European Review for Medical and Pharmacological Sciences 2020; 24: 8008-8016

Long non-coding RNA FOXP4-AS1 acts as an adverse prognostic factor and regulates proliferation and apoptosis in nasopharyngeal carcinoma

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Abstract. – OBJECTIVE: Nasopharyngeal carcinoma (NPC) is one of the most common malignancies worldwide. In The Cancer Genome Atlas (TCGA) database, the expression level of lncRNA forkhead box P4 antisense RNA 1 (FOXP4-AS1) is higher in NPC samples than in normal samples.

PATIENTS AND METHODS: Quantitative Real-time PCR and Western blotting were performed to detect the expression level of RNA and protein. Luciferase reporter assay ran to test the interactions between FOXP4-AS1 and miR-423-5p and STMN1. Subcellular fractionation assay was used to determine the subcellular localization of FOXP4-AS1. The tumor-promotion functions of FOXP4-AS1 were determined by both *in vitro* and *in vivo* assays.

RESULTS: The expression of FOXP4-AS1 was up-regulated in 80 cases with NPC, and these patients with a poor prognosis. Functionally, high expression of FOXP4-AS1 in NPC was connected with promoted cell proliferation and inhibited apoptosis. Moreover, FOXP4-AS1 is located in the cytoplasm of CNE1 (NPC cell lines). Mechanistically, FOXP4-AS1 up-regulated STMN1 on post-transcriptional regulation by means of miR-423-5p.

CONCLUSIONS: Our present study demonstrated that high expression of FOXP4-AS1 in NPC portended poor outcomes. FOXP4-AS1up-regulated STMN1 by interacting with miR-423-5p as a competing endogenous RNA (ceRNA) to promote NPC progression.

Key Words:
Nasopharyngeal carcinoma, LncRNA FOXP4-AS1, MiR-423-5p, STMN1.

Introduction

Nasopharyngeal carcinoma (NPC), which derives from the nasopharynx epitheliums, is one of the highest prevalence malignancies in East and Southeast Asia[1,2]. Despite remarkable advances in clinical diagnosis and treatment strategies, the prognosis of patients with advanced stage of nasopharyngeal carcinoma is still poor. Therefore, a better understanding of the regulatory mechanisms underlying nasopharyngeal carcinoma pathogenesis is urgently needed in clinical management[3]. Long non-coding RNAs (lncRNAs) are a new class of RNA transcripts longer than 200 nucleotides and lacking protein coding potential. LncRNAs according to a specific way are expressed in cell nucleus or cytoplasm. The lncRNAs play a vital role on expression of disease related genes[4] and exert pivot roles in all kinds of human cancers, including breast cancer, glioma, liver cancer and so on[5]. Of note, FOXP4-AS1, a 24.727 kb lncRNA, was found to be highly expressed in human nasopharyngeal carcinoma and was correlated with tumor progression and poor prognosis[6,7]. However, the potential role of FOXP4-AS1 in the development of nasopharyngeal carcinoma has not been studied. Herein, we aimed to explore the role of FOXP4-AS1 in nasopharyngeal carcinoma.

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Patients and Methods

Patient Specimens and Cell Culture

80 matched nasopharyngeal tumor and adjacent non-tumor nasopharyngeal tissues were collected from preliminary diagnosed patients with NPC in the department of ENT of the Affiliated Traditional Chinese Medical Hospital of Southwest Medical University from January 2010 to January 2016. All tissues were taken by nasopharyngoscopy and stored at −80°C. All participants signed informed consents. Our study was authorized by the Research Ethics Committee of the department of ENT of the Affiliated Traditional Chinese Medical Hospital of Southwest Medical University. According to the median of the expression level of FOXP4-AS1, miR-423-5p or STMN1, 80 NPC tissues were classified into high-expression group (n = 40) and low-expression group (n = 40).

Human NPC cell line (CNE1, HONE1, C666-1, CNE2) and human normal nasopharyngeal epithelial cell line (NP69) were presented by Professor Chaonan Qian (Sun Yat-sen University Cancer Center, Guangzhou, China) and cultured in Dulbecco's Modified Eagle's Medium (DMEM; Invitrogen, Carlsbad, CA, USA) supplemented with 10% fetal bovine serum (FBS, Gibco, Rockville, MD, USA) at 37°C, 5% CO_2 incubator. All cell lines used in this study have been certified by a professional organization (Suzhou Genetic Testing Biotechnology Co., Ltd, China).

Quantitative Real-Time PCR

Total RNA was collected from tissue samples or cell lines utilizing TRIzol reagent (Invitrogen, Carlsbad, CA, USA). RNA (2 mg) was reverse transcribed into cDNA using random primers and Reverse Transcription Kit (Tiangen, China), cDNA productions were stored at -20°C. qRT-PCR was performed on Roche 480 Real-Time PCR system (Roche, Basel, Switzerland), and SYBR Green PCR Master Mix (Roche, Basel, Switzerland) was used to detect the expression of lncRNA or miRNA. The primers of miR-423 and U6 were purchased from GeneCopoeia (GeneCopoeia Inc. Rockville, MD, USA). Relative expression was normalized to 18S or U6 and was determined by the $2^{-\Delta\Delta Ct}$ method. All the primer sequences used in this study were listed in Supplementary **Table S1**.

