MicroRNA-223-5p suppresses the progression of nasopharyngeal carcinoma by targeting DCLK1

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Abstract. The aim of the present study was to investigate the function of microRNA (miR)-223-5p in the malignant biological behavior of nasopharyngeal carcinoma (NPC) and elucidate the underlying molecular mechanism. The expression levels of miR-223-5p and doublecortin-like kinase 1 (DCLK1) were detected via reverse transcription-quantitative PCR analysis. Cell viability was evaluated using Cell Counting Kit-8 assay. Cell migration and invasion were measured via Transwell assays, while a luciferase reporter assay was conducted to identify the interaction between miR-223-5p and DCLK1. The results demonstrated that miR-223-5p expression was significantly downregulated, whereas DCLK1 expression was significantly upregulated in NPC tissues and cells. Moreover, both miR-223-5p overexpression and DCLK1 silencing markedly suppressed the progression of NPC. It was also observed that miR-223-5p directly targeted DCLK1 and decreased its expression. Furthermore, it was suggested that DCLK1 overexpression may partially reverse the suppressive effects of miR-223-5p on the progression of NPC. Collectively, the results of the present study indicated that miR-223-5p may suppress NPC progression by targeting DCLK1, thereby indicating a novel potential approach to the diagnosis and treatment of NPC.

Introduction

Nasopharyngeal carcinoma (NPC) is a type of cancer that originates from epithelial cells in the nasopharynx (1). According to GLOBOCAN 2012, 86,691 new NPC cases and 50,831 NPC-associated deaths were reported worldwide (2). Although NPC is relatively rare in the rest of the world, its prevalence is relatively high in Southern China and Southeast Asian countries (3). Epidemiological data indicate that the NPC incidence rate has gradually decreased and the mortality rate of NPC has significantly declined in recent years due to the advances in diagnostic and therapeutic strategies (4). However, the 5-year survival rate of patients with NPC remains very low due to recurrence and metastasis, particularly in cases with late-stage disease (5). Therefore, it is necessary to identify new and efficient methods for NPC treatment.

MicroRNAs (miRNAs/miRs) are short-chain non-coding RNAs that are 19-24 nucleotides in length. Previous studies have reported that miRNAs play key roles in various cellular functions, such as proliferation, metastasis, apoptosis and chemoresistance (6). miRNAs usually bind to the 3'-untranslated region (UTR) of their target mRNAs and inhibit translation (7). Numerous studies have revealed that miR-223-5p is significantly downregulated in cancer tissues and cells, suggesting its inhibitory effect on tumor progression. For example, miR-223-5p may inhibit the progression of non-small cell lung cancer by modulating E2F transcription factor 8 (8), suppress the malignant phenotype of prostate cancer cells via modulating ETS transcription factor ERG (9) and repress the aggressiveness of bladder cancer cells (10). However, the potential regulatory role of miR-223-5p in NPC has not been extensively investigated.

Doublecortin-like kinase 1 (DCLK1) is a kinase highly expressed in various types of cancer (11) and has been identified as a potential oncogene implicated in the progression of human cancers, such as pancreatic cancer (12), lung squamous cell carcinoma (13), ovarian clear cell carcinoma (14), intestinal tumors (15) and basal-like breast cancer (16). In addition, DCLK1 has been reported to contribute to the tumorigenic process of colorectal cancer by downregulating miR-200c (17). However, whether DCLK1 is implicated in NPC progression and the underlying mechanism remain to be investigated.

The present study was undertaken to investigate miR-223-5p and DCLK1 expression levels in NPC tissues and cells, and to determine the effects of these factors on the viability, migration and invasion of NPC cells. The aim was to elucidate whether miR-223-5p can suppress NPC progression by downregulating DCLK1 expression, in the hope of providing a novel approach to the diagnosis and treatment of NPC.

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Materials and methods

Clinical tissues. A total of 28 paired samples of NPC and adjacent normal nasopharyngeal tissues were obtained from patients undergoing surgery at The Third Affiliated Hospital of Soochow University (Changzhou, China) between March 2016 and January 2019. All the tissues were reviewed by two pathologists blinded to the clinicopathological information and were immediately frozen in liquid nitrogen after surgical excision. None of the patients with NPC had received anticancer treatments prior to surgery. The present study was approved by the Ethics Committee of The Third Affiliated Hospital of Soochow University, and written informed consent was obtained from each patient enrolled in the study.

Cell culture and transfection. Human NPC cell lines (6-10B, 5-8F, SUNE-1, SUNE-2 and C666-1) and a human nasopharyngeal-derived epithelial cell line (NP69) were obtained from the Cell Bank of the Chinese Academy of Science. Cells were cultured in DMEM (Gibco; Thermo Fisher Scientific, Inc.) containing 12 U/l gentamicin and 100 ml/l inactivated FBS (Gibco; Thermo Fisher Scientific, Inc.) in a humidified atmosphere containing 5% CO_2 at 37˚C, and were passaged once every 2 days.

NPC cells ($5x10^4$) were transfected with 100 nM miR-223-5p mimics (5'-UGUCAGUUUGUCAAAUACCCCA-3'), 100 nM corresponding scrambled negative control (NC mimics; 5'-UUC UCCGAACGUGUCACGUUU-3'), 100 nM miR-223-5p inhibitor (5'-UGGGGUAUUUGACAAACUGACA-3'), 100 nM corresponding scrambled NC (NC inhibitor; 5'-CAG UACUUUUGUGUAGUACAA-3'), 0.2 μM small interfering (si)RNA targeting DCLK1 (si-DCLK1; 5'-UUUCAGAGC AUACUCUCUAGC-3'), 0.2 μM scrambled siRNA NC (si-NC; 5'-UUCUCCGAACGUGUCACGUUU-3'), 2 μl pcDNA3.1 (empty vector; Ctrl) or 2 μl pcDNA3.1-DCLK1 (DCLK1) using Lipofectamine® 2000 (Invitrogen; Thermo Fisher Scientific, Inc.) at room temperature for ~20 min. The transfected cells were cultured for 48 h and were then used in subsequent experiments. The transfection efficiency was confirmed by reverse transcription-quantitative (RT-q)PCR.

