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Dysregulation of microRNA-106a-5p-RUNX1 axis associates with clinical progression and prognosis of osteosarcoma patients

Kun Chen[a], Guobiao Pan[b,*]

[a] Beijing Chaoyang Emergency Medical Rescuing Center, Beijing 100026, China
[b] Department of Orthopedic Oncology, Hangzhou Cancer Hospital, Hangzhou 310002, Zhejiang Province, China

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ABSTRACT

MicroRNA-106a-5p (miR-106a-5p) functions as a tumor suppressor in osteosarcoma cells. Here, we aimed to identify novel target genes of miR-106a-5p in osteosarcoma, as well as to investigate their prognostic value and the biological functions. At first, the mammalian runt-related factor 1 (RUNX1) was identified as one of the target genes of miR-106a-5p in osteosarcoma cells by luciferase reporter gene assay, real-time quantitative RT-PCR and Western blot analysis. Then, the expression levels of miR-106a-5p and RUNX1 in osteosarcoma tissues were detected, and their associations with clinicopathological features and patients' prognosis were statistically analyzed. Compared with adjacent non-cancerous tissues, miR-106a-5p and RUNX1 mRNA/protein expression in osteosarcoma tissues were significantly decreased and increased, respectively (all $P < 0.01$). Low miR-106a-5p, high RUNX1 and miR-106a-5p-low/RUNX1-high expression in osteosarcoma tissues were all significantly associated with advanced Enneking stage, positive metastasis and shorter overall survival (all $P < 0.05$). Moreover, miR-106a-5p and RUNX1 expression, alone or in combination, were identified as independent prognostic factors for osteosarcoma patients' overall survival. Functionally, the enforced expression of miR-106a-5p significantly suppressed proliferation and invasion of osteosarcoma cells, while the overexpression of RUNX1 effectively reversed its suppressive roles. In conclusion, our findings show the dysregulation of miR-106a-5p-RUNX1 axis in human osteosarcoma tissues and suggest its crucial roles in cancer progression and patients' prognosis. More interestingly, miR-106a-5p may function as a tumor suppressor in osteosarcoma cells via regulating its target gene RUNX1.

1. Introduction

Osteosarcoma represents the most frequent primary malignant bone tumor with high prevalence in children, adolescents, and young adults [1]. Surgery combined with chemotherapy is the main therapeutic strategy for the treatment of osteosarcoma patients, and has markedly improved the five-year survival rate of patients with localized tumor [2]. However, the clinical outcome of osteosarcoma patients with metastasis is very unfavorable, with an approximate five-year survival rate of 20–30% [3]. Therefore, it is urgently needed to identify new diagnostic markers and novel therapeutic targets for improving the treatment and prognosis in patients with osteosarcoma.

An increasing number of research have been focus on the molecular mechanisms of osteosarcoma. Among them, the aberrant expression of microRNAs (miRNAs) has been reported to be involved in the carcinogenesis and cancer progression of many cancers. miRNAs represent a class of non-coding small RNAs with 18–24 nucleotides in length [4]. miRNAs exert biological functions by negatively regulating the expression of the corresponding target genes at the post-transcriptional and/or translational level via binding to complimentary sequences in the 3′-untranslated regions (UTRs) of target mRNAs [5]. Growing evidence show that the miRNAs may involve the tumor suppressive or oncogenic functionality [6–8]. The miR-17 family, including miR-17-5p, miR-20a, miR-20b, miR-106a-5p, miR-106b and miR-93, is divided into three clusters (the miR-17-92a, miR-106b-25 and miR-106a-363 clusters) according to consensus seed regions [9]. miR-106a-5p belongs to the miR-106a-363 cluster and is located on Xq26.2 [10]. The aberrant expression of miR-106a-5p has been observed in various human cancers. It is overexpressed in breast, non-small cell lung, gastric and colorecal cancers, while is downregulated in astrocytoma, squamous cell carcinoma and osteosarcoma, implying this miRNA may exert either oncogenic role or tumor suppressive role in different cancers [11–17]. Especially, miR-106a-5p expression was previously observed to be decreased in osteosarcoma tissues compared with adjacent normal

* Corresponding author.
E-mail address: guobiaop_hz@163.com (G. Pan).

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tissues, and also associated with tumor size, Enneking stage, distant metastasis, and lung metastasis [17]. However, the prognostic value of miR-106a-5p in osteosarcoma and the underlying mechanisms have not been fully elucidated.

To address this problem, in the current study, we firstly identified the candidate target gene of miR-106a-5p by collecting from the miRNA-target interaction database-miRTarBase [18], as well as verifying though luciferase reporter gene assay, real-time quantitative RT-PCR and western blot analysis based on two osteosarcoma cell lines. Then, the expression levels of miR-106a-5p and its target gene in 100 self-paired specimens of osteosarcoma and adjacent non-cancerous tissues were detected by real-time quantitative RT-PCR and western blot analysis, and their associations with clinicopathological features and patients' prognosis were statistically analyzed. The functions of miR-106a-5p-target gene axis in osteosarcoma cells were further investigated by cell counting kit-8 (CCK-8) and transwell assays.

2. Materials and methods

2.1. Patients and tissue samples

Our study was approved by the medical ethics committee of Hangzhou Cancer Hospital, China (Approved Number: 20190301A). Prior informed consent was obtained from the patients for collecting tissue specimens according to the guidelines of Hangzhou Cancer Hospital, China. A total of 100 osteosarcoma patients (80 male, 20 female; aged 10–60 years, median 25 years) were enrolled for this study between January 2005 and December 2010 from Department of Orthopedic Oncology, Hangzhou Cancer Hospital, China. The clinical data were collected by reviewing medical records and patient inquiries. The clinical staging was based on the Enneking staging system [19]. All patients were performed follow-up every 3 months for the first year and then every 6 months for the next 2–5 years. The overall survival was defined as the time period from the start date of follow-up to the date of death or the end date of follow-up. Table 1 summarized the clinicopathological characteristics of all 100 osteosarcoma patients enrolled in this study.

