ab9845, Abcam) and p53 (ab131442, Abcam) at 4°C for 18 h. After that, membranes were further incubated with IgG-HRP goat anti rabbit (MyBioSource) at 24°C for 2 h. Signals were developed using ECL (Sigma-Aldrich) and quantity one was used to normalize the signals.

Statistical Analyses

Three independent biological replicates were included in each experiment. Data were expressed as mean values. Comparisons between two groups were performed by unpaired *t*-test. Differences between LSCC and non-tumor tissues were analyzed by paired *t*-test. ANOVA (one-way) and Tukey's test were used to explore differences among multiple groups. The 60 LSCC patients were divided into high and low IUR level groups (n = 30) with the median expression value of IUR in LSCC tissues as cutoff score. K-M method was used to plot survival curves and Log rank test was used to compare survival curves. $p < 0.05$ was statistically significant.

Results

Downregulation of IUR in LSCC Predicted Poor Survival

Differential expression of IUR in LSCC was determined by measuring the expression levels of IUR, p53 and miR-24 in both LSCC and non-tumor tissues collected from the

60 patients with LSCC. Paired *t*-test showed that the expression levels of IUR and p53 were significantly lower in LSCC tissues than that in non-tumor tissues (Figure 1A and C, $p < 0.001$), while the expression levels of miR-24 were higher in LSCC tissues than that in non-tumor tissues. Survival curves showed that the overall survival rate of patients in the low IUR level group was significantly lower than that of patients in the high IUR level group (Figure 1B, $p < 0.05$). And Chi-squared test showed that the expression levels of IUR were not significantly correlated with clinical stages ($p = 0.87$). See Table 1 for details.

Table 1 Association of the IUR Expression in Different Stages of Patients

LSCC	Cases	High	Low	χ^2	P value
I	12	5	7	0.69	0.87
II	15	8	7		
III	18	10	8		
IV	15	7	8		

IUR and miR-24 Can Interact with Each Other

The potential base pairs formed by IUR and miR-24 were predicted using IntaRNA (http://rna.informatik.uni-freiburg.de/IntaRNA/Input.jsp). It was observed that IUR and miR-24 can form strong base pairs between each other (Figure 2A). Dual luciferase reporter assay was performed to further analyze the interaction between IUR and miR-24. Compared to cells transfected with IUR and miRNA NC (NC group), cells transfected with IUR and miR-24 mimic (miR-24 group) showed significantly lower relative luciferase activity (Figure 2B, $p < 0.05$).

Overexpression of IUR Inhibited Cell Migration and Invasion Through miR-24

Wound healing assay (Figure 3A and C) and transwell assay (Figure 3B and D) were performed to study the effects of the interactions between IUR and miR-24 of cell migration and invasion. Compared to C, NC miRNA and PcDNA3.1, overexpression of miR-24 led to promoted, while overexpression of IUR inhibited the cell migration and invasion. In addition, overexpression of miR-24 reduced the inhibited effects of IUR to LSCC cells.