RT-qPCR assay. Total RNA was extracted from tissues and cells using TRIzol® reagent (Invitrogen; Thermo Fisher Scientific, Inc.) and was reverse-transcribed into cDNA using a RT kit (Takara Bio, Inc.) following the manufacturer's protocol. Subsequently, qPCR was performed with SYBR Select Master Mix (Thermo Fisher Scientific, Inc.) on a ViiA™ 7 Real-Time PCR System (Thermo Fisher Scientific, Inc.) to measure the mRNA expression levels, according to the manufacturer's instructions. The thermocycling conditions were: 95˚C for 10 min, followed by 40 cycles at 95˚C for 10 sec and 55˚C for 10 sec, and a final step at 72˚C for 30 sec. The $2^{-\Delta\Delta Cq}$ method (18) was used to calculate the relative DCLK1 and miR-223-5p expression levels, with GAPDH and U6 as internal controls. The primers used were as follows: miR-223-5p forward, 5'-GCAGCGTGTATTTGA CAAG-3' and reverse, 5'-CTCAACTGGTGTCGTGGA-3'; DCLK1 forward, 5'-CAGCGCCATCAAATACCTGC-3' and reverse, 5'-TGGTCATCACCACTTCCACG-3'; U6 forward, 5'-GCTTCGGCAGCACATATACTAAAAT-3' and reverse, 5'-CGCTTCACGAATTTGCGTGTCAT-3'; and GAPDH forward, 5'-GGAGTCCACTGGCGTCTT-3' and reverse, 5'-ATCTTGAGGCTGTTGTCATAC-3'.

Cell Counting Kit (CCK)-8 assay. After 48 h of transfection, the cells were plated into 96-well plates at 5,000 cells per well. Then, 20 μl CCK-8 reagent (Dojindo Molecular Technologies, Inc.) was added into each well after the cells were cultured for 0, 48, 72 and 96 h at 37˚C with 5% CO_2. Subsequently, the cells were incubated at room temperature for 4 h. The optical density of each well was measured using a microplate reader (Bio-Rad Laboratories, Inc.) at a wavelength of 450 nm. The cell viability was considered to be directly proportional to the optical density of the cells in this assay.

Transwell assay. Cell migratory and invasive abilities were analyzed in 96-well Transwell chambers with an 8-μm pore membrane (Corning, Inc.) following the manufacturer's instructions. A total of 100 μl cell suspension ($1x10^5$ cells/ml) was added to the upper chamber, and 600 μl medium supplemented with 10% FBS was added to the lower chamber. After incubation at 37˚C overnight, non-migrating cells in the upper chamber were removed with a cotton swab, and the upper chamber was washed three times with PBS. Then, the cells were fixed with 4% paraformaldehyde at room temperature for 30 min and stained with 0.1% crystal violet solution at room temperature for ~20 min. In order to observe the migrating cells attached to the lower surface of the chamber, five fields of view were randomly selected and the cells were counted under a light microscope (Olympus Corporation; magnification, x200). For the detection of cell invasion, the upper surface of the chamber was pre-coated with Matrigel® for 1 h at room temperature, and the following experimental steps were the same as those described for the detection of cell migration.

Target prediction and luciferase reporter assay. TargetScan (http://www.targetscan.org/vert_72/) was used to predict the binding sites between miR-223-5p and DCLK1. psiCHECK2 vectors (Promega Corporation) were used to construct psiCHECK2-DCLK1-wild-type (WT) and psiCHECK2-DCLK1-mutant (MUT) plasmids (containing WT and MUT 3'-UTR, respectively), according to the manufacturer's instructions. DCLK1 MUT luciferase reporter containing MUT miR-223-5p-binding sites were produced using GeneArt™ Site-Directed Mutagenesis System (Invitrogen; Thermo Fisher Scientific, Inc.). Subsequently, miR-223-5p mimics were co-transfected into 6-10B cells together with psiCHECK2-DCLK1-WT or psiCHECK2-DCLK1-MUT using Lipofectamine® 2000 (Invitrogen; Thermo Fisher Scientific, Inc.). After 48 h of transfection, luciferase activity was evaluated using a Dual-Luciferase Reporter Analysis system (Promega Corporation). Firefly luciferase activity was normalized to *Renilla* (Promega Corporation) luciferase gene activity.

Immunohistochemistry (IHC). The tissue expression of DCLK1 was evaluated via IHC based on the intensity and the proportion of positively stained cells as previously described (19). Sections were incubated with primary antibody against DCLK1 (1:1,000; cat. no. 62257; Cell Signaling

Technology, Inc.) overnight at 4˚C, followed by incubation with goat anti-rabbit secondary antibody (cat. no. ab205718; 1:2,000; Abcam) at room temperature for 20 min. The sections were stained with 3,3'-diaminobenzidine and the nuclei were counterstained with hematoxylin for 5 min at room temperature. Images were captured under a light microscope (Olympus Corporation; magnification, x200).

