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# miR-421 induces cell proliferation and apoptosis resistance in human nasopharyngeal carcinoma via downregulation of FOXO4

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ABSTRACT

microRNAs have been demonstrated to play important roles in cancer development and progression. Hence, identifying functional microRNAs and better understanding of the underlying molecular mechanisms would provide new clues for the development of targeted cancer therapies. Herein, we reported that a microRNA, miR-421 played an oncogenic role in nasopharyngeal carcinoma. Upregulation of miR-421 induced, whereas inhibition of miR-421 repressed cell proliferation and apoptosis resistance. Furthermore, we found that upregulation of miR-421 inhibited forkhead box protein O4 (FOXO4) signaling pathway following downregulation of p21, p27, Bim and FASL expression by directly targeting *FOXO4* 3′UTR. Additionally, we demonstrated that FOXO4 expression is critical for miR-421-induced cell growth and apoptosis resistance. Taken together, our findings not only suggest that miR-421 promotes nasopharyngeal carcinoma cell proliferation and anti-apoptosis, but also uncover a novel regulatory mechanism for inactivation of FOXO4 in nasopharyngeal carcinoma.



## 1. Introduction

Nasopharyngeal carcinoma (NPC) is an Epstein–Barr virus-associated head and neck cancer that is most common in eastern Asia [1]. Despite advances in diagnostic and treatment modalities, NPC is leading to significant morbidity and mortality in southern China [2]. Statistic analysis revealed that Guangdong province in China and Hong Kong displayed the highest incidence rate around the World, with incidences ranging from 20 to 22 per 100,000 men 8–10 per 100,000 females [3]. Although it is widely accepted that Epstein–Barr virus infection, environmental factors, and genetic susceptibility play important roles in NPC pathogenesis [1], the molecular mechanism of its development and progression remains poorly understood, and thus far, no effective prognostic or therapeutic targets have been developed. Therefore, it is of great clinical value to identify and better understanding molecular mechanisms of novel targets involved in NPC pathogenesis.

The FoxO family of transcription factors, including FOXO 1 (FKHR), FOXO3 (FKHRL1), FOXO4 (AFX) and FOXO6, belongs to an evolutionarily conserved transcription factor family that are characterized by a highly conserved forkhead domain with a DNA-binding motif [4]. The FOXO signaling plays a central role in diverse biological processes, including cellular proliferation, apoptosis, differentiation, energy storage and DNA damage repair [5]. FOXO can perform pleiotropic functions via transcriptional regulation of its taget genes. It have been reported that activation of each member of the FOXO family in cells can inhibit cell growth by upregulating cell-cycle inhibitors $p21^{Cip1}$ and $p27^{Kip1}$, and downregulation of Cyclin D1/D2 [6–8]. Meanwhile, FOXO protein can induce cellular apoptosis via upregulation of multiple pro-apoptotic proteins, including Bim, FASLand TRAIL [9–11]. Hence, FOXO transcriptional factors are considered key tumor suppressors in cancer pathogenesis. Indeed, downregulation or inactivation of FOXO4 has been demonstrated to contribute to progression of various cancer types, including breast, cholangiocarcinoma, colon cancers and leukaemia [12–15]. Therefore, better understanding of the mechanisms that regulate FOXO4 activity may provide clues of novel targets for therapeutic intervention.

MicroRNAs are a class of 21–24-nucleotides (nt) noncoding RNA molecules which have been demonstrated to play oncogenic or tumor suppressive roles in cancer progression by specifically interacting with their target mRNA molecules [16,17]. Herein, we found that miR-421 play as an onco-miR to promote cell proliferation and inhibit cell apoptosis in NPC. As described below, upregulation of miR-421 induced whereas inhibition of miR-421 repressed NPC cell proliferation and apoptosis resistance. Moreover, we found that FOXO4 was the direct and functional target of miR-421. Upregulation of miR-421 inhibited FOXO4 signaling following downregulation of p21, p27, Bim and FASL expression.

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Taken together, our results suggest that miR-421 play oncogenic roles in NPC progression and uncover a novel mechanism for FOXO4 signaling inactivation in NPC.

