LncRNA KCNQ1OT1 contributes to the cisplatin resistance of tongue cancer through the KCNQ1OT1/miR-124-3p/TRIM14 axis

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Abstract. – OBJECTIVE: Tongue cancer is a common malignant tumor in the oral and maxillofacial region, most of which is squamous cell carcinoma. Cisplatin (DDP) is one of the chemotherapy drugs for patients with tongue squamous cell carcinoma (TSCC). However, DDP resistance has become a major obstacle to its clinical application. Our study aimed to investigate the effects of long non-coding RNA (lncRNA) KCNQ1 overlapping transcript 1 (KCNQ1OT1) on DDP resistance of tongue cancer and the underlying mechanism.

PATIENTS AND METHODS: The levels of KCNQ1OT1, miR-124-3p, and tripartite motif containing 14 (TRIM14) were detected by quantitative Real Time-Polymerase Chain Reaction (qRT-PCR). The maximum size of tumor (MTS) assay was used to detect the cell survival rates. Furthermore, the cell proliferation was detected by 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2-H-tetrazolium bromide (MTT) assay. Transwell assay was performed to detect the cell migration and invasion. Western blot assay was used to detect the protein levels of Vimentin, N-cadherin, E-cadherin, and TRIM14. The functional targets of KCNQ1OT1 and miR-124-3p, miR-124-3p and TRIM14 were predicted by starBase 3.0 and TargetScan. The relationship between KCNQ1OT1 and miR-124-3p was confirmed by Dual-Luciferase reporter assay, RNA immunoprecipitation (RIP) and RNA pull-down. Further, the relationship between miR-124-3p and TRIM14 was verified by Dual-Luciferase reporter assay. Animal experiment revealed the effect of KCNQ1OT1 on DDP resistance of tongue cancer cells *in vivo*.

RESULTS: KCNQ1OT1 was upregulated in DDP-resistant tongue cancer tissues and cells, and mainly expressed in cytoplasm. Functionally, the knockdown of KCNQ1OT1 inhibited the survival rate, proliferation, migration, invasion, and EMT of the DDP-resistant tongue cancer cells. Of note, miR-124-3p acted as a target of KCNQ1OT1 and KCNQ1OT1 could reduce the expression of miR-124-3p. Moreover, miR-124-3p targeted TRIM14 and the downregulation of TRIM14 reduced the DDP resistance of tongue cancer cells. Importantly, KCNQ1OT1 regulated the TRIM14 expression by targeting miR-124-3p. Furthermore, KCNQ1OT1 knockdown reduced the DDP-resistant tumor growth and weight through the miR-124-3p/TRIM14 axis *in vivo*.

CONCLUSIONS: LncRNA KCNQ1OT1 promotes the DDP resistance of tongue cancer by sponging miR-124-3p to regulate TRIM14 expression.

Key Words:
Tongue cancer, Cisplatin resistance, KCNQ1OT1, MiR-124-3p, TRIM14.

Introduction

Tongue cancer is one of the most common oral diseases, with a growing incidence in young people and a significant geographic specificity[1]. The survival of tongue cancer has not significantly improved in recent years[2]. Cisplatin has demonstrated efficacy in a variety of solid tumors, including tongue cancer, during chemotherapy[3]. In the early stage of oral cancer, about 80.6% of patients responded to platinum drugs,

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but the drug resistance rate of DDP after subsequent treatment may still be higher than 70%[4,5]. Therefore, finding a molecular mechanism that attenuates DDP resistance is necessary for the treatment of tongue cancer.

Long non-coding RNAs (RNAs) are non-protein coding transcripts longer than 200 nucleotides, and their expression plays a role in the pathological processes of tumor growth, differentiation, and metastasis[6]. For example, the lncRNA metastasis-associated lung adenocarcinoma transcript 1 (MALAT1) increased the proliferation and metastasis of tongue cancer through miR-124/jagged1 (JAG1) axis was reported[7]. Wang et al[8] also indicated that lncRNA urothelial cancer-associated 1 (UCA1) knockdown might significantly promote DDP induced tongue squamous cell carcinoma (TSCC), cell apoptosis, and chemical sensitivity by suppressing the DDP activated phosphatidylinositol 3'-kinase (PI3K)/Akt signaling pathway. Moreover, Zhang et al[9] showed that lncRNA KCNQ1 overlapping transcript 1 (KCNQ1OT1) regulates tumor growth and DDP resistance in tongue cancer by sponging miR-211-5p to mediate Ezrin/focal adhesion kinase (FAK)/steroid receptor coactivator (Src) signaling pathway. But the precise regulation mechanism of KCNQ1OT1 on the DDP resistance in tongue cancer remains poorly investigated.

In some reports, bioinformatics data and comprehensive analysis have confirmed that lncRNA, miRNA, and mRNA interactions are involved in the formation of competing endogenous RNA (ceRNA), such as in papillary thyroid cancer[10], metastatic melanoma[11], hepatocellular carcinoma[12], and oral tongue squamous cell carcinoma (OSCC)[13]. For instance, lncRNA-MALAT1 regulated the cell proliferation and metastasis of hepatocellular carcinoma by affecting the expression of Slug (SNAI2) and by sponging miR-124-3p[14]. Moreover, a previous study indicated that the downregulation of the expression of miR-200b and miR-15b promoted the DDP-induced epithelial-mesenchymal transition (EMT) in tongue cancer cells[15]. Zhang et al[16] suggested that lncRNA UCA1 affected the transforming growth factor-β1 (TGFβ1)-induced EMT and invasion by regulating the miR-124 through jagged 1 (JAG1)/Notch signaling in tongue cancer cells. These findings indicated that miR-124-3p might have a potential function in the DDP resistance of tongue cancer.

Tripartite motif containing 14 (TRIM14), which contains a B-box, a coiled-coil region, and a C-terminal PRYSPRY region, involve in cell apoptosis, cell cycle regulation, and viral response[17]. Hu et al[18] indicated that TRIM14 promoted the proliferation and inhibited the apoptosis of human breast cancer cells and might be used as an indicator of cancer prognosis in breast cancer patients. Wang et al[19] showed that TRIM14 induced the formation of cancer-initiating cells and EMT in TSCC regulated by miR-15b, and TRIM14 overexpression facilitated the DDP resistance in TSCC. However, whether TRIM14 involved in the regulations of cell development and drug resistance was not fully reported.

In this study, the functional effects of KCNQ1OT1 on proliferation and metastasis of DDP-resistant tongue cancer cells were detected by gain-of-function and loss-of-function experiments. The targeting relationship between KCNQ1OT1 and miR-124-3p or miR-124-3p and TRIM14 was firstly verified in our research.

Patients and Methods

Patients and Specimens

Tongue cancer samples were classified as cisplatin sensitive or insensitive according to the patient's prognosis[20]. The classification of tumor samples was determined by two pathologists according to WHO standards (2004)[21]. The tissues of chemo-sensitive patients and chemo-insensitive patients were taken from the School and Hospital of Stomatology, Jilin University. The written consent has been informed by every tongue cancer patient. The research obtained approval from the Ethics Committee of the School and Hospital of Stomatology, Jilin University.

