ORIGINAL RESEARCH

Long Noncoding RNA UCA1 Accelerates Nasopharyngeal Carcinoma Cell Progression By Modulating miR-124-3p/ITGB1 Axis

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Background: Nasopharyngeal carcinoma (NPC) is a common collision cancer that is distributed particularly in Southeastern Asia. To vious radies have manifested that long noncoding RNA urothelial carcinoma associated 1 (1611) was in olved in NPC tumorigenesis and metastasis. However, the biological mechanist of ULA1 for NPC cell progression requires further investigation.

Methods: The expression levels of UCA1, UR-124-3p, integrin beta-1 (ITGB1) were detected by qRT-PCR. Proton expression of httpB1 was determined by Western blot assay. Cell proliferation, a gration and divasion were evaluated by CCK8 and transwell assay, respectively. The interaction between miR-124-3p and UCA1 or ITGB1 was determined by luciferase reported system, Fur and RNA pull-down assay. Mice model was established by success pously injecting SUNE1 cells stably transfected with sh-UCA1 and sh-NC.

Results The expression of UCA1 was up-regulated in NPC tissues and cells. However, UC1 knoclown hadered NPC cell growth, migration and invasion. In addition, the intraction haveen mig-124-3p and UCA1 or ITGB1 was confirmed by luciferase reporter system of P and RNA pull-down assay. Besides, miR-124-3p inhibitor abrogated UCA1 silencing mediated suppression on cell progression in NPC. Moreover, UCA1 accelerated NPC cell progression through modulating ITGB1 via sponging miR-124-3p. In vivo experiments revealed the interference of UCA1-inhibited tumor growth by regulating miR-124-3p/ITC-1 axis.

Conclusion: UCA1 acts as an oncogene to promote NPC cell proliferation by up-regulating ITGB1 through suppressing miR-124-3p in vitro and in vivo, providing a potential target for NPC diagnosis and treatment.

Keywords: NPC, proliferation, migration, UCA1, miR-124-3p, ITGB1

Introduction

Nasopharyngeal carcinoma (NPC) which originated from nasopharyngeal epithelial cells is one of the most malignant squamous cell carcinomas with high metastasis.¹

It geographically distributes in Southeastern Asia and has high incidence in Southern China.² Clinically, the most prevalent strategies for NPC are still chemotherapy and radiotherapy; however, multidrug resistance and chemotherapy sensitivity could hinder the treatment efficiency and lead to high recurrence, poor therapeutic and prognosis outcomes.^{3–5} The pathogenesis is complex, including dietary, genetic susceptibility, virus infection and carcinogen hazards.⁶ Therefore,

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Long noncoding RNAs (lncRNAs) are RNAs that comprise of over 200 nucleotides in length. Typically, they are involved in multiple biological processes, including cellular signal transmission, chromosome imprinting, hormonal control and genetic translation, therefore imbalance of can trigger different diseases.^{7,8} Shin lncRNAs Matsubara et al reported that lncRNA-Amhr2 which is located in the cell nucleus is capable of modulating folliculogenesis by activating Amhr2 gene in ovarian granulosa cells.⁹ In addition, lncRNA-H19 enhanced mesenchymal stem cells angiogenesis and survival by sponging miR-199a-5p.¹⁰ Urothelial carcinoma associated 1 (UCA1), derived from bladder cancer, has been identified as oncogenic lncRNA with strong carcinogenic activity.¹¹ Song et al investigated the regulatory mechanism of UCA1 and found UCA1 can positively accelerate colon cancer cells' progression by regulating miR-28-5p/ HOXB3 axis.¹² However, it remains suspicious whether UCA1 is regulating NPC cell behavior by modulating the specific miRNA.