## 2. Materials and methods

### 2.1. Plasmid, siRNA, microRNA mimic and inhibitor

The region of human FOXO4 3′UTR, from 45 to 561, was generated by PCR amplification and subcloned into the pGL3 vector plasmid (Promega, Madison, WI). The primers are as the following: forward, FOXO4-3′UTR-luc-up, 5′-GCCCCGCGGGCGTGTTCATATCTACTCTTT-3′; FOXO4-3′UTR-luc-dn, 5′-GCCCCGCGGAAGCCTTTCTGTTATCTGC-3′; FOXO4-3′UTR-mut-luc-up, 5′-GGTCTGGTCACACACAGGTGTTCTAGAAATTATAAAGATAAAGCT-3′; FOXO4-3′UTR-mut-luc-dn, 5′-AGCTTTATCTTTATAATTTCTAGAACACCTGTGTGTGACCAGACC-3′. FOXO4 siRNA: CCUGGAGUGUGACAUGGAUAA. The miR-421 mimic, inhibitor and negative control (NC) were purchased from RiboBio (Guangzhou, Guangdong, China). miR-421 mimic are chemically modified double-stranded RNAs that mimic endogenous miR-421, and the miR-421 inhibitor is a LNA/OMe modified antisense oligonucleotide designed to specifically bind to and inhibit endogenous miR-421. Transfection of the plasmids, microRNA mimic, inhibitor and siRNA were performed using Lipofectamine 2000 (Invitrogen) according to the manufacturer's instructions.

### 2.2. Colony formation assay

Cells were plated in 6-well plate at the density of 1000/well and cultured for 10 days. The colonies were stained with 1.0% crystal violet for 30 s after fixation with 10% formaldehyde for 5 min.

### 2.3. Terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling (TUNEL) assay

Apoptotic DNA fragmentation was examined using the *in situ* Dead-EndTM Fluorometric TUNEL System assay kit (Promega, Madison, WI, USA) according to the manufacturer's protocol. Briefly, cells were plated in 24-well flat-bottom plates at a density of $1 \times 10^5$ cells/well and treated with 10 μM cisplatin for 24 h. Cells were fixed in 4% paraformaldehyde at 4 °C for 30 min. Fixed cells were then permeabilized in 0.1% Triton X-100, and labeled with fluorescein-12-dUTP using terminal deoxynucleotidyl-transferase. The localized green fluorescene of apoptotic cells (fluorescein-12-dUTP) was detected by fluorescence microscopy (Zeiss Axiovert100 M, Carl Zeiss, Germany).

### 2.4. Annexin V-FITC/propidium iodide (PI)-stained fluorescence-activated cell sorting (FACS)

Cells were harvested by trypsinization, washed in ice-cold PBS and centrifuged at 1,000*g* for 5 min. The supernatant was discarded and the pellet was resuspended in binding buffer at a density of $1.0 \times l0^6$ cells/ml. Following this, 100 μl of the sample solution was incubated with 5 μl of FITC-conjugated Annexin V and 5 μl of PI for 15 min at room temperature in the dark. Subsequently, 400 μl of binding buffer was added to each sample and the samples were analyzed by FACS.

### 2.5. Real-time quantitative PCR

Total RNA from cells was extracted using the Trizol reagent (Invitrogen) according to the manufacturer's instruction. 2 μg of RNA from each sample was used for cDNA synthesis primed with random hexamers. The primers are: p21, forward, AGTCAGTTCCTTGTGGAGCC, and reverse, CATGGGTTCTGACGGACAT; p27, forward, AAGAAGCCTGGCCTCAGAAG, and reverse, TTCATCAAGCAGTGATGTATCTGA; Bim, forward, CCTCCCTACAGACAGAGCCA, and reverse, GATAGTGGTTGAAGGCCTGG; FASL, forward, GCACACAGCATCATCTTTGG, and reverse, GGACCTTGAGTTGGACTTGC. Expression data were normalized to the geometric mean of housekeeping gene GAPDH (forward, 5′-GACTCATGACC ACAGTCCATGC-3′, and reverse, 5′-AGAGGCAGGGATGATGTT CTG-3′) to control the variability in expression levels and calculated as $2-[(Ct^{of\ target}) - (Ct^{of\ GAPDH})]$, where Ct represents the threshold cycle for each transcript. Reverse transcription and quantification of miR-421 expression was assesed with the RiboBio miRNA kit. The expression of miRNA was defined based on the threshold cycle (Ct), and relative expression levels were calculated as $2^{-[(Ct\ of\ miR\text{-}421) - (Ct\ of\ U6)]}$ after normalization with reference to expression of U6 small nuclear RNA.

### 2.6. Western blotting

Cells were harvested in cell lysis buffer (Cell Signaling Technology; Cat#: 9803) and heated for 5 min at 100 °C. Equal quantities of denatured protein samples were resolved on 10% SDS–polyacrylamide gels, and then transferred onto polyvinylidene difluoride membranes (Roche). After blocking with 5% non-fat dry milk in Tris–buffered saline/0.05% Tween 20 (TBST), the membrane was incubated with a specific primary antibody, followed by the horseradixh peroxidase-conjugated secondary antibody. Proteins were visualized using ECL reagents (Pierce). The anti-GAPDH, anti-FOXO4, anti-p21, anti-p27, anti-Bim and anti-FASL antibodies were purchased from abcam (Cambridge, MA).