Cell Culture

Tongue cancer cell lines CAL27 and SCC9 were purchased from the Beijing Concorde Cell Library (Beijing, China). Dulbecco's Modified Eagle's Medium (DMEM; HyClone, South-Logan, UT, USA) and 10% fetal bovine serum (FBS; HyClone, South-Logan, UT, USA) were used for providing nutrients for cell growth, and CAL27 and SCC9 cells were cultured in an incubator at 37°C with 5% CO_2. To establish cisplatin-resistant tongue cancer cells, we routinely added cisplatin concentrations to the medium every other day to treat the cells and remove them before the experiment.

Cell Transfection

Small interfering RNA (siRNA) targeting KCNQ1OT1#1 (si-KCNQ1OT1#1), si-KCNQ1OT1#2, si-KCNQ1OT1#3, siRNA targeting TRIM14 (si-TRIM14) and siRNA negative control (si-NC), miR-124-3p inhibitor and the negative control (inhibitor-NC), the miR-124-3p mimics (miR-124-3p) and the negative control mimics (miR-NC), short hairpin RNA (shRNA) targeting KCNQ1OT1 (sh-KCNQ1OT1) and shRNA negative control (sh-NC) were constructed by Biomics Biotechnology (Jiangsu, China). Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA) was performed for the transfection of these oligonucleotides.

RNA Isolation and Quantitative Real Time-Polymerase Chain Reaction (qRT-PCR)

The extraction of the total RNA from tongue cancer patients and cell lines was performed by the TRIzol reagent (TaKaRa, Dalian, China). Then, Nanodrop 2000 (Thermo Fisher Scientific, Waltham, MA, USA) and gel electrophoresis (Sigma-Aldrich, St. Louis, MO, USA) were used to test the quality and quantity of the total RNAs. RT-PCR kit (Invitrogen, Carlsbad, CA, USA) was used for reverse transcription experiments. The qRT-PCR was carried out with the ABI SYBR Green Master Mix (Invitrogen, Carlsbad, CA, USA). The primers used were as follows: Forward 5'-AGGGTGACAGTGTTTCATAGGCT-3' and reverse 5'-GAGGCACATTCATTCGTTGGT-3' for KCNQ1OT1. Forward 5'-AGCACAGAGCCTCGCCTTTG-3' and reverse 5'-CTTCTGACCCATGCCCACCA-3' for β-actin. Forward 5'-CGATACAGAGAAGATTAGCATGGC-3' and reverse 5'-AACGCTTCACGAATTTGCGT-3' for U6[22]. Forward 5'-TGTGATGAAAGACGGCACAC-3' and reverse 5'-CTTCCTTTGGGTATTGTTTGG-3' for miR-124-3p[23]. Forward 5'-GGATTTGTGTCTCCGTTCTG-3' and reverse 5'-TCTGTCTGCCTGGTATTCTG-3' for tripartite motif containing 14 (TRIM14)[24].

Cell Proliferation Assay

Cell proliferation was detected by the maximum size of tumor (MTS) assay and 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2-H-tetrazolium bromide (MTT) assay. For the MTS assay, the MTS assay kit (Abcam, Cambridge, MA, USA) was performed. In briefly, the cells were inoculated into a 96-well plate and were cultivated for 6 d at 37°C; then, each well was added to 20 µl MTS solution and incubated for 1 h at 37°C. Finally, the microplate reader (Bio-Rad, Hercules, CA, USA) was employed to detect the optical density at 490 nm. For the MTT assay, the cells were seeded in 96-well plates and were incubated for 24 h; after that, 20 µL (5 mg/mL) MTT (Invitrogen, Carlsbad, CA, USA) was added, and 200 µL dimethyl sulfoxide (DMSO; Invitrogen, Carlsbad, CA, USA) was used for dissolving the intracellular formazan crystals. Finally, the microplate reader was used to detect the absorbance value at 490 nm.

Transwell Assay

After 48 h of transfection, the CAL27/DDP and SCC9/DDP cells were collected and suspended in the serum-free medium. To detect cell invasion, Matrigel (Corning, Corning, NY, USA) was used to cover the upper transwell chamber (Corning, Corning, NY, USA), while for the cell migration detection, these chambers do not require any treatment. Then, CAL27/DDP and SCC9/DDP cells were cultured into the upper chamber and incubated for 12 h. Subsequently, the cells were incubated with 0.1% crystal violet (Corning, Corning, NY, USA) for 20 min, and then, the cells on the upper layer of the chamber were removed. Finally, the inverted optical microscope was used to detect the number of cells with three random fields.

Western Blot Analysis

The RIPA lysis buffer (Sangon Biotech, Shanghai, China) was taken to dissolve protein samples. Total protein extraction was performed based on the instructions. After protein quality and quantity measurement, the proteins were loaded into freshly prepared sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Then, the proteins were blotted onto the membranes (Thermo Fisher Scientific, Waltham, MA, USA) and incubated with 5% skimmed milk, and the membrane was incubated with primary anti-β-actin antibody (1:1000, ab8227, Abcam, Cambridge, MA, USA), anti-Vimentin antibody (1:1000, ab92547, Abcam, Cambridge, MA, USA), anti-N-cadherin antibody (1:1000, ab18203, Abcam, Cambridge, MA, USA), anti-E-cadherin antibody (1:1000, ab202413, Abcam, Cambridge, MA, USA), anti-TRIM14 an-

tibody (1:1000, ab185349, Abcam, Cambridge, MA, USA). Finally, the goat anti-rabbit secondary antibody (1:5000, ab150077, Abcam, Cambridge, MA, USA) was added and the chemiluminescent image was viewed on Kodak film developer (Fujifilm, Tokyo, Japan).

Dual-Luciferase Reporter Assays

The wild type KCNQ1OT1 sequences (WT-KCNQ1OT1), mutant type KCNQ1OT1 sequences (MUT-KCNQ1OT1), wild type 3'Untranslated Region (UTR) TRIM14 sequence (WT-TRIM14) or mutant type 3'UTR TRIM14 sequence (MUT-TRIM14) were constructed and inserted into pGL-3 plasmid (GenomediTech, Shanghai, China), Then, all the vectors were transfected into CAL27/DDP and SCC9/DDP cells with miR-124-3p or miR-NC by Lipofectamine 2000 (Thermo Fisher Scientific, Waltham, MA, USA). Finally, the Dual-Luciferase reporter assay system (GenomediTech, Shanghai, China) was used to detect Luciferase activity.

RNA Immunoprecipitation (RIP) and RNA Pull-Down

The Magna RIP RNA-binding Protein Immunoprecipitation Kit (Gzscbio, Guangzhou, China) was used to confirm the relationship between KCNQ1OT1 and miR-124-3p. In brief, the magnetic beads and anti-Argonaute-2 (Ago2) antibody (ab32381, Abcam, Cambridge, MA, USA) were added into the cells. After 24 h incubation, the proteinase K and the phenol-chloroform-isoamyl alcohol reagent were used for purifying RNAs. Finally, the KCNQ1OT1 enrichment was detected by qRT-PCR.

For the RNA pull-down assay, firstly, the biotin label joins miR-124-3p to establish the Bio-miR-124-3p and Bio-miR-NC as the negative control. Then, the Radio-Immunoprecipitation Assay (RIPA) lysis buffer (Sangon, Shanghai, China) and RNase inhibitor (Invitrogen, Carlsbad, CA, USA) were used for cell lysing, the Bio-miR-124-3p or Bio-miR-NC was added to the supernatant, and then, streptavidin beads were incubated (Invitrogen, Carlsbad, CA, USA) and proteinase K was used to isolating RNA. Finally, qRT-PCR was used to detect the KCNQ1OT1 enrichment.

