Inhibition of miR-24 suppresses malignancy of human non-small cell lung cancer cells by targeting WWOX in vitro and in vivo

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Abstract

Background: We investigated the effect of micro-RNA 24 (miR-24) and WWOX on non-small cell lung cancer (NSCLC) cell proliferation and migration in vitro and in vivo.

Methods: We performed bioinformatics analysis and 3’ untranslated region luciferase assay to investigate the direct target of miR-24. Proliferation, apoptosis, and transwell invasion assays were employed to evaluate the effect of WWOX overexpression with pcDNA3-WWOX and knocking down miR-24 with miR-24 small interfering RNA. Quantitative real-time PCR, Western blot, and immunohistochemistry were also used to investigate miR-24 and c-Kit expression, and apoptosis and invasion-related proteins. Finally, we constructed a tumor xenograft model in nude mice to confirm the effect of miR-24 on NSCLC cell proliferation in vivo.

Results: According to our experimental data, miR-24 inhibition could induce apoptosis by activating caspase 3 and suppress the viability and proliferation of NSCLC cells in vitro and in vivo. MiR-24 downregulation could reduce the invasive ability of NSCLC cells by downregulating MMP9. WWOX was identified as a functional target of miR-24. WWOX overexpression generated the same effect with antagonizing miR-24, while blocking WWOX counteracted the tumor suppressive effect caused by miR-24 inhibition. MiR-24 may function as an onco-gene and play an important role in the cell growth and migration of NSCLC.

Conclusions: Our findings enhance understanding of the miR-24 regulatory network and the molecular mechanism that underlies the oncogenesis and development of NSCLC. Suppressing the effect of miR-24 on cancer cells using a miR-24 inhibitor may be an attractive therapeutic strategy against NSCLC.
miR-24 inhibition suppresses NSCLC

X.-h. Wang et al.

The WWOX gene spans the FRA16D common chromosomal fragile site and encodes a member of the short-chain dehydrogenases/reductases (SDR) protein family. Expression of WWOX-encoded protein induces apoptosis, while defects in this gene are associated with multiple types of cancer. However, the role of WWOX in regulating NSCLC cell proliferation and motility has not yet been elucidated.

Apoptosis is a well-orchestrated and programmed cell death that occurs in multicellular organisms. Certain kinds of damage trigger a series of biochemical steps, leading to characteristic cell morphology and death.\(^1\) It seems clear that the tight regulation of apoptotic function through miRNAs is critical to many cellular processes and the development of cancer. However, the relationship between miR-24 and NSCLC cell proliferation and apoptosis is not clear.

In this study, we performed a 3′ untranslated region (UTR) luciferase assay and observed that luciferase activity was increased after co-transfection of the miR-24 inhibitor and WWOX 3′UTR vector. MiR-24 binds directly to the 3′-UTR of WWOX to suppress gene expression. Inhibition of miR-24 induces apoptosis and suppresses the cell proliferation and migration ability of NCI-H358 and NCI-H1299 human NSCLC cells. Moreover, inhibition of miR-24 also suppresses the tumor growth of mice with severe combined immunodeficiency in a tumor xenograft model. WWOX overexpression showed the same effect with antagonizing miR-24. In summary, our findings suggest that miR-24 regulates the viability and migration of NSCLC cells via the direct targeting of WWOX. This may eventually provide practical information for the management of NSCLC.

Methods

Patient samples

Fifteen patients (7 men, 8 women; mean age 45.5 years), confirmed by pathological examination with NSCLC at the Sichuan Academy of Medical Sciences and Sichuan Provincial People’s Hospital were enrolled in the study. Informed consent was obtained for the use of tissue samples. Three patients were at tumor node metastasis (TNM) stage I, four at stage II, six at stage III, and two at stage IV. TNM staging was performed according to American Joint Committee on Cancer (6th edition) criteria.