MicroRNAs (miRNAs) are short-chain noncoding RNAs with 16–22 nucleotides. They participate in variety cancer cell functions, for example, cell proliferation, dia ferentiation and apoptosis, by regulating the domestream gene at post-transcriptional level.¹³ For instance, UCA1 has been reported to promote cell progression via submessing miR-28-5p and up-regulating HGXB3 uncession in colon cancer.¹⁴ Binbin Liu et al clusted that mul24-3p could accelerate intrahepatic charagio accinoma cen progression through regulating UHRF1.¹⁵ The role of miR-124-3p in NPC requires a depthetemptic characteristic.

Integrin beta-1 (IT R1), coundispensable member of integrin beta submit is a colved in the acceleration of cancer cells a desion survivation metastasis by interacting with expacellul constrix components fibronectin and laminin.¹⁶ The appromotion effects of ITGB1 on cancer cells are regulated by activating intracellular signaling molecules FAK and CSrc to compound pl30Cas and paxillin proteins with kinase activity.¹⁷ ITGB1 was verified to stimulate gallbladder cancer (GBC) cells metastasis, while ITGB1 knockdown played an inhibitory role in GNC cell infiltration, proliferation and migration.¹⁸ However, the regulatory mechanism of ITGB1 for NPC cell progression is unclear.

In his study, we attempted to illuminate the regulatory effects of UCA1 on NPC tumor growth. The expression of

UCA1, miR-124-3p and ITGB1 in NPC was investigated by qRT-PCR. The interaction of miR-124-3p and UCA1 or ITGB1 was validated by dual-luciferase reporter assay. Moreover, animal experiments were conducted to reveal the function of UCA1 in vivo.

Materials And Methods

Tissue Samples

A total of 30 NPC patients were recruited from Jining First People's Hospital of Shandong Province. NPC patients have not received preoperative therapy. TPC tissues and the adjacent normal tissues were collected from those patients by surgery. All the patient have signed informed consent, and the investigation was approved by the Ethics Committee of Jining First People's Hospital of Shandong Province, in accordance without Decletation of Helsinki.

Quantitative real Time rolymerase Chain Reaction (qRT-F-R)

Total RNA extraction was conducted by incubating NPC tisses and cells with Trizol reagent (Invitrogen). RNA transcript in reaction was performed using M-MLV revel reverse otase kit (Invitrogen). qRT-PCR was perusing SYBR green (Applied Biosystems). The pri-Ť0. ers for miR-124-3p, UCA1 and ITGB1 were listed as follows: UCA1, (Forward, 5'-CTCTCCATTGGGTTCACC TTC-3'; Reverse, 5'-CTCTCCATTGGGTTCACCATTC-3'); miR-124-3p, (Forward, 5'-CTCAACTGGTGTCGTG GAGTCGGCAATTCAGTTGAGGGCATTCA-3'; Reverse, 5'-ACACTCCAGCTGGGTAAGGCACGCGGTGAATGC C-3'); ITGB1 (Forward, 5'-AGGGCCAAATTGTGGGTG G-3'; Reverse, 5'-TGCTGTTCCTTTGCTACGGT-3').

Cell Transfection

C666-1 cells were purchased from Kalang Biomart, SUNE1 cells and human immortalized nasopharyngeal epithelial cells NP69 were purchased from Sbj-Bio Life Science. SUNE1, C666-1 and NP69 cells were maintained in RPMI-1640 medium (Gibco) supplemented with 10% FBS and 0.05% penicillin/streptomycin. Small interfering RNA (siRNA) targeting UCA1 (si-UCA1, including si-UCA1#1, si-UCA1#2 and si-UCA1#3), siRNA targeting ITGB1 (si-ITGB1), siRNA negative control (si-NC), pcDNA and pcDNA-ITGB1 overexpression vector (ITGB1) were synthesized by Genepharma. The miRNA mimic (miR-124-3p), miR-124-3p inhibitor (in-miR-124-3p), miRNA negative control (miR-NC) and miRNA negative

control inhibitor (in-miR-NC) were purchased from RIBOBIO. SUNE1 and C666-1 cells were transfected with those plasmids using Lipofectamine 2000 (Invitrogen).