Cell Transfection

The small interfering RNAs (siRNAs) against FOXP4-AS1 (si-FOXP4-AS1#1/2) which were designed by GeneCopoeia (GeneCopoeia, China) were transfected into CNE1 cells at 80-90% confluence utilizing the Lenti-Pac™ HIV Expression Packaging Kit (GeneCopoeia, China) in 50 mm dishes. MiR-423-5p mimics and miR-423-5p inhibitor were bought from GeneCopoeia and were transfected into CNE1 cell lines utilizing the aforementioned kit to upregulate or silence miR-423-5p. Similarly, the whole sequence of STMN1 was cloned into pcDNA3.1 vectors (GeneCopoeia, China). Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA) was used for transfection following the protocol. RNA was collected after 48 h and protein were obtained after 72 h from successful transfected CNE1 cells for following experiments.

Cell Proliferation Assays

A total of 1×10^3 transfected CNE1 cell lines were plated into 96-well plates. The cell growth rate was analyzed by Cell Counting CCK-8 kit (Dojindo Molecular Technologies, Kumamoto, Japan) according to the guideline. For foci formation assay, 1×10^3 CNE1 cells were plated in six-well plates for 2 weeks. Colonies comprised of > 50 cells were fixed in 4% paraformaldehyde for 10 min and dyed in 0.1% crystal violet for 30 min. Finally, colonies were counted and recorded under 20× microscope. By the above experiments. Triplicate independent tests were repeated.

Cell Cycle Analysis

Cells were synchronized by starving in G0/G1 phase, then fixed in pre-cooled 75% ethanol and stained with propidium iodide (PI; Sigma-Aldrich, St. Louis, MO, USA). The DNA content of G0/G1, S, G2/M phase in cell cycle was examined by CytoFLEX (Beckman Coulter, Brea, CA, USA) and calculated with CytoExpert and ModFitLT software. Triplicate independent tests were repeated.

Apoptosis Assay

Cells with starvation treatment were obtained and stained with Annexin V/PI Staining Kit (KeyGEN BioTECH, Shanghai, China). Apoptosis was examined by CytoFLEX (Beckman Coulter, Brea, CA, USA). Triplicate independent tests were repeated.

Subcellular Fractionation Assay

The subcellular fractionation assay was performed by utilizing the Cytoplasmic and Nuclear RNA Purification Kit (Norgenbiotek Corpora-

tion, Thorold, ON, Canada) according to the principles of the guideline. The isolated RNA was quantified and calculated by quantitative real-time PCR. 18S and U6 as standardized indicators respectively represented cytoplasmic and nuclear fractions.

Luciferase Reporter Assay

The amplified FOXP4-AS1 or STMN1 was cloned into the plasmids in pmirGLO Luciferase vector (GeneChem, Shanghai, China). The wild type of FOXP4-AS1 or STMN1 3'-UTR (FOXP4-AS1-WT or STMN1-WT) was constructed. The site-directed mutation of miR-423-5p binding sites in FOXP4-AS1 or STMN1 3'-UTR (FOXP4-AS1-MUT or STMN1-MUT) was generated using the GeneTailor™ Site-Directed Mutagenesis System (Invitrogen, Carlsbad, CA, USA). MiR-423-5p mimics or miR-NC were separately cotransfected with the aforementioned plasmids in CNE1 cells. At last, a Dual-Luciferase Reporter Assay System (Promega, Madison, WI, USA) was used to evaluate Luciferase activity.

Animal Experiments

All experimentation on animals were authorized and carried out with the principles of the Welfare of Experimental Animals in the Department of ENT of the Affiliated Traditional Chinese Medical Hospital of Southwest Medical University. 5×10^6 si-NC group cells, si-FOXP4-AS1#1 group cells, si-FOXP4-AS1#1 + miR-423-5P inhibitor group cells and si-FOXP4-AS1#1+ PC DNA3.1 group cells were subcutaneously injected into the dorsal flanks of nude BALB/c mice (n = 6, 4-5 week-old, male). The xenografts were measured every week. Xenograft volume was defined by V = 0.5 × L (length of tumor) × W2 (width of tumor).

Statistical Analysis

Statistical analysis used SPSS 22.0 (IBM SPSS, Armonk, NY, USA) and GraphPad PRISM 6 (GraphPad, San Diego, CA, USA). Kaplan-Meier analysis was utilized to analyze the overall survival rate of patients with NPC. Differences between groups were analyzed by Student's *t*-test or one-way ANOVA. Bonferroni test method was used as post-hoc test to validate ANOVA. $p< 0.05$ was recognized to be significant. All data were presented as the mean ±standard deviation in triplicate independent experiments.

