miRNA-22-3p inhibits cell viability and metastasis of nasopharyngeal carcinoma by targeting FOXP1

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Abstract. Nasopharyngeal carcinoma (NPC) is a malignant tumor with a high incidence rate in certain regions. MicroRNA (miRNA/miR)-22-3p is implicated in the regulation of tumorigenesis and progression. However, the biological role of miRNA-22-3p in the progression of NPC remains unclear. The present study aimed to assess the effects of miRNA-22-3p overexpression on the cell viability and migration of NPC cells. The cell viability and migration of HK-1 cells was evaluated using Transwell, wound healing and Cell Counting Kit-8 assays. To assess the epithelial-mesenchymal transition ability of NPC cells, the expression of E-cadherin, vimentin and N-cadherin was evaluated using western blot analysis. The results revealed expression of miRNA-22-3p was significantly decreased in NPC tissues compared with para-cancerous tissues. Decreased expression of miRNA-22-3p was also observed in NPC cell lines (C666-1 and HK-1). The overexpression of miRNA-22-3p reduced HK-1 cell viability and migration. In addition, a dual luciferase reporter assay revealed that miRNA-22-3p functioned as a molecular sponge for forkhead box protein 1 (FOXP1). Notably, FOXP1 overexpression counteracted the suppressive effects induced by transfection with miRNA-22-3p mimic on HK-1 cell viability and migration. Therefore, these data indicate that miRNA-22-3p may be a clinically valuable biomarker for the therapy of NPC.

Introduction

Nasopharyngeal carcinoma (NPC) is a malignant neoplasm originating from the parietal epithelial cells of the nasopharynx (1). It is the most prevalent type of cancer in otorhinolaryngology, with age-standardized rates typically <1 per 100,000 person-years (2). NPC is distinguished by its localized distribution, complex etiology, subtle onset, extensive metastasis and high invasiveness (1). The primary clinical strategy for the management of NPC is radiotherapy, albeit with numerous adverse effects (1,3). While the combination of chemo-radiotherapy yields a satisfactory 5-year survival rate of 85-90%, a recurrence and tumor metastasis still occur in 8-10% of patients (4). Hence, exploring its pathogenesis and identifying novel drugs and therapeutic targets is of utmost importance.

MicroRNAs (miRNAs/miRs) are a class of small RNA molecules (19-24 nucleotides in length) that exert regulatory control over gene expression by selectively binding and impeding the translation of specific mRNA molecules (5-7). These molecules occupy a pivotal position in a multitude of biological processes, encompassing developmental events, cellular signaling cascades and metabolic pathways (5,8). In tumorigenesis, miRNAs can function as either oncogenes or tumor suppressors, serves a crucial role in the regulation of cell proliferation, apoptosis, invasion and metastasis (9). The dysregulation of miRNA expression (such as miRNA-106a-5p, miRNA-9 and miRNA-194) has been observed in NPC, contributing to aberrant cellular growth and the development of NPC (10,11).

Among the numerous miRNAs identified, miRNA-22-3p has emerged as a novel cancer-associated miRNA. A previous study indicated that repression of miRNA-22-3p expression resulted in the suppression of the proliferative ability and the arrest of cell cycle progression, both of which were subsequently restored upon the overexpression of cyclin dependent kinase inhibitor 2C (CDKN2C) (12). In another study on patients diagnosed with glioblastoma, a marked increased expression of miRNA-22-3p was observed compared with that in healthy controls (13). Additionally, miRNA-22-3p has been reported to suppress human hepatocellular carcinoma cell proliferation and metastasis by modulating the activity of methylenetetrahydrofolate reductase (14). However, the role of miRNA-22-3p in NPC remains largely unclear. Thus, the present study aimed to elucidate the function and underlying molecular mechanisms of miRNA-22-3p in NPC.

Forkhead box protein 1 (FOXP1), a forkhead box transcription factor, has garnered significant attention due to its

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association with cancer development and progression. The altered expression of FOXP1 has been observed in several malignancies, including lymphomas, breast cancer, prostate cancer and others (15). Depending on the cancer type and cellular context, FOXP1 can function as either a tumor suppressor or oncogene, highlighting its complex role in tumorigenesis (16). The aberrant expression of FOXP1 is often associated with aggressive tumor phenotypes, worse prognoses and resistance to therapy. Therefore, elucidating the precise mechanisms through which FOXP1 regulates cancer development and progression holds promise for identifying novel therapeutic targets. The aim of the present study was to explore the role and mechanisms of action of miRNA-22 and FOXP1 in the occurrence and development of NPC.

Materials and methods

Bioinformatics analysis. StarBase (targetscan.org/vert_80/) and TargetScan (rnasysu.com/encori/) was used to predict binding sites between miRNAs and target genes.

