European Review for Medical and Pharmacological Sciences 2020; 24: 189-199

Inhibitory effect of the Notch pathway-inhibitor DAPT on invasion and metastasis of tongue cancer *via* lncRNA-KAT14 regulation

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Abstract. – OBJECTIVE: We aimed to identify a reliable biomarker for tongue squamous cell carcinoma (TSCC), the most common oral cancer with no established biomarkers, to predict prognosis and to select the optimal treatment.

MATERIALS AND METHODS: To investigate whether DAPT exhibited antitumor functions, CAL-27 and SCC-9 cells were treated with DAPT (5 μM or 10 μM) for different times. Further, qRT-PCR was used to determine the mRNA expression levels of lncRNA-KAT14 after treatment with DAPT or si-KAT14 and both combined. Moreover, the treated cells were cultured for different times to investigate their antitumor function. The Wound-healing and Transwell assay were carried out to evaluate the migration and invasion viability of cancer cells, respectively. Finally, the Western blots were performed to determine the expression of EMT-related proteins after transfection with si-KAT14 or treatment with DAPT to investigate the effects of DAPT on EMT-related proteins.

RESULTS: Proliferation was inhibited after treatment with DAPT, and the expression of lncRNA-KAT14 was upregulated. To investigate the correlation of DAPT and lncRNA-KAT14 on the metastasis and invasion in tongue cancer, the following cellular processes were assessed: proliferation, invasion, and migration ability. The Western blots were used to determine the expression of E-cadherin, N-cadherin, Vimentin, and Snail, showing that DAPT or lncRNA-KAT14 suppressed all these processes, inducing a decreased expression of N-cadherin, Vimentin, and Snail, and increased expression of E-cadherin, compared with the control group. Once transfection with si-KAT14 occurred, the evaluated cellular processes were enhanced, being this attenuated by the treatment with DAPT.

CONCLUSIONS: Our results suggest that DAPT suppresses invasion and metastasis of tongue cancer by regulating lncRNA-KAT14.

Introduction

An increasingly amount of patients are diagnosed worldwide with oral cancer, and even more than half of them die annually because of this disease[1,2]. The tongue squamous cell carcinoma (TSCC) accounts for up to 90% of all cases and became one of the major causes of cancer-related mortality of oral cancer all around the world[3]. Bad prognosis, high metastasis rate, and short survival time are the most common characteristics in the majority of patients with TSCC, despite having received a systematic treatment[4]. Hence, accumulating evidence suggests that the metastasis capability of tumor plays an important role in these events.

It is reported that the epithelial-mesenchymal transition (EMT), which is a process characterized by the loss of polarity and cell adhesion in the epithelial cells[4,5], is a driver of invasion and metastasis in cancer, promoting motility in the cells. Recently, some studies[4-6] on tumorigenesis showed that some signal pathways involved in EMT, including Wnt/β-catenin, mitogen-activated protein kinase (MAPK), transforming growth factor (TGF-β), protein kinase B (AKT), and so on, are related to drug resistance. Thus, it suggests that EMT could be one of the initial and major factors in tumor progression.

Long non-coding RNAs (lncRNAs) are a group of unique RNAs, being > 200 nt long and having no protein-coding properties. However, they mediate the regulation of various processes in the cells including cell proliferation, differentiation, and apoptosis and hence they mediate tumorigenesis or anti-tumoral pro-

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cesses[7-9]. In fact, lncRNA-SBF2-AS1 promotes hepatocellular carcinoma metastasis via regulation of EMT[10], and MALAT1 also promotes tumorigenesis in the tongue cancer cells[9,11]. Moreover, H19 and TUG1 promote osteogenic differentiation in multiple kinds of stem cells[12,13], while lncRNA-MIR31HG exhibits an inhibitory effect on the differentiation of human adipose-derived stem cells[14]. In summary, several investigations[7,15] on the functions of lncRNAs show that they play a significant role in the regulation of cellular process and emerged as a new important factor in tumorigenesis.

DAPT (N-[(3,5-Difluorophenyl)-acetyl]-L-alanyl-2-phenyl]-glycine-1,1-dimethylethylester) is a new kind of γ-secretase inhibitor that can interdict the Notch pathway[16-18]. A previous study showed that in tongue cancer cells the production of lncRNA-KAT14 was increased after treatment with DAPT. However, there is not any study yet about the correlation of KAT14 and DAPT in TSCC patients or to find out whether KAT14 and DAPT played an important role in tumor metastasis. Therefore, the aim of this study was to investigate the relationship between DAPT and lncRNA-KAT14 in TSCC, and their functional relevance in cell viability, proliferation, invasion, and migration ability in the tongue cancer cells. We also observed that EMT was both related to the dosage of DAPT and expression of lncRNA-KAT14 in the tongue cancer cells.