Animal Experiments

The 4-week-old BALB/c male nude mice (Guangdong Medical Lab Animal Center, Guangzhou, China) were raised in a sterile environment for operations. Then, the cells (2×10^6) transfected with sh-KCNQ1OT1 were suspended by phosphate-buffer saline (PBS), and the cells inoculated subcutaneously into the nude mice. Finally, the xenografted tongue tumor volume was detected every 7 d and the tumor weight was detected in 35 d. The animal experiment was approved by the Animal Experimentation Ethics Committee of School and Hospital of Stomatology, Jilin University.

Statistical Analysis

The software GraphPad Prism 7 (La Jolla, CA, USA) was used to statistical analysis and the data was shown as mean ± standard deviation (SD). The Student's *t*-test was employed for data comparison between two groups and One-way analysis of variance (ANOVA) for comparisons among at least three groups. $*p<0.05$ was considered as statistically significant.

Results

KCNQ1OT1 was Upregulated in DDP-Resistant Tongue Cancer Tissues and Cells, and Mainly Expressed in Cytoplasm

To begin with, qRT-PCR was performed to detect the level of KCNQ1OT1 in DDP chemo-insensitive tongue cancer tissues and cells. The results showed that KCNQ1OT1 was significantly upregulated in chemo-insensitive tissues, DDP-resistant CAL27 (CAL27/DDP) cells, and DDP-resistant SCC9 (SCC9/DDP) cells compared with that in chemo-sensitive tissues, CAL27 cells and SCC9 cells (Figure 1A and B). Furthermore, the KCNQ1OT1 was mainly expressed in cytoplasm of CAL27/DDP and SCC9/DDP cells, which was detected by qRT-PCR (Figure 1C and D). These results suggested that KCNQ1OT1 might involve in the regulation of the resistance in DDP-resistant tongue cancer cells.

KCNQ1OT1 Knockdown Reduced the DDP Resistance of Tongue Cancer Cells

To further explore the functional effect of KCNQ1OT1 in DDP-resistant tongue cancer cells, the si-KCNQ1OT1#1, si-KCNQ1OT1#2, and si-KCNQ1OT1#3 were obtained, and qRT-PCR was used to detect the transfection efficiency of si-KCNQ1OT1#1, si-KCNQ1OT1#2, and si-KCNQ1OT1#3 in CAL27/DDP and SCC9/DDP cells (Figure 2A and B). Further, we selected the si-KCNQ1OT1#3 for the next exper-

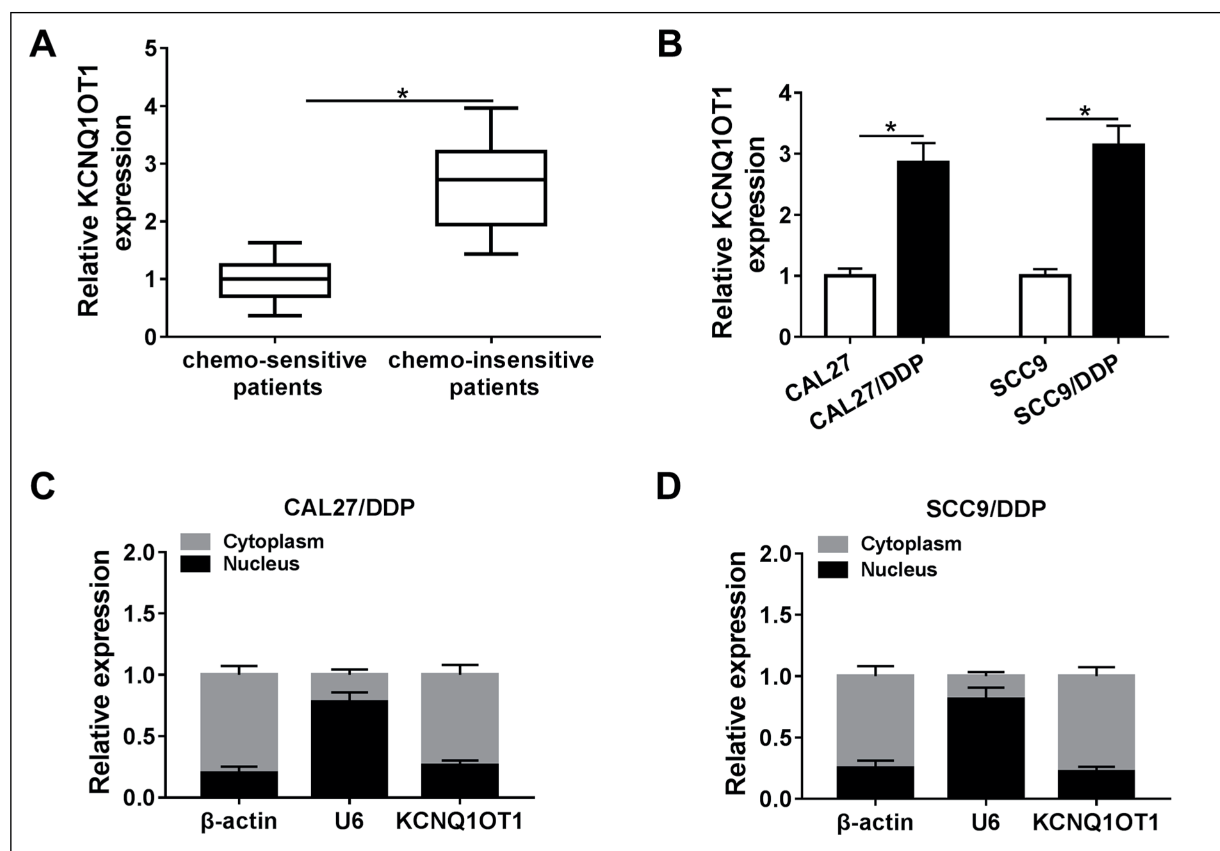

Figure 1. KCNQ1OT1 was upregulated in DDP-resistant tongue cancer tissues and cells, and mainly expressed in cytoplasm. **A,** The expression of KCNQ1OT1 in chemo-sensitive tissues (n = 30) and chemo-insensitive tissues (n = 30) was detected by qRT-PCR. **B,** qRT-PCR was used to measure the level of KCNQ1OT1 in CAL27 cells, DDP-resistant CAL27 (CAL27/DDP) cells, SCC9 cells and DDP-resistant SCC9 (SCC9/DDP) cells. **C,** and **D,** The proportion of KCNQ1OT1 in CAL27/DDP and SCC9/DDP cells was detected by qRT-PCR. *p<0.05.

iment. The MTS survival assay revealed that KCNQ1OT1 knockdown was significantly related with a lower rate of overall survival of CAL27/DDP and SCC9/DDP cells (Figure 2C and D). In addition, the MTT assay was performed. The results showed that the proliferation was downregulated in CAL27/DDP and SCC9/DDP cells transfected with si-KCNQ1OT1#3 (Figure 2E and F). Moreover, transwell assay demonstrated that KCNQ1OT1 silencing markedly decreased the migration rate and invasion rate of CAL27/DDP and SCC9/DDP cells (Figure 2G-J). Western blot was used to detected the protein levels of EMT-related proteins in CAL27/DDP and SCC9/DDP cells transfected with si-KCNQ1OT1#3, and the results found that the epithelial marker E-cadherin was upregulated while the mesenchymal markers N-cadherin and Vimentin were significantly downregulated (Figure 2K and L). These data revealed that KCNQ1OT1 knockdown reduced the CAL27/DDP and SCC9/DDP cells survival, proliferation, migration, invasion, and EMT process.