Western blotting. Total protein was isolated from transfected cells using RIPA lysis buffer (Beyotime Institute of Biotechnology) and the protein concentrations were determined using a BCA kit (Thermo Fisher Scientific, Inc.). Subsequently, 10 μg protein/lane was separated via SDS-PAGE on 10% gels (Bio-Rad Laboratories, Inc.) and was electrotransferred to PVDF membranes. After blocking with 5% non-fat milk for 2 h at room temperature, the membranes were first incubated with primary antibodies against Notch receptor 1 (Notch1; 1:1,000; cat. no. ab52627; Abcam) and GAPDH (1:1,000; cat. no. ab8245; Abcam) overnight at 4˚C, followed by incubation with corresponding HRP-conjugated secondary antibody (1:1,000, cat. nos. ab6789 and ab205718; Abcam) for 1 h at room temperature. Blots were visualized with an enhanced chemiluminescent detection system (EMD Millipore). Protein expression was measured using Image-Pro® Plus software (version 6.0; Media Cybernetics, Inc.).

Statistical analysis. All experiments were repeated at least 3 times. All the data collected in the experiments were analyzed with GraphPad Prism 7 (GraphPad Software, Inc.). Data are presented as the mean ± SD. The association between miR-223-5p or DCLK1 expression and clinicopathological characteristics of patients with NPC was analyzed using χ^2 tests. Both paired and unpaired Student's t-tests were used for comparisons between two groups, and one-way ANOVA followed by Tukey's post hoc test was conducted for comparisons among multiple groups. P<0.05 was considered to indicate a statistically significant difference.

Results

miR-223-5p and DCLK1 expression levels in NPC. miR-223-5p expression was measured via RT-qPCR, and the results demonstrated that miR-223-5p expression was decreased in NPC tissues and cell lines (6-10B, 5-8F, SUNE-1, SUNE-2 and C666-1), compared with that in adjacent normal nasopharyngeal tissues and NP69 human nasopharyngeal-derived epithelial cells (Fig. 1A and B). DCLK1 expression was also examined in the present study. The RT-qPCR results indicated that DCLK1 expression was significantly increased in NPC tissues and cell lines (6-10B, 5-8F, SUNE-1, SUNE-2 and C666-1) compared with that in adjacent normal nasopharyngeal tissues and NP69 cells (Fig. 1C and D). Moreover, the expression of DCLK1 in NPC tissues was higher compared with that in adjacent normal nasopharyngeal tissues, as determined via IHC (Fig. 1E). It was observed that low miR-223-5p or high DCLK1 expression was associated with distant metastasis and TNM stage, while there was no significant association with sex or age in patients with NPC (Table I). These results suggested that miR-223-5p expression was downregulated and DCLK1 expression was upregulated in NPC tissues and cell lines. 5-8F and 6-10B cells were selected for subsequent experiments due to their high DCLK1 expression.

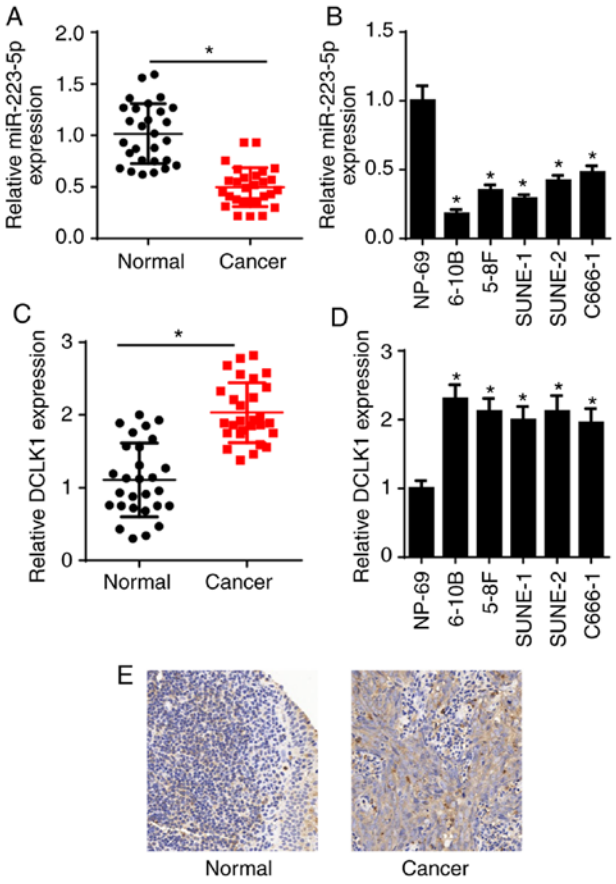

Figure 1. miR-223-5p and DCLK1 expression in NPC. (A) miR-223-5p expression in NPC tissues and adjacent normal nasopharyngeal tissues was measured via RT-qPCR. (B) miR-223-5p expression in NPC cell lines and human nasopharyngeal-derived epithelial cells was measured via RT-qPCR. (C) DCLK1 expression in NPC tissues and adjacent normal nasopharyngeal tissues was measured via RT-qPCR. (D) DCLK1 expression in NPC cell lines and human nasopharyngeal-derived epithelial cells was measured via RT-qPCR. (E) Representative immunohistochemical staining images of DCLK1 in NPC tissues and adjacent normal nasopharyngeal tissues. DCLK1 expression in NPC tissues was positive, with a brown/yellow signal detected in the cytoplasm (magnification, x200). *P<0.05 vs. NP-69 or as indicated. miR, microRNA; DCLK1, doublecortin-like kinase 1; NPC, nasopharyngeal carcinoma; RT-qPCR, reverse transcription-quantitative PCR.

miR-223-5p overexpression suppresses viability, migration and invasion of NPC cells. To investigate the biological role of miR-223-5p in NPC, 5-8F and 6-10B cells were transfected with NC mimics and miR-223-5p mimics. The RT-qPCR results demonstrated that miR-223-5p expression was significantly elevated following transfection with miR-223-5p mimics (Fig. 2A). The CCK-8 and Transwell assay results indicated that cell viability, migration and invasion were inhibited following transfection of 5-8F and 6-10B cells with miR-223-5p mimics (Fig. 2B-D), suggesting that miR-223-5p overexpression NPC cell viability, migration and invasion.