Materials and Methods

Cell Lines and Cell Cultures

The CAL-27 and SCC-9 cell lines were purchased from ScienCell Research Laboratories (Carlsbad, CA, USA). CAL-27 cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM), from Gibco (Rockville, MD, USA) containing 10% of fetal bovine serum (FBS) from Gibco (Rockville, MD, USA) and 1% antibiotics (penicillin and Streptomycin), also from Gibco (Rockville, MD, USA) at 37°C in an incubator containing 5% CO_2. The SCC-9 cells were cultured in 1:1 mixture of DMEM and Ham's F12 medium [DMEM-F12 (1:1)] from Gibco (Rockville, MD, USA) supplemented with 10% FBS, 400 ng/mL hydrocortisone obtained from Gibco (Rockville, MD, USA) and 1% antibiotics at 37°C in an incubator containing 5% CO_2. The mediums were both changed once every two days.

Cell Counting Kit-8 (CCK-8) Assay

The cell viability of both cell lines was measured by CCK-8 assay, produced by Dojindo (Kumamoto, Japan). 7×10^3 cells/well were seeded in 96-well plates. The culture mediums were then discarded and the fresh medium containing 5 μM or 10 μM of DAPT was added into the wells. After treatments for 24, 48, and 72 hours, the mediums were discarded and the fresh medium containing 10% of CCK-8 was added per well and stood for 4 hours. Then, the absorbance at 450 nm was determined by Multiscan Spectrum produced by Thermo Fisher Scientific (Waltham, MA, USA). The cell viability assay was carried out three times.

Small Interfering RNA Transfection

The cells were seeded in 6-well plates with an initial density of 5×10^5 cells/ml in the serum-free medium. The transfections were then carried out using Lipofectamine™ 2000 reagent purchased from Invitrogen (Carlsbad, CA, USA), with KAT14 small interfering RNA (si-KAT14), negative control si-RNA (si-NC), or empty vehicle (Veh), according to the manufacturer's protocol. The si-KAT14 and si-NC were ordered from GenePharma Co. Ltd (Suzhou, China). The sequence of si-KAT14 was made according to that of lncRNA-KAT14: sense: 5′-AAATCTGGGTGATAAACG-3′, antisense: 5′-GCTGAGCAATCCGAAC-3′, and synthesized by Life Technology (Invitrogen, Carlsbad, CA, USA).

Colony Formation Assay

The cells were seeded in 6-well plates with an initial density of 500 cells/well, and gently rotated to spread the cells around. Then, the cells were cultured for 8-10 days at 37°C in an incubator containing 5% CO_2, and the mediums were not changed throughout the course of the experiment. When the macroscopic clones, which could be observed, appeared in the 6-well plates, the supernatant was discarded and carefully rinsed with PBS twice. Then, 5 mL 4% paraformaldehyde was added per well for 15-20 min, and then stained with 0.2% crystal violet. The staining solution was slowly washed away with water, inverted the plate, and counted the number of clones.

Wound-Healing Assay

The cells were seeded in 6-well plates for 24 hours and once they reached the 80-90% confluence, a 200 μL pipette tip was used to make a straight artificial wound. The pipette tip should be vertical and not tilted. Then, we washed the 6-well plates three times with Phosphate-Buffered Saline

(PBS) so that the cell debris was removed, and the serum-free medium was added. We placed them at 37°C in an incubator containing 5% CO_2, and took pictures at 0, 6, 12, 24 hours.

Migration Assay

The migration assay was performed utilizing the transwell chambers. The cells (5×10^4 cells) were seeded into the upper chamber of the transwell chamber, produced by BD Bioscience (Franklin Lakes, NJ, USA) in a medium without serum, and a medium containing 10% FBS was added to the lower chamber as a chemoattractant. After 48 hours incubation, the cells without migration through the membrane were washed out and the membranes were fixed and stained with 0.5% crystal violet. Three random fields were counted per chamber using an inverted microscope produced by Olympus (Tokyo, Japan). Each experiment was repeated three times.

Western blot

The total protein content was extracted from the cells by lysis with radioimmunoprecipitation assay (RIPA) buffer from Beyotime (Shanghai, China). The protein content of the lysate was determined using the bicinchoninic acid (BCA) kit also from Beyotime (Shanghai, China) according to the manufacturer's instruction. And then, 20 mg of the cell lysate were separated by 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). The separated proteins were transferred to a polyvinylidene difluoride (PVDF) membrane from Millipore (Billerica, MA, USA). The membranes were blocked with 5% BSA for 2 hours and then incubated with primary antibodies overnight at 4°C. The primary antibodies were anti-rabbit or anti-mouse IgG antibodies that recognized either E-cadherin, N-cadherin, Vimentin, snail, or GAPDH. The immune complexes were then immunoblotted with an HRP-conjugated anti-mouse or anti-rabbit immunoglobulin-G antibody from CWBio (Beijing, China) at a dilution of 1:2000. The immunodetection was performed using the enhanced chemiluminescence reagents (ECL) from Fdbio (Hangzhou, China). All experiments were repeated three times.