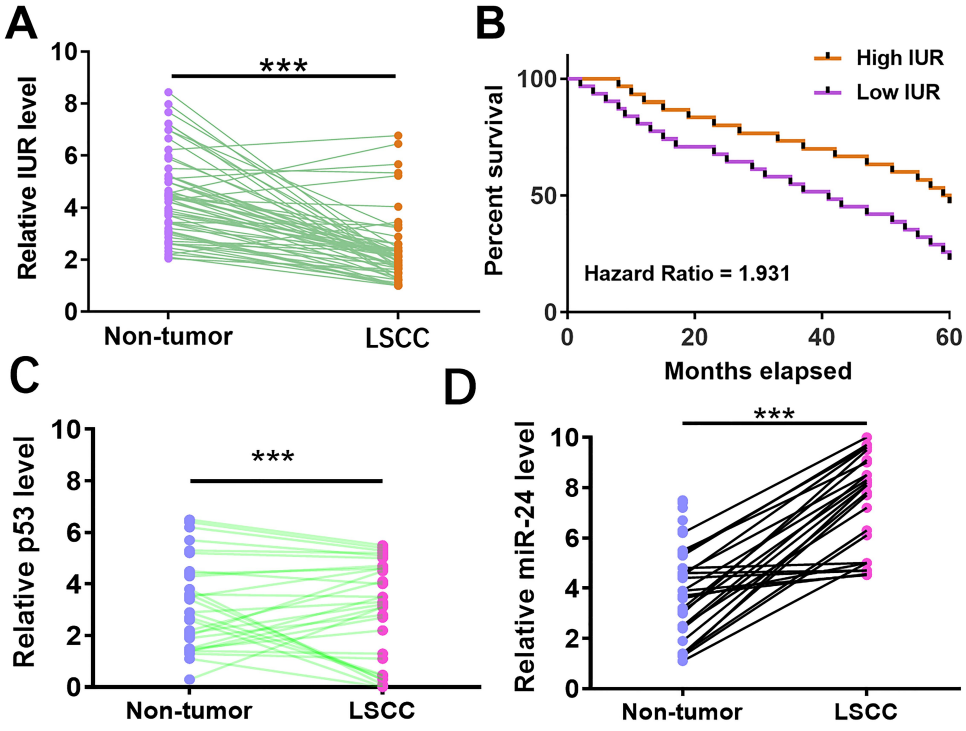

Figure 1 Downregulation of IUR in LSCC predicted poor survival. Differential expression of IUR (**A**), p53 (**C**), miR-24 (**D**) in LSCC was analyzed by measuring the expression levels of IUR in both LSCC and non-tumor tissues collected from the 60 patients with LSCC. Data were compared by paired *t*-test. PCR reactions were repeated 3 times and mean values were presented ***$p < 0.001$. The 60 LSCC patients were divided into high and low IUR level groups (n = 30) with the median value of IUR expression in LSCC tissues as cutoff score. K-M method was used to plot survival curves and Log rank test was used to compare survival curves (**B**).

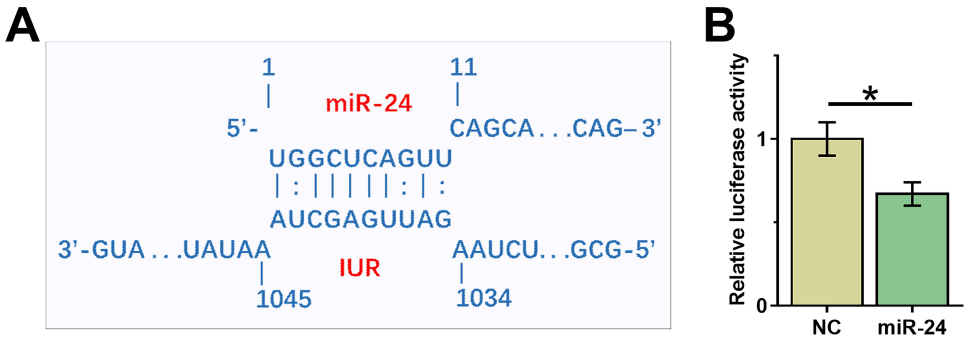

Figure 2 IUR and miR-24 can interact with each other. The potential base pairs formed by IUR and miR-24 were predicted by using IntaRNA (**A**) Dual luciferase reporter assay was performed by transfecting IUR and miRNA NC (NC group) or IUR and miR-24 mimic (miR-24 group) into UM-SCC-17A cells. Relative luciferase activity was compared between unpaired *t*-test (**B**). Experiments were repeated 3 times and data were expressed as mean values. *$p < 0.05$.