### 2.7. Statistical analysis

The two-tailed Student's *t*-test was used to evaluate the significance of the differences between two groups of data in all pertinent experiments; A $P$ values <0.05 was considered significant.

## 3. Results

### 3.1. Upregulation of miR-421 promotes cell proliferation and renders apoptosis resistance in human nasopharyngeal carcinoma

Realtime PCR analysis revealed that the expression of miR-421 in 13 freshly-frozen NPC tissues of varying clinical stages was dramatically upregulated as compared to that in 2 human normal nasopharyngeal tissues (Fig. 1A). Importantly, our results revealed that miR-421 expression positively correlated with NPC clinical stage (Fig. 1A), suggesting a possible link between miR-421 upregulation and progression of human NPC.

To further investigate the effect of miR-421 on NPC progression, CNE2 cells was transduced with miR-421 mimic to overexpress miR-421. As shown in Fig. 1B and Fig. 1C, upregulation of miR-421 significantly increased the growth rate of CNE2 cells, analyzed by MMT and colony formation assays. Moreover, overexpressing miR-421 also augmented the apoptosis resistance of nasopharyngeal carcinoma cells to treatment of chemotherapeutic agent cisplatin as indicated by the Annexin V and TUNEL staining assays (Fig. 1D and E). These results suggest that miR-421 induces cell proliferation and apoptosis resistance in nasopharyngeal carcinoma cells *in vitro*.

### 3.2. Inhibition of miR-421 reduces cell proliferation and induces apoptosis in human nasopharyngeal carcinoma

We further examined the effect of miR-421 inhibition on nasopharyngeal carcinoma progression. Consistent with abovementioned results, MTT and colony formation assays showed that miR-421 suppression dramatically inhibited the growth rate of