MicroRNA (miR) target prediction

We predicted the putative downstream messenger RNA (mRNA) target of miR-24 using the most frequently used prediction algorithms, TargetScan version 7.0 (Whitehead Institute for Biological Research, Cambridge, UK) and PicTar (Rajewsky Lab, New York, NY, USA).

Plasmid and oligonucleotides

MiR-24 inhibitor and WWOX small interfering RNA (siRNA) were commercially synthesized with antisense oligonucleotide (OriGene, Beijing, China). The 3′-UTR of the WWOX gene carrying the predicted miR-24 binding site was cloned by PCR. We inserted this fragment upstream of the reporter gene in the pGL3-basic/luciferase vector and tested the luciferase activity using the Dual-Luciferase Reporter Assay system (Promega, Madison, WI, USA), following the manufacturer’s instructions. To construct a WWOX overexpression plasmid, we amplified the full-length human WWOX gene (without the 3′-UTR) using a complementary (DNA) clone as a template and inserted it into the pcDNA3 vector. The insertions were verified by DNA sequencing.

Cell culture and transfection

NCI-H358 cells were cultured in RPMI-1640 (Gibco, Grand Island, NY, USA) supplemented with 10% fetal bovine serum (FBS) and 1000 U/ml penicillin/streptomycin (P/S). NCI-H1299 cells were grown in Dulbecco’s modified Eagle medium supplemented with 10% FBS and 50 μg/mL kanamycin. The two human NSCLC cell lines were incubated in a humidified atmosphere at 37°C with 5% CO₂. Transfection was performed using a Lipofectamine 2000 Reagent (Invitrogen, Carlsbad, CA, USA), following the manufacturer’s instructions.

RNA isolation and quantitative real-time PCR

RNA was extracted from cells using TRIzol (Invitrogen). In miRNA quantitation, complementary DNA was generated with the stem-loop reverse transcript primer and Moloney murine leukemia virus (M-MLV) reverse transcriptase (Promega) using 1 μg of small RNA as a template. To detect the WWOX level, complementary DNA was generated with oligo(dT) primers and M-MLV reverse transcriptase (Promega) using 4 μg of large RNA as a template. PCR amplification was performed using a SYBR Premix Ex Taq II (Perfect Real-Time) kit (Takara Bio, Shiga, Japan) and an ABI PRISM 7300 Sequence Detection system (Applied Biosystems, Foster City, CA, USA). U6 and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) were used as an endogenous control. The primers used were as follows: U6 forward 5′-GCTTCCGAGCACATATAACTAAAAT-3; reverse 5′-CGGTTCAGGAATTTGCGTGAT-3; GAPDH forward 5′-CTCCTCGTGATTTCCAGGC-3; reverse
5'-CCCAATACGACAAATCCGT-3'; WWOX forward 5'-TCCTCAGAGTCCCATCGATT-3'; reverse 5'-GGGCA GCAGTGTGTAAGTA-3'.

Western blot
Cells were lysed and the protein was harvested 48 hours after transfection. Immunoblot assays were performed using antibodies against WWOX, MMP-9, and caspase 3, as well as GAPDH. All antibodies were purchased from Beijing Bios Biotechnology, Inc. (Beijing, China). Lab-Works image acquisition and analysis software (UVP, LLC; Analytik Jena AG, Upland, CA, USA) was used to acquire images of bands of interest and to quantify protein intensities.

Proliferation assay
To evaluate the viability of NSCLC cells, 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl-tetrazolium bromide (MTT) assay was performed. Ten microliters of MTT (0.5%) was added into the culture solution at 24, 48, and 72 hours after transfection. The absorbance at 570 nm was measured using the μQuant universal microplate spectrophotometer (Bio-Tek Instruments, Winooski, VT, USA).

Apoptosis assay
NCI-H358 and NCI-H1299 cells were transfected with synthesized oligonucleotides or control vectors. After an additional incubation of 36 hours, the cells were harvested, stained with 7-Aminoactinomycin D and phycoerythrin-labeled anti-annexin-V antibody, and analyzed by fluorescence-activated cell sorting (FACS).