LSCC Sponges miR-24 to Upregulate P53

UM-SCC-17A cells were transfected with IUR expression vector or miR-24 mimic to further evaluate the interaction between IUR and miR-24. Overexpression of IUR and miR-24 was confirmed by qPCR at 24 h post-transfection (Figure 4A, $p < 0.05$). Comparing to C and NC groups, overexpression of IUR and miR-24 did not alter the expression of each

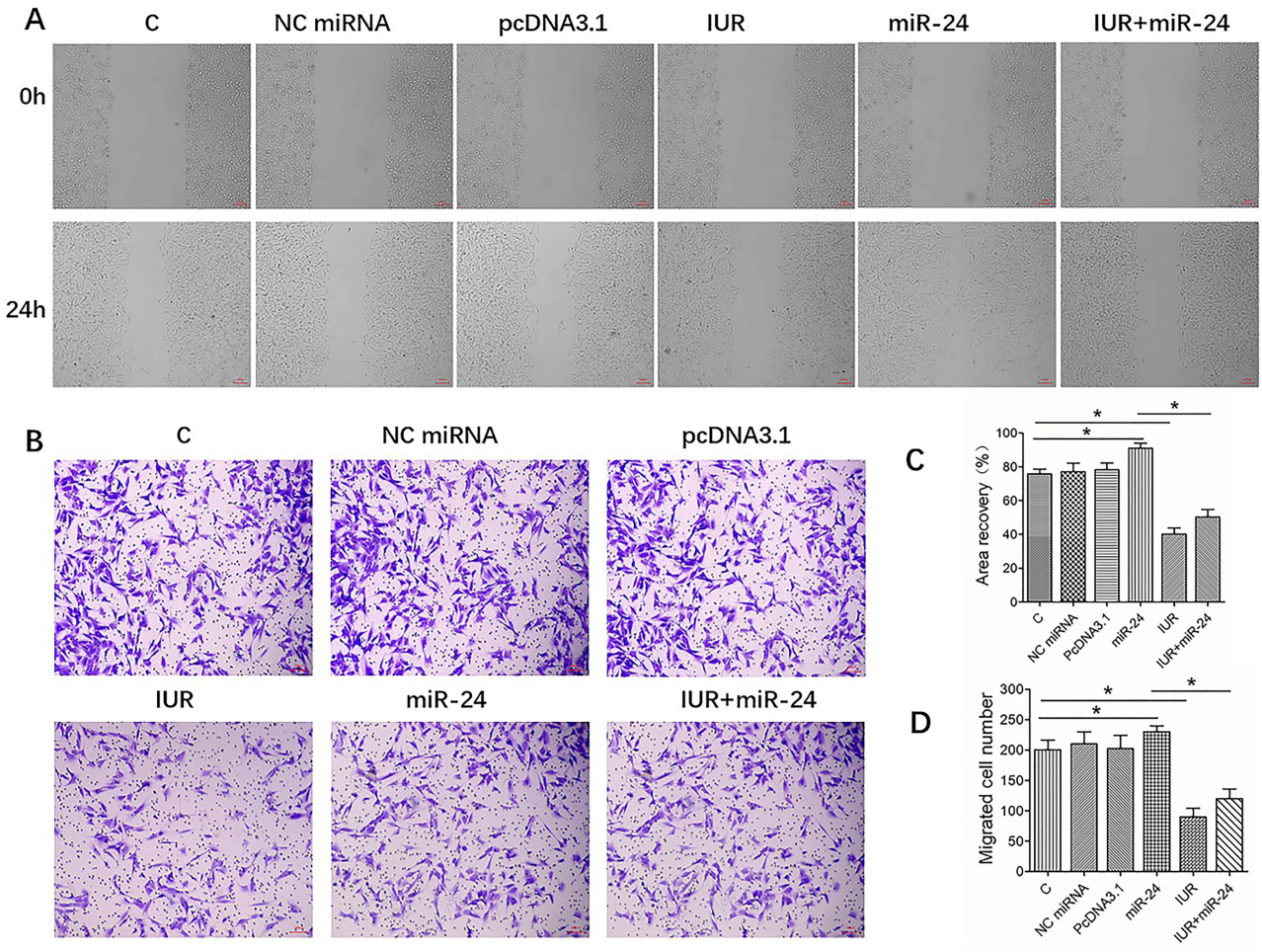

Figure 3 IUR regulated miR-24/p53 axis to suppress the invasion and migration of UM-SCC-17A cells. Wound healing assay was used to illustrate the migration of UM-SCC-17A cells (**A**). Transwell assays were carried to show the invasion of cells (**B**). Experiments were repeated 3 times and data were expressed as mean values. The effects of IUR and miR-24 expression on the expression of p53 in UM-SCC-17A cells were analyzed by qPCR and western blot at mRNA (**C**) and protein (**D**) levels, respectively. Comparing to C and NC groups, overexpression of miR-24 led to downregulated p53 while IUR reversed the effects of overexpression of miR-24.*$p < 0.05$. Scale bar = 100μM.