Cell Viability

After transfected with relative vectors for 24 hrs, SUNE1 and C666-1 cells were seeded in 96-well plates (5000 cells/well) and cultured for another 24 hrs, 48 hrs and 72 hrs. Then, cells were reacted with 10 μ L CCK-8 reagent (Beyotime) for 2 hrs. The optical density (OD) value at 450 nm was measured by a microplate reader.

Transwell Assay

Transfected cells were resuspended $(2 \times 10^5 \text{ cells/mL}, 200 \,\mu\text{L})$ and seeded in the upper chamber pre-treated with Matrigel (Becton Dickinson). The bottom chamber was filled with 600 μL medium containing 10% FBS. After 24 hrs incubation, invasive cells in the lower chamber through the membranes were stained with 0.1% crystal violet (Sigma) for 10 mins and counted using a microscope.

Luciferase Reporter Assay

Wild-type and mutant-type luciferase vectors (UCA1WT, UCA1 MUT, ITGB1 3'-UTR-WT, ITGB1 3'-UTR-WT) were constructed, then SUNE1 and C666-1 cells were cotransfected with those luciferase vectors and n 2-124-0 mimics or miR-NC for 24 hrs using Lapofectar and 2000 transfection reagent. Subsequently, he for a converse were evaluated by dual-luciferate assay system.

RNA Immunopre-ipitation (RIP)

For Ago2 RIP assay a UNE1 and C666-acells were transfected with miR-12-3p or niR-NC and the analysis was performed using EZ-200gna RIPT RNA-Binding Protein Immunoprospitation Kn. Mill pore). In brief, cells were lysed von RIP k offer 24 hrs post-transfection, centrifuged at 10,000 p.f.r. 5 mins, resuspended and incubated with magnetic beacts conjugated with Ago2 or IgG antibody. The immunopre-pitated RNA was subjected to qRT-PCR to detect the enrichment of UCA1.

RNA Pull-Down Assay

Biotinylated UCA1 WT (Bio-UCA1 WT), UCA1 MUT (Bio-UCA1 MUT) and negative control (Bio-NC) that purchased from Sangon were transfected into SUNE1 and C666-1 cells. The cells were lysed and collected 24 hrs post-transfection. After incubation with Dynabeads M-280

Streptavidin (Invitrogen) for 10 mins, the bound RNAs were subjected to qRT-PCR for quantification and analysis.

Murine Xenograft Assay

Male Balb/c nude mice were obtained from Vital River Laboratory Animal Technology and all the animal experiments were conducted in accordance with the guidelines provided by the National Animal Care and Ethics Institution. Animal experiments were approved by the animal experimental ethics committee of Jining First People's Hospital of Shandong pince. Mice were divided into 2 groups (n=6) readomly and ubcutaneously injected with SUNE1 cells (10^6 cells) 0 μL) stably transfected with sh-UC f or sh-Tume volumes were recorded every weel for 4 weeks and culated according to the equation: volume $f(m^3) = \text{length} \times \text{width} \times \text{width}/2$. e sacrift 1 at 28 Jays post-treatment, and The mice w the tumor we weighed collected for the subsequent biological analy

tatistical Analysis

Il the experiments were repeated at least 3 times and the data were are tyzed by SPSS software and GraphPad Prism 7. Student's *t*-test and one-way ANOVA were used to compare two more than two groups. The survival rate was analyzed using the Kaplan-Meier method. The correlation between miR-124-3p and UCA1 or ITGB1 was calculated by Pearson's correlation coefficient. P value less than 0.05 (P<0.05) was considered statistically significant.