Transwell invasion assay
Transwell invasion assay was conducted by planting 5 × 10^4 NCI-H358 cells coated with 40 μL of Matrigel diluted to 4 μg/μL with RPMI-1640 medium or 9 × 10^4 NCI-H1299 cells with 1 μg/μL of Dulbecco’s modified Eagle medium on the upper chamber of each insert (Corning, Cambridge, MA, USA); 800 μL of medium supplemented with 20% FBS was added to the lower chambers. Cells attached to the lower surface were stained for 25 minutes with crystal violet and then photographed for counting.

Tumor xenograft model in nude mice
NCI-H358 cells were suspended in 150 μL of serum-free RPMI 1640 and subcutaneously injected into the flank of five-week-old female nude mice. The mice with severe combined immunodeficiency were randomly divided into two groups of seven. The two groups were treated with miR-24 inhibitor or scrambled oligo through local injection of the xenograft tumor at multiple sites every five days for 25 days. Tumor size was measured every three days after 10 days of injections. The tumor volume was calculated as follows: length × width^2 × 1/2. All experiments were performed according to American Association for the Accreditation of Laboratory Animal Care guidelines for human treatment of animals.

Immunohistochemistry
The sections were pretreated with microwave irradiation, blocked, and incubated using polyclonal rabbit anti-human c-Kit (Beijing Bios Biotechnology Inc.). Staining intensity was assessed.

Statistical analysis
The results were reported as the mean ± standard deviation of at least three independent experiments. The data was processed using GraphPad Prism 5 software (GraphPad Software, Inc., San Diego, CA, USA) with the two-tailed Student’s t-test. A P value of < 0.05 was considered significant.

Results
Identification of WWOX as a direct target of miR-24
To identify the potential downstream molecules regulated by miR-24, we used TargetScan and PicTar to predict the target genes. WWOX was chosen for further study. Base-pairing complementation revealed that the 3' UTR of WWOX had significant complementarity with miR-24 (Fig 1a), and the species conservation information of the interaction of miR-24 and WWOX were predicted by TargetScan software (Fig 1b). We then examined the expression of miR-24 and WWOX in 15 pairs of human NSCLC tissue by quantitative real-time PCR. Spearman’s rank correlation analysis revealed a negative correlation between miR-24 and WWOX expression (Fig 1c). To further demonstrate that WWOX was directly targeted by miR-24 in NSCLC cells, dual-luciferase reporter assay was performed to observe the interaction between miR-24 and 3'-UTR of WWOX mRNA. The miR-24 inhibitor obviously reduced the miR-24 level (Fig 1e) and elevated WWOX luciferase activity (Fig 1d) and protein levels (Fig 1f) in NCI-H358 and NCI-H1299 cells. These results revealed that miR-24
Figure 1 MiR-24 downregulates WWOX by binding to its 3' untranslated region (UTR). (a) Predicted miR-24 binding sites in the 3'UTR of WWOX. (b) Species conservation information of the interaction between miR-24 and WWOX was predicted using TargetScan software. (c) The correlation between miR-24 and WWOX expression. MiR-24 and WWOX messenger RNA (mRNA) levels in 15 pairs of human non-small cell lung cancer (NSCLC) tissues were measured by quantitative real-time PCR (qRT-PCR); U6 and β-Actin were used for normalization. (d) Luciferase reporter assays in NSCLC cells, following co-transfection with the WWOX 3'UTR and miR-24 inhibitor. The data shows fold changes (mean ± standard deviation) of three independent experiments. (a) Ctrl+WWOX-3'UTR, (b) miR-24 inhibitor+WWOX-3'UTR, (c) Ctrl+WWOX-3'UTRmut, and (d) miR-24 inhibitor +WWOX-3'UTRmut. (e) The miR-24 mRNA level after treatment with the miR-24 inhibitor, by qRT-PCR. (f) WWOX protein level after treatment with the miR-24 inhibitor, by Western blot. (g) Ctrl and (h) miR-24 inhibitor. *P < 0.05.
binds directly to the 3’ UTR of WWOX to suppress gene expression.