DCLK1 silencing suppresses viability, migration and invasion of NPC cells. To investigate the function of DCLK1 in NPC tumorigenesis, si-DCLK1 or si-NC were transfected into 5-8F

Table I. Association between miR-223-5p or DCLK1 expression and clinicopathological characteristics in nasopharyngeal carcinoma.

		miR-223-5p expression			DCLK1 expression		
Characteristics	Patients, n	Low	High	P-value	Low	High	P-value
Sex				0.625			0.573
Male	17	9	8		7	10	
Female	11	6	5		5	6	
Age, years				0.452			0.362
<45	13	6	7		5	8	
≥45	15	9	6		7	8	
Distant metastasis				0.005			0.032
No	18	7	11		9	9	
Yes	10	8	2		3	7	
TNM stage				0.013			0.011
I + II	16	7	9		9	7	
III + IV	12	8	4		3	9	

miR, microRNA; DCLK1, doublecortin-like kinase 1.

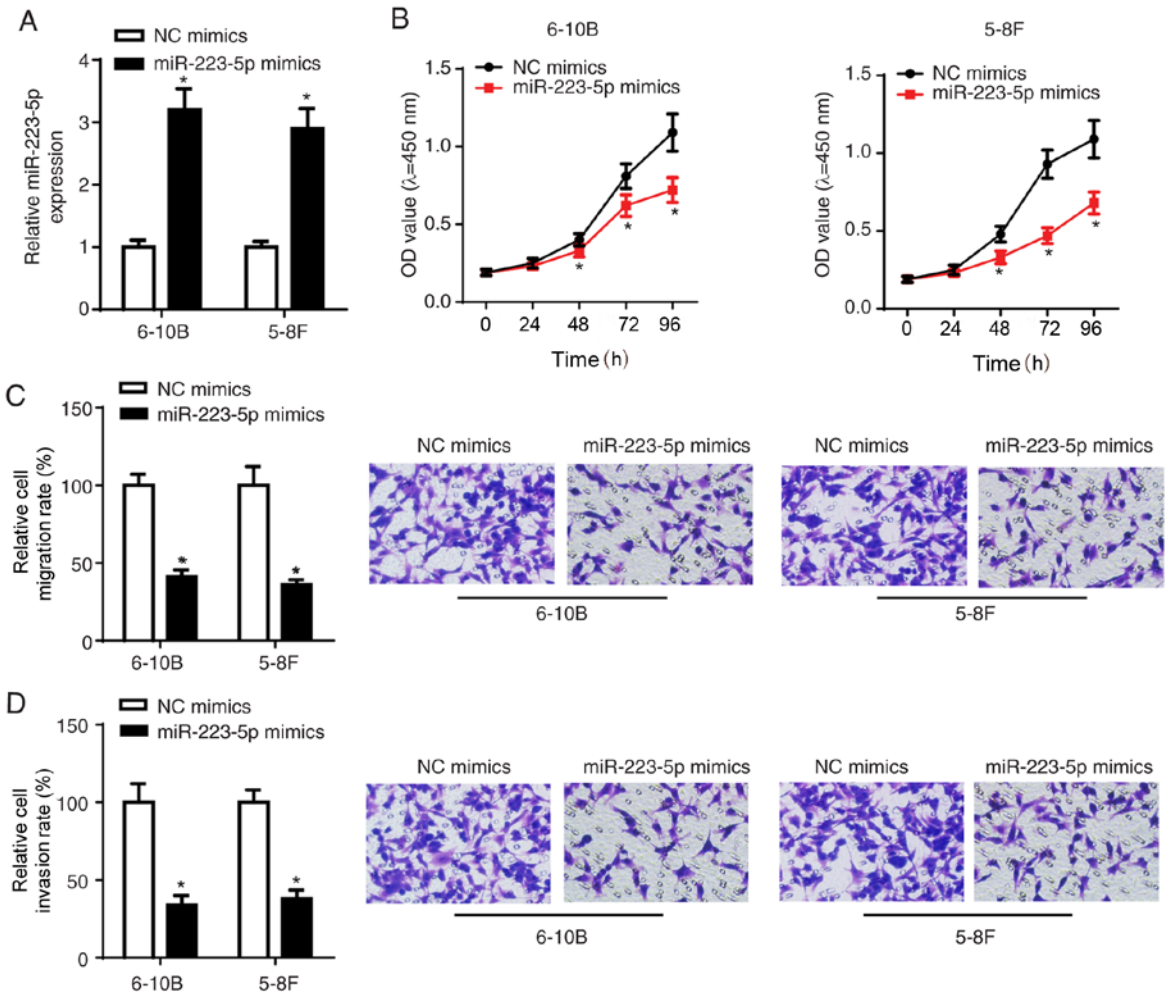

Figure 2. miR-223-5p overexpression suppresses cell viability, migration and invasion in nasopharyngeal carcinoma cells. 5-8F and 6-10B cells were transfected with NC mimics or miR-223-5p mimics. (A) Effect of transfection of miR-223-5p overexpression plasmid on miR-223-5p expression in 5-8F and 6-10B cells as determined via RT-qPCR. (B) Effect of miR-223-5p overexpression on the activity of 5-8F and 6-10B cells as determined by Cell Counting Kit-8 assay. (C and D) Effect of miR-223-5p overexpression on the migration and invasion of 5-8F and 6-10B cells as analyzed via Transwell assays (magnification, x200). *P<0.05 vs. NC mimics. miR, microRNA; RT-qPCR, reverse transcription-quantitative PCR; NC, negative control.