Results

UCA1 Was Up-Regulated In NPC Tissues And Cells

The role of UCA1 in NPC was identified by a series of experiments. As displayed in Table 1, NPC patients at stage III and IV possessed higher UCA1 expression than that of patients at stage I and II. High level of UCA1 was associated with cell metastasis in NPC. UCA1 expression levels in 30 pairs of NPC tissues and corresponding healthy tissues were analyzed by qRT-PCR. The expression of UCA1 was significantly up-regulated in NPC tissues compared with the adjacent normal tissues (P<0.05) (Figure 1A). Similarly, the expression of UCA1 in SUNE1 and C666-1 NPC cells was extremely higher than that in immortalized nasopharyngeal epithelial NP69 cells (Figure 1C). Furthermore, high level of UCA1 showed the opposite

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Variable	Patients, n	UCAI Expressi	P-value	
		Low	High	
Sex				0.897
Male	14	6	8	
Female	11	5	6	
Age, years				0.812
<45	15	7	8	
≥45	10	4	6	
Tumor stage				0.012
I, II	10	6	4	
III, IV	15	4	11	
Lymph node status				0.005
Metastasis	16	5	11	
No metastasis	9	5	4	

Table	L	Analysis	Of 1	The Co	rrelation	Between	Expression	Of
UCAI	In	Primary	NPC	And It	s Clinico	pathologic	al Paramete	ers

result (Figure 1B). Those results collectively manifested that UCA1 was correlated with NPC tumorigenesis.

UCAI Knockdown Suppressed NPC Cell Proliferation, Migration And Invasion

To elucidate the biological function of UCA1 in NPC cel progression, SUNE1 and C666-1 cells were fected with si-NC, si-UCA1#1, si-UCA1#2 and side JCA1# As showed in Figure 2A and B, the expression] UCA1 decreased in SUNE1 and C67 -1 cells ansfected with si-UCA1#1, si-UCA1#2 ap UCA1#3 npared with their corresponding control group. Due to the relatively high knockdown efficiency, SUNL and C666-1 cells transfected with JCA1[#] were recruited for the subsequent cellular ex ime s. CCK8 results exhibited ked N C cell proliferation that UCA1 kng wn s

ability (Figure 2C and D). In consistent with CCK8 results, transwell assay showed that the migration and invasion ability of SUNE1 and C666-1 cells transfected with si-UCA1 reduced largely (Figure 2E and F). Taken together, UCA1 knockdown attenuated cell proliferation, migration and invasion in NPC.

UCAI Directly Interacted With miR-124-3p

Accumulating evidences have identified lncRNA exerts its regulatory function by interfering with miRNA and further triggering the post-transcriptional pro-**Pioinformatics** analysis prediction by LncBase Predicted v.2 realed that there were putative binding sit, between CA1 and miR-124-3p (Figure 3A). ual-luch ase rer rter assay in SUNE1 and C666-1 ells revaled by ne luciferase activity of cells co-transfecter with miR-124-3p mimic A1 (UCA WT) as reduced, whereas and wild-type U the luciferase ity of cells ransfected with mutant UCA1 (UCA1-MU, remains unchanged (Figure 3B further determine the interaction between and UC and miR-124-3p, RIP assay was performed. As 3D, the enrichment of UCA1 was sho n in Figur d significantly in SUNE1 and C666-1 cells transenhan sted who miR-124-3p in comparison with cells transfe as ith miR-NC. Similarly, RNA pull-down assay evealed that the expression level of miR-124-3p was upegulated in SUNE1 and C666-1 cells transfected with CA1-WT (Figure 3E and F). Then, we discovered that the expression of miR-124-3p was low in NPC tumor tissues and cells compared with the normal tissues and cells (Figure 3G and H), indicating that miR-124-3p functions as a suppressor in NPC cell progression. Subsequently, we found UCA1 was inversely correlated with miR-124-3p (Figure 3I). Furthermore, we detected the expression of miR-124-3p in SUNE1 and C666-1



Figure I UCAI was up-regulated in NPC tissues and cell lines.

Notes: (A) The expression of UCA1 was detected by qRT-PCR in 30 pairs of NPC tissues and adjacent normal tissues. (B) The survival rate of NPC patients with different UCA1 levels (high UCA1: n=14, low UCA1: n=11) in 60 months. P<0.01. (C) The expression of UCA1 in SUNE1, C666-1 cells compared with NP69 cells. *P<0.05.



Figure 2 The effects of UCA1 knockdown on the proliferation, migration and invasion of NPC cells.