KCNQ1OT1 Targeted MiR-124-3p and Inhibited the MiR-124-3p Expression

Of note, the online software starBase 3.0 detected the potential binding sites between KCNQ1OT1 and miR-124-3p (Figure 3A). Next, to confirm their relationship the Dual-Luciferase reporter assay, RIP assay, and RNA pull-down assay were performed. The results showed that the Luciferase activity of WT-KCNQ1OT1 was reduced by the miR-124-3p, but the MUT-KCNQ1OT1 was not changed in CAL27/DDP and SCC9/DDP cells (Figure 3B and C). Similarly, the RIP and RNA pull-down assay further demonstrated the directly relationship between KCNQ1OT1 and miR-124-3p because a large amount of KCNQ1OT1 was measured in these

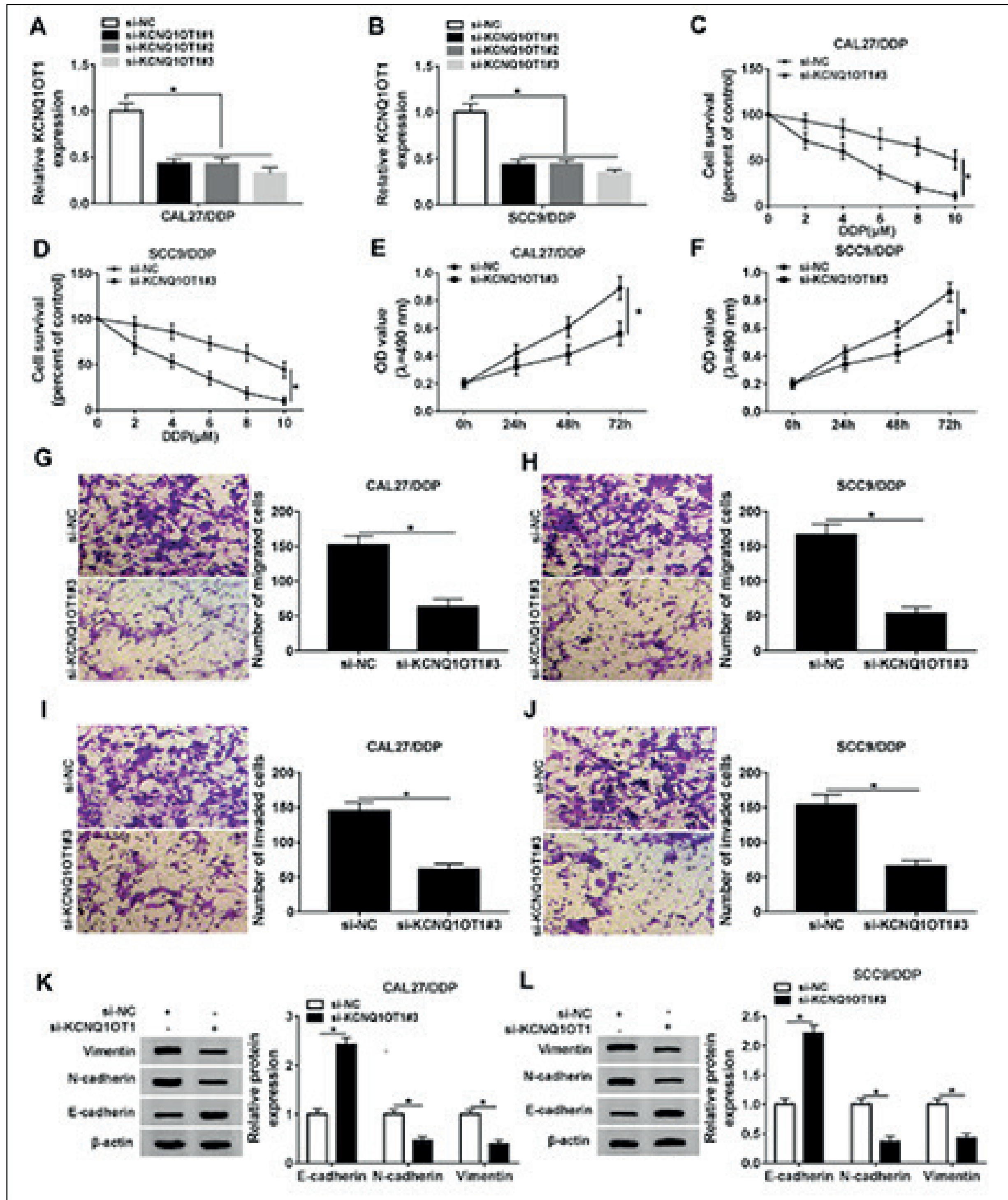

Figure 2. KCNQ1OT1 knockdown reduced the DDP resistance of DDP-resistant tongue cancer cells. **A,** and **B,** qRT-PCR was used to detect the transfection efficiency of si-KCNQ1OT1#1, si-KCNQ1OT1#2 and si-KCNQ1OT1#3 in CAL27/DDP and SCC9/DDP cells. **C,** and **D,** MTS assay was used to measure the survival rates of CAL27/DDP and SCC9/DDP cells transfected with si-NC or si-KCNQ1OT1#3. **E,** and **F,** The proliferation of CAL27/DDP and SCC9/DDP cells transfected with si-NC or si-KCNQ1OT1#3 was detected by MTT assay. **G-J,** Transwell assay was performed to measure the migration and invasion of CAL27/DDP and SCC9/DDP cells transfected with si-NC or si-KCNQ1OT1#3 (×200). **K,** and **L,** The EMT-related proteins (E-cadherin, N-cadherin and Vimentin) in CAL27/DDP and SCC9/DDP cells transfected with si-NC or si-KCNQ1OT1#3 were detected by Western blot. * $p<0.05$.