**Downregulation of miR-24 suppresses the proliferation and invasion of non-small cell lung cancer (NSCLC) cells in vitro**

To determine the effects of miR-24 on the malignant behavior of NSCLC cells, miR-24 inhibitor was transfected into NCI-H358 and NCI-H1299 cells. The viability decreased in cells that had low levels of miR-24 compared to control cells, as observed in MTT assay (Fig 2a). To further investigate cell viability suppression caused by the miR-24 inhibitor, we performed FACS to detect the impact of miR-24 on NSCLC cell apoptosis. The miR-24 inhibitor significantly increased apoptosis in NCI-H358 and NCI-H1299 cells compared to the control (Fig 2b). Transwell invasion assay revealed that the passing of DNA in cells transfected with the miR-24 inhibitor decreased compared to the control (Fig 2c,d). The two NSCLC cell lines showed similar results. The data indicated that downregulation of miR-24 suppressed NSCLC cell viability and invasion in vitro.

**WWOX could induce apoptosis and suppress invasion of NSCLC cells**

To investigate the function of WWOX on the phenotype of NSCLC cells, we constructed a WWOX overexpression vector. After the efficiency of the vector was confirmed by Western blot (Fig 3a), we transfected it into the two NSCLC cell lines...
and found that apoptosis was increased in the WWOX overexpression group compared to the control (Fig 3b). Meanwhile, the number of passed NSCLC cells was decreased by WWOX expression, as observed in transwell invasion assay (Fig 3c,d). These results revealed that WWOX could induce NSCLC cell apoptosis and inhibit invasion in vitro.

**Knockdown of WWOX restored the influence of the miR-24 inhibitor on apoptosis and invasion**

To confirm that the function of miR-24 is mediated by repression of WWOX and not by the targeting of other cellular molecules, a rescue experiment was performed. WWOX siRNA and miR-24 inhibitor were co-transfected into NCI-H358 and NCI-H1299 cells. The miR-24 inhibitor group expressed a higher level, while the co-transfection group expressed a lower level of WWOX by Western blot (Fig 4a). WWOX siRNA counteracted the effect of the miR-24 inhibitor on WWOX expression. As is shown in the FACS and transwell invasion assay, WWOX siRNA also counteracted the effects of the miR-24 inhibitor on apoptosis (Fig 4b) and cell invasion (Fig 4c,d). These results demonstrated the mechanism of miR-24, at least partially, is to targeting WWOX in NSCLC.

![Image](image_url)
miR-24 and WWOX influence caspase 3 and MMP9 expression in NSCLC cells

In order to explore the mechanisms underlying miR-24 and WWOX regulation of apoptosis and invasion of NSCLC cells, we detected the manner of expression of apoptosis and invasion-related proteins (caspase 3 and MMP9). According to Western blot results, the protein level of activated caspase 3 increased when miR-24 expression was inhibited (Fig 5a) or when WWOX expression was ectopic (Fig 5b) in NCI-H358 and NCI-H1299 cells. However, MMP9 protein levels were reduced in the same experiment (Fig 5a,b). WWOX siRNA counteracted the effect of the miR-24 inhibitor on caspase 3 and MMP9 expression (Fig 5c). These results suggested that miR-24 downregulation induces apoptosis, possibly by activating caspase 3, and suppresses invasion by inhibiting MMP9.

miR-24 downregulation suppresses NSCLC cell proliferation in vivo

To further confirm the effect of miR-24 on NSCLC cell proliferation in vivo, we constructed a tumor xenograft model in nude mice. Mice were treated with miR-24 inhibitor or scrambled oligo through local injection of the xenograft tumor; the tumor nodules are shown in Figure 6a. The tumor volumes were smaller in the miR-24 inhibitor group than in the control (Fig 6b). To verify that the decrease in the tumor volume of xenograft nodules was caused by miR-24 downregulation, we tested the levels of miR-24 and WWOX in the xenograft tumors. The miR-24 mRNA levels were lower and the WWOX protein levels higher in the miR-24 inhibitor group than in the control (Fig 6c,d). Immunohistochemical assay showed that c-Kit (a well-known oncogene) expression was lower in the...
miR-24 inhibitor group (Fig 6e), confirming that the miR-24 inhibitor has a tumor suppressive function. The result revealed that downregulation of miR-24 suppresses NSCLC cell proliferation in vivo.