Quantitative Real Time-Polymerase Chain Reaction (qRT-PCR)

The total RNA was extracted with TRIzol reagent purchased from Invitrogen (Carlsbad, CA, USA) according to the manufacturer's protocol. Total RNA was quantified by Nanodrop 2000 spectrophotometry produced by Thermo Fisher Scientific (Waltham, MA, USA). The first strand of the complementary deoxyribose nucleic acid (cDNA) was synthesized using the Superscript RT Kit from Biouniquer Technology (Nanjing, China) following the manufacturer's instructions. qPCR was conducted with SYBR Green I also from Biouniquer Technology (Nanjing, China) based on the LightCycler 480 II System from Roche (Basel, Switzerland). The primer sequences were as follows: lncRNA-KAT14: sense: 5′-AAATCTGGGTGATAAACG-3′, antisense: 5′-GCTGAGCAATCCGAAC-3′. And β-actin: sense primer 5′-GTCCCTCACCCTCCCAAAAG-3′, antisense primer 5′-GCTGCCTCAACACCTCAACCC-3′. The $2^{-\Delta\Delta Ct}$ method was used for the data analysis normalized to β-actin.

Statistical Analysis

All data were analyzed using the Statistical Product and Service Solutions (SPSS) 23.0 software (IBM Corp., Armonk, NY, USA), and performed using the One-way analysis of variance (ANOVA) followed by the post-hoc test (Least Significant Difference) and the unpaired Student's *t*-test for the statistical evaluation. All experiments in the study were repeated for three times, and all data were represented as means ± SD (standard deviation) and $p < 0.05$ was considered significant.

Results

DAPT Suppressed the Cell Viability and Proliferation of Tongue Cancer Cells

In order to investigate whether DAPT exhibited antitumor functions, the CAL-27 and SCC-9 cells were both respectively treated with DAPT at 5 μM or 10 μM for different times. As shown in Figures 1A and 1B, the cell viability of CAL-27 and SCC-9 cells was gradpually increased in the time. However, compared to the control group, the cell viability of the tongue cancer cells was attenuated by DAPT. In addition, compared to the control, the result of the colony formation assay showed that the number of clones was significantly reduced after treatment with 5 μM DAPT ($p < 0.05$, Figures 1C and 1D) and 10 μM DAPT ($p < 0.01$, Figures 1C and 1D). The results indicate that DAPT significantly suppresses cell viability and proliferation in the tongue cancer cells.

DAPT Suppressed the Migration and Invasion of Tongue Cancer Cells

To further investigate whether DAPT played a role in the progression of the tongue cancer cells,