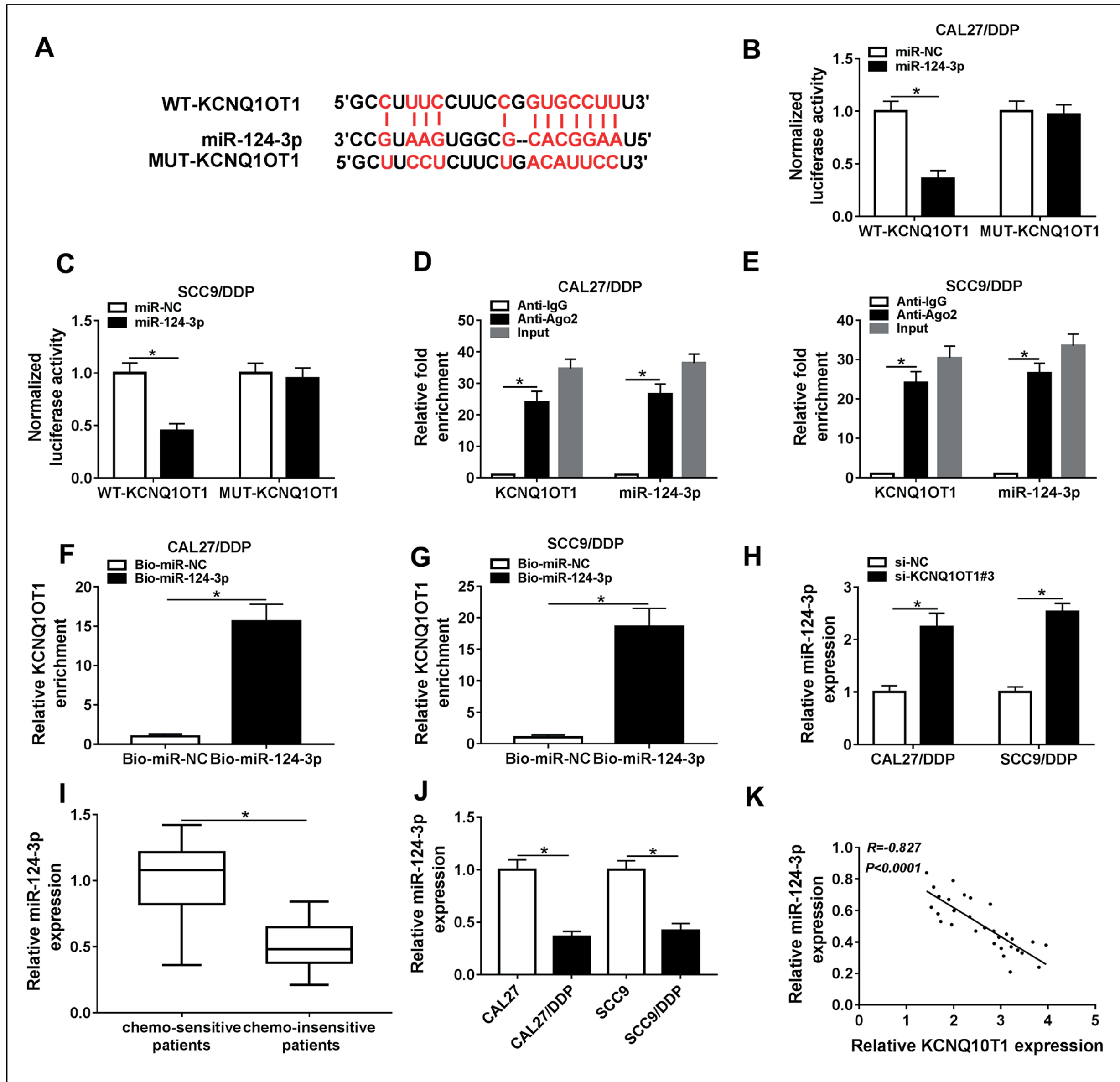

Figure 3. KCNQ1OT1 targeted miR-124-3p and inhibited the miR-124-3p expression. **A,** The potential binding target between KCNQ1OT1 and miR-124-3p was predicted by starBase 3.0. **B,** and **C,** The Dual-Luciferase reporter assay was used to verify the relationship between KCNQ1OT1 and miR-124-3p. **D-G,** RIP assay and RNA pull-down assay were performed to confirm the interaction of KCNQ1OT1 and miR-124-3p. (H) The expression of miR-124-3p in CAL27/DDP and SCC9/DDP cells transfected with si-NC or si-KCNQ1OT1#3 was detected by qRT-PCR. **I,** and **J,** The level of miR-124-3p in chemo-sensitive tissues (n = 30), chemo-insensitive tissues (n = 30), CAL27 cells, CAL27/DDP cells, SCC9 cells and SCC9/DDP cells was detected by qRT-PCR. **K,** A negative correlation between the expression of KCNQ1OT1 and miR-124-3p was analyzed by Pearson's correlation coefficient (R = -0.827, p<0.0001). * p<0.05.

two assays (Figure 3D-G). Thus, our research also focused on the function of miR-124-3p in CAL27/DDP and SCC9/DDP cells. Notably, the expression of miR-124-3p in CAL27/DDP and SCC9/DDP cells transfected with si-KCNQ1OT1#3 was up-regulated (Figure 3H). However, the expression of miR-124-3p was reduced in both chemo-insensitive tissues and CAL27/DDP and SCC9/DDP cells (Figure 3I and J). A striking negatively correlation between the expression of KCNQ1OT1 and miR-124-3p was also observed (R = -0.827, p<0.0001) (Figure 3K).

TRIM14 Acted as a Target of MiR-124-3p

The online software TargetScan showed the TRIM14 3'Untranslated Region (UTR) contain-

ing the potential binding site of miR-124-3p (Figure 4A). Then, the relationship between miR-124-3p and TRIM14 was verified by Dual-Luciferase reporter assay; the results revealed that the Luciferase activity of WT TRIM14 3'UTR Luciferase vector was significantly reduced by miR-124-3p in CAL27/DDP and SCC9/DDP cells, while the Luciferase activity of MUT TRIM14 3'UTR Luciferase vector was not changed (Figure 4B and C). More importantly, qRT-PCR and Western