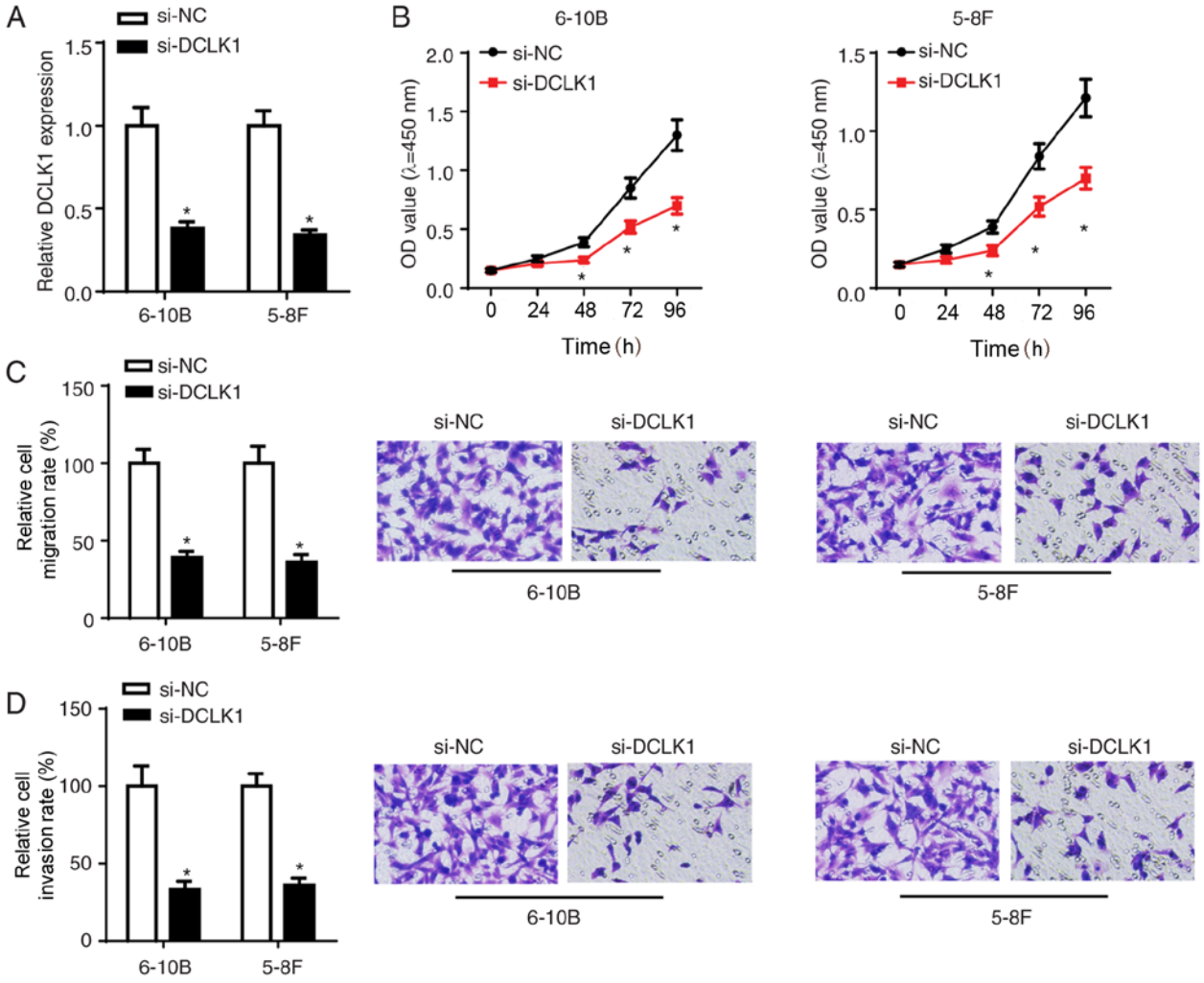

Figure 3. DCLK1 silencing suppresses the viability, migration and invasion of nasopharyngeal carcinoma cells. si-DCLK1 or si-NC were transfected into 5-8F and 6-10B cells. (A) Effect of DCLK1 silencing on DCLK1 expression in 5-8F and 6-10B cells as determined via reverse transcription-quantitative PCR. (B) Proliferative activity of 5-8F and 6-10B cells as determined by Cell Counting Kit-8 assay. (C and D) Effect of DCLK1 silencing on the migration and invasion of 5-8F and 6-10B cells as determined using Transwell assays (magnification, x200). *P<0.05 vs. si-NC. DCLK1, doublecortin-like kinase 1; si, small interfering RNA; NC, negative control.

and 6-10B cells. The transfection efficiency was confirmed via RT-qPCR (Fig. 3A). The CCK-8 assay results demonstrated that DCLK1 knockdown inhibited the viability of NPC cells (Fig. 3B). Moreover, the Transwell assay results further confirmed that DCLK1 silencing suppressed the migration and invasion of NPC cells (Fig. 3C and D).