Results

FOXP4-AS1 is Significantly Up-Regulated in Nasopharyngeal Carcinoma Tissues and Cell Lines

The expression level of FOXP4-AS1 was detected in nasopharyngeal carcinoma and adjacent specimens which collected from 80 patients with nasopharyngeal carcinoma. As expected, FOXP4-AS1 was expressed at a higher level in nasopharyngeal carcinoma specimens in contrast to adjacent normal specimens (Figure 1A). Consistently, FOXP4-AS1 was higher expressed in nasopharyngeal carcinoma cell lines compared to human normal nasopharyngeal epithelial cell NP69 (Figure 1B). Furthermore, to analyze the prognostic correlation of FOXP4-AS1 in patients with nasopharyngeal carcinoma, Kaplan-Meier

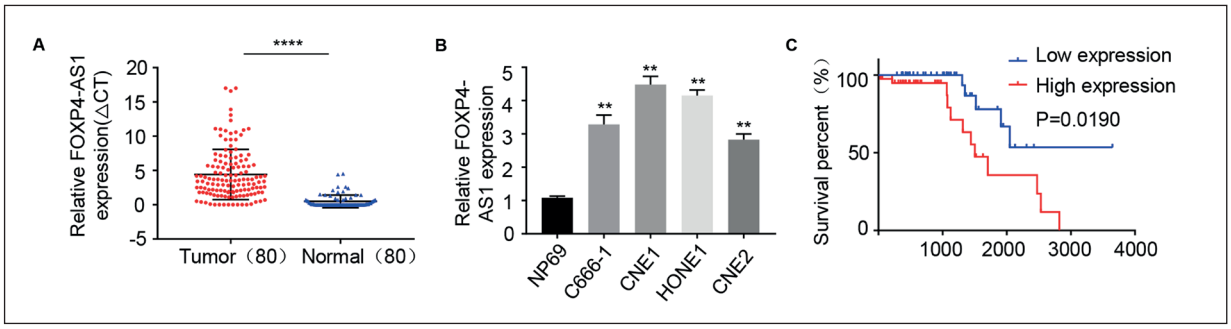

Figure 1. FOXP4-AS1 is significantly up-regulated in nasopharyngeal carcinoma tissues and cell lines. **A**, The expression level of FOXP4-AS1 was examined in nasopharyngeal carcinoma samples and adjacent samples collected from 80 patients with nasopharyngeal carcinoma. **B**, Expression level of FOXP4-AS1 in nasopharyngeal carcinoma cell lines and one human normal nasopharyngeal epithelial cell NP69. **C**, Overall survival of 80 patients with nasopharyngeal carcinoma with high (n=40) or low (n=40) FOXP4-AS1 level was analyzed. FOXP4-AS1 median expression value is cut-off value. Kaplan-Meier survival curve to assess overall survival in both groups. $^{**}p < 0.01$, $^{***}p < 0.0001$.

surviving curves were plotted. After analysis, we determined that high level of FOXP4-AS1 has been associated with adverse outcomes in nasopharyngeal carcinoma patients (Figure 1C).

Silencing of FOXP4-AS1 Inhibits NPC Cell Proliferation and Induces Cell Apoptosis

To validate the functions of FOXP4-AS1 in nasopharyngeal carcinoma cellular, we designed functional experiments in nasopharyngeal carcinoma cell line. On the basis of the data in Figure 1B, CNE1 cell presented the highest level of FOXP4-AS1 expression. Thus, we silenced FOXP4-AS1 in CNE1 cell (Figure 2A). Primarily, we implemented the cell proliferation assay (CCK-8 assay) to detect the growing ability of cells with up or down regulated of FOXP4-AS1. The results indicated that downregulation of FOXP4-AS1 led to decreased cell viability ($p < 0.01$, ANOVA; Figure 2B). Cell growing ability was inhibited more efficiently when cells were transfected with si-FOXP4-AS1#2. Meanwhile, via foci formation assays, we determined that knockdown of FOXP4-AS1 led to markedly inhibit cell proliferation ($p < 0.01$, ANOVA; Figure 2C,