Discussion

Only one third of the patients diagnosed with NSCLC are eligible for surgery\(^\text{12}\) and post-surgical survival is limited. Emerging evidence suggests that the deregulation of miRNAs and their target genes constitutes a complex interacting network that controls cancer progression, including NSCLC. For example, miR-130a targets MET and induces TRAIL-sensitivity in NSCLC by downregulating miR-221 and 222,\(^\text{13}\) and miR-195 suppresses NSCLC by targeting CHEK1.\(^\text{14}\) Other miRNAs related to NSCLC include miR-29b,\(^\text{15}\) miR-582-3p,\(^\text{16}\) miR-326\(^\text{17}\) and miR-205.\(^\text{18}\)

We focused on miR-24, which has been reported as an oncogene in many cancers, including gastric,\(^\text{19}\) pancreatic,\(^\text{20}\) and breast cancers.\(^\text{21,22}\) However, the role of miR-24 in NSCLC has not previously been well illustrated. Based on our experimental data, low expression of miR-24 induced apoptosis and inhibited cell proliferation and migration in NSCLC cells by directly targeting WWOX, indicating the oncogenic property of miR-24 in NSCLC. Meanwhile, Zhao et al. found that a high level of miR-24 promotes cell proliferation by targeting NAIF1 in NSCLC,\(^\text{23}\) which is in accordance with our observation. One miRNA may regulate many target genes, and one gene may be targeted by many miRNAs. To determine which downstream targets are also regulated by miR-24 requires further study. Researchers have discovered that miR-24 functions as a tumor suppressor in nasopharyngeal carcinoma and prostate cancer,\(^\text{24,25}\) which does not match our observation. One possible explanation is that miRNA expression

Figure 5 Influence of miR-24 and WWOX on caspase 3 and MMP9. (a) Regulation of the miR-24 inhibitor on caspase 3 and MMP9 by Western blot. Ctl and miR-24 inhibitor. (b) The influence of WWOX on caspase 3 and MMP9. pcDNA3 and pcDNA3-WWOX. WWOX small interfering RNA (siRNA) restored the effect of the miR-24 inhibitor on caspase 3 and MMP9. miR-24 inhibitor, miR-24 inhibitor+siRNA-NC and miR-24 inhibitor+WWOX-siRNA. Error bars indicate the mean ± standard deviation of three independent experiments, \(^*P < 0.05\). GAPDH, glyceraldehyde 3-phosphate dehydrogenase; NC, negative control.
depends on the cellular context, and the function of miRNA is tumor-type specific.26

WWOX, generally known as a tumor suppressor gene,27,28 is located at a common fragile region, FRA16D,29 and is reported to be involved in several tumor types, including ovary, breast, and esophagus tumors.30–32 Sai et al. demonstrated that the WWOX gene is altered by deletion and/or aberrant expression in a significant number of lung cancers and may contribute to the pathogenesis of NSCLC.33 Baykara et al. reported that hypermethylation of the WWOX gene promoter and mutations in this gene may be related to lung carcinogenesis. By contrast, our results showed that miR-24 binds directly to the WWOX mRNA to suppress its expression.34 Thus, WWOX may be involved in the oncogenesis and development of NSCLC at both transcriptional and post-transcriptional levels.