DCLK1 is a target of miR-223-5p. Since DCLK1 was reported to act as an oncogene and promote tumorigenesis in different types of human cancer (20,21), TargetScan was used to predict the binding sites between miR-223-5p and DCLK1. The potential binding sites are shown in Fig. 4A. Luciferase reporter analysis demonstrated that the luciferase activity was reduced in 6-10B cells co-transfected with DCLK1-WT and miR-223-5p mimics, while there was little change in the DCLK1-MUT group (Fig. 4B), suggesting a specific interaction between miR-223-5p and DCLK1. RT-qPCR revealed that miR-223-5p expression was significantly downregulated in 6-10B cells transfected with miR-223-5p inhibitor (Fig. 4C). In addition, the effect of miR-223-5p on DCLK1 expression was examined (Fig. 4D) and the results indicated that miR-223-5p overexpression suppressed DCLK1 expression in 6-10B cells, while miR-223-5p knockdown resulted in increased expression of DCLK1.

miR-223-5p overexpression suppresses NPC cell viability, migration and invasion via the DCLK1/Notch1 signaling pathway. To examine whether miR-223-5p overexpression suppressed cell viability, migration and invasion in NPC via DCLK1, 5-8F and 6-10B cells were transfected with NC mimics, miR-223-5p mimics, miR-223-5p + Ctrl and miR-223-5p + DCLK1, and then DCLK1 expression, viability, migration and invasion were measured in 5-8F and 6-10B cells. DCLK1 expression was found to be markedly increased in NPC cells transfected with DCLK1 overexpression plasmid (Fig. 5A). In addition, it was found that miR-223-5p overexpression not only decreased DCLK1 expression in 5-8F and 6-10B cells, but also inhibited the viability, migration and invasion of 5-8F and 6-10B cells. However, DCLK1 overexpression could partially reverse the suppressive effects of miR-223-5p overexpression on the viability, migration and invasion of 5-8F and 6-10B cells (Fig. 5B-E).

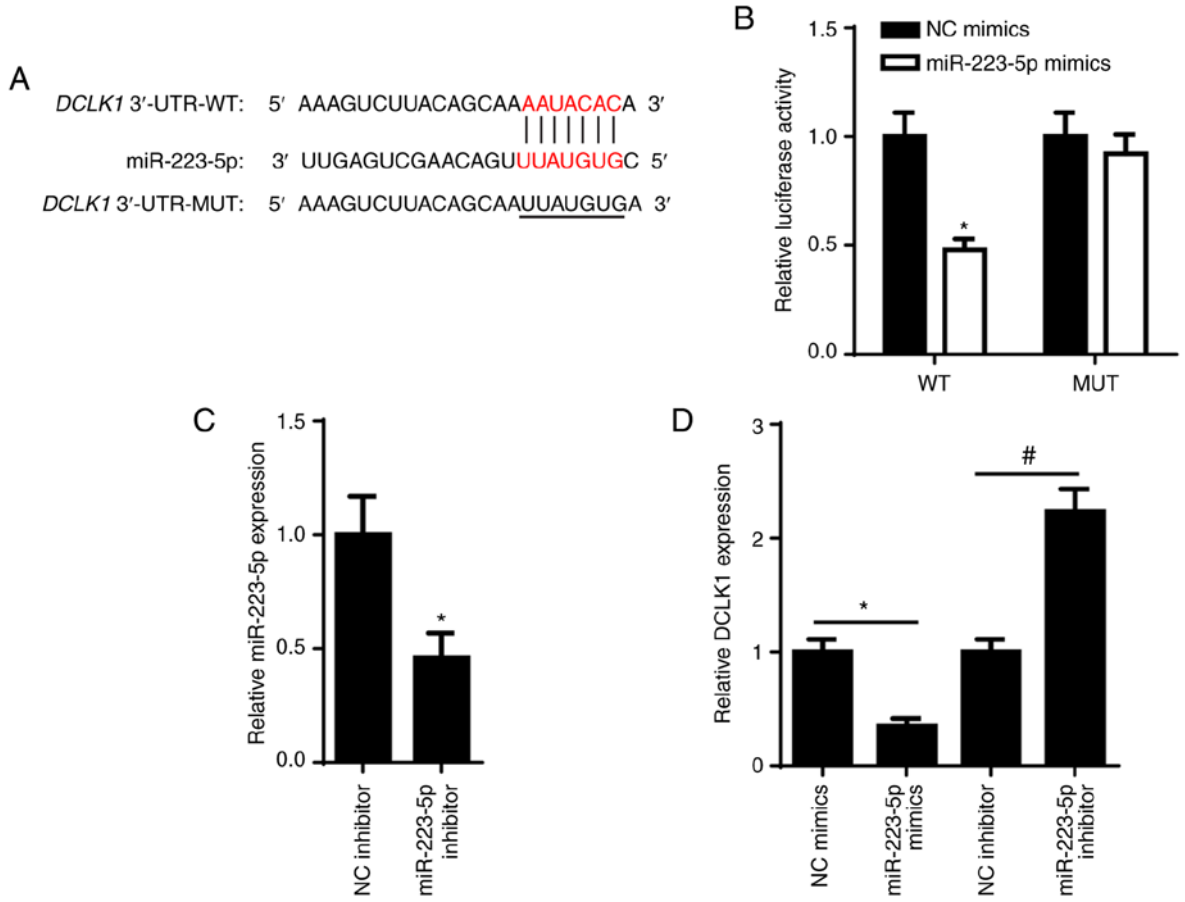

Figure 4. DCLK1 is a target of miR-223-5p. 6-10B cells were transfected with NC mimics, miR-223-5p mimics, NC inhibitor or miR-223-5p inhibitor. (A) Predicted binding sites between miR-223-5p and DCLK1. (B) Luciferase activity was analyzed in 6-10B cells transfected with NC mimics and miR-223-5p mimics. (C) miR-223-5p expression in 6-10B cells transfected with NC inhibitor or miR-223-5p inhibitor was measured using RT-qPCR. (D) DCLK1 expression in 6-10B cells was measured using RT-qPCR. *P<0.05 vs. NC mimics; #P<0.05 vs. NC inhibitor. DCLK1, doublecortin-like kinase 1; miR, microRNA; RT-qPCR, reverse transcription-quantitative PCR; NC, negative control; WT, wild-type; MUT, mutant.