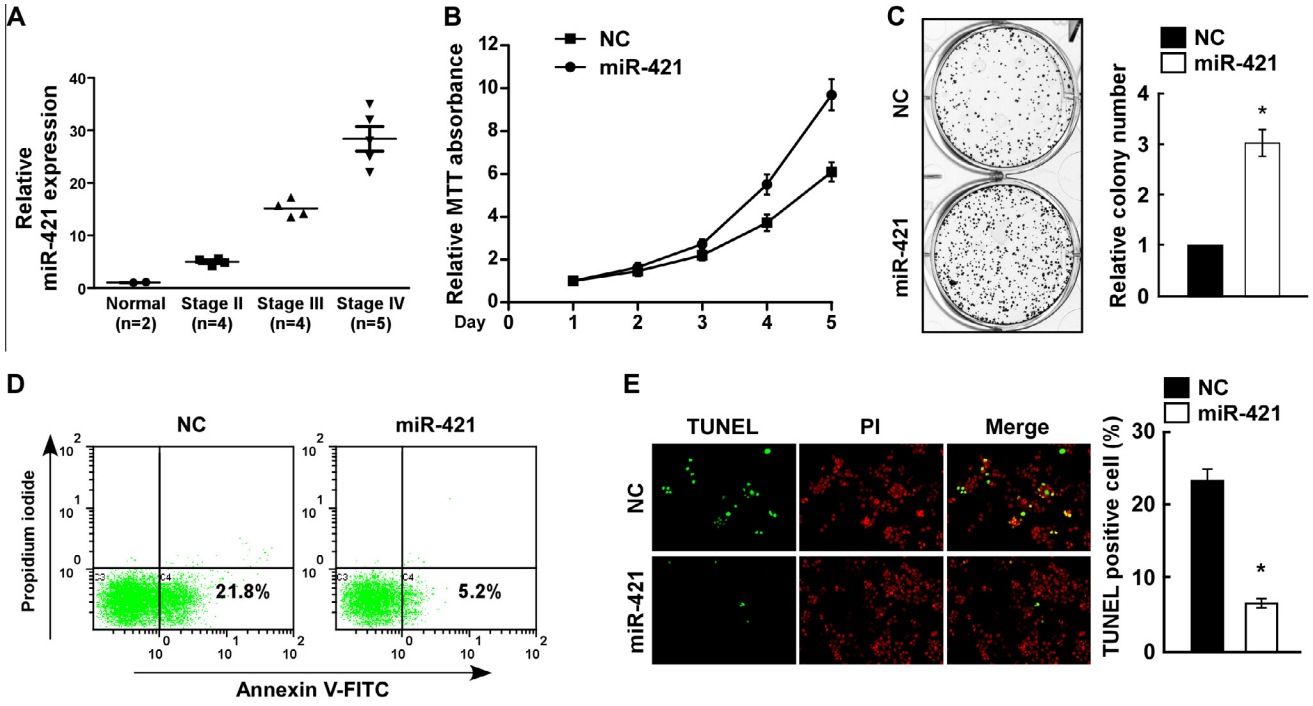


**Fig. 1.** Upregulation of miR-421 promotes cell proliferation and renders apoptosis resistance of nasopharyngeal carcinoma cell line CNE2. (A) Realtime PCR analysis revealed that the expression of miR-421 in 2 freshly-frozen human normal nasopharyngeal tissues and 13 NPC tissues of varying clinical stages, including 4 clinical stage II, 4 stage III and 5 stage IV samples. (B) MTT assays revealed that upregulation of miR-421 promoted cell growth of CNE2 cells. (C) Representative micrographs (left) and quantification (right) of crystal violet stained cell colonies, indicating that upregulation of miR-421 promoted cell growth of CNE2 cells. (D) Annexin V-FITC/PI staining of indicated CNE2 cells treated with cisplatin (20 μM) for 12 h. (E) Representative micrographs (left) and quantification of TUNEL positive cells in indicated CNE2 cells treated with cisplatin (20 μM) for 36 h $^{*}P < 0.05$.

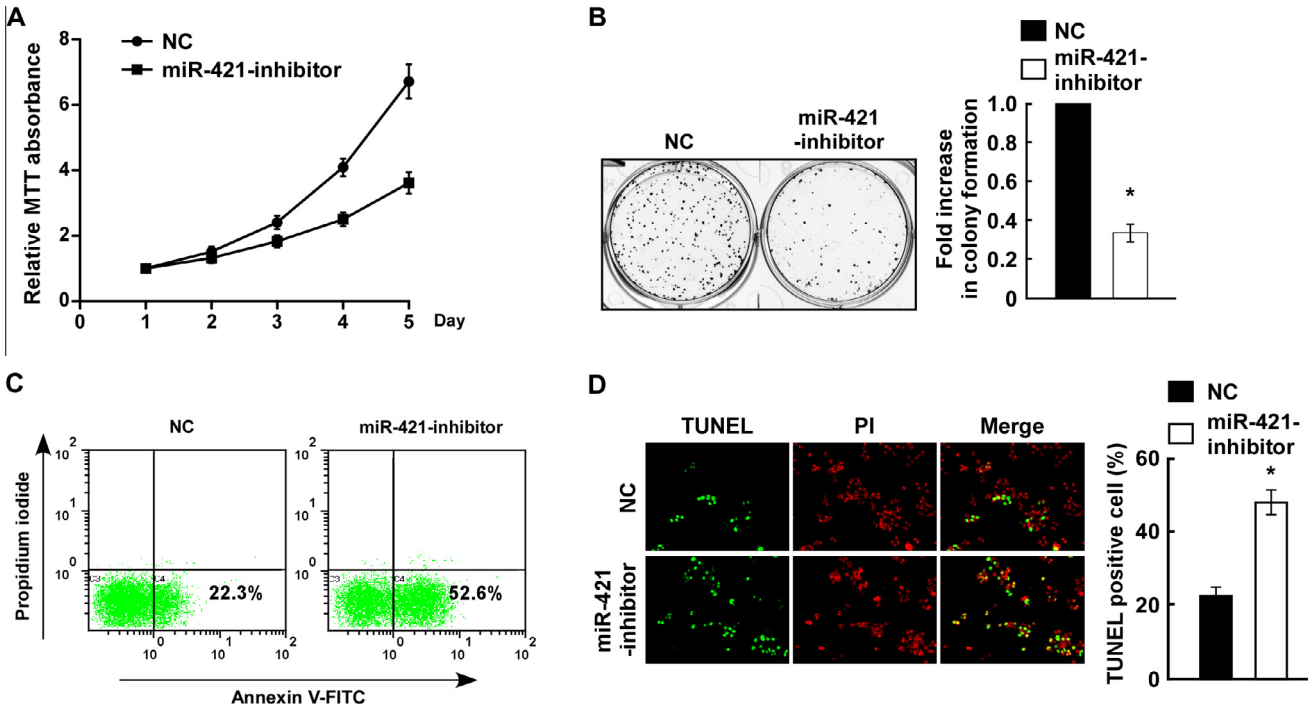


**Fig. 2.** Inhibition of miR-421 suppresses cell proliferation and induces apoptosis of nasopharyngeal carcinoma cell line CNE2. (A) Representative micrographs (left) and quantification (right) of crystal violet stained cell colonies, indicating that inhibition of miR-421 induced apoptosis of nasopharyngeal carcinoma cell line CNE2. (B) Annexin V-FITC/PI staining of indicated CNE2 cells treated with cisplatin (20 μM) for 12 h. Representative micrographs (left) and quantification of TUNEL positive cells in indicated CNE2 cells treated with cisplatin (20 μM) for 36 h $^{*}P < 0.05$.