Clinical specimens. The study protocols were approved by the Ethics Committee of the Affiliated Hospital of Guizhou Medical University (Guiyang, China; approval no. 2021-019). A total of 15 pairs of NPC tissues and para-cancerous tissue samples were collected from patients (10 male and 5 female patients; age, 54.60±11.01 years; range, 36-72 years) who underwent surgery at the Affiliated Hospital of Guizhou Medical University between February 2011 and October 2022. Written informed consent was obtained from all patients prior to the collection of samples. The tumor specimens were obtained from surgical resections of the patients, and none of the patients had undergone chemotherapy or radiotherapy prior to tumor excision. Inclusion criteria were as follows: 1. All patients must be pathologically diagnosed with NPC. 2. Include patients with untreated, newly diagnosed NPC. Exclusion Criteria: 1. Exclude patients with a history of other malignant tumors to avoid confounding factors. 2. Exclude patients with severe complications or systemic diseases, such as severe liver or kidney dysfunction, heart disease, etc. 3. Exclude patients who have received treatment.

Cells and cell culture. Human nasopharyngeal epithelial NP69SV40T cell lines were purchased from Procell Life Science & Technology Co., Ltd. Human NPC cell lines (C666-1 and HK-1) were purchased from iCell Bioscience, Inc. The cells were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS; Gibco; Thermo Fisher Scientific, Inc.) at a temperature of 37˚C with 5% CO_2 in a humidified incubator.

Transfection. Lipofectamine 3000® transfection reagent (Thermo Fisher Scientific, Inc.) was used for the transient transfection of pcDNA-negative control (NC), pcDNA-FOXP1 (100 nM), NC mimic or miRNA-22-3p mimic (50 nM) into HK-1 cells ($1.0x10^5$ cells/well). NC mimic (cat. no. B04002) and miRNA-22-3p mimic (cat. no. B02001) were synthesized by Shanghai GenePharma Co., Ltd. miRNA-22-3p mimics sequence were as follows: Sense, 5'-AAGCUGCCAGUUGAAGAACUG U-3'; antisense, 5'-AGUUCUUCAACUGGCAGCUUUU-3'. NC mimics sequences were as follows: Sense, 5'-UUCUCCGAA CGUGUCACGUTT-3'; antisense, 5'-ACGUGACACGUUCGG AGAATT-3'. Following transfection at 37˚C for 20 min, the cells were cultured for 48 h prior to further experiments.

Reverse transcription (RT)-quantitative polymerase chain reaction (qPCR). Total RNA was extracted from the NP69SV40T, C666-1 and HK-1 cells or NPC tissues using TRIzol™ reagent (Thermo Fisher Scientific, Inc.). cDNA was synthesized using a RT kit (cat. no. 11904018; Invitrogen™; Thermo Fisher Scientific, Inc.). The RT reaction conditions were as follows: 95˚C for 30 sec, followed by 40 cycles of 95˚C for 5 sec and 60˚C for 30 sec. The relative levels of target gene RNA transcriptome were determined using qPCR using the SYBR Premix Ex Taq kit (cat. no. RR820; Takara Bio Inc.). The thermocycling conditions were as follows: Initial denaturation at 95˚C for 10 min, followed by 40 cycles of 95˚C for 15 sec and 60˚C for 60 sec. The relative gene expression level was calculated using the $2^{-\Delta\Delta Cq}$ method (17) using ABI software (Veriti 96-Well; Thermo Fisher Scientific, Inc.). The following primer sequences were used for RT-qPCR: FOXP1 forward, 5'-TCCAGAAAAGCAGCTAACACTA-3' and reverse, 5'-TTC TACTCGCACAAAACACTTG-3'; GAPDH forward, 5'-TGA CTTCAACAGCGACACCCA-3' and reverse, 5'-CACCTG TTGCTGTAGCCAAA-3'; miRNA-22-3p forward, 5'-AAG CTGCCAGTTGAAGAACTGTA-3' and reverse, 5'-GCT GTCAACGATACGCTACGTA-3'; U6 forward, 5'-ACTTCA GCAGCACATATACTAAAAA-3' and reverse, 5'-CGCTTC ACGAATTTGCATGTCAT-3'. cDNA was synthesized using the Mir-X miRNA FirstStrand Synthesis Kit (cat. no. 638315; Takara Bio Inc.) The relative levels of target miRNA transcripts were determined by RT-qPCR using the Mir-X miRNA qRT-PCR TB Green Kit (cat. no. 638316; Takara Bio Inc.).

Western blot analysis. The HK-1 cell line was used for western blot analysis. The cell lysis solution was prepared using RIPA buffer from Cell Signaling Technology, Inc. The protein concentration was determined using a BCA assay. A total of 30 μg protein/lane was separated using 10% SDS-PAGE and transferred onto nitrocellulose membranes. The membranes were then blocked with 5% non-fat dried milk overnight at 4˚C and incubated with the following corresponding protein antibodies: E-cadherin (1:2,000; cat. no. A3044; ABclonal Biotech Co., Ltd.), N-cadherin (1:2,000; cat. no. BS-1172R; BIOSS), vimentin (1:2,000; cat. no. A19607; ABclonal Biotech Co., Ltd.), FOXP1 (1:2,000; cat. no. ab134055133595; Abcam) and β-actin (1:50,000; cat. no. AC026; ABclonal Biotech Co., Ltd.) overnight at 4˚C.