A previous study reported that DCLK1 regulated tumor metastasis and epithelial-to-mesenchymal transition of gastric cancer cells via the Notch1 signaling pathway (22). Therefore, it was hypothesized that miR-223-5p and DCLK1 may regulate NPC progression via Notch1 signaling. The results suggested that overexpression of miR-223-5p decreased the protein expression of Notch1, which was partially restored following overexpression of DCLK1 in 5-8F and 6-10B cells (Fig. 5F). These results indicated that the effects of miR-223-5p and DCLK1 on NPC progression may be mediated via the Notch1 signaling pathway.

Discussion

Multiple factors, such as Epstein-Barr virus infection, environmental factors and genetic susceptibility genes, have been reported to contribute to the development of NPC (23). Since the two major causes of mortality among patients with NPC are recurrence and metastasis, it is crucial to further elucidate the molecular mechanism underlying NPC progression. As a result, additional personalized treatments may be developed and provided to patients with NPC. The present study demonstrated that miR-223-5p was downregulated in NPC tissues and cells, and acted as a tumor suppressor by suppressing the malignant behavior of NPC cells.

Abnormally expressed miRNAs may play key roles in NPC progression by facilitating cell proliferation, invasion and angiogenesis. For example, miR-216b was found to suppress NPC growth by downregulating KRAS expression (24), and miR-26a inhibited NPC cell proliferation and cell cycle by suppressing enhancer of zeste 2 polycomb repressive complex 2 subunit (25). miR-142-3p silencing may contribute to NPC progression via modulating zinc finger E-box binding homeobox 2 (26). Moreover, miR-223 was found to be involved in various types of cancer. For example, miR-223 expression was found to be suppressed in hepatocellular carcinoma and facilitated Stathmin1 expression (27). miR-223 also suppressed the progression of prostate cancer via regulating integrin subunit α3/integrin subunit β1 signaling (28). Additionally, miR-223 suppressed cell proliferation and migration in NPC via targeting MAF bZIP transcription factor B (29). The aforementioned studies indicated the important role of miR-223 in NPC progression. miR-223-5p is the passenger strand of the miR-223 duplex (10). Consistent with all these studies, the present study demonstrated that miR-223-5p expression was downregulated in NPC tissues compared with that in adjacent normal nasopharyngeal tissues. In the present study, RT-qPCR analysis revealed low miR-223-5p expression in NPC tissues and cells. The results of the CKK-8 and Transwell assays suggested that miR-223-5p overexpression may suppress