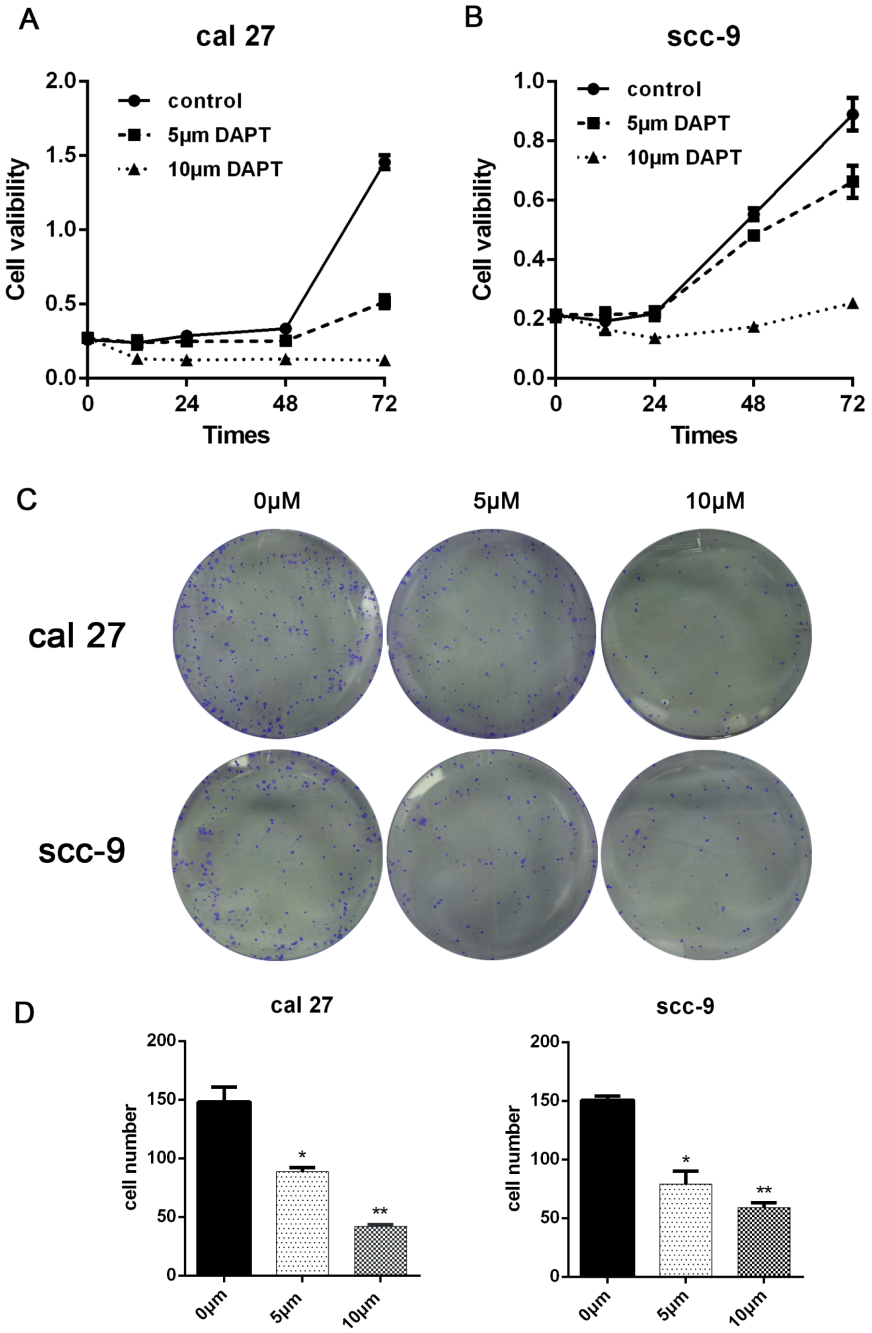

Figure 1. DAPT, a Notch pathway inhibitor, suppressed the cell viability and proliferation of the tongue cancer cells. **A-B,** CCK-8 assay detecting the cell viability of cal-27 cell and SCC-9 cell after treated with DAPT (0, 5 μM, 10 μM) for 24 h, 48 h, and 72 h. **C,** Colony formation assay detecting the cell proliferation of cal-27 cell and SCC-9 cell after treated with DAPT (0, 5 μM, 10 μM) Magnification ×40. **D,** The cell number of colony formation assay of cal-27 cell and SCC-9 cell. *p<0.05, **p<0.01 *vs.* control group.

the Wound-healing assay and the Transwell assay were performed to assess the migration and invasion viability, respectively. Then, semi-quantitative analysis was used to count the cell number and the migration rate. Figures 2A and 2B show that the cell number was significantly decreased in the DAPT-treated groups compared with the control group, in a concentration-dependent manner ($p < 0.01$). Additionally, as shown in Figures 2C and 2D, the migration rate was also gradually reduced ($p < 0.01$) with a DAPT increased concentration. The results of the wound-healing

assay and Transwell assay in CAL-27 cells were both concordant to those performed with SCC-9 cells. Altogether, the results demonstrate that DAPT significantly suppresses cell migration and invasion viability in the tongue cancer cells. Also, given these results, DAPT at a concentration of 10 μM was chosen to carry out the subsequent experiments.

Effect of DAPT on Expression of Epithelial-Mesenchymal Transition (EMT)-Related Proteins in Tongue Cancer Cells

To investigate the effect of DAPT on EMT in the tongue cancer cells, the Western Blots were performed to determine the expression of EMT-related proteins in CAL-27 and SCC-9 cells after treating with DAPT for 24 hours. The results showed that DAPT induced a decreased expression of N-cadherin and increased expression of E-cadherin, and less expression of Vimentin and Snail compared with the control group (Figure 2E). The changes in the expression of EMT-related proteins in CAL-27 cells were like those observed in SCC-9 cells. The results suggest that DAPT plays an inhibitory effect on the migration and invasion viability in tongue cancer cells through the regulation of the expression of key proteins in EMT-related pathways.

LncRNA-KAT14 Mediates Cell Viability and Proliferation in Tongue Cancer Cells

At first, to investigate the interrelation between DAPT with lncRNA-KAT14, the qRT-PCR was used to determine the mRNA expression of lncRNA-KAT14 after treatment with DAPT and/or transfection with si-KAT14. Our result showed that the mRNA expression of lncRNA-KAT14 was upregulated after the treatment with DAPT in a concentration-dependent manner ($p < 0.01$, Figures 3A and 3B). In addition, after transfecting with si-KAT14, the mRNA expression of lncRNA-KAT14 was significantly downregulated compared to the si-NC group. However, in the cells transfected with si-KAT14 and then treated with DAPT, the inhibitory effect on mRNA expression of lncRNA-KAT14 could be reversed and significantly upregulated ($p < 0.01$, Figure 3B). The changes in the mRNA expression of lncRNA-KAT14 in CAL-27 cells were consistent with those observed in SCC-9 cells.

In order to further investigate the relationship between DAPT with lncRNA-KAT14 on antitumor functions, the cells were transfected with si-KAT14 and cultured for different times. As shown in Figure 3C, the cell viability of CAL-27 and SCC-9 cells in the si-KAT14 group was significantly increased, and then significantly decreased after treatment with DAPT. Similarly, compared to the si-NC group, the result of the colony formation assay showed that the number of clones was significantly increased after transfection with si-KAT14 ($p < 0.05$, Figures 3D and 3E), while remarkably decreased after treatment with DAPT. Altogether, this suggests that there is a positive correlation between DAPT and lncRNA-KAT14, and both of them have an inhibitory effect on cell viability and proliferation in the tongue cancer cells.

LncRNA-KAT14 Mediates the Migration and Invasion of Tongue Cancer Cells

Subsequently, to further investigate whether lncRNA-KAT14 also played an inhibitory effect on the progression of the tongue cancer cells, the Wound-healing assay and Transwell assay were carried out to evaluate the migration and invasion viability, respectively. After that, the semi-quantitative analysis was used to count the cell number and the migration rate in both assays, respectively. Figures 4A and 4D show that the cell number and migration rate were both significantly increased in the si-KAT14 groups compared with si-NC group ($p < 0.01$ for CAL-27 cells, $p < 0.05$ for SCC-9 cells), while significantly decreased after treatment with DAPT ($p < 0.01$ for CAL-27 cells, $p < 0.01$ for SCC-9 cells). Consequently, the results demonstrate that DAPT significantly suppresses cell migration and invasion viability in the tongue cancer cells by regulating lncRNA-KAT14.