Notes: (A and B) UCA1 expression in SUNE1, C666-1 cells transfected with si-NC, si-UCA1#1, si-UCA1#2 and si-UCA1#3 was measured by qRT-PCR. (C and D) Cell viability of SUNE1 and C666-1 cells transfected with si-NC and si-UCA1 was measured by CCK-8 assay. (E and F) The migration and invasion ability of SUNE1 and C666-1 cells were conducted 24 hrs post-transfection by transwell assay. *P<0.05.





Notes: (A) The putative binding sites of UCA1 and miR-124-3p were predicted by LncBase Predicted v.2. (B and C) Luciferase activity of SUNE1 and C666-1 cells cotransfected with miR-124-3p or miR-NC and UCA1 WT or UCA1 MUT was determined by dual-luciferase assay. (D) The expression of UCA1 extracted by RIP assay was evaluated in samples bound to Ago2 or IgG. (E and F) The interaction between UCA1 and miR-124-3p in SUNE1 and C666-1 cells transfected with UCA1 WT and UCA1 MUT was verified by RNA pull-down assay. (G and H) Expression levels of miR-124-3p in NPC tissues and cells compared with normal tissues and cells. (I) Certification of correlation between UCA1 and miR-124-3p (R=-0.9003, P<0.0001). (J) Expression levels of miR-124-3p in SUNE1 and C666-1 cells transfected with si-NC and si-UCA1. *P<0.05. cells transfected with si-NC and si-UCA1, and observed up-regulation of miR-124-3p level in UCA1 knockdown cells (Figure 3J). Collectively, UCA1 could directly interact with miR-124-3p.

miR-124-3p Inhibitor Abolished UCA1 Silencing-Mediated Regulatory Effects On Cell Proliferation, Migration And Invasion In NPC

Next, to explore the influences of UCA1/miR-124-3p axis on NPC cell proliferation, migration and invasion, SUNE1 and C666-1 cells were transfected with si-NC, si-UCA1, si-UCA1+in-miR-NC and si-UCA1+in-miR-124-3p. Cell viability results showed that the inhibition of miR-124-3p altered UCA1 silencing-mediated inhibitory effects on SUNE1 and C666-1 cell proliferation (Figure 4A and B). In addition, transwell assay exhibited that miR-124-3p inhibitor rescued UCA1 silencing-induced inhibition of cell migration and invasion (Figure 4C and D). Those data demonstrating UCA1 could regulate NPC cell growth by suppressing miR-124-3p expression.

miR-124-3p Exerted Its Function By Targeting ITGB1

Previous studies have identified that miRNA executed their specific regulatory functions through binding to their corresponding target genes. According to bioinformatics analysis, ITGB1 was the potential target gene of miR-124-3p (Figure 5A). Dual cefferal reporter system analysis further validated the prediction sh e the luciferase activity reduced endmous. in SUNF and C666-1 TGB1 3'-UTR cells co-transfected y n miR-124 ap WT compared with cells of annsfected with miR-NC and Figure ITGB1 3'-UT MU and C). Then, we of GB1 by qRT-PCR and detected 1 expression observe that TGB1 expression was up-regulated in



Figure 4 miR-124-3p inhibitor attenuated UCA1 silencing-mediated repression on NPC cell progression.

Notes: (A and B) Cell proliferation, (C and D) migration and invasion ability were verified in SUNE1 and C666-1 cells transfected with si-NC, si-UCA1+in-miR-NC and si-UCA1+in-miR-124-3p using CCK8 assay and transwell assay, respectively. *P<0.05.



Figure 5 ITGBI was a target gene of miR-124-3p.