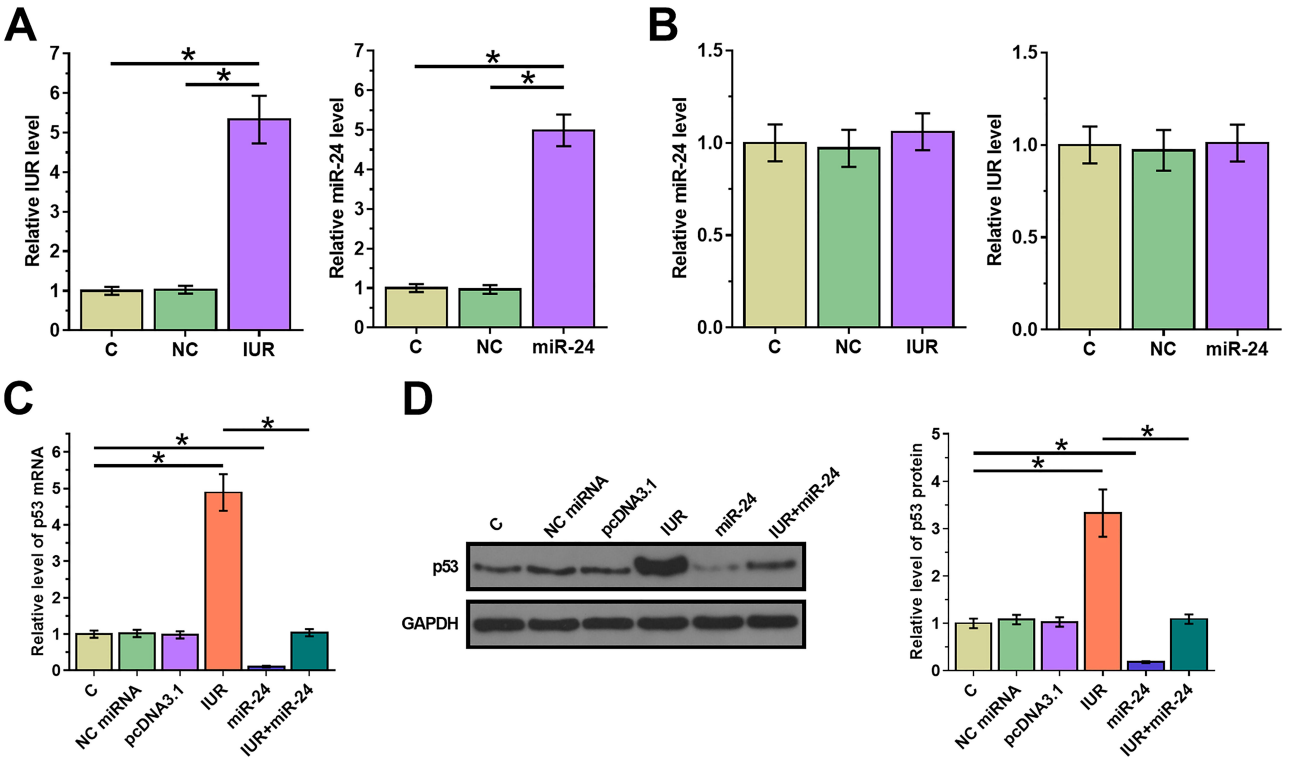

Figure 4 LSCC sponges miR-24 to upregulate p53. UM-SCC-17A cells were transfected with IUR expression vector or miR-24 mimic to further analyze the interaction between IUR and miR-24. Over expression of IUR and miR-24 was confirmed by qPCR at 24h post-transfection (**A**). The effects of IUR and miR-24 overexpression on the expression of each other were also analyzed by qPCR at 24h post-transfection (**B**). The effects of IUR and miR-24 expression on the expression of p53 in UM-SCC-17A cells were analyzed by qPCR and Western blot at mRNA (**C**) and protein (**D**) levels, respectively. Experiments were repeated 3 times and data were expressed as mean values. *$p < 0.05$.

other (Figure 4B). Then, the effects of IUR and miR-24 on the expression of p53 in UM-SCC-17A cells were assessed by qPCR and Western blot at mRNA (Figure 4C) and protein (Figure 4D) levels, respectively. Comparing to C and NC groups, overexpression of miR-24 led to downregulated p53. In contrast, overexpression of IUR played an opposite role and reduced the effects of overexpression of miR-24 ($p < 0.05$).

IUR Regulated miR-24/P53 Axis to Suppress the Proliferation of UM-SCC-17A Cells

CCK-8 assay was performed to analyze the effects of transfections on the proliferation of UM-SCC-17A cells. Comparing to C group, overexpression of IUR and p53 led to decreased proliferation rate of LSCC cells, while overexpression of miR-24 led to increased rate of LSCC cell proliferation. In addition, overexpression of miR-24 attenuated the effects of overexpression of IUR on the expression of p53 and cancer cell proliferation (Figure 5, $p < 0.05$).