DAPT Affects the EMT Expression of Tongue Cancer Cells by Regulating LncRNA-KAT14

Finally, to investigate whether DAPT affected the expression of EMT-related proteins in tongue cancer cells via regulating lncRNA-KAT14, Western blots were performed to determine the expression of EMT-related proteins in CAL-27 and SCC-9 cells after transfection with si-KAT14 and treatment with DAPT. The result showed that the expression of N-cadherin, Vimentin, and Snail were both upregulated, and the expression of E-cadherin was downregulated after transfection with si-KAT14, however, this effect on the expression of EMT-related proteins in the si-KAT14 group could be reversed by DAPT (Figure 4E). The change of the expression of EMT-related proteins in CAL-27 cells was consistent with that observed in the SCC-9 cells. Hence, our results

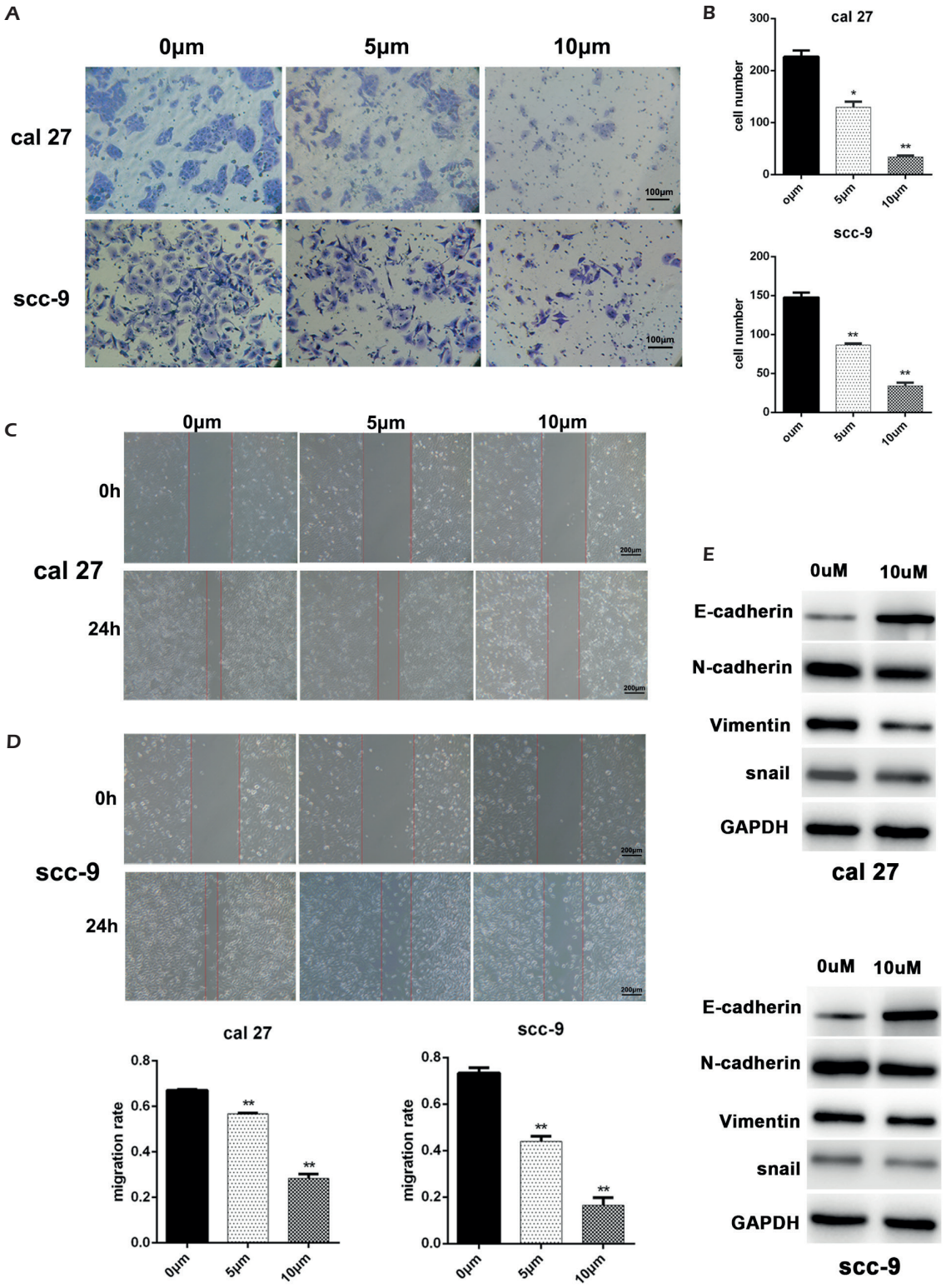

Figure 2. DAPT, a Notch pathway inhibitor, suppressed the cell migration ability and EMT expression of tongue cancer cells. **A,** Migration assay detecting the migration ability of cal-27 cell and SCC-9 cell after treated with DAPT (0, 5 μM, 10 μM) for 48 h. Magnification ×40. **B,** The cell number of migration assay of cal-27 cell and SCC-9 cell. **C,** Wound-healing assay detecting the migration rate of cal-27 cell and SCC-9 cell after treated with DAPT (0, 5 μM, 10 μM) for 48 h. Magnification, ×20. **D,** The migration rate of wound-healing assay of cal-27 cell and SCC-9 cell. ***E,*** The Western blot analysis of expression of E-cadherin, N-cadherin, Vimentin and Snail in cal-27 cell and SCC-9 cell treated with DAPT for 48 h. **p*<0.05, ***p*<0.01 *vs.* control group.