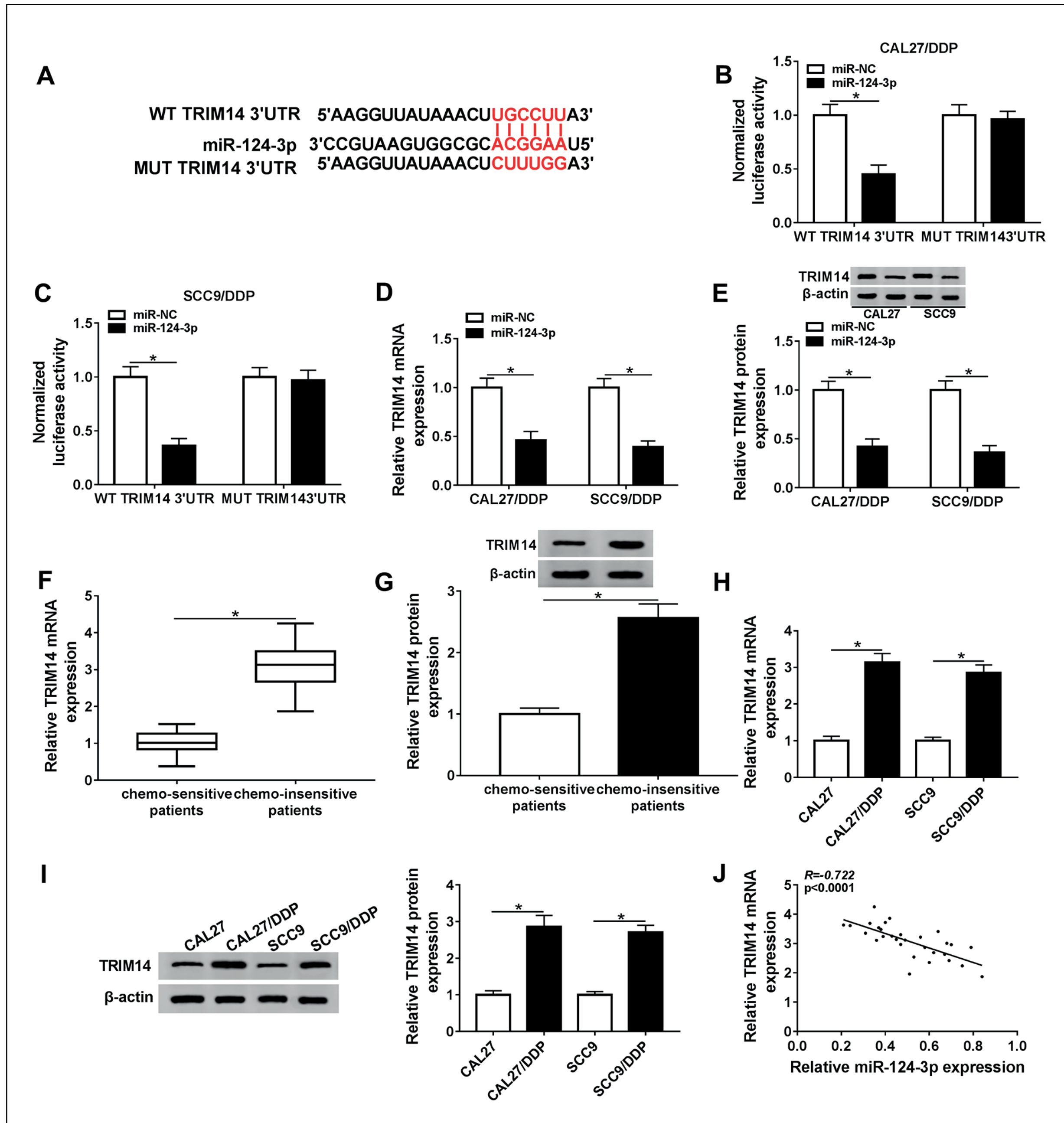

Figure 4. TRIM14 acted as a target of miR-124-3p. **A,** The online software TargetScan revealed the potential binding site between TRIM14 3'UTR and miR-124-3p. **B,** and **C,** The relationship between TRIM14 3'UTR and miR-124-3p was verified by the dual-luciferase reporter assay. **D,** and **E,** The expression of TRIM14 in CAL27/DDP and SCC9/DDP cells transfected with miR-124-3p was detected by qRT-PCR. **F-I,** The mRNA and protein level of TRIM14 in chemo-sensitive tissues (n = 30), chemo-insensitive tissues (n = 30), CAL27 cells, CAL27/DDP cells, SCC9 cells and SCC9/DDP cells was detected by qRT-PCR and Western blot. **J,** Pearson's correlation coefficient was used to analysis the correlation between the expression of TRIM14 and miR-124-3p (R = -0.722, p<0.0001). * $p < 0.05$.

blot demonstrated that the expression of TRIM14 was inhibited by miR-124-3p in CAL27/DDP and SCC9/DDP cells (Figure 4D and E). Moreover, the mRNA and protein levels of TRIM14 were increased in chemo-insensitive tissues and CAL27/DDP and SCC9/DDP cells (Figure 4F-I). Finally, the negatively correlation between the expression of miR-124-3p and TRIM14 was also determined (Figure 4J).

Downregulation of TRIM14 Reduced the Survival Rate, Proliferation and Metastasis of CAL27/DDP and SCC9/DDP Cells

Based on this evidence, it was of great significance to further study the function of TRIM14. Firstly, qRT-PCR and Western blot were used to detect the transfection efficiency of si-TRIM14 in CAL27/DDP and SCC9/DDP cells (Figure 5A and B). Then, the MTS assay revealed that the cell survival of CAL27/DDP and SCC9/DDP cells transfected with si-TRIM14 was gradually reduced as the DDP content was increased (Figure 5C and D). In addition, MTT assay and transwell assay were performed; the results showed that the proliferation, migration, and invasion of CAL27/DDP and SCC9/DDP cells transfected with si-TRIM14 were decreased (Figure 5E-H). Western blot assay indicated that the expression level of E-cadherin was upregulated, while the levels of N-cadherin and Vimentin were downregulated in CAL27/DDP and SCC9/DDP cells transfected with si-TRIM14 (Figure 5I-L), thus suggesting that TRIM14 inhibition suppressed the EMT process in DDP-resistant tongue cancer cells.

KCNQ1OT1 Regulated the TRIM14 Expression Through Targeting MiR-124-3p

We aimed to confirm whether KCNQ1OT1 affected DDP-resistant tongue cancer cells progression through a miR-124-3p/ TRIM14 axis. MiR-124-3p inhibitor was constructed, and the expression level of TRIM14 was detected by qRT-PCR and Western blot. The results revealed that TRIM14 was significantly downregulated in CAL27/DDP and SCC9/DDP cells transfected with si-KCNQ1OT1#3. However, miR-124-3p inhibitor reversed the inhibitory effect of si-KCNQ1OT1#3 on TRIM14 expression in CAL27/DDP and SCC9/DDP cells (Figure 6A-D). These data demonstrated that KCNQ1OT1 affected the expression of TRIM14 by directly targeting miR-124-3p.

KCNQ1OT1 Knockdown Reduced the DDP-Resistant Tumor Growth and Weight Through the MiR-124-3p/TRIM14 Axis In Vivo

To further explore the functional effects of KCNQ1OT1 on DDP resistance of tongue cancer cells *in vivo*, the xenograft tumor model was established. The tumor volume was measured every 7 d, and the results showed that it was markedly reduced in DDP treated cells transfected with sh-KCNQ1OT1 (Figure 7A). Next, the tumor weight in DDP treated cells transfected with sh-KCNQ1OT1 resulted in a significant downregulation detected in 35 days (Figure 7B). Finally, qRT-PCR was used to detect the expression of KCNQ1OT1, miR-124-3p, and TRIM14; the results showed that the expression of KCNQ1OT1 and TRIM14 was decreased, while miR-124-3p was raised in DDP treated cells transfected with sh-KCNQ1OT1 compared with DDP treatment cells or control cells *in vivo* (Figure 7C).

Discussion

Although there have been many advances in radiotherapy and surgery for patients with tongue cancer, the poor prognosis can have a significant impact on their quality of life[25]. In the treatment of tongue cancer resection, the reconstruction of the free flap of the oral tongue or the base of the tongue becomes a difficult problem, and after the operation, the early voice and swallowing function significantly decreased and slowly recovered[26]. DDP is considered to be one of the most effective antineoplastic chemotherapy drugs; however, because of the DDP toxicity of normal tissue and the body resistance, its application is restricted[27]. Therefore, it is of considerable significance to investigate the molecular mechanism of reducing cisplatin resistance in patients with tongue cancer.