Notes: (A) The putative binding sites of miR-124-3p and ITGBI were predicted by TargetScan. (B and C) Luciferase activity of SUNEI and C666-1 cells co-transfected with miR-124-3p or miR-NC and UCAI WT or UCAI MUT was determined by dual-luciferase assay. (D and E) The expression of ITGBI in NPC tissues and cells was measured by qRT-PCR. (F and G) The expression of ITGBI protein in SUNEI and C666-1 cells transfected with miR-NC, miR-124-3p, si-NC, and si-UCAI. (H) ITGBI was negatively correlated with miR-124-3p (r=-0.8917, P<0.0001). (I) UCAI was positively correlated with ITGBI (r=0.8745, P<0.0001). *P<0.051.

tumor tissues and NPC cells (SUNE1 and C666-1 cell lines) compared with the adjacent normal tissues and cells (Figure 5D and E). Subsequently, Western blot assay showed that miR-124-3p up-regulation inhibited ITGB1 protein expression whereas UCA1 down-regulation decreased ITGB1 protein expression (Figure 5F and G), revealing that UCA1 regulated ITGB1 by sponging miR-124-3p. Figure 5H and I presented that ITGB1 was positively correlated with UCA1 while inversely correlated with miR-124-3p. Therefore, we considered that ITGB1 was a target of miR-124-3p.

UCA1 Promoted Cell Progression By Up-Regulating ITGB1 Via Sponging miR-124-3p

To verify the regulatory effects of UCA1/miR-124-3p/ ITGB1 axis on NPC cell proliferation, migration and invasion, SUNE1 and C666-1 cells were transfected with si-ITGB1+in-miR-124-3p, si-ITGB1+in-miR-NC, si-ITGB1, si-UCA1, si-UCA1+pcDNA, si-UCA1 +ITGB1 and si-NC. CCK8 assay and transwell assay indicated that miR-124-3p inhibitor reversed ITGB1 silencing-mediated inhibitory effects on SUNE and C666-1 cell proliferation, migration and in sion (Figure 6A–D). Similarly, up-regulation of ITGB1 nuated UCA1 silencing-induced inhibitory fects SUNE1 and C666-1 cell progress (Figu 6E-H Taken together, UCA1 could property PC ell deven pment by regulating ITGB1 via **P**-124-3p. oonging n

Interference Of UCAI Suppressed SUNEI Xenograft Tumor Growth

Xenograft model was constructed by subcutaneously injecting SUNE1 cells stably transfected with sh-UCA1 or sh-NC. Tumor growth was suppressed largely in sh-UCA1 xenograft mice compared with sh-NC xenograft mice (Figure 7A). Tumor weight was measured when the mice were sacrificed after 28 days. Reduction of tumor weight was observed in sh-UCA1 xenograft mice (Figure 7B). Furthermore, UCA1 expression was reduced while miR-124-3p expression was called in sh-UCA1 group compared with sh-NC group (Figure 7C and D). Western blot result revealed the UTGB1 price in level was reduced in sh-UCA1 knock own hir ered turner growth in vivo.

Discussion

Recently inckers have been proposed as promising biomarkers and therap etic targets of genopathy and cancer due to its variability and abundance.¹⁹ Despite lacking proteinincoding capecity, lncRNAs play essential roles in cancer thogenesis nevelopment and malignancy via participating in genomession and transcription.^{20–22} Previous research is confirmed lncRNA LL22NC03-N64E9.1 as lung cancer prognostic biomarker since its sufficiency enhanced lung cancer cells' proliferation.²³ Likewise, lncRNA NPCCAT1 overexpression in NPC amplified NPCCAT1 genomic copy number by binding to 3'UTR YY1, resulting in accelerating cell adhesion in vivo and in vitro, while NPCCAT1



Figure 6 UCA1 promoted NPC cell progression by regulating miR-124-3p/ITGB1 axis.

Notes: (A-D) miR-124-3p inhibitor alleviated ITGB1 silencing-induced suppression on SUNE1 and C666-1 cells proliferation, migration and invasion. (E-H) Up-regulation of ITGB1 relieved UCA1 silencing-induced inhibitory effects on SUNE1 and C666-1 cell proliferation, migration and invasion. *P<0.05.