Subsequently, the membranes underwent a washing process with Tris-buffered saline/0.1% Tween (TBST) and were subjected to a 1.5-h incubation period at room temperature with a HRP goat anti-rabbit IgG (1:5,000; cat. no. S0001; Affinity Biosciences, Ltd.). The bands were visualized using an ECL detection system (ECL Plus; Cytiva), with β-actin serving as the internal control. The net optical density was semi-quantified using Quantity One software (V4.6.2; Bio-Rad Laboratories, Inc.).

Dual-luciferase reporter assay. Wild-type (Wt) and mutant (Mut) FOXP1-3' untranslated region (3'UTR) sequences were cloned into the luciferase reporter plasmid psiCHECK-2 vector

(cat. no. C8021; Promega Corporation). Subsequently, the luciferase reporter gene plasmid and either miRNA-22-3p mimic or NC mimic were co-transfected into 293T cells (Procell Life Science & Technology Co., Ltd.; $4x10^4$ cells/well) using Lipofectamine 3000® (Thermo Fisher Scientific, Inc.). The dual-luciferase activity was measured 48 h after transfection. The dual-Luciferase reporter system (cat. no. E1910; Promega Corporation) was used to quantify luciferase activities according to manufacturer's protocol. Luciferase activity was standardized by comparison with Renilla luciferase activity.

Cell counting kit-8 (CCK-8) assay. The viability of the HK-1 cells was assessed using the CCK-8 assay (Thermo Fisher Scientific, Inc.) following the manufacturer's guidelines. CCK-8 was added into each well and incubated for 3 h. The absorbance was measured at 450 nm.

Wound-healing assay. HK-1 cells were cultured in 96-well plates until they reached confluency in DMEM supplemented with 10% FBS at 37˚C. The cell monolayers were gently scratched using a 200-μl pipette tip and the cells were incubated in serum-free DMEM for 24 h at 37˚C. To remove any detached cells, the wells of the plate were gently washed with fresh medium. The distance between edges of the wound was measured under a light microscope (Olympus Corporation), and multiple visual fields were selected for observing each well. After 24 h, the wound channel distance was measured again for analysis. The wound area was measured using Image J software (Version 1.48; National Institutes of Health). Wound-healing assay results were presented as migration rate (%)=(initial wound area-wound area at 24 h)/initial wound area x100.

Transwell assay. A concentration of $1x10^5$ HK-1 cells/ml was suspended in DMEM, and 200 μl of the cell suspension was plated into the upper chambers of 24-well Transwell plate precoated with Matrigel (BD Biosciences) at room temperature for 24 h. The lower chambers were filled with 600 μl DMEM supplemented with 10% FBS (Gibco; Thermo Fisher Scientific, Inc.). Subsequently, the cells were incubated in a 5% CO_2 and 37˚C incubator for 48 h. Following this, the cells were fixed with 4% paraformaldehyde at room temperature for 20 min and stained with 0.1% crystal violet at room temperature for 15 min. The total number of cells in five randomly selected fields of view was observed using an inverted light microscope (Olympus Corporation) and the mean number of cells was calculated.

Statistical analysis. The data are presented as the mean ± standard deviation. Statistical analysis was performed using SPSS 20.0 software (IBM Corp.). Multiple groups were compared using one-way analysis of variance followed by Tukey's post hoc test, and two groups were compared using unpaired Student's t-test. $P<0.05$ was considered to indicate a statistically significant difference.

Results

Expression of miRNA-22-3p and FOXP1 differs between NPC tissues and para-cancerous tissues. The expression of miRNA-22-3p was demonstrated to be significantly reduced in NPC tissues compared with para-cancerous tissues, as shown by RT-qPCR (Fig. 1A). Moreover, significantly decreased expression of miRNA-22-3p also observed in the NPC cell lines, HK-1 and C666-1 compared with normal nasopharyngeal epithelial cells (Fig. 1B). In addition, the expression levels of FOXP1 were significantly increased in NPC issues compared with para-cancerous tissues (Fig. 1C).

Overexpression of miRNA-22-3p reduces the malignant behaviors of NPC cells. miRNA-22-3p mimic was constructed to enhance miRNA-22-3p expression. Compared with the control group, the miRNA-22-3p levels significantly increased effectively by transfection with miRNA-22-3p mimic in HK-1 cells (Fig. 2A). Furthermore, the results revealed that compared to the control group, miRNA-22-3p mimic significantly inhibited the viability of HK-1 cells (Fig. 2B). Moreover, compared to the control group, the overexpression of miRNA-22-3p significantly suppressed the migration of HK-1 cells (Fig. 2C-F). Compared to the control group, the expression of FOXP1 and the epithelial-mesenchymal transition-related proteins, vimentin and N-cadherin, was significantly reduced, and the expression of E-cadherin was significantly induced in miRNA-22-3p mimic-transfected HK-1 cells (Fig. 2G-K).