Li et al[28] showed that lncRNA KCNQ1OT1 facilitated the resistance of oxaliplatin by regulating autophagy-related gene 4B (ATG4B) through miR-34a in colon cancer cells. KCNQ1OT1 also contributed to the methotrexate resistance of colorectal cancer by regulating the gene coding for dopamine-regulated and cAMP-regulated phosphoprotein (PPP1R1B) by sponging miR-760[29]. Our study found that the KCNQ1OT1 expression was increased in DDP-resistant tongue cancer tissues and cells, and KCNQ1OT1 was mainly expressed in the cytoplasm. On the func-

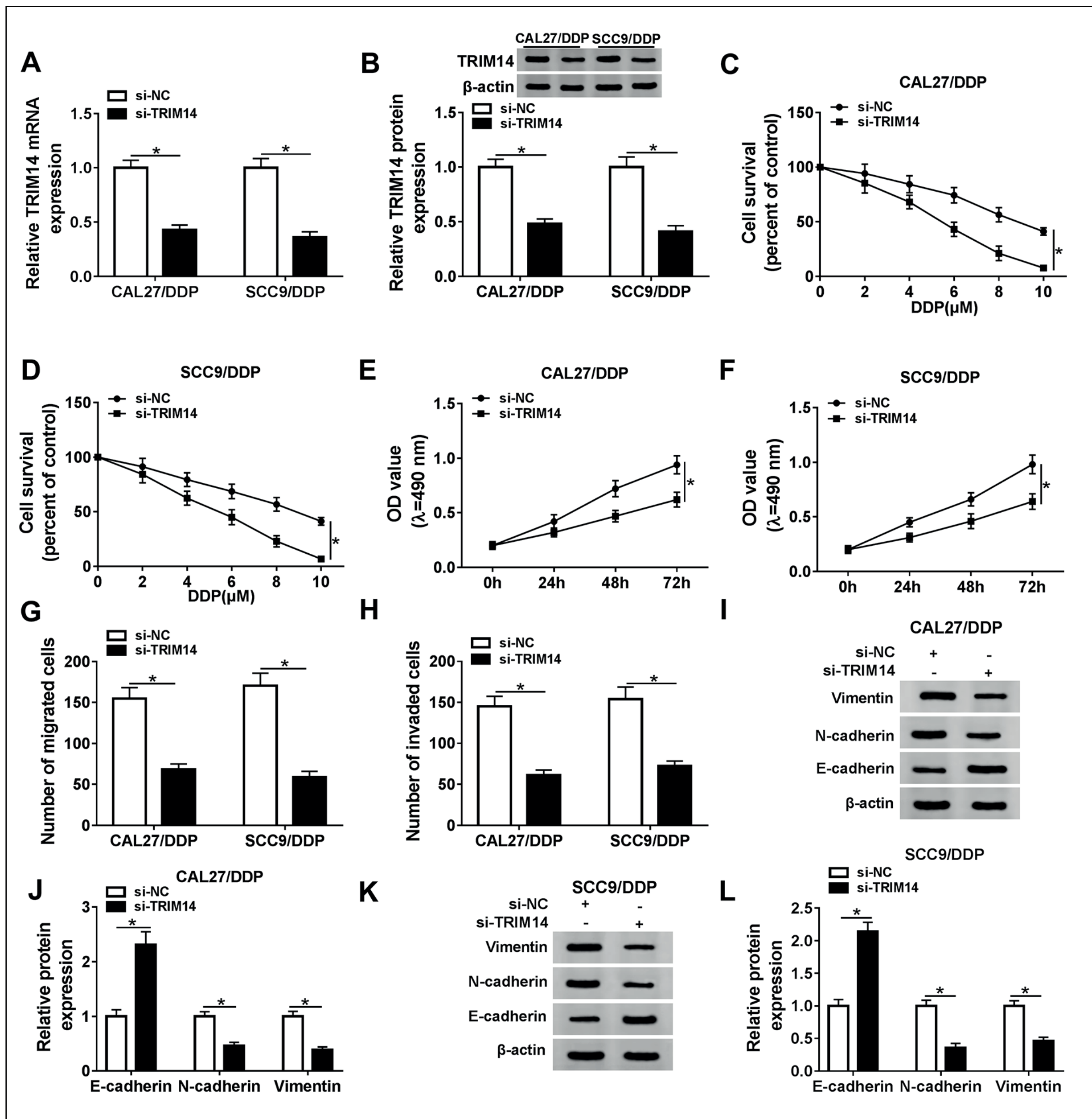

Figure 5. Downregulation of TRIM14 reduced the survival rate, proliferation and metastasis of CAL27/DDP and SCC9/DDP cells. **A,** and **B,** The transfection efficiency of si-TRIM14 in CAL27/DDP and SCC9/DDP cells was detected by qRT-PCR and Western blot. **C,** and **D,** MTS assay was used to detect the survival rates of CAL27/DDP and SCC9/DDP cells transfected with si-TRIM14. **E,** and **F,** The proliferation of CAL27/DDP and SCC9/DDP cells transfected with si-TRIM14 was measured by MTT assay. **G,** and **H,** Transwell assay was used to detect the migration and invasion of CAL27/DDP and SCC9/DDP cells transfected with si-TRIM14. **I-L,** Western blot was used to test the levels of E-cadherin, N-cadherin and Vimentin in CAL27/DDP and SCC9/DDP cells transfected with si-TRIM14. *p<0.05.

tion, the downregulation of KCNQ1OT1 inhibited the CAL27/DDP and SCC9/DDP cells survival, proliferation, migration, and invasion. Kalluri et al[30] indicated that the epithelial marker E-cadherin and the mesenchymal markers N-cadherin and Vimentin were thought to be the marker proteins of EMT. The results also demonstrated that the KCNQ1OT1 knockdown suppressed the EMT process of DDP resistant tongue cancer cells.

LncRNA has been described as an endogenous sponge, which directly binds to miRNA and regulates the molecular function[31,32]. To further