Based on our observations, miR-24 inhibitor-mediated apoptosis suppresses NSCLC cell proliferation. To explore the intermediate signaling molecule, we examined the level of caspase 3 expression, a member of the cysteine-aspartic acid protease family. As expected, the protein level of activated caspase 3 increased when miR-24 expression was
inhibited, indicating the important role of caspase 3 in cell apoptosis, caused by the miR-24 inhibitor in NSCLC cells. However, whether other members of caspase family also participate in miR-24-related apoptosis requires further investigation. Proteins of the matrix metalloproteinase (MMP) family are involved in extracellular matrix breakdown in normal physiological processes, such as embryonic development, reproduction, and tissue remodeling, as well as in disease processes, such as arthritis and metastasis. Surprisingly, we discovered that MMP9 is an important mediator in the miR-24-related migration process of NSCLC cells. The miR-24 inhibitor suppresses NSCLC cell migration by downregulating MMP9.

Collectively, the results of our study provide evidence of the coordination of miR-24 and WWOX in the regulation of cell apoptosis, proliferation, and migration during NSCLC development. Inhibition of miR-24 expression may serve as an alternative treatment modality for NSCLC.

**Disclosure**

No authors report any conflict of interest.

**References**

1. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2015. *CA Cancer J Clin* 2015; 65: 5–29.
2. Bartel DP. MicroRNAs: Target recognition and regulatory functions. *Cell* 2009; 136: 215–33.
3. Chen JF, Mandel EM, Thomson JM et al. The role of microRNA-1 and microRNA-133 in skeletal muscle proliferation and differentiation. *Nat Genet* 2006; 38: 228–33.
4. Cheng AM, Byrom MW, Shelton J, Ford LP. Antisense inhibition of human miRNAs and indications for an involvement of miRNA in cell growth and apoptosis. *Nucleic Acids Res* 2005; 33: 1290–7.
5. Cho WJ, Shin JM, Kim JS et al. miR-372 regulates cell cycle and apoptosis of ags human gastric cancer cell line through direct regulation of LATS2. *Mol Cells* 2009; 28: 521–7.
6. Adams CM, Eischen CM. Histone deacetylase inhibition reveals a tumor-suppressive function of MYC-regulated miRNA in breast and lung carcinoma. *Cell Death Differ* 2016; 23: 1312–21.
7. Xie Y, Todd NW, Liu Z et al. Altered miRNA expression in sputum for diagnosis of non-small cell lung cancer. *Lung Cancer* 2010; 67: 170–6.
8. Xiong F, Wu C, Chang J et al. Genetic variation in an miRNA-1827 binding site in MYCL1 alters susceptibility to small-cell lung cancer. *Cancer Res* 2011; 71: 5175–81.
9. Lal A, Pan Y, Navarro F et al. miR-24-mediated downregulation of H2AX suppresses DNA repair in terminally differentiated blood cells. *Nat Struct Mol Biol* 2009; 16: 492–8.
10. Neilson JR, Zheng GX, Burge CB, Sharp PA. Dynamic regulation of miRNA expression in ordered stages of cellular development. *Genes Dev* 2007; 21: 578–89.
11. Kerr JF, Wyllie AH, Currie AR. Apoptosis: A basic biological phenomenon with wide-ranging implications in tissue kinetics. *Br J Cancer* 1972; 26: 239–57.
12. Predina JD, Puc MM, Bergey MR et al. Improved survival after pulmonary metastasectomy for soft tissue sarcoma. *J Thorac Oncol* 2011; 6: 913–9.
13. Acunzo M, Visone R, Romano G et al. miR-130a targets MET and induces TRAIL-sensitivity in NSCLC by downregulating miR-221 and 222. *Oncogene* 2012; 31: 634–42.
14. Liu B, Qu J, Xu F et al. MiR-195 suppresses non-small cell lung cancer by targeting CHEK1. *Oncotarget* 2015; 6: 9445–56.
15. Langsch S, Baumgartner U, Haemmig S et al. miR-29b mediates NF-kB signaling in KRAS-induced non-small cell lung cancers. *Cancer Res* 2016; 76: 4160–9.
16. Fang L, Cai J, Chen B et al. aberrantly expressed miR-582-3p maintains lung cancer stem cell-like traits by activating Wnt/beta-catenin signalling. *Nat Commun* 2015; 6: 8640.
17. Li D, Du X, Liu A, Li P. Suppression of nucleosome-binding protein 1 by miR-326 impedes cell proliferation and invasion in non-small cell lung cancer cells. *Oncol Rep* 2016; 35: 1117–24.
18. Lei L, Huang Y, Gong W. miR-205 promotes the growth, metastasis and chemoresistance of NSCLC cells by targeting PTEN. *Oncol Rep* 2013; 30: 2897–902.
19. Zhang H, Duan J, Qu Y et al. Onco-miR-24 regulates cell growth and apoptosis by targeting BCL2L11 in gastric cancer. *Protein Cell* 2016; 7: 141–51.
20. Liu R, Zhang H, Wang X et al. The miR-24-Bim pathway promotes tumor growth and angiogenesis in pancreatic carcinoma. *Oncotarget* 2015; 6: 43831–42.
21. Lu K, Wang J, Song Y et al. miRNA-24-3p promotes cell proliferation and inhibits apoptosis in human breast cancer by targeting p27Kip1. *Oncol Rep* 2015; 34: 995–1002.
22. Sochor M, Basova P, Pesta M et al. Oncogenic microRNAs: miR-155, miR-19a, miR-181b, and miR-24 enable monitoring of early breast cancer in serum. *BMC Cancer* 2014; 14: 448.
23. Zhao G, Liu L, Zhao T et al. Upregulation of miR-24 promotes cell proliferation by targeting NAI1 in non-small cell lung cancer. *Tumour Biol* 2015; 36: 3693–701.
24. Li YQ, Lu JH, Bao XM, Wang XF, Wu JH, Hong WQ. miR-24 functions as a tumor suppressor in nasopharyngeal carcinoma through targeting FSCN1. *J Exp Clin Cancer Res* 2015; 34: 130.
25. Lynch SM, McKenna MM, Walsh CP, McKenna DJ, miR-24 regulates CDKN1B/p27 expression in prostate cancer. *Prostate* 2016; 76: 637–48.
26. Meltzer PS. Cancer genomics: Small RNAs with big impacts. *Nature* 2005; 435: 745–6.
27 Bednarek AK, Keck-Waggoner CL, Daniel RL et al. WWOX, the FRA16D gene, behaves as a suppressor of tumor growth. *Cancer Res* 2001; **61**: 8068–73.