IUR Regulated miR-24/P53 Axis to Suppress the Growth of Tumor

The xenograft tumor formation assay was performed to assess the growth-inhibitory effect of IUR in vivo. We found that IUR significantly reduced tumor growth while miR-24 increased tumor growth (Figure 6A). Both the average tumor volume and the tumor weight were obviously lower in IUR group mice compared with that in other groups (Figure 6A). In addition, in vivo results also showed similar trend which already performed in cell level that overexpression of miR-24 could reverse the inhibited effect of IUR in tumor growth. These results indicated that IUR suppressed laryngeal tumor growth in vitro and in vivo. And we further demonstrated that IUR regulated miR-24/p53 axis to suppress the growth of tumor. The results of WB assay showed that p53 was upregulated with the overexpression of IUR, but downregulated with the overexpression of miR-24 (Figure 6B).

Discussion

This study mainly investigated the roles of IUR in LSCC. We found that IUR was downregulated in LSCC and predicted

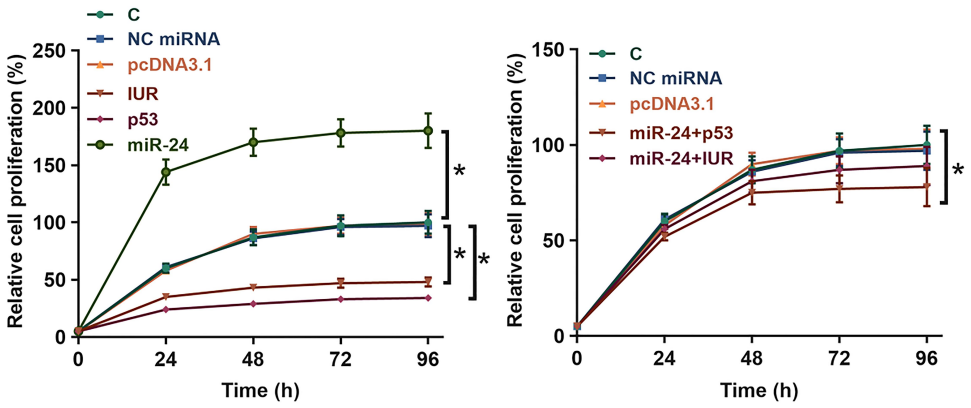

Figure 5 IUR regulated miR-24/p53 axis to suppress the proliferation of UM-SCC-17A cells. CCK-8 assay was performed to analyze the effects of transfections on the proliferation of UM-SCC-17A cells. Experiments were repeated 3 times and data were expressed as mean values. *$p < 0.05$.