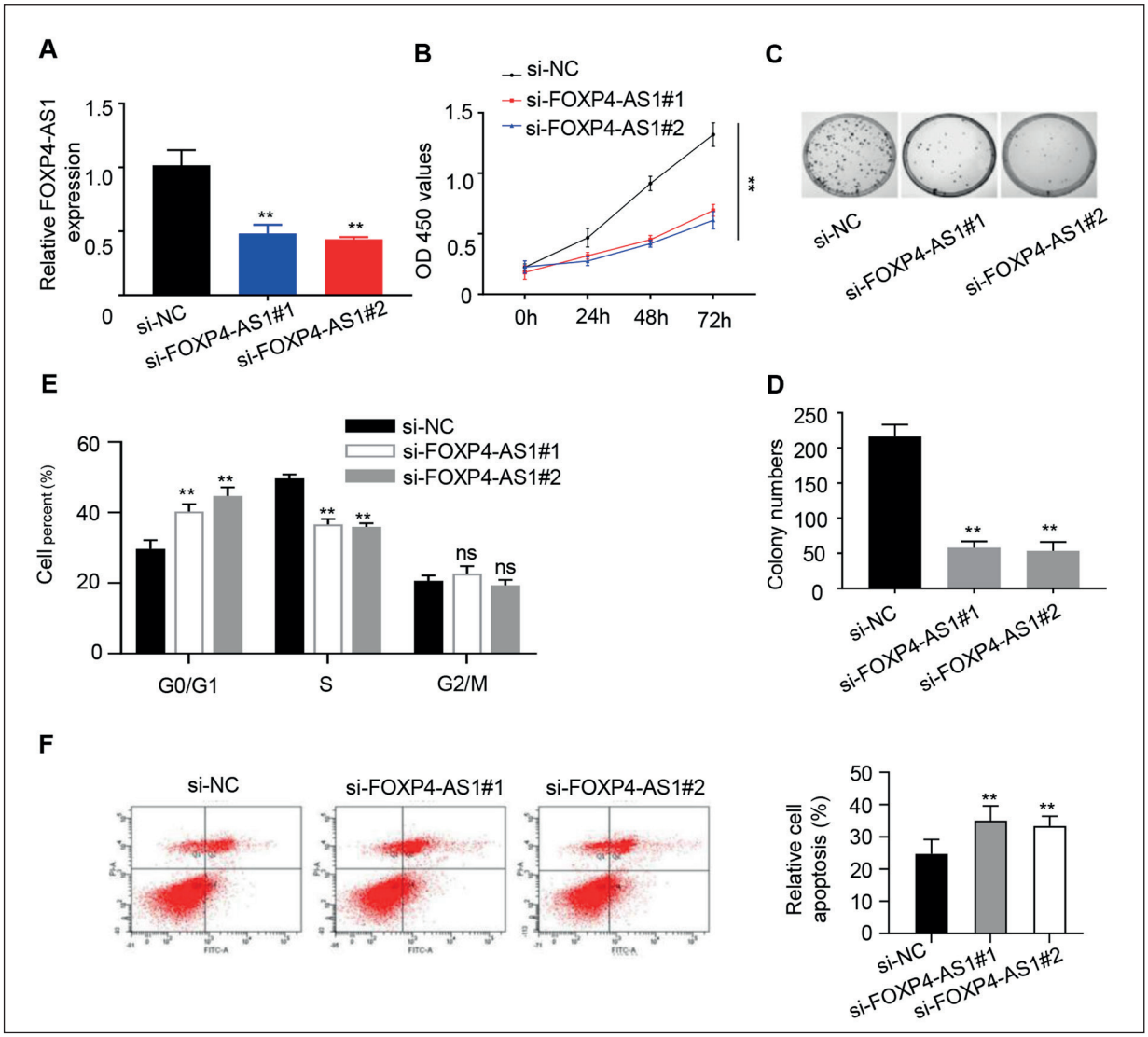

Figure 2. Silencing of FOXP4-AS1 inhibits NPC cell proliferation and induces cell apoptosis. **A**, Knockdown efficiency for FOXP4-AS1S (si-FOXP4-AS1#1 and si- FOXP4-AS1#2) in CNE1 cells. **B**, The CCK-8 assay was used to detect cell viability after knockdown of FOXP4-AS1. **C, D**, Proliferative ability of CNE1 cell transfected with si-FOXP4-AS1 was assessed by the colony formation assay (100×). **E**, Summary of DNA content of si-FOXP4-AS1#1, si-FOXP4-AS1#2 and corresponding control cells detected by flow cytometry. **F**, Representatives and summary of apoptosis index of si-FOXP4-AS1#1, si-FOXP4-AS1#2 and corresponding control cells detected by FACS(C). Triplicate independent assays were repeated and summarized, $^{**}p < 0.01$.

D). For exploring the mechanism of FOXP4-AS1 suppressing tumor cell proliferation, we used flow cytometry to compare cell distribution ratio in cell cycle between FOXP4-AS1 downregulation cells and vector cells. The results suggested that si-FOXP4-AS1#1 and si-FOXP4-AS1#2 cells were arrested at G0/G1 checkpoint ($p < 0.01$, ANOVA; Figure 2E). Because FOXP4-AS1 was reported to play a role in apoptosis[5]. We tested whether FOXP4-AS1 could affect apoptosis in nasopharyngeal carcinoma cells. The apoptotic index increased in si-FOXP4-AS1#1 and si-FOXP4-AS1#2 cells compared with corresponding control cells ($p < 0.01$, ANOVA; Figure 2F).

FOXP4-AS1 Directly Interacts with MiR-423-5p in NPC

LncRNAs can give play to functions by transcriptionally or post-transcriptionally regulating their downstream genes. To research the regulatory mechanism of FOXP4-AS1 in nasopharyngeal carcinoma cells, we did the subcellular fractionation assay in CNE1. The experimental result revealed that FOXP4-AS1 was mainly located in the cytoplasm of nasopharyngeal carcinoma cells ($p < 0.001$, Figure 3A). Interaction between miRNAs and long noncoding RNAs was critical for lncRNAs regulation[6,7]. By exploring bioinformatic databases, LncBase (http: // carolina. imis. athena-innovation. gr), we found miR-423-5p was a putative miRNA targeting FOXP4-AS1 (Figure 3B). To further verify whether FOXP4-AS1 is a direct target of miR-432-5p, we established two constructs, wild-type FOXP4 (FOXP4-WT) and mutant-type FOXP4 (FOXP4-MUT). Empty Luciferase reporter construct served as a control. The relative Luciferase activity in CNE1 cells appeared significant diminishment after co-transfection of miR-432-5p mimics and FOXP4-WT, while the Luciferase activity was not affected in empty vector group or FOXP4-MUT group ($p < 0.01$, Figure 3C). Then, we tested the Luciferase activity of FOXP4-WT or FOXP4-MUT in CNE1 cells transfected with miR-423-5p mimics. The results indicated that the Luciferase activity was decreased in CNE1 FOXP4-WT cells by miR-3184-5p mimics ($p < 0.01$, Figure 3C). Upregulation of miR-423-5p was identified significantly higher in si-FOXP4-AS1#1, si- FOXP4-AS1#2 cells compared with control cells ($p < 0.01$, Figure 3D). Further, linear