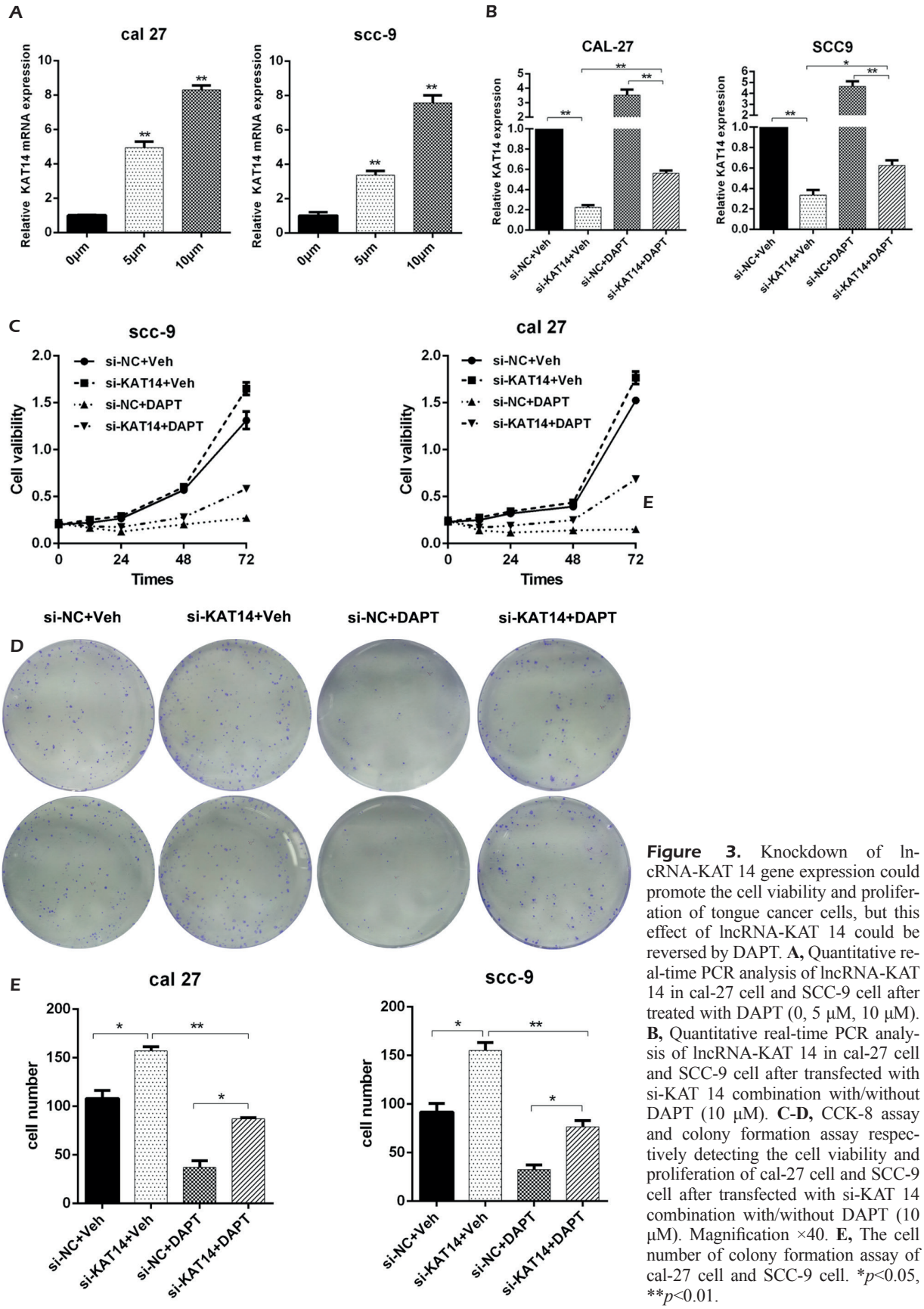

Figure 3. Knockdown of lncRNA-KAT 14 gene expression could promote the cell viability and proliferation of tongue cancer cells, but this effect of lncRNA-KAT 14 could be reversed by DAPT. **A,** Quantitative real-time PCR analysis of lncRNA-KAT 14 in cal-27 cell and SCC-9 cell after treated with DAPT (0, 5 μM, 10 μM). **B,** Quantitative real-time PCR analysis of lncRNA-KAT 14 in cal-27 cell and SCC-9 cell after transfected with si-KAT 14 combination with/without DAPT (10 μM). **C-D,** CCK-8 assay and colony formation assay respectively detecting the cell viability and proliferation of cal-27 cell and SCC-9 cell after transfected with si-KAT 14 combination with/without DAPT (10 μM). Magnification ×40. **E,** The cell number of colony formation assay of cal-27 cell and SCC-9 cell. **p*<0.05, ***p*<0.01.