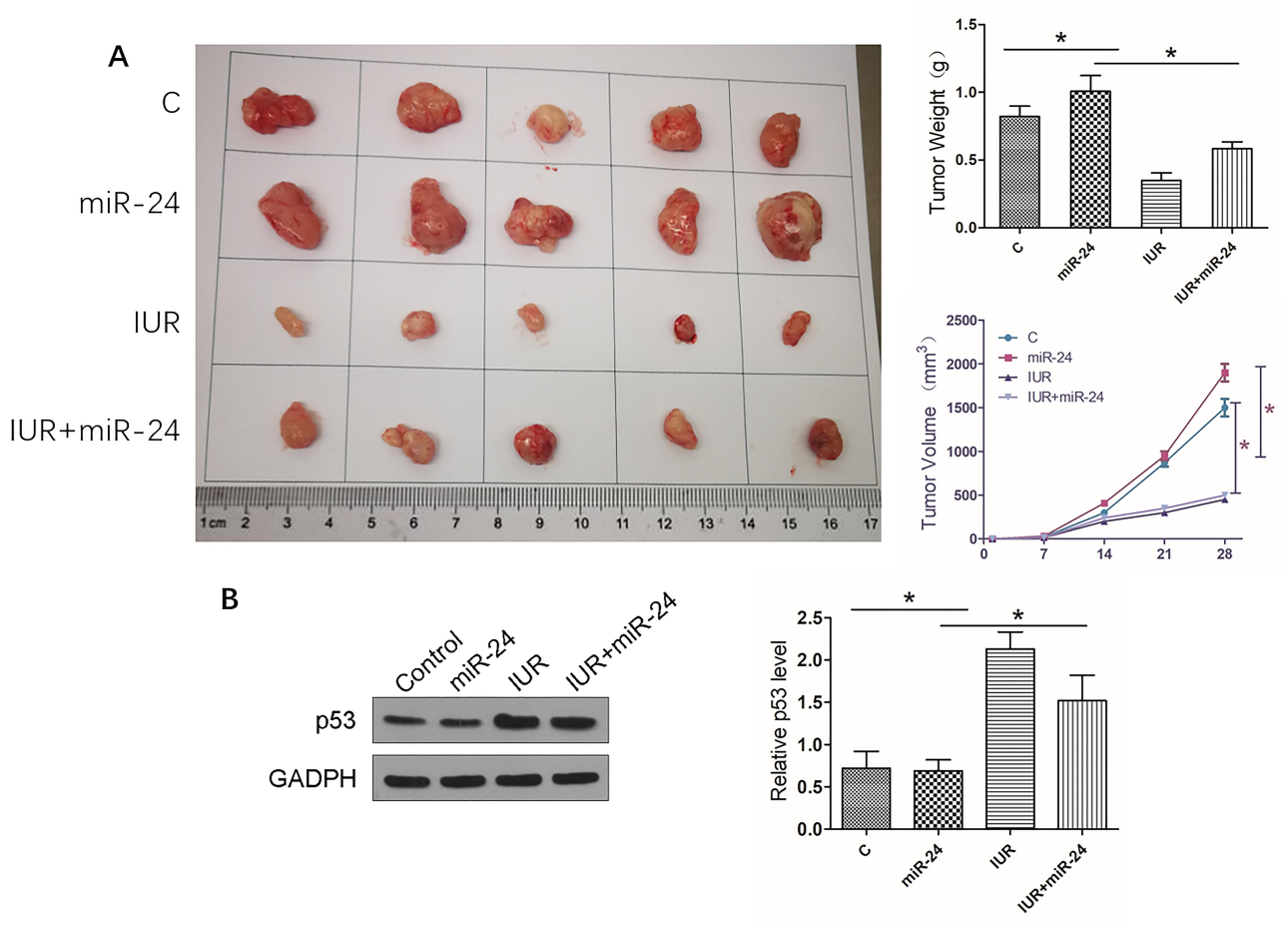

Figure 6 IUR sponges miR-24 to upregulate p53 in subcutaneous tumor model and inhibiting tumor growth. Photographs of tumor xenografts and tumor weight and volume showed the effects of IUR and miR-24 expression in tumor growth (**A**) scale bar = 1mm. On the expression of p53 in tumor were analyzed by Western blot at protein levels (**B**). Experiments were repeated 5 times and data were expressed as mean values. *$p < 0.05$.

poor survival of LSCC patients. In addition, IUR may regulate miR-24/p53 axis to regulate the proliferation of LSCC cells.

The functions of IUR in cancer biology have only been reported in leukemia.[12] IUR is downregulated in leukemia and its overexpression suppresses Bcr-Abl-induced tumorigenesis.[12] Our study is the first to report the downregulation of IUR in LSCC. In addition, we also observed the reduced proliferation rate, migration,

and invasion of LSCC cells after overexpression of IUR. We also proved that IUR can block tumor growth. Therefore, IUR is likely a tumor suppressor in LSCC.

Accurate prognosis is critical for the determination of therapeutic approaches and the development of post-operative care system, therefore affects the survival of patients. Previous studies have developed a considerable number of prognostic factors, such as BMI and BCL2L12 for LSCC.[13,14] However, these prognostic factors are limited by the low accuracy and reliability.[13,14] This study showed that low expression levels of IUR were closely correlated with the poor survival of LSCC patients. Therefore, measurement of the expression levels of IUR before therapies may assist the prognosis of LSCC. However, more studies are needed to further confirm the accuracy.