Binding association between miRNA-22-3p and FOXP1. Using the bioinformatics databases, StarBase and TargetScan, miRNA-22-3p was predicted to bind to the 3'UTR of FOXP1 (Fig. 3A). The miRNA-22-3p mimic significantly suppressed the luciferase activity of the FOXP1 wild-type (Wt) reporter compared with the NC mimic, but not that of the mutant (Mut) reporter in 293T cells (Fig. 3B). Moreover, specific primers were designed to perform RT-qPCR analysis of the expression of FOXP1 in 15 pairs of NPC tissues and para-cancerous tissues, and it was demonstrated that the mRNA level of FOXP1 was significantly increased in NPC tissues compared with the para-cancerous tissues (Fig. 3C).

FOXP1 overexpression inhibits miRNA-22-3p mimic-mediated NPC progression. Subsequently, the present study aimed to investigate the effects of FOXP1 on the miRNA-22-3p-mediated progression of NPC. Notably, compared with the control, transfection with miRNA-22-3p mimic resulted in significant inhibition of FOXP1 protein expression, which was subsequently reversed upon transfection with pcDNA-FOXP1 (Fig. 4A and B). Furthermore, similar trends were observed in the protein expression levels of vimentin and N-cadherin, as well as E-cadherin (Fig. 4C-E). Notably, the overexpression of FOXP1 in HK-1 cells in miRNA-22-3p + FOXP1 significantly counteracted the suppressive effects of miRNA-22-3p mimic on cell viability compared to the miRNA-22-3p group (Fig. 4F). Consistently, co-treatment with pcDNA-FOXP1 in the miRNA-22-3p + FOXP1 group markedly abrogated the inhibitory effects exerted by miRNA-22-3p mimic on NPC cell migration, as compared to the miRNA-22-3p group (Fig. 4G-I). Collectively, these findings indicate that miRNA-22-3p mimics suppress NPC cell viability and migration through the negative regulation of FOXP1 expression.

Discussion

The regulation of target gene expression by miRNAs involves the specific binding of miRNAs to their complementary target