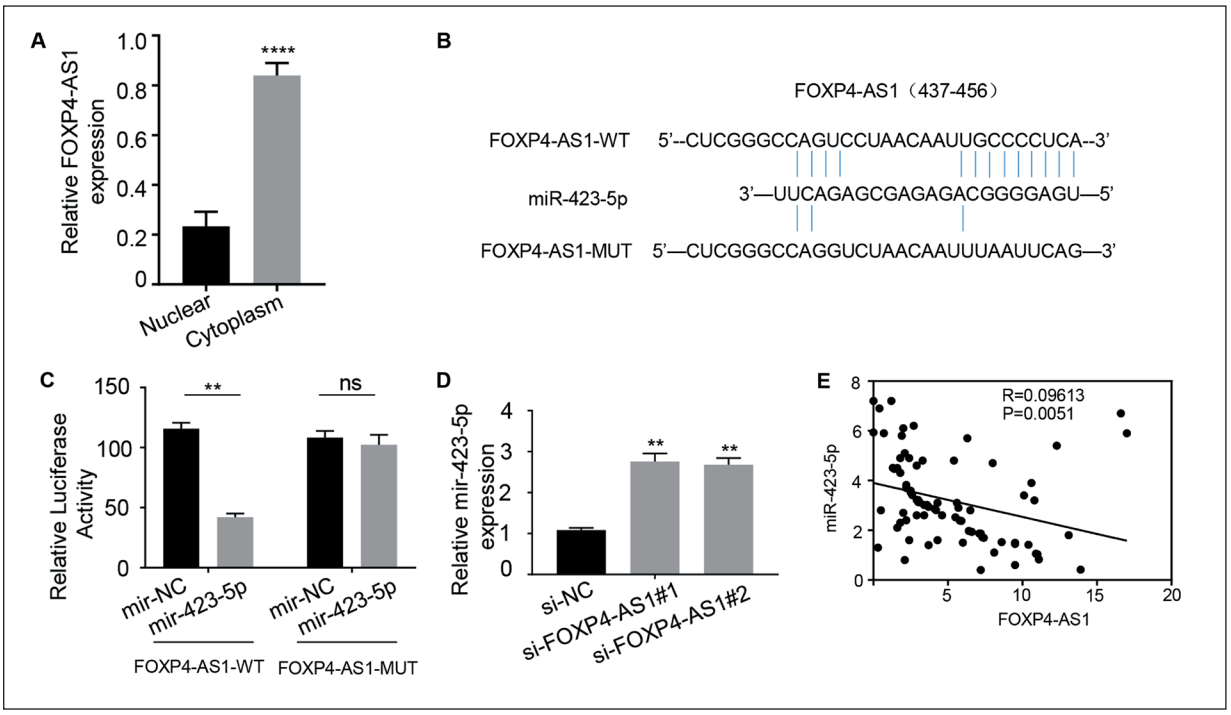

Figure 3. FOXP4-AS1 directly interacts with miR-423-5p in NPC. **A**, FOXP4-AS1 was predominantly located in the cytoplasm of nasopharyngeal carcinoma cells CNE1. **B**, MiR-423-5p and the complementary sequences among FOXP4-AS1-WT, miR-423-5p and FOXP4-AS1-MUT predicted by LncBase. **C**, The Luciferase reporter assay was conducted in cells transfected with miR-423-5p mimics or miR-NC to examine the Luciferase activity of FOXP4-AS1-WT and FOXP4-AS1-MUT. **D**, The expression level of miR-423-5p was examined in si-FOXP4-AS1#1, si- FOXP4-AS1#2 and corresponding control cells. **E**, Expression association between miR-423-5p and FOXP4-AS1 in 80 nasopharyngeal carcinoma samples.**$p < 0.01$, ***$p < 0.001$.

regression analyses showed the expression of miR-423-5p was negatively correlated with FOXP4-AS1 expression in 80 nasopharyngeal carcinoma specimens using qRT-PCR (R = 0.09613, p =0.0051, Figure 3E).

STMN1 Is a Direct Target Gene of MiR-423-5P in NPC

In the same way, we performed experiments to give evidence of the mutual effect between miR-423-5p and STMN1. In the first place, the binding sequence of miR-423-5p to wild-type STMN1 (STMN1-WT) and mutant-type STMN1 (STMN1-MUT) 3′UTR was predicted and acquired (Figure 4A). Then, we tested the Luciferase activity of STMN1-WT or STMN1-MUT in CNE1 cells transfected with miR-423-5p mimics. The results indicated that the Luciferase activity was decreased in CNE1 STMN1-WT cells by miR-3184-5p mimics (Figure 4B). Then, we examined the expression level of STMN1 in mir-423-5p inhibited group, mir-423-5p overexpression group and miR-NC group. Unsurprisingly, miR-423-5p can inhibit the expression of STMN1 (Figure 4C). The same results were obtained by Western blotting (Figure 4D). Further, linear regression analyses showed the expression of miR-423-5p was negatively correlated with STMN1 expression in 80 nasopharyngeal carcinoma specimens using qRT-PCR (R = 0.07364, p =0.0149, Figure 4E).