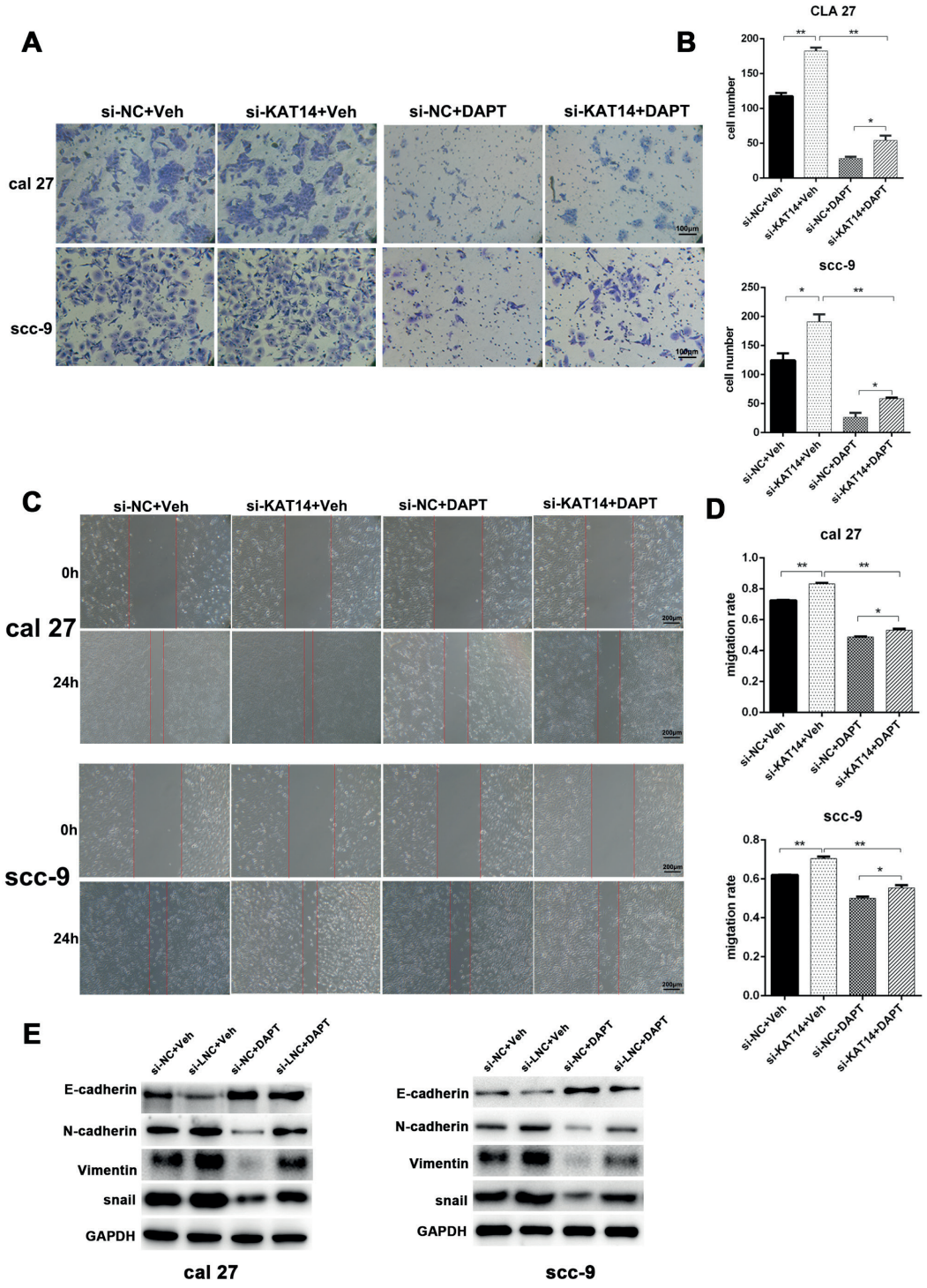

Figure 4. Knockdown of lncRNA-KAT 14 gene expression could promote the migration and EMT expression of the tongue cancer cells, but this effect of lncRNA-KAT 14 could be reversed by DAPT. **A,** Migration assay detecting the migration ability of cal-27 cell and SCC-9 cell after transfected with si-KAT 14 combination with/without DAPT (10 μM). Magnification, ×40. **B,** The cell number of migration assay of cal-27 cell and SCC-9 cell. **C,** Wound-healing assay detecting the migration rate of cal-27 cell and SCC-9 cell after transfected with si-KAT 14 combination with/without DAPT (10 μM). Magnification, ×20. **D,** The migration rate of wound-healing assay of cal-27 cell and SCC-9 cell. **E,** The Western blot analysis of expression of E-cadherin, N-cadherin, Vimentin, and Snail in cal-27 cell and SCC-9 cell after transfected with si-KAT 14 combination with/without DAPT (10 μM). *p<0.05, **p<0.01 *vs.* control group.

indicate that DAPT upregulates the expression of E-cadherin and downregulates the expression of N-cadherin, Vimentin, and Snail by regulating lncRNA-KAT14, playing an inhibitory effect on the migration and invasion viability in tongue cancer cells.