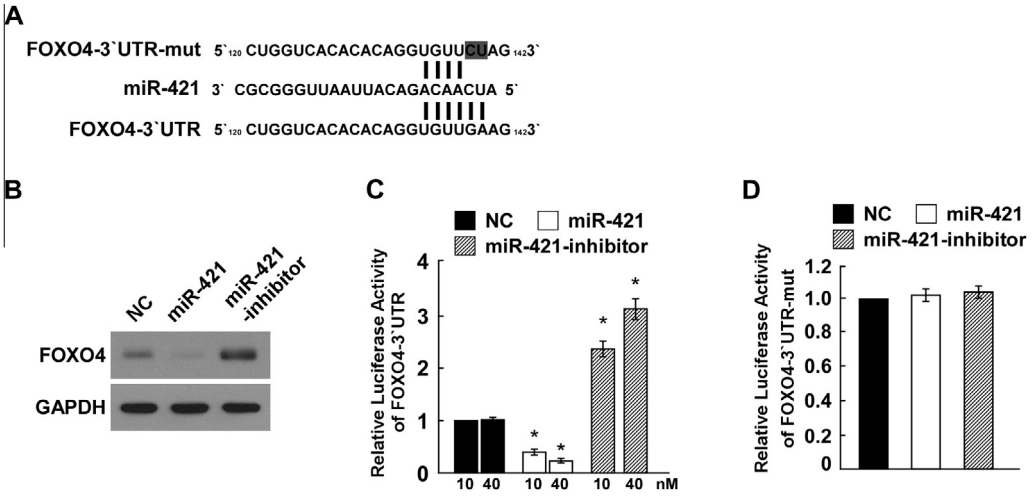


**Fig. 3.** miR-421 Suppresses FOXO4 expression via directly targeting FOXO4-3′UTR. (A) Predicted miR-421 target sequence in the 3′UTR of FOXO4 (FOXO4-3′UTR) and mutant containing two mutated nucleotides in 3′-UTR of FOXO4 (FOXO4-3′UTR-mut). (B) Western blotting analysis of FOXO4 expression in miR-421-transduced or miR-421-inhibited CNE2 cells. GAPDH served as the loading control. (C) Luciferase assays of pGL3-FOXO4-3′UTR reporter in miR-421-transduced or miR-421-inhibited CNE2 cells. (D) Luciferase assays of pGL3-FOXO4-3′UTR-mut reporter in miR-421-transduced or miR-421-inhibited cells $^{*}P < 0.05$.

CNE2 cells as compared with control cells (Fig. 2A and B). Moreover, miR-421 inhibition could also sensitize nasopharyngeal carcinoma cells to cisplatin treatment as indicated by Annexin V and TUNEL staining analysis (Fig. 2C and D).

### 3.3. miR-421. directly targets FOXO4

FOXO4, one of the FoxO family member of transcription factors, is considered to be a tumor-suppressor protein and is implicated in the proliferation and apoptosis of tumor progression [18,19,5]. Interestingly, target prediction using TargetScan program tool showed that FOXO4 is the potential target of miR-421 (Fig. 3A). Immunoblotting analysis revealed that the expression of FOXO4 was decreased in miR-421 transfected cells and increased in miR-421 inhibitor-transfected cells, as compared with that in control cells, respectively (Fig. 3B). To examine whether miR-421-induced FOXO4 downregulation was mediated by the 3′-untranslated region (3′UTR) of FOXO4, we subcloned the FOXO4 3′UTR fragment, containing the miR-FOXO4 binding site into pGL3 dual luciferase reporter vectors. As shown in Fig. 3C, overexpression of miR-421 decreased and inhibition of miR-421 increased the luciferase activity of the FOXO4–3′UTR in a dose dependent manner. Furthermore, point mutations in the tentative miR-421-binding seed region in FOXO4 3′-UTR abrogated the aforementioned repressive effect of miR-421 (Fig. 3D), suggesting that miR-421 specifically targets the 3′-UTR of FOXO4. Collectively, our results demonstrate that FOXO4 is a *bona fide* target of miR-421.