FOXP4-AS1 Promotes NPC Progression by Activating STMN1 Pathway Through Sponging MiR-423-5P

We explored the relationship between STMN1 and FOXP4-AS1 in CNE1 cells. qRT-PCR resulted that mRNA and protein level of STMN1 were negatively regulated in si-FOXP4-AS1#1 group, and the expression level partially recovered when CNE1 cells co-transfected with si-FOXP4-AS1#1 and mir-423-5p inhibitor or si-FOXP4-AS1#1 and PC DNA3.1 STMN1 (Figure 5A, B). Subsequently, linear regression analyses showed the expression of STMN1 was

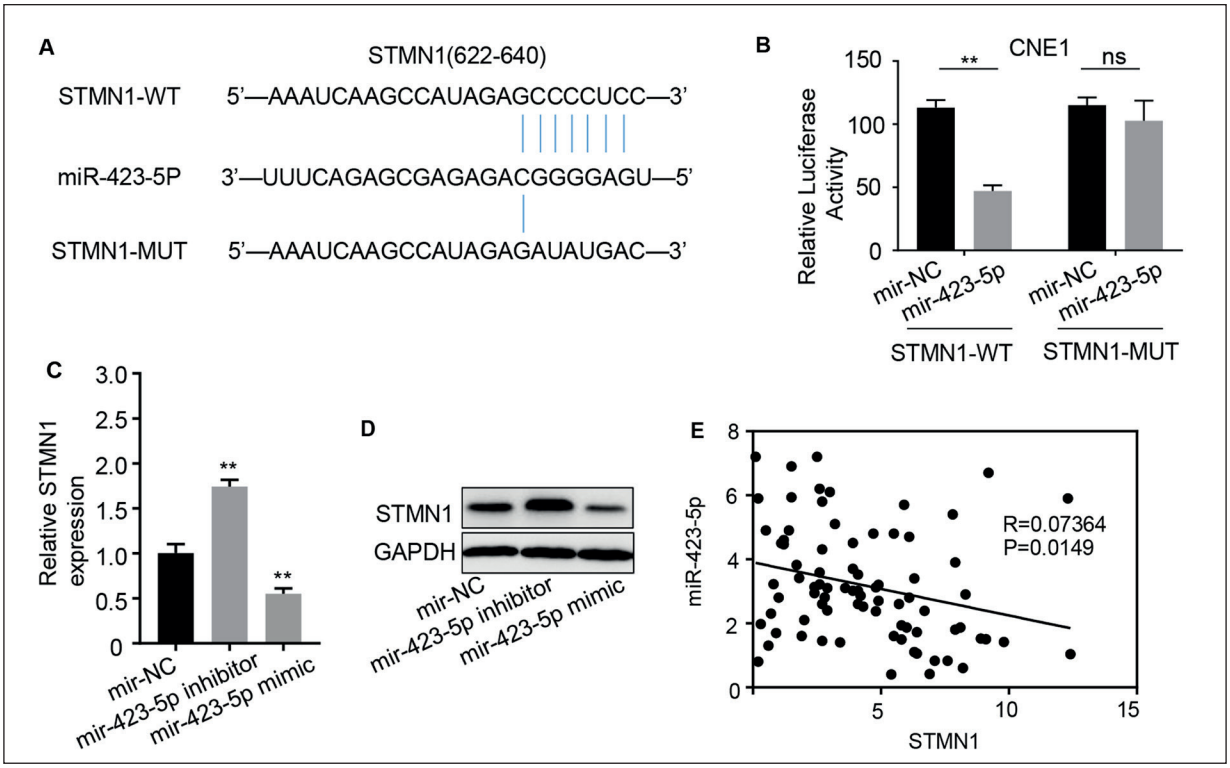

Figure 4. STMN1 is a direct target gene of miR-423-5P in NPC. **A**, Analysis of miRwalk online software revealed a potential miR-423-5P binding site in the 3' non-coding region of the STMN1 gene. **B**, The Luciferase reporter assay was conducted in cells transfected with miR-423-5p mimics or miR-NC to examine the Luciferase activity of STMN1-WT and STMN1-MUT. **C**, The expression level of STMN1 by qRT-PCR was examined in mir-423-5p inhibited group, mir-423-5p overexpression group and miR-NC group. **D**, The expression level of STMN1 by Western blotting was examined in mir-423-5p inhibited group, mir-423-5p overexpression group and miR-NC group. **E**, Expression association between miR-423-5p and STMN1 in 80 nasopharyngeal carcinoma samples. $^{**}p < 0.01$.