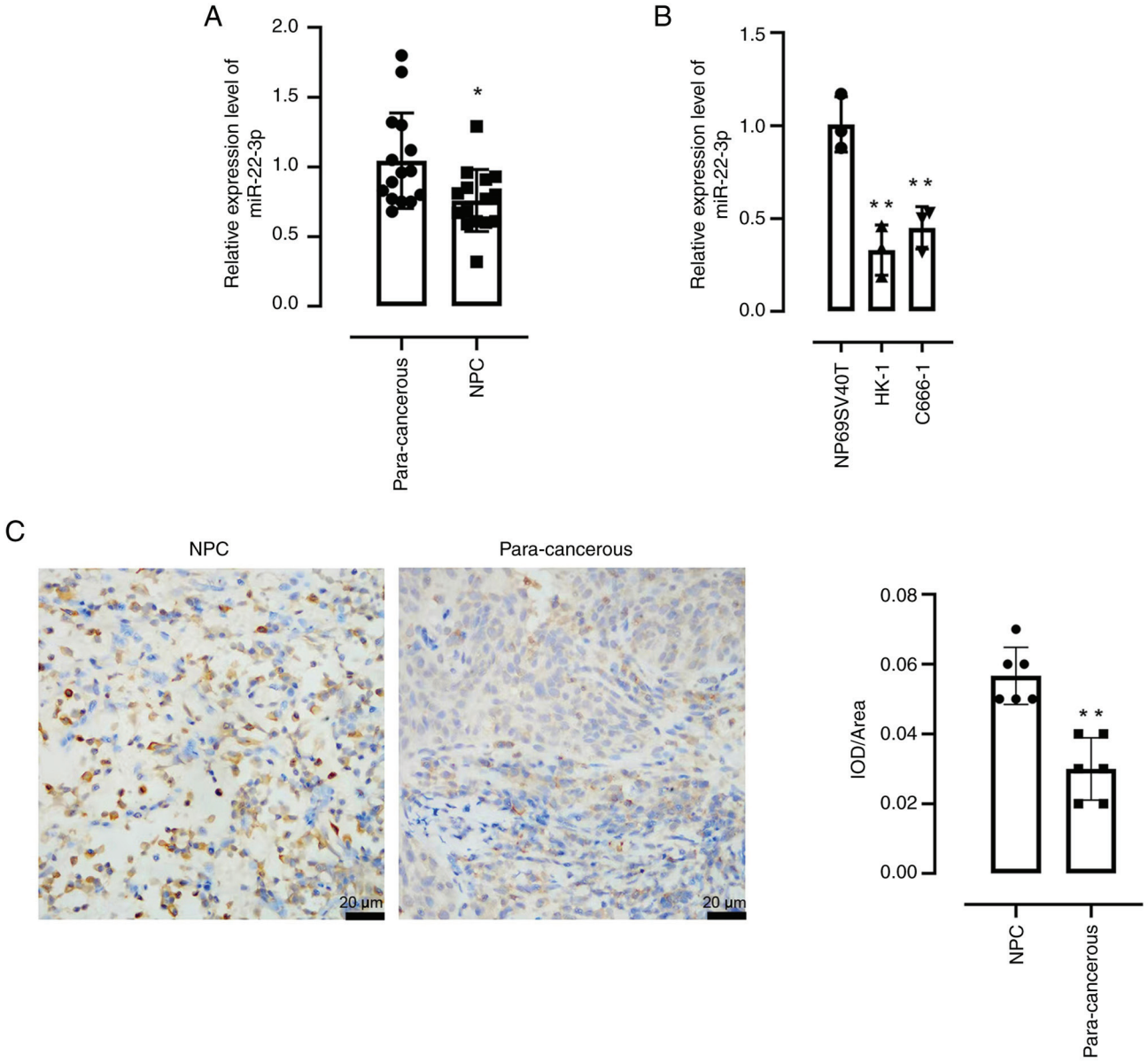

Figure 1. Expression of miRNA-22-3p and FOXP1. (A) Reverse transcription-quantitative polymerase chain reaction of miRNA-22-3p expression in NPC tissues and para-cancerous tissues. (B) Expression of miRNA-22-3p in NPC cell lines. (C) Expression of FOXP1 in NPC tissues and para-cancerous tissues. *P<0.05; **P<0.01. NPC, nasopharyngeal carcinoma; miRNA/miR, microRNA; FOXP1, forkhead box protein 1; IOD, integrated optical density.

mRNAs, resulting in the inhibition of target gene translation or degradation of the target mRNAs (18). In cancer cells, the aberrant expression of miRNAs can lead to dysregulated target gene expression, thereby affecting the development and progression of cancer (19,20). Numerous investigations have been performed to explore the regulatory role of miRNAs in NPC. Notably, a previous study reported that the expression of miRNA-194 was markedly decreased in both NPC tissue and cells, resulting in the suppression of proliferation and invasion in NPC cells through the direct targeting of MAP3K3 (11). Furthermore, another study reported that miRNA-146a enhanced NPC progression by modulating Epstein-Barr virus latent membrane protein 1 (21). In addition, the decreased expression of miRNA-506 has been observed in NPC, and it functions as a potent tumor suppressor by facilitating apoptosis and suppressing invasion and migration of NPC cells through the direct targeting of EZH2 (22). Thus, miRNAs are of utmost importance in the progression of NPC, rendering them promising targets for both fundamental and applied research into this ailment.

In the present study, the data revealed that the expression of miRNA-22-3p was significantly decreased in NPC tissues and cells, and the overexpression of miRNA-22-3p inhibited the cell viability and migration of NPC cells *in vitro* by directly targeting FOXP1. It has been reported that miRNA-22-3p exhibits diverse biological functions by regulating several target genes. By targeting MAPK14, miRNA-22-3p has been reported to suppress the proliferation and differentiation, while enhancing the apoptosis, of $CD14^{+}$ peripheral blood mononuclear cells (23). Targeting of high mobility group box 1 by miRNA-22-3p in arteriosclerosis obliterans leads to suppression of arterial smooth muscle cell proliferation and migration, as well as a reduction in neointimal hyperplasia (24). The long non-coding RNA metastasis-associated