### 3.4. miR-421 inhibits the FOXO4 signaling pathway

Consistently, realtime PCR and immunoblotting analysis revealed that miR-421 overexpression downregualted, whereas miR-421 inhibition increased the expression of FOXO4 downstream genes, such as p21, p27, Bim and FASL (Supplementary Figure 1). Thus, our results indicated that miR-421 inhibits the FOXO4 signaling pathway.

### 3.5. FOXO4 expression is critical for miR-421-induced cell growth and anti-apoptosis

We then attempt to understand the role of FOXO4 repression in miR-421-induced cell proliferation and anti-apoptosis. As shown in Fig. 4A and B, we found that the miR-421 inhibition induced cell growth arrest and apoptosis promotion could be abolished by downregulation of FOXO4, indicating that FOXO4 plays an important role in miR-421-mediated biological functions.

### 3.6. miR-421 expression correlates with FOXO4 expression in nasopharyngeal carcinoma tissues

Finally, we examined whether the miR-421-induced FOXO4 repression identified in our study is clinically relevant. As shown in Fig. 4C, we found that miR-421 levels in 7 freshly collected nasopharyngeal carcinoma tissue samples inversely correlated with the FOXO4 expression levels ($r = -0.798$, $P = 0.032$). Taken together, our results suggest that miR-421 upregulation inactivates FOXO4 signaling, and consequently leads to cell proliferation and apoptosis resistance in nasopharyngeal carcinoma.

## 4. Discussion

The key finding of our study is that miR-421 expression is drastically upregulated in NPC tissues as compared to that in normal nasopharyngeal tissues. Ectopic expression of miR-421 promotes, whereas inhibition of miR-421 repressed NPC cell proliferation and apoptosis resistance. Furthermore, we found that miR-421 was involved in modulation of the FOXO signaling pathway and downregulation of FOXO4 expression by directly targeting the FOXO4 3′UTR. Taken together, our results suggest that upregulation of miR-421 plays an important role in the proliferation and anti-apoptosis of NPC.

FOXO4/AFX has been recognized as one of the most pleiotropic transcription factors and a key tumor suppressor in the regulation of tumorgenesis [12–15]. Several mechanisms have been reported to be mediated with FOXO4 downregulation or inactivation. Borkhardtet al. reported that in acute leukemias, the FOXO4 gene undergoes a t(X;11)(q13;q23) chromosome translocation, resulting the inactivation of FOXO4 and oncogenic activation of MLL-FOXO4 fusion protein [20]. Leeet al. identified an N-terminally deleted form of AFXalpha (alpha198–505), which resulted in loss-of-function in the apoptosis-inducing of FOXO4 to facilitate tumorigenesis [21]. Moreover, Oteizaet al. reported that FOXO4 protein stability and transcriptional activity could be downregulated by the viral Tax oncoprotein via the ubiquitin–proteasome [22].