positively correlated with FOXP4-AS1 expression in 80 nasopharyngeal carcinoma specimens using qRT-PCR (R = 0.6315, p <0.0001, Figure 5C). These data further showed the regulatory network of FOXP4-AS1/miR-423-5p/STMN1. Based on the acquired data, we confirmed that FOXP4-AS1 can play functions in CNE1 by decreased expression of miR-423-5p, then the over expression of FOXP4-AS1 could upregulate the gene STMN1. To reinforce our point, we performed rescue assays in CNE1 cells. CCK8 proliferation assays, foci formation assays and subcutaneous xenograft tumor model *in vivo* demonstrated that inhibition of

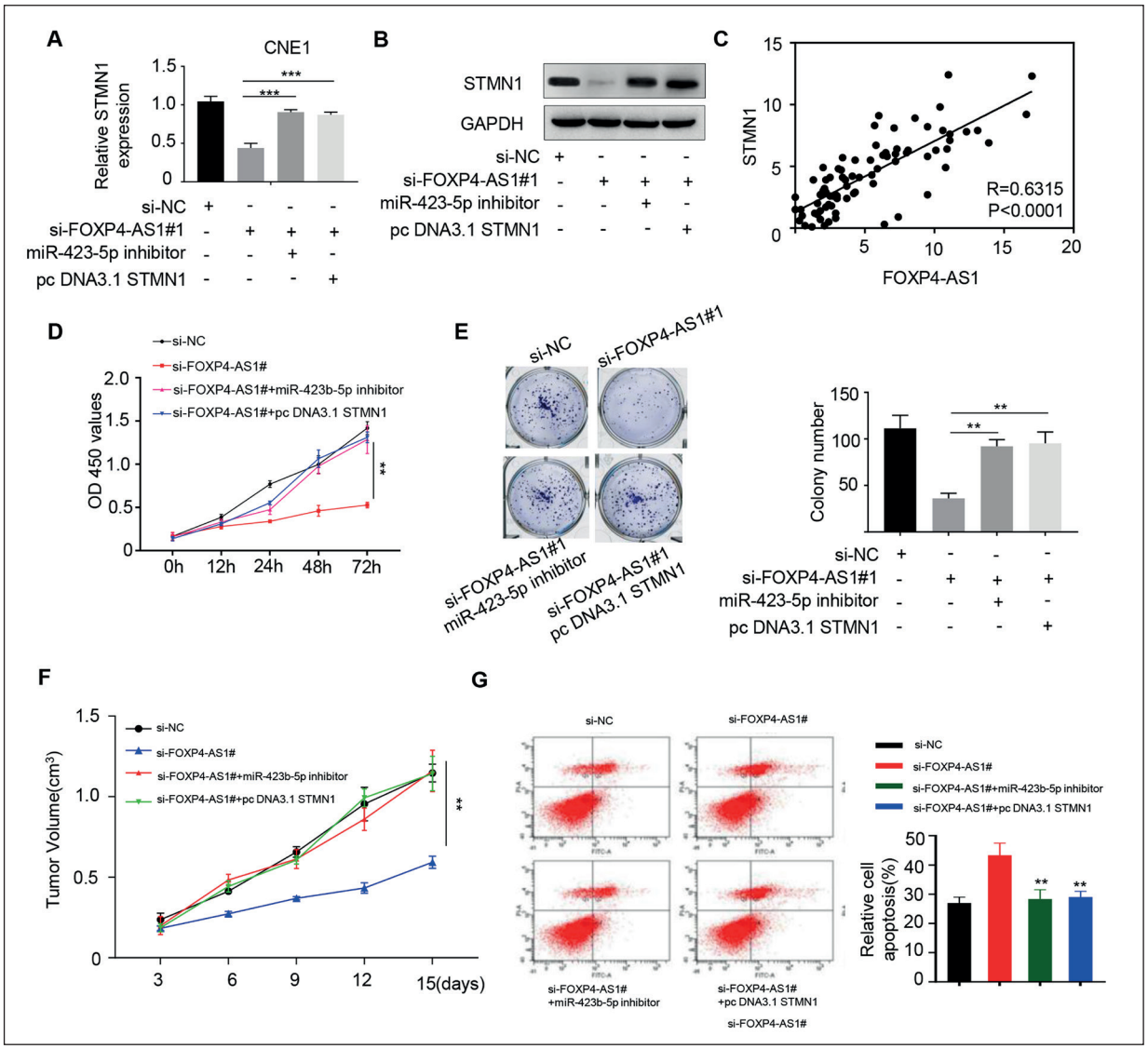

Figure 5. FOXP4-AS1 promotes NPC progression by activating STMN1 pathway through sponging miR-423-5P. **A**, The expression level of STMN1 by qRT-PCR was examined in si-NC group, si- FOXP4-AS1#1 group, si-FOXP4-AS1#1+miR-423-5P inhibitor group and si- FOXP4-AS1#1+ PC DNA3.1 group. **B**, The expression level of STMN1 by Western blotting was examined in si-NC group, si- FOXP4-AS1#1 group, si-FOXP4-AS1#1+miR-423-5P inhibitor group and si- FOXP4-AS1#1+ PC DNA3.1 group. **C**, Expression association between FOXP4-AS1 and STMN1 in 80 nasopharyngeal carcinoma samples. ***p < 0.0001. **D**, CCK8 proliferation assays were used to compare cell growth rates among si-NC group, si- FOXP4-AS1#1 group, si-FOXP4-AS1#1+miR-423-5P inhibitor group and si- FOXP4-AS1#1+ PC DNA3.1 group. **p < 0.001. **E**, Foci formation assays were used to compare colony formation abilities among si-NC group, si-FOXP4-AS1#1 group, si-FOXP4-AS1#1 + miR-423-5P inhibitor group and si-FOXP4-AS1#1+ PC DNA3.1 group (100×). **p < 0.01. **F**, Tumor growth curves were summarized in the line chart. The average tumor volume was expressed as the mean standard deviation of 6 mice. **G**, Apoptosis was analyzed among si-NC group, si-FOXP4-AS1#1 group, si-FOXP4-AS1#1 + miR-423-5P inhibitor group and si-FOXP4-AS1#1+ PC DNA3.1 group by FACS(C). **p < 0.01.