MiR-24 has been proved to play oncogenic roles in several types of cancer, such as colon cancer and lung cancer.[15,16] However, the involvement of miR-24 in LSCC is unknown. A recent study reported that miR-24 targeted p53 to promote the development of liver cancer.[11] Consistently, our study also observed downregulation of p53 in LSCC cells after overexpression of miR-24, indicating that miR-24 can also target p53 in LSCC cells. In addition, miR-24 overexpression led to the increased proliferation rate, migration, and invasion of LSCC cells. From in vivo model, we also illustrated the overexpression of miR-24 downregulated the expression of p53. Therefore, miR-24 plays oncogenic roles in LSCC.

In this study, we showed that IUR is likely an endogenous sponge of miR-24. This speculation is based on: i) IUR and miR-24 can directly interact with each other; ii) IUR and miR-24 failed to affect the expression of each other; iii) overexpression of IUR led to the upregulation of p53, the target of miR-24. Therefore, we identified a novel IUR/miR-24/p53 pathway in LSCC.

In conclusion, IUR is downregulated in LSCC and may regulate miR-24/p53 axis to suppress the proliferation of LSCC cells.

Abbreviations

LSCC, Laryngeal squamous cell carcinoma.

Data Sharing Statement

The analyzed data sets generated during the study are available from the corresponding author on reasonable request.

Ethics Approval and Consent to Participate

The present study was approved by the Ethics Committee of Guangxi Medical University College of Stomatology, Wuming Hospital of Guangxi Medical University. The research has been carried out in accordance with the World Medical Association Declaration of Helsinki. All patients provided written informed consent prior to their inclusion within the study.

Acknowledgments

We appreciate the good cooperations supported by the Guangxi Key Laboratory of Oral and Maxillofacial Rehabilitation and Reconstruction, Guangxi Universities and Colleges Key Laboratory of Oral and Maxillofacial Surgery Disease Treatment & Guangxi Clinical Research Center for Craniofacial Deformity.

Author Contributions

All authors contributed to data analysis, drafting or revising the article, gave final approval of the version to be published, and agree to be accountable for all aspects of the work.