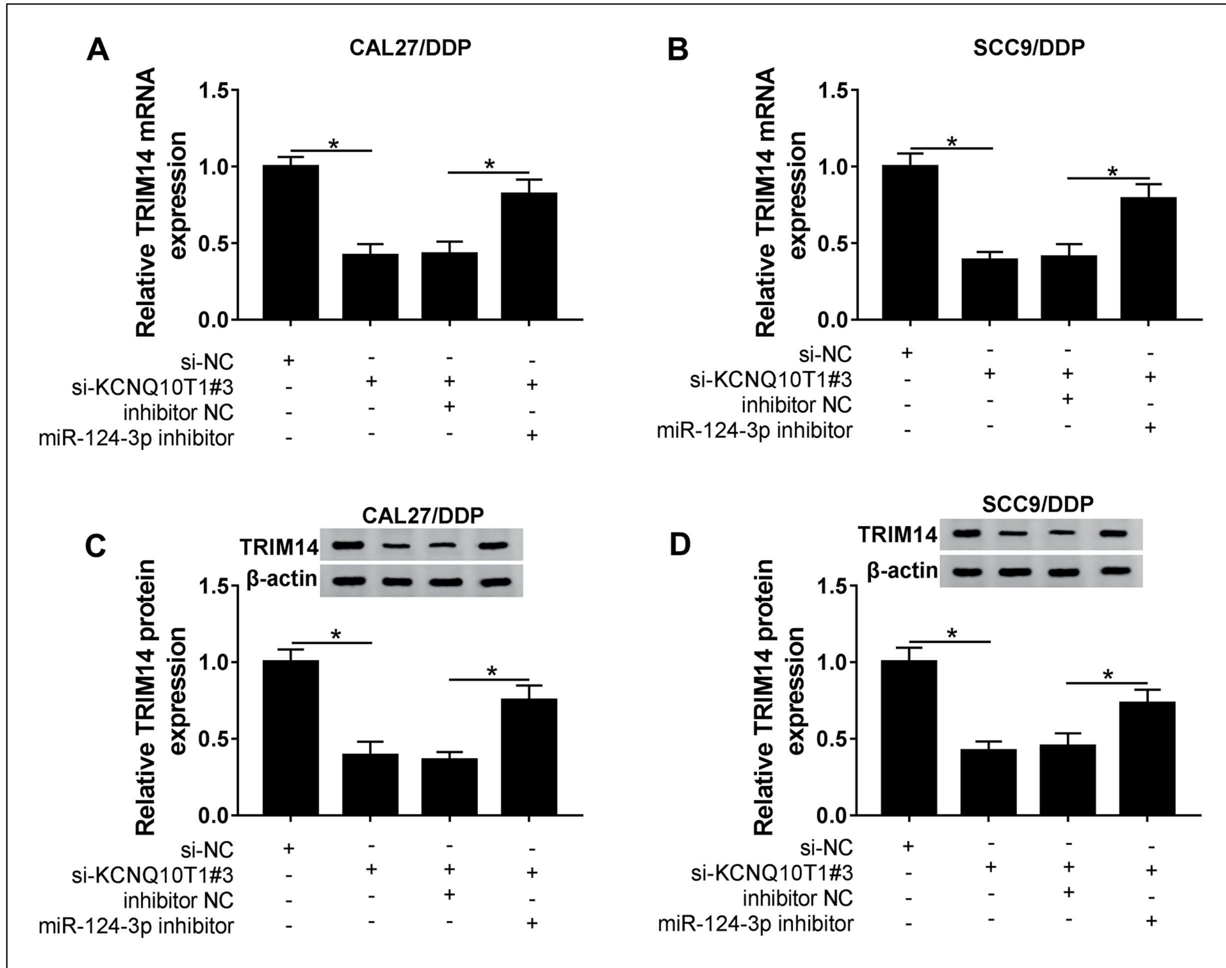

Figure 6. KCNQ1OT1 regulated the TRIM14 expression through targeting miR-124-3p. **A,** and **B,** qRT-PCR was used to detect the expression of TRIM14 in CAL27/DDP and SCC9/DDP cells transfected with si-KCNQ1OT1#3 or si-KCNQ1OT1#3 + miR-124-3p inhibitor. **C,** and **D,** The protein levels of TRIM14 in CAL27/DDP and SCC9/DDP cells transfected with si-KCNQ1OT1#3 or si-KCNQ1OT1#3 + miR-124-3p inhibitor were measured by Western blot. *$p<0.05$.

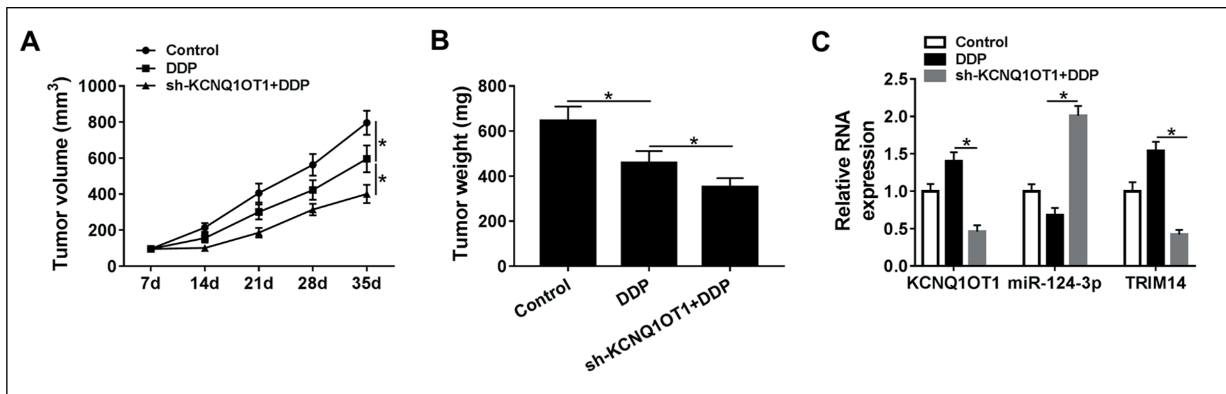

Figure 7. KCNQ1OT1 knockdown reduced the DDP-resistant tumor growth and weight through the miR-124-3p/TRIM14 axis *in vivo*. CAL27/DDP cells transfected with sh-KCNQ1OT1 were inoculated subcutaneously into the nude mice to establish the xenograft tumor. Then, the PBS or 3 mg/kg DDP were injected subcutaneously into the mice every 3 d. **A,** The tumor volume was measured every 7 d after injection. **B,** Tumor weight was measured on d 35. **C,** The expression of KCNQ1OT1, miR-124-3p and TRIM14 in xenograft tumor was detected by qRT-PCR. *$p < 0.05$.

explore whether KCNQ1OT1 acted as a sponge of miRNA, the starBase 3.0 was performed. The results indicated that KCNQ1OT1 contained binding sites of miR-124-3p, which was then verified by the Dual-Luciferase reporter assay. A previous document showed that miR-124-3p played a tumor suppressor role in breast cancer and Cbl proto-oncogene; E3 ubiquitin protein ligase (CBL) acted as its target gene to participate in the inhibition of cell proliferation and invasion[33]. Furthermore, it was found that KCNQ1OT1 negatively regulated the miR-124-3p expression, and TRIM14 acted as a target of miR-124-3p. Feng et al[34] indicated that TRIM14 facilitated the EMT process in glioblastoma cells. Similar to these findings, the data in our research showed that the TRIM14 knockdown suppressed the survival rate, proliferation, migration, invasion, and EMT of CAL27/DDP and SCC9/DDP cells. More importantly, KCNQ1OT1 regulated the TRIM14 expression by targeting miR-124-3p. In animal experiments, the downregulation of KCNQ1OT1 expression inhibited the DDP-resistant tumor growth and weight through the miR-124-3p/TRIM14 axis.

Conclusions

This study indicated that the expression of KCNQ1OT1 in DDP-resistant tongue cancer tissues and cells was higher than tongue cancer tissues and cells, while the KCNQ1OT1 expression inhibition resulted in a suppression of DDP-resistant tongue cancer cells survival rate, proliferation, and metastasis, suggesting that KCNQ1OT1 knockdown decreased the DDP resistance of tongue cancer cells. Moreover, miR-124-3p acted as a target of KCNQ1OT1, which could inhibit the expression of miR-124-3p. Of note, miR-124-3p directly targeted TRIM14 and the downregulation of TRIM14 inhibited the survival rate, proliferation, migration, invasion, and EMT process of CAL27/DDP and SCC9/DDP cells. More importantly, KCNQ1OT1 regulated the TRIM14 expression by sponging miR-124-3p. Furthermore, KCNQ1OT1 inhibition also reduced the DDP-resistant tumor volume and weight through the miR-124-3p/TRIM14 axis *in vivo*. Collectively, the study revealed that KCNQ1OT1 promoted the cisplatin resistance of tongue cancer by affecting TRIM14 mediated by miR-124-3p. These results provide references for reducing cisplatin resistance and developing new diagnostic and therapeutic tools for tongue cancer.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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