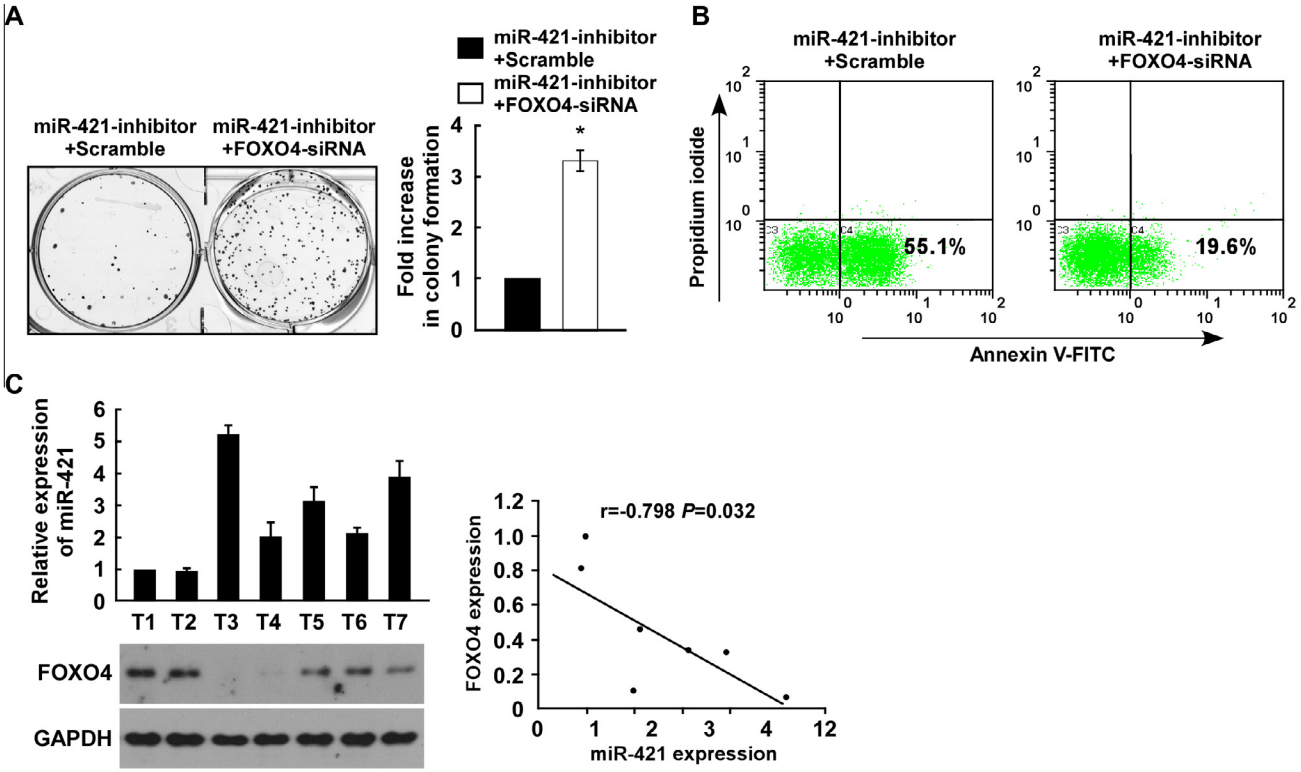


**Fig. 4.** FOXO4 expression is critical for miR-421-induced cell growth and anti-apoptosis. (A and B) Colony formation (A) and TUNEL (B) assays indicated the proliferation and anti-apoptosis abilities of indicated cells transfected with miR-421 inhibitor, or miR-421 inhibitor plus FOXO4-siRNA. (C) Analysis (left) and correlation (right) between miR-421 expression and FOXO4 expression levels in 7 freshly collected human nasopharyngeal carcinoma tissue samples. GAPDH was used as loading control.

However, the microRNA regulatory mechanism of FOXO4 remains unclear. In the current study, we demonstrated that FOXO4 is directly downregulated by miR-421 by different methods. Western blotting analysis showed that ectopic expression of miR-421 reduced FOXO4 protein expression. Realtime PCR analysis determined that the downstream targets of FOXO4, including cell cycle inhibitors p27 and p21, apoptosis inducers Bim and FASL were significantly downregulated by miR-421 overexpression. The luciferase activity assay and point mutation analysis demonstrated that the repression of FOXO4 was mediated by miR-421 through the FOXO4 3′UTR. Taken together, our results suggest that FOXO4 is a *bona fide* target of miR-421 and provide a novel mechanism of FOXO4 downregulation in tumors.

Previously, miR-421 has been reported to be upregulated in multiple human cancers, such as gastric, pancreatic, hepatocellular and biliary tract cancers [23–26]. Herein, we found that miR-421 is upregulated in NPC and its expression positively correlated with the clinical stage. Moverover, overexpression of miR-421 significantly induces NPC cell proliferation and apoptosis resistance. Thus, integrating previous studies and our results suggest that miR-421 might function as an oncogenic miRNA and its upregulation contributes to the progression of multiple cancers. miR-421 may be useful as a potential therapeutic target for cancers.

## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bbrc.2013.05.056.