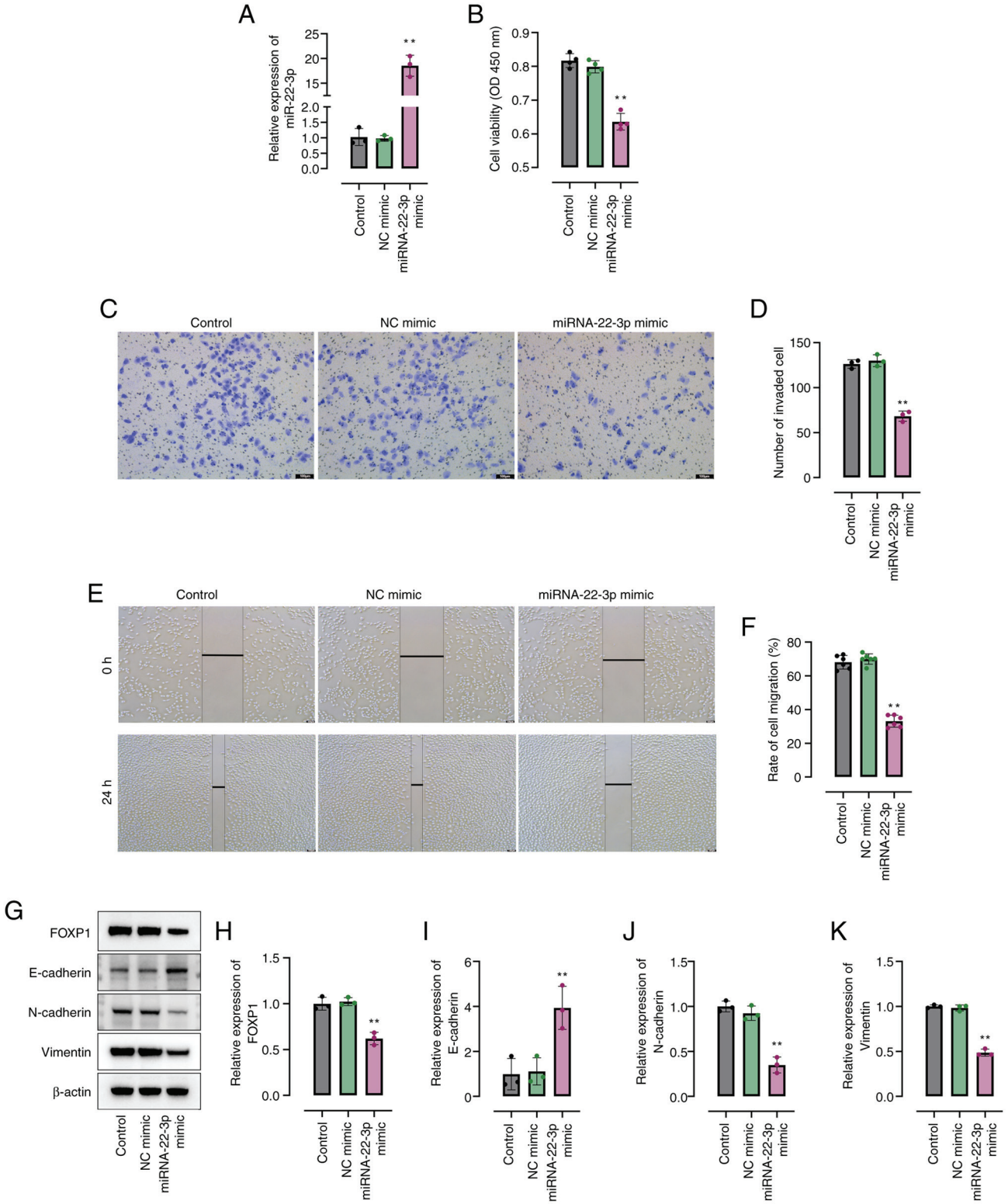

Figure 2. Overexpression of miRNA-22-3p reduces the malignant behaviors of nasopharyngeal carcinoma cells. (A) miRNA-22-3p expression was determined using reverse transcription-quantitative polymerase chain reaction. (B) Effects of miRNA-22-3p mimic on the cell viability of HK-1 cells detected using Cell Counting Kit-8 assay. (C) A Transwell assay was performed using HK-1 cells transfected with miRNA-22-3p mimic. (D) Number of invaded cells. (E) Effects of miRNA-22-3p overexpression on HK-1 cell migration detected using a wound-healing assay. (F) Rate of cell migration. (G) Western blot analysis used to determine the expression of (H) FOXP1, (I) vimentin, N-cadherin and E-cadherin. (J) Relative expression level of N-cadherin. (K) Relative expression level of E-cadherin. β-actin was used as the reference protein. **P<0.01 vs. NC mimic. miRNA/miR, microRNA; NC, negative control; OD, optical density; FOXP1, forkhead box protein 1.

lung adenocarcinoma transcript 1 safeguards endothelial function against oxidized low-density lipoprotein-induced dysfunction by enhancing the expression of miRNA-22-3p target genes, C-X-C motif chemokine receptor 2 and AKT (25). Notably, miRNA-22-3p promotes the occurrence and development of hepatocellular carcinoma by targeting CDKN2C (14)