Co-First Author: Cen Wei and Huaqing Wei make equal contribution to this article.

Funding

This work was supported by Project of Clinical Research Foundation of Western Stomatology Chinese Stomatological Association (grant number CSA-W2018 -06) & National Natural Science Foundation of China (grant number 81360407).

Disclosure

The authors declare that they have no competing interests.

References

1. Bray F, Ferlay J, Soerjomataram I, et al. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin*. 2018;68(6):394–424. doi:10.3322/caac.21492
2. Du L, Li H, Zhu C, et al. Incidence and mortality of laryngeal cancer in China, 2011. *Chin J Cancer Res*. 2015;27(1):52–58.
3. Cahoon EK, Preston DL, Pierce DA, et al. Lung, laryngeal and other respiratory cancer incidence among Japanese atomic bomb survivors: an updated analysis from 1958 through 2009. *Radiat Res*. 2017;187 (5):538–548. doi:10.1667/RR14583.1
4. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2019. *CA Cancer J Clin*. 2019;69(1):7–34. doi:10.3322/caac.21551
5. Nguyen N, Bellile E, Thomas D, et al. Tumor infiltrating lymphocytes and survival in patients with head and neck squamous cell carcinoma. *Head Neck*. 2016;38(7):1074–1084. doi:10.1002/hed.24406

6. Brandstorp-Boesen J, Falk RS, Evensen JF, et al. Risk of recurrence in laryngeal cancer. *PLoS One*. 2016;11(10):e0164068.
7. Zhou J, Yang Y, Zhang D, et al. Genetic polymorphisms and plasma levels of BCL11A contribute to the development of laryngeal squamous cell carcinoma. *PLoS One*. 2017;12(2):e0171116. doi:10.1371/journal.pone.0171116
8. Shen Z, Ren W, Bai Y, et al. DIRC3 and near NABP1 genetic polymorphisms are associated laryngeal squamous cell carcinoma patient survival. *Oncotarget*. 2016;7(48):79596–79604. doi:10.18632/oncotarget.12865
9. Ludwig ML, Birkeland AC, Hoesli R, et al. Changing the paradigm: the potential for targeted therapy in laryngeal squamous cell carcinoma. *Cancer Biol Med*. 2016;13(1):87–100.
10. Guimaraes DP, Hainaut P. TP53: a key gene in human cancer. *Biochimie*. 2002;84(1):83–93. doi:10.1016/S0300-9084(01)01356-6
11. Chen L, Luo L, Chen W, et al. MicroRNA-24 increases hepatocellular carcinoma cell metastasis and invasion by targeting p53: miR-24 targeted p53. *Biomed Pharmacother*. 2016;84:1113–1118. doi:10.1016/j.biopha.2016.10.051
12. Wang X, Yang J, Guo G, et al. Novel lncRNA-IUR suppresses Bcr-Abl-induced tumorigenesis through regulation of STAT5-CD71 pathway. *Mol Cancer*. 2019;18(1):84.
13. Te Riele R, Dronkers EAC, Wieringa MH, et al. Influence of anemia and BMI on prognosis of laryngeal squamous cell carcinoma: development of an updated prognostic model. *Oral Oncol*. 2018;78:25–30. doi:10.1016/j.oraloncology.2018.01.001
14. Giotakis AI, Lazaris AC, Kataki A, et al. Positive BCL2L12 expression predicts favorable prognosis in patients with laryngeal squamous cell carcinoma. *Cancer Biomark*. 2019;25(2):141–149. doi:10.3233/CBM-181772
15. Zhang H, Guo J, Mao L, et al. Up-regulation of miR-24-1-5p is involved in the chemoprevention of colorectal cancer by black raspberry anthocyanins. *Br J Nutr*. 2018;30:1–25.
16. Pan Y, Wang H, Ma D, et al. miR-24 may be a negative regulator of menin in lung cancer. *Oncol Rep*. 2018;39(5):2342–2350.