28 Fabbri M, Iliopoulos D, Trapasso F et al. WWOX gene restoration prevents lung cancer growth in vitro and in vivo. *Proc Natl Acad Sci U S A* 2005; **102**: 15611–6.

29 Bednarek AK, Laflin KJ, Daniel RL, Liao Q, Hawkins KA, Aldaz CM. WWOX, a novel WW domain-containing protein mapping to human chromosome 16q23.3-24.1, a region frequently affected in breast cancer. *Cancer Res* 2000; **60**: 2140–5.

30 Driouch K, Prydz H, Monese R, Johansen H, Lidereau R, Fresgen E. Alternative transcripts of the candidate tumor suppressor gene, WWOX, are expressed at high levels in human breast tumors. *Oncogene* 2002; **21**: 1832–40.

31 Kuroki T, Trapasso F, Shiraishi T et al. Genetic alterations of the tumor suppressor gene WWOX in esophageal squamous cell carcinoma. *Cancer Res* 2002; **62**: 2258–60.

32 Paige AJ, Taylor KJ, Taylor C et al. WWOX: A candidate tumor suppressor gene involved in multiple tumor types. *Proc Natl Acad Sci U S A* 2001; **98**: 11417–22.

33 Yendamuri S, Kuroki T, Trapasso F et al. WW domain containing oxidoreductase gene expression is altered in non-small cell lung cancer. *Cancer Res* 2003; **63**: 878–81.

34 Baykara O, Demirkaya A, Kaynak K, Tanju S, Toker A, Buyru N. WWOX gene may contribute to progression of non-small-cell lung cancer (NSCLC). *Tumour Biol* 2010; **31**: 315–20.