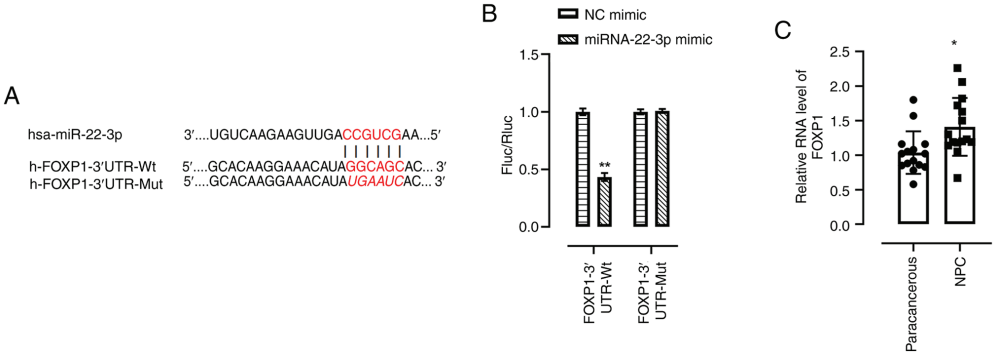

Figure 3. Binding association between miRNA-22-3p and FOXP1. (A) The predicted miRNA-22-3p binding site in the 3'UTR sequences of FOXP1. (B) miRNA-22-3p negatively regulated the luciferase activity of FOXP1-3'UTR-Wt, but not FOXP1-3'UTR-Mut in 293T cells. (C) FOXP1 expression in NPC tissues was analyzed using reverse transcription-quantitative polymerase chain reaction. *P<0.05; **P<0.01 vs. NC mimic. miRNA/miR, microRNA; NC, negative control; Wt, wild type; Mut, mutant; UTR, untranslated region; Rluc, *Renilla* luciferase; fluc, firefly luciferase; FOXP1, forkhead box protein 1; NPC, nasopharyngeal carcinoma.