Figure 7 UCA1 depletion hindered tumor growth in vivo. Notes: (A) Tumor volume of SUNE1 xenograft mice was measured every were (P) Tumor weige to the survey of survey were sacrificed after 28 days. (C-E) The expression of UCA1, miR-124-3p and ITGB1 in tumor tissues s expression of Western blot. *P<0.05.

knockdown reversed the promotion effects ever, LncRNA-AF147447 functioned as a supp sor to hibit gastric cancer formation by binding t 22 gasun cancer.²⁵ UCA1 is located in 19p13 chromosche region and involved in cancer deterior on r cess, for e. mple, UCA1 acts as promoter to indice EMT process and enhance glioma cell invasion by spring miR-204-5p up-regulate ZEB1.²⁶ However, the ole of CA1 in NPC cell growth requires further exploratio

In our study, we exploit the underlying regulatory mechanismen UCA1 for NPC cert progression. The expression level of UCA1 was elevated in NPC tumor tissue and cell lines measure by qRT-PCR. However, UCA1 silencing led to an obvious suppression on NPC cell proliferation, suggesting that UCA1 regulated NPC cell progression positively. Dual-luciferase reporter assays' results confirmed that UCA1 exerted its function by interacting with miR-124-3p. The interaction between UCA1 and miR-124-3p was further determined by RIP assay and RNA pull-down assay. RIP assay displayed that transfection of miR-124-3p resulted in promoted enrichment of UCA1 in SUNE1 and C666-1 cells compared with transfection of miR-NC. Meanwhile, enhanced miR-124-3p expression was observed in NPC ells transfected with Bio-UCA1 WT in comparison with cells transfected with Bio-UCA1 MUT and Bio-NC. By calculation, we discovered that the UCA1 was correlated with miR-124-3p inversely. Next, rescue experiments revealed that miR-124-3p inhibitors could reverse the inhibitory effects on NPC cells induced by UCA1 silencing. Subsequently, the bioinformatics analysis prediction exhibited that the target gene of miR-124-3p was ITGB1.

Numerous evidences have revealed that ITGB1 acts as an oncogene and participates in the development of many cancers, like lung cancer, hepatocellular carcinoma and glioma.^{27–29} For instance, Zheng et al elucidated that the up-regulation of ITGB1 in clear cell renal cell carcinoma (ccRCC) has facilitated cell migration via binding the specific site of Mcl-1.³⁰ However, the decreasing of ITGB1 expression inversely reduced trophoblast cell infiltration in preeclampsia patients.³¹ Therefore, exploration of the potential mechanism of ITGB1 in NPC cell growth and migration possesses great clinical significance.

We further investigated whether miR-124-3p regulates NPC cell development by targeting ITGB1 and altering its

post-transcription process. Specifically, overexpression of ITGB1 was observed in NPC tissues and cells, suggesting the oncogenic role of ITGB1. To examine the association between miR-124-3p and ITGB1, Western blot was recruited to detect ITGB1 protein level, resulting in reduced ITGB1 protein expression in SUNE1 and C666-1 cells transfected with miR-124-3p, clarifying that ITGB1 was regulated by miR-124-3p. Similarly, ITGB1 protein expression was downregulated after UCA1 silencing, implying that UCA1 might function by modulating ITGB1 via miR-124-3p sponging. Moreover, CCK8 assay and wound healing assay revealed that up-regulation of ITGB1attenuated UCA1 silencingmediated inhibitory effect on NPC cell proliferation, migration and invasion. In vivo experiments further confirmed that UCA1 silencing inhibited NPC cells' progression through regulating miR-124-3p/TGB1 axis. In short, we discovered that UCA1 could facilitate TGB1 expression by sponging miR-124-3p and further accelerate cell progression in NPC.

Conclusion

Our study suggested that UCA1 acted as an oncogene to promote NPC cell progression by up-regulating TGB1 via sponging miR-124-3p. It is the first time to elucidate the biological mechanism of UCA1/miR-124-3p/TGB1 act for promoting NPC cell growth. Therefore, this study provided promising therapeutical targets for TCC prevtion and treatment.

Disclosure

The authors report no conflict to interest in this work.

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