miR-423-5p expression or overexpression of STMN1 enhanced the impact of si-FOXP4-AS1 on CNE1 cell proliferation (Figure 5D, E, F). FACS(C) were also tested in cells transfected with indicated plasmids. The experimental results proved that the positive effect of silenced FOXP4-AS1 on cell apoptosis was attenuated in CNE1 cell co-transfected with miR-31423-5p inhibitor or overexpression of STMN1 (Figure 5G). Corporately, we indicated that FOXP4-AS1 promoted CNE1 growth by regulating miR-423-5p/STMN1 axis.

Discussion

Recently, mutations and dysregulation of long noncoding RNAs (lncRNA), appear to play crucial roles in tumorigenesis, metastasis and be closely correlated with the overall survival (OS) of cancer patients[8-10]. Some lncRNAs act as oncogenes, including PCA3, PCGEM1, PCAT1, PCAT18, CTBP-AS, SCHLAP1, HOTAIR, ANRIL, MALAT1, NEAT1, H19, KCNQ1OT1, lncTCF-7, HOTTIP, HULC, HEIH, TUG1, UCA1, PVT1, and LSINCT5[11]. Whereas others act as tumor suppressors, such as GAS5, MEG3, DILC, NBAT-1, DLEU1, DLEU2, TERRA, BGL3, and others. Novel lncRNAs are still being discovered[12]. LncRNA FOXP4-AS1 as a prognostic marker in prostate cancer has been proved[13]. In this study we explored that FOXP4-AS1 was overexpressed in collected NPC samples. In addition, high expression of FOXP4-AS1 by survival analysis is a poor prognostic indicator in NPC patients.

Functionally, upregulated lncRNAs can facilitate tumorigenesis[14,15]. For instance, overexpression of H19 expression leads to the activation of oncogenes, then triggering malignancies by means of angiogenesis, cell survival and proliferation[16-18]. In addition to this, PCGEM1 as a 1.6-kb long lncRNA is overexpressed in prostate cancer and promotes cell proliferation and colony formation[19]. In our functional experiment, we discovered that overexpression of FOXP4-AS1in CNE1 cells could promote cell proliferation and restrained cell apoptosis, revealing that FOXP4-AS1 as an oncogene played a part in NPC cells. *In vivo* animal experiment further verified that FOXP4-AS1 could accelerate NPC growth. These data indicated that FOXP4-AS1 exerted oncogenic function in the tumorigenesis of NPC.

LncRNAs can give play to function in human cancers by means of regulating microRNA[20,21]. According to online search databases, we located in miR-423-5p. To investigate the potential relationship between FOXP4-AS1 and miR-423-5p, we tested the expression of miR-423-5p in CNE1 cells with knocking down FOXP4-AS1. According to the result, FOXP4-AS1 negatively regulate the miR-423-5p. The same methods validate that STMN1 as the target gene is reversed regulated by miR-423-5p. Mechanistically, we produced the result FOXP4-AS1 was localized dominantly in the cytoplasm of NPC cells. Both functional assays and mechanism research revealed that FOXP4-AS1 and STMN1 could interact with miR-423-5p to form a ceRNA network. SMN1 (stathmin 1), also known as LAP18, is affirmed to regulate microtubule-associated protein (MAP) whose activity directly was incapacitated stabilization of microtubule dynamics. STMN1 is highly expressed in nearly all human carcinomas, including nasopharyngeal carcinoma (NPC)[22-27]. Therefore, we speculated that FOXP4-AS1/miR-423-5p/STMN1 axis is an oncogenic pathway in NPC by promoting cell growth.

Conclusions

Our research demonstrated that FOXP4-AS1 was highly expressed in NPC samples and showed poor outcome as an independent prognostic indicator. FOXP4-AS1 upregulated STMN1 by post-transcriptionally regulating miR-423-5p. All our findings may contribute to investigate the molecular mechanism associated with NPC tumorigenesis and will provide new insights in exploring the novel diagnostic or therapeutic biomarker for NPC.

Conflict of Interest

The Authors declare that they have no conflict of interests.

Authors' Contribution

Conception and design: LZ, JZ, XH, BH, XZ, JZ, JL, YQ; Development of methodology: LZ and JZ; Acquisition of data: LZ, JZ and YQ; Analysis and interpretation of data: LZ, JZ and YQ; Writing, review, and/or revision of the manuscript: LZ, JZ and YQ; Administrative, technical, or material support: LZ, JZ and YQ; Study supervision: YQ.

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