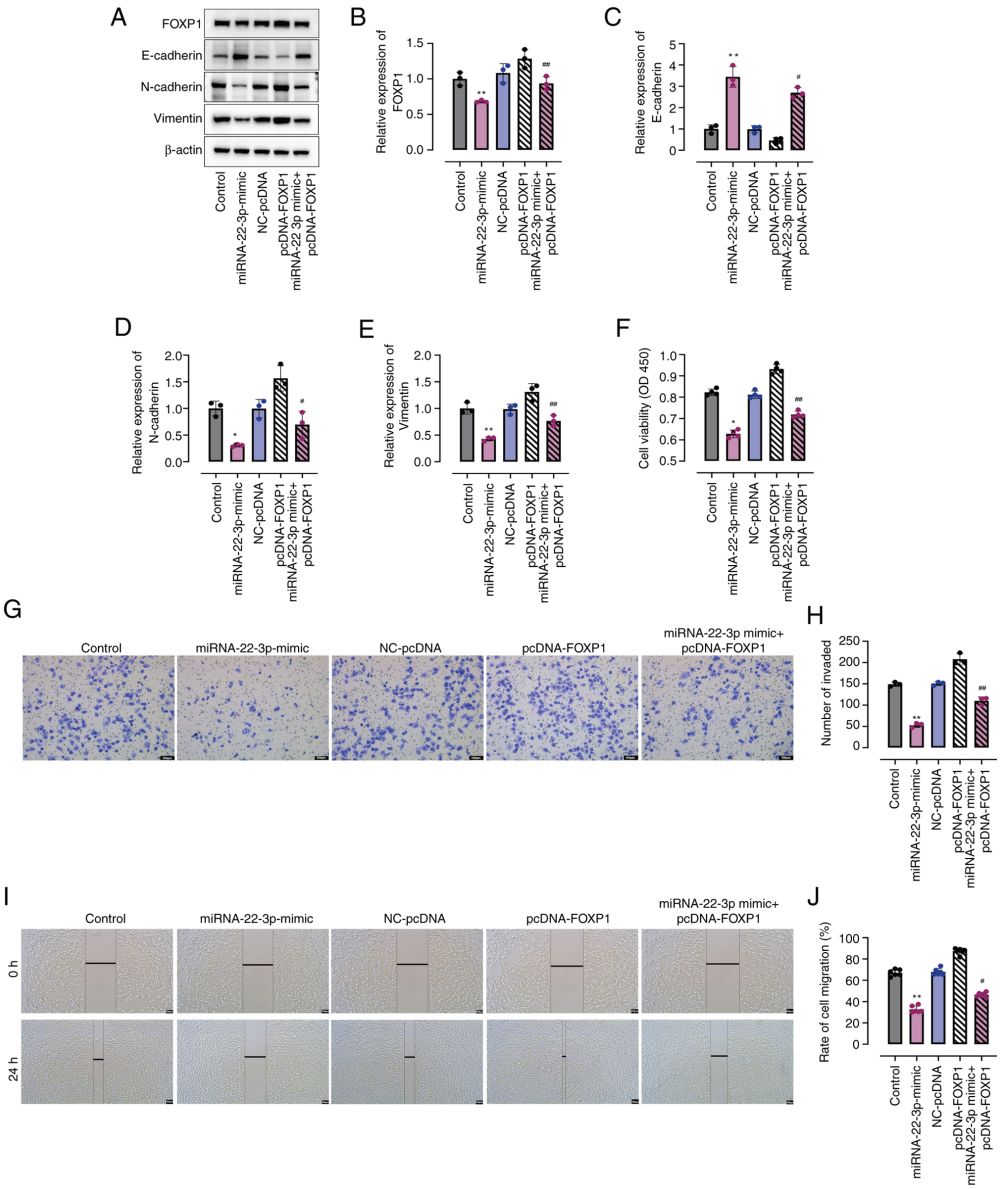

Figure 4. FOXP1 overexpression inhibits the suppressive effects of miRNA-22-3p mimic on NPC progression. (A) Western blot analysis was used to determine the expression of (B) FOXP1, vimentin, N-cadherin and E-cadherin. β-actin was used as the reference protein. (C) Relative expression level of vimentin. (D) Relative expression level of N-cadherin. (E) Relative expression level of E-cadherin. (F) Viability of HK-1 cells was assessed using the Cell Counting Kit-8 assay. (G) A Transwell assay was used to evaluate HK-1 cell migration. (H) Number of invaded cells. (I) HK-1 cell migration was detected using a wound-healing assay. (J) Rate of cell migration. *P<0.05; **P<0.01 vs. Control. $^{\#}$P<0.05; $^{\#\#}$P<0.01 vs. pcDNA-FOXP1. NC, negative control; miRNA, microRNA; FOXP1, forkhead box protein 1.

and methylenetetrahydrofolate reductase proteins (16). In triple-negative breast cancer, miRNA-22-3p exerts tumor suppressive effects by selectively targeting clinically relevant oncogenic signaling pathways, including the eEF2K/PI3K/Akt and Src signaling cascades (26). In the present study, another target gene of miRNA-22-3p, FOXP1, was identified, and the role of miRNA-22-3p in inhibiting the progression of NPC was reported for the first time. In addition, the bidirectional regulatory effect of miRNA-22-3p on the progression of different cancers may be due to the fact that, in different types of cancer cells, miRNA-22-3p targets different genes and thus performs different functions. Furthermore, the influence of the cellular environment and signaling pathways may also lead to changes in the action of miRNA-22-3p. Therefore, further studies are required to explore the possibility that miRNA-22-3p modulates changes in signaling pathways in NPC cells.

The biological function of the FOX transcription factor family proteins lies in their ability to modulate gene expression through specific DNA binding, thereby exerting regulatory control over target genes and ultimately influencing cellular growth, differentiation and development (27,28). Among these, FOXP1 exhibits a diverse array of biological functions, encompassing the regulation of B-cell development and the multifaceted differentiation of monocytes (29,30). Furthermore, FOXP1 has been implicated as either an oncogenic or tumor suppressor gene in several malignancies. The 3p14.1 position of FOXP1 was identified as a potential tumor suppressor binding site due to the loss of heterozygosity at the 3p position of chromosomes in a variety of human tumors (31). The decreased expression of FOXP1 has been reported in several solid tumors, such as bowel cancer and lung cancer (32). In addition, it has been reported that a high expression of FOXP1 is predictive of a good prognosis in patients with non-small cell lung cancer, suggesting a tumor-suppressive effect of FOXP1 (33,34). By contrast, other studies have reported that the increased expression of FOXP1 in patients with hepatocellular carcinoma, gastric mucosa-associated lymphoma and B-cell lymphoma is associated with a poor prognosis (35-37). As an oncogene, FOXP1 can widely inhibit the expression of numerous pro-apoptotic genes in B-cell lymphoma, such as tumor protein 63, Ras association domain family member 6 and tumor protein P53 inducible nuclear protein 1 (36). A previous study has substantiated the role of FOXP1 in enhancing the activity of the Wnt/β-catenin signaling pathway in B-cell lymphoma (38). Consequently, activation of the Wnt signaling pathway has been implicated in facilitating tumor growth (38). However, little is known about the expression level and role of FOXP1 in NPC. In the present study, it was found that FOXP1 reversed the suppressive effects of miRNA-22-3p mimic on NPC cell viability and migration. The results presented in the present study indirectly demonstrate the oncogenic role of FOXP1 in NPC.

In conclusion, the present study demonstrates that miRNA-22-3p directly inhibits the expression of FOXP1, thereby inhibiting the cell viability and migration of NPC cells. The results confirmed that miRNA-22-3p serves a role as a tumor suppressor in NPC, suggesting that miRNA-22-3p may be a novel therapeutic target for NPC. However, the present study has limitations. Firstly, the clinical sample size was relatively small and more patients need to be included in subsequent studies. Secondly, the present study has not been validated in animal models. Further *in vivo* studies are required to clarify the anti-NPC effects of miRNA-22-3p and its mechanisms with the intention of clinical applications in detail. Finally, further studies are required to determine other downstream mechanisms of miRNA-22-3p in NPC, such as some signaling pathways.

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Availability of data and materials

The data generated in the present study may be requested from the corresponding author.

Authors' contributions

YJin, ZW, YL and YJia wrote the manuscript and performed experiments. FY and TZ analyzed and interpretation of data. All authors have read and approved the final manuscript. YJin and TZ confirm the authenticity of all the raw data.

Ethics approval and consent to participate

The study protocols were approved by the Ethics Committee of the Affiliated Hospital of Guizhou Medical University (Guiyang, China; approval no. 2021-019). Written informed consent was obtained from all patients prior to the collection of samples.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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