Discussion

It is known that TSCC has become the most common malignant oral tumor in patients all around the world and the number of patients dying of this cancer is increasing every year [1-3,19]. Poor prognosis and bad survival and high metastasis rates are common characteristics in the majority of patients with TSCC[4,20]. Therefore, TSCC is to concern when it comes to developing treatments in order to improve people's health[21]. Studies that aim to elucidate the molecular mechanisms underlying the suppression of cell proliferation, invasion, and migration are the key to inhibit the progression of cancer in patients. However, there is still little available information about tongue cancer tumorigenesis and metastasis.

In oral cancer patients, metastasis is the most common characteristic, which directly leads to high mortality rates in humans[7,22]. EMT is a pivotal factor in metastasis, as it is often observed during tumor progression, and promotes cancer cells spreading to other organs[5,22]. An increasing number of studies demonstrate that the epithelial cells loss polarity and cell adhesion ability during EMT in a process mediated by the downregulation of E-cadherin, acquiring mesenchymal properties, thus leading to enhanced cell motility and invasion ability[4,5,23]. In addition, the upregulation of Vimentin, N-cadherin, and Snail, another EMT features, could also promote metastasis and resistance to apoptosis[4,22]. Consequently, it is possible to argue that EMT is one of the initial and major factors in tumor progression.

DAPT is an effective inhibitor of the Notch signaling pathway, which plays a critical role in neural differentiation, cell adhesion, and morphology[16-18]. In the current study, we found that DAPT suppresses the cell viability of CAL-27 and SCC-9 cells in a concentration-dependent manner, and also that DAPT plays an inhibitory effect on cell proliferation, invasion, and migration of the tongue cancer cells. In addition, our results show that DAPT induces an increased expression of E-cadherin and a decreased expression of N-cadherin, Vimentin, and Snail compared with the control group. Our findings indicate that DAPT suppresses EMT-related proteins expression and cell proliferation, invasion, and migration, suggesting that it ultimately limits the progression of tongue cancer. In our previous research we showed that the gene expression of lncRNA-KAT14 was upregulated in the tongue cancer cells after treatment with DAPT. However, little is known about the effect of lncRNA-KAT14 on tumorigenesis.

An increasing number of findings demonstrate that lncRNAs play a critical role in cell proliferation, invasion, and migration, in tumor growth and metastasis[7,24-27]. In fact, lncRNA-HOTAIR and lncRNA-ANRIL promote carcinogenesis[28,29], whereas lncRNA-MEG3 and lncRNA-GAS5 suppress tumorigenesis[30,31]. Zhang et al [10] found that lncRNA-SBF2-AS1 was overexpressed in hepatocellular carcinoma and that it is correlated with poor prognosis. Also, it induces E-cadherin expression and reduces the expression of fibronectin and Vimentin, promoting the metastasis of the hepatocellular carcinoma cells. Liang et al[9] demonstrated that the metastasis-associated lung adenocarcinoma transcript 1 (MALAT1) induces cell invasion and migration and EMT-related proteins expression in TSCC, and it also inhibits apoptosis by regulating the Wnt/β-catenin signaling pathway. Jing et al[32] showed that lncRNA-RP11 suppressed the inhibitory effect of artesunate on cell proliferation, migration, invasion, adhesion, and EMT in liver cancer cells, promoting metastasis and leading to poor survival rates in patients. In the current study, we found that DAPT induces lncRNA-KAT14 overexpression in the tongue cancer cells. When lncRNA-KAT14 production was blocked in CAL-27 and SCC-9 cells, the cell viability, proliferation, invasion, and migration were enhanced in both cell lines compared to the control groups (si-NC). To investigate whether DAPT could reverse the effect of lncRNA-KAT14 on tongue cancer cells, the CAL-27 and SCC-9 cells were treated with DAPT after transfection with si-KAT1 and we found that DAPT partly reverses the effect of blocking lncRNA-KAT14 on cell proliferation, invasion, and migration of the tongue cancer cells. Additionally, the results show that the expression of N-cadherin, Vimentin, and Snail are upregulated, whereas the expression of E-cadherin was downregulated after transfection with si-KAT14. However, the expression of EMT-related proteins in the si-KAT14 group could be reversed after the treatment with DAPT.

Conclusions

It has been found that DAPT significantly induces lncRNA-KAT14 overexpression, and further inhibits the tongue cancer cells proliferation, invasion, and migration. Moreover, DAPT also induces a decreased expression of N-cadherin and an increased expression of E-cadherin, Vimentin, and Snail by regulating lncRNA-KAT14. Our findings indicated that lncRNA-KAT14 may be a reliable prognostic biomarker to identify patients with high a risk of recurrence and poor treatment in TSCC and may become an effective target for the treatment of oral cancer.

Conflict of Interests

The Authors declare that they have no conflict of interests.

Funding Acknowledgements

This work was financially supported by the Science and Technology Project of Guangzhou city (201802020018).

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