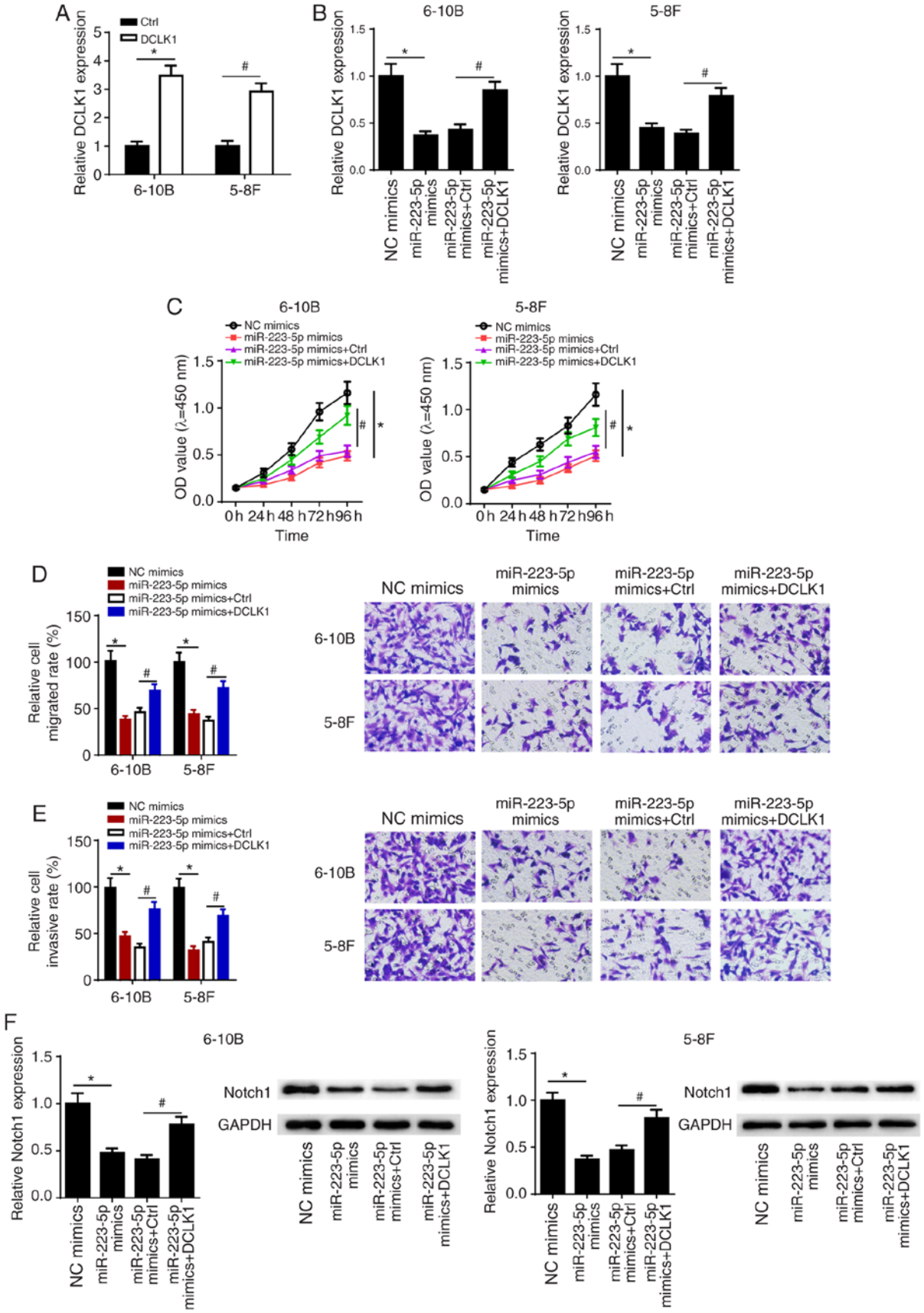

Figure 5. miR-223-5p overexpression suppresses nasopharyngeal carcinoma cell viability, migration and invasion via the DCLK1/Notch1 signaling pathway. (A) RT-qPCR was used to measure the DCLK1 expression in 5-8F and 6-10B cells transfected with Ctrl or DCLK1 overexpression plasmid. (B) DCLK1 expression in 5-8F and 6-10B cells transfected with NC mimics, miR-223-5p mimics, miR-223-5p mimics + Ctrl or miR-223-5p mimics + DCLK1 was measured via RT-qPCR. (C) Proliferative activity of 5-8F and 6-10B cells as determined by Cell Counting Kit-8 assay. (D and E) Transwell assays were performed to detect the migration and invasion of 5-8F and 6-10B cells (magnification, x200). (F) RT-qPCR and western blotting were performed to measure the expression of Notch1 expression at mRNA and protein levels in 5-8F and 6-10B cells. *P<0.05 vs. Ctrl or NC mimics; #P<0.05 vs. Ctrl or miR-223-5p mimics + Ctrl group. miR, microRNA; DCLK1, doublecortin-like kinase 1; RT-qPCR, reverse transcription-quantitative PCR; NC, negative control; Ctrl, pcDNA3.1.

cell viability, migration and invasion in NPC. Therefore, the present study demonstrated the inverse association between miR-223-5p expression and NPC tumor progression.

Previous studies have reported that aberrant DCLK1 expression may be closely associated with the malignant biological properties of tumors. For example, miR-613 was found to suppress the growth and invasion of human hepatocellular carcinoma by inhibiting DCLK1 (30), whereas miR-137 inhibited the malignant behavior of colon cancer via downregulation of DCLK1 (31). miR-424 also suppressed the viability and invasion of neuroblastoma cells by directly modulating DCLK1 (32). In addition, high expression of DCLK1 has been demonstrated to promote the progression of human pancreatic cancer (33). In the present study, DCLK1 was predicted as a functional target gene of miR-223-5p in NPC. Subsequently, DCLK1 expression was measured in NPC tissues and cells, and it was observed that DCLK1 was highly expressed in NPC. The effect of DCLK1 knockdown on NPC was similar to that of miR-223-5p overexpression. Moreover, DCLK1 overexpression was able to reverse the inhibitory effect of miR-223-5p on NPC cell viability, migration and invasion.

Furthermore, DCLK1 has been reported to be involved in the progression of human cancers via several signaling pathways. For example, Wang *et al* (34) reported that DCLK1 facilitated progression of breast cancer via the Wnt/β-catenin signaling pathway. Liu *et al* (22) indicated that DCLK1 promoted the epithelial-to-mesenchymal transition of gastric cancer cells through Notch1 signaling. The inhibition of the Notch1 signaling pathway is considered as an effective target for human cancer treatment (35). In the present study, it was observed that overexpression of miR-223-5p decreased the expression of Notch1, while this effect was partially reversed by the overexpression of DCLK1. Therefore, these results suggested that miR-223-5p and DCLK1 may regulate the tumorigenic process of NPC via the Notch1 signaling pathway.

In conclusion, the present study demonstrated that miR-223-5p expression was downregulated in NPC tissues and cells, and miR-223-5p functioned as a tumor suppressor in NPC. miR-223-5p overexpression may decrease the viability, migration and invasion of NPC cells, and suppress tumor progression via downregulating DCLK1. The present findings may improve our understanding of the mechanism involved in the progression of NPC mediated by miR-223-5p, and prompt further investigation of novel targeted therapies based on miRNA-mRNA networks for patients with NPC.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

ZZ and CC designed the study. HL, JY, HX and XT performed the experiments, analyzed the data and prepared the figures. ZZ and CC drafted the initial manuscript and revised the manuscript. ZZ, HL, JY, HX, XT and CC confirm the authenticity of all the raw data. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The present study was approved by the Ethics Committee of The Third Affiliated Hospital of Soochow University, and written informed consent was obtained from each patient enrolled in the study.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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