## References

[1] Y. Pan, F.X. Claret, Targeting Jab1/CSN5 in nasopharyngeal carcinoma, Cancer Lett. 326 (2012) 155–160.
[2] W. Fang, X. Li, Q. Jiang, et al., Transcriptional patterns, biomarkers and pathways characterizing nasopharyngeal carcinoma of Southern China, J. Transl. Med. 6 (2008) 32.
[3] W. Gao, J.Z. Li, W.K. Ho, et al., Biomarkers for use in monitoring responses of nasopharyngeal carcinoma cells to ionizing radiation, Sensors (Basel). 12 (2012) 8832–8846.
[4] M. Katoh, M. Igarashi, H. Fukuda, et al., Cancer genetics and genomics of human FOX family genes, Cancer Lett. 328 (2013) 198–206.
[5] H. Huang, D.J. Tindall, Dynamic FoxO transcription factors, J. Cell Sci. 120 (2007) 2479–2487.
[6] G.J. Kops, R.H. Medema, J. Glassford, et al., Control of cell cycle exit and entry by protein kinase B-regulated forkhead transcription factors, Mol. Cell Biol. 22 (2002) 2025–2036.
[7] R.H. Medema, G.J. Kops, J.L. Bos, et al., AFX-like Forkhead transcription factors mediate cell-cycle regulation by Ras and PKB through p27kip1, Nature 404 (2000) 782–787.
[8] M. Schmidt, S. Fernandez de Mattos, A. van der Horst, et al., Cell cycle inhibition by FoxO forkhead transcription factors involves downregulation of cyclin D, Mol. Cell Biol. 22 (2002) 7842–7852.
[9] P.F. Dijkers, R.H. Medema, J.W. Lammers, et al., Expression of the pro-apoptotic Bcl-2 family member Bim is regulated by the forkhead transcription factor FKHR-L1, Curr. Biol. 10 (2000) 1201–1204.
[10] J.Y. Yang, W. Xia, M.C. Hu, Ionizing radiation activates expression of FOXO3a, Fas ligand, and Bim, and induces cell apoptosis, Int. J. Oncol. 29 (2006) 643–648.
[11] V. Modur, R. Nagarajan, B.M. Evers, et al., FOXO proteins regulate tumor necrosis factor-related apoptosis inducing ligand expression. Implications for PTEN mutation in prostate cancer, J. Biol. Chem. 277 (2002) 47928–47937.
[12] S.H. Kim, F.R. Miller, L. Tait, et al., Proteomic and phosphoproteomic alterations in benign, premalignant and tumor human breast epithelial cells and xenograft lesions: biomarkers of progression, Int. J. Cancer 124 (2009) 2813–2828.
[13] M.J. Lee, G.R. Yu, H.J. Yoo, et al., ANXA8 down-regulation by EGF-FOXO4 signaling is involved in cell scattering and tumor metastasis of cholangiocarcinoma, Gastroenterology 137 (2009) 1138–1150.
[14] I.K. Kwon, R. Wang, M. Thangaraju, et al., PKG inhibits TCF signaling in colon cancer cells by blocking beta-catenin expression and activating FOXO4, Oncogene 29 (2010) 3423–3434.
[15] C.W. So, M.L. Cleary, MLL-AFX requires the transcriptional effector domains of AFX to transform myeloid progenitors and transdominantly interfere with forkhead protein function, Mol. Cell Biol. 22 (2002) 6542–6552.
[16] D.P. Bartel, MicroRNAs: genomics, biogenesis, mechanism, and function, Cell 116 (2004) 281–297.

[17] C.S. William, MicroRNAs in cancer – from research to therapy, Biochim. Biophys. Acta 2010 (1805) 209–217.
[18] M. Katoh, M. Katoh, Human FOX gene family (Review), Int. J. Oncol. 25 (2004) 1495–1500.
[19] H. Huang, D.J. Tindall, FOXO factors: a matter of life and death, Future Oncol. 2 (2006) 83–89.
[20] A. Borkhardt, R. Repp, O.A. Haas, et al., Cloning and characterization of AFX, the gene that fuses to MLL in acute leukemias with a t(X;11)(q13;q23), Oncogene 14 (1997) 195–202.
[21] E.J. Lee, J.M. Kim, M.K. Lee, et al., Splice variants of the forkhead box protein AFX exhibit dominant negative activity and inhibit AFXalpha-mediated tumor cell apoptosis, PLoS ONE 3 (2008) e2743.
[22] A. Oteiza, N. Mechti, The human T-cell leukemia virus type 1 oncoprotein tax controls forkhead box O4 activity through degradation by the proteasome, J. Virol. 85 (2011) 6480–6491.
[23] Z. Jiang, J. Guo, B. Xiao, et al., Increased expression of miR-421 in human gastric carcinoma and its clinical association, J. Gastroenterol. 45 (2010) 17–23.
[24] J. Hao, S. Zhang, Y. Zhou, et al., MicroRNA 421 suppresses DPC4/Smad4 in pancreatic cancer, Biochem. Biophys. Res. Commun. 406 (2011) 552–557.
[25] Y. Zhang, W. Gong, S. Dai, et al., Downregulation of human farnesoid X receptor by miR-421 promotes proliferation and migration of hepatocellular carcinoma cells, Mol. Cancer Res. 10 (2012) 516–522.
[26] X.Y. Zhong, J.H. Yu, W.G. Zhang, et al., MicroRNA-421 functions as an oncogenic miRNA in biliary tract cancer through down-regulating farnesoid X receptor expression, Gene 493 (2012) 44–51.