2.2. Cell culture and cell transfection

Human osteosarcoma cell lines U2OS and MG63 were purchased from the Cell Bank, China Academy of Sciences (Shanghai, China), and were cultured in RPMI 1640 medium (Gibco BRL, Carlsbad, CA, USA) supplemented with 10% fetal bovine serum (Gibco BRL) and incubated in a humidified 37 °C incubator supplemented with 5% CO_2.

miR-106a-5p mimics (miR-106a-mimics, sense, 5′-AAA AGU GCU UAC AGU GCA GGU AG-3′, antisense, 5′-ACC UGC ACU GUA AGC ACU UUU UU-3′), mimic control (NC-mimics, sense, 5′-UUC UCC GAA CGU GUC ACG UTT-3′, antisense, 5′-ACG UGA CAC GUU CGG AGA ATT-3′), RUNX1 expression vector (RUNX1-ex) and contro vector (NC-ex) were purchased from Guangzhou RiboBio Co., Ltd. (Guangzhou, China). Following the cell amount reached 70–80% confluency, both U2OS and MG63 cells were transfected with miR-106a-mimics, NC-mimics, RUNX1-ex or NC-exs using Lipofectamine 2000 reagent (Invitrogen; Thermo Fisher Scientific, Inc.), according to the manufacturer's protocol. Following the transfection for 48 h, the cells were harvested and used for further experiments.

2.3. Dual luciferase reporter gene assay

To verify the binding site of miR-106a-5p and RUNX1 mRNA, dual luciferase reporter gene assay was performed. In brief, the RUNX1 3′-UTR sequences were subcloned into the pGL3-basic luciferase reporter vector (Promega Corporation, Madison, WI, USA). Both U2OS and MG63 cells were seeded into 12-well plates and co-transfected with RUNX1 3′-UTR, RUNX1 mut 3′-UTR and NC-mimics or miR-106a-mimics using Lipofectamine 2000 reagent (Invitrogen; Thermo Fisher Scientific, Inc.). At the end of 48 h transfection, the dual luciferase reporter gene assay was performed using the Dual Luciferase Reporter kit (Promega Corporation) based on the users' instruction. The relative luciferase activity was normalized to Renilla luciferase activity. For each sample, all experiments were done in triplicate.

2.4. CCK-8 assay

CCK-8 assay was used to assess the proliferative ability of U2OS and MG63 cells transfected with miR-106a-5p mimics (miR-106a-mimics), mimic control (NC-mimics), RUNX1 expression vector (RUNX1-ex) and contro vector (NC-ex) using CCK-8 kit (Beyotime, China). Following the transfection for 24 h, the 5000 transfected osteosarcoma cells were seeded into 96-well plates with 100 μL of culture media. The medium of each well was replaced with 100 μL fresh culture media contained 10% CCK-8 at different times (24, 48, 72 and 96 h). The absorbance was measured at a wavelength of 450 nm using microplate reader (Multiscan FC, Thermo Scientific).

2.5. Transwell assay

Transwell assay was used to assess the invasive abilities of U2OS and MG63 cells transfected with miR-106a-5p mimics (miR-106a-mimics), mimic control (NC-mimics), RUNX1 expression vector (RUNX1-ex) and contro vector (NC-ex) using Transwell filters (Costar, USA),

Table 1
Associations of miR-106a-5p expression with clinicopathological features of osteosarcoma (osteosarcoma) patients.

Clinical variables	No. of patients (n, %)	miR-106a-5p expression (n, %)		P	RUNX1 expression (n, %)		P	miR-106a-5p/RUNX1 (n, %)	P
		Low	High		Low	High		Low/high	
Gender									
Male	65 (65.00)	36 (55.38)	29 (44.62)	0.33	33 (50.77)	32 (49.23)	0.35	22 (33.85)	0.32
Female	35 (35.00)	16 (45.71)	19 (54.29)		16 (45.71)	19 (54.29)		14 (40.00)	
Age (year)									
≤18	58 (58.00)	30 (51.72)	28 (48.28)	0.42	30 (51.72)	28 (48.28)	0.40	20 (34.48)	0.40
> 18	42 (42.00)	22 (52.38)	20 (47.62)		19 (45.24)	23 (54.76)		16 (38.10)	
Tumor size									
≤5 cm	48 (48.00)	24 (50.00)	24 (50.00)	0.29	24 (50.00)	24 (50.00)	0.30	16 (33.33)	0.29
> 5 cm	52 (52.00)	28 (53.85)	24 (46.15)		25 (48.08)	27 (51.92)		20 (38.46)	
Enneking stage									
I-II	40 (40.00)	12 (30.00)	28 (70.00)	< **0.05**	10 (25.00)	30 (75.00)	< **0.05**	10 (25.00)	< **0.05**
III-IV	60 (60.00)	40 (66.67)	20 (33.33)		39 (65.00)	21 (35.00)		26 (43.33)	
Metastasis									
M0	41 (41.00)	16 (39.02)	25 (60.98)	< **0.05**	13 (31.70)	28 (68.30)	< **0.05**	10 (24.39)	< **0.05**
M1	59 (59.00)	36 (61.02)	23 (38.98)		36 (61.02)	23 (38.98)		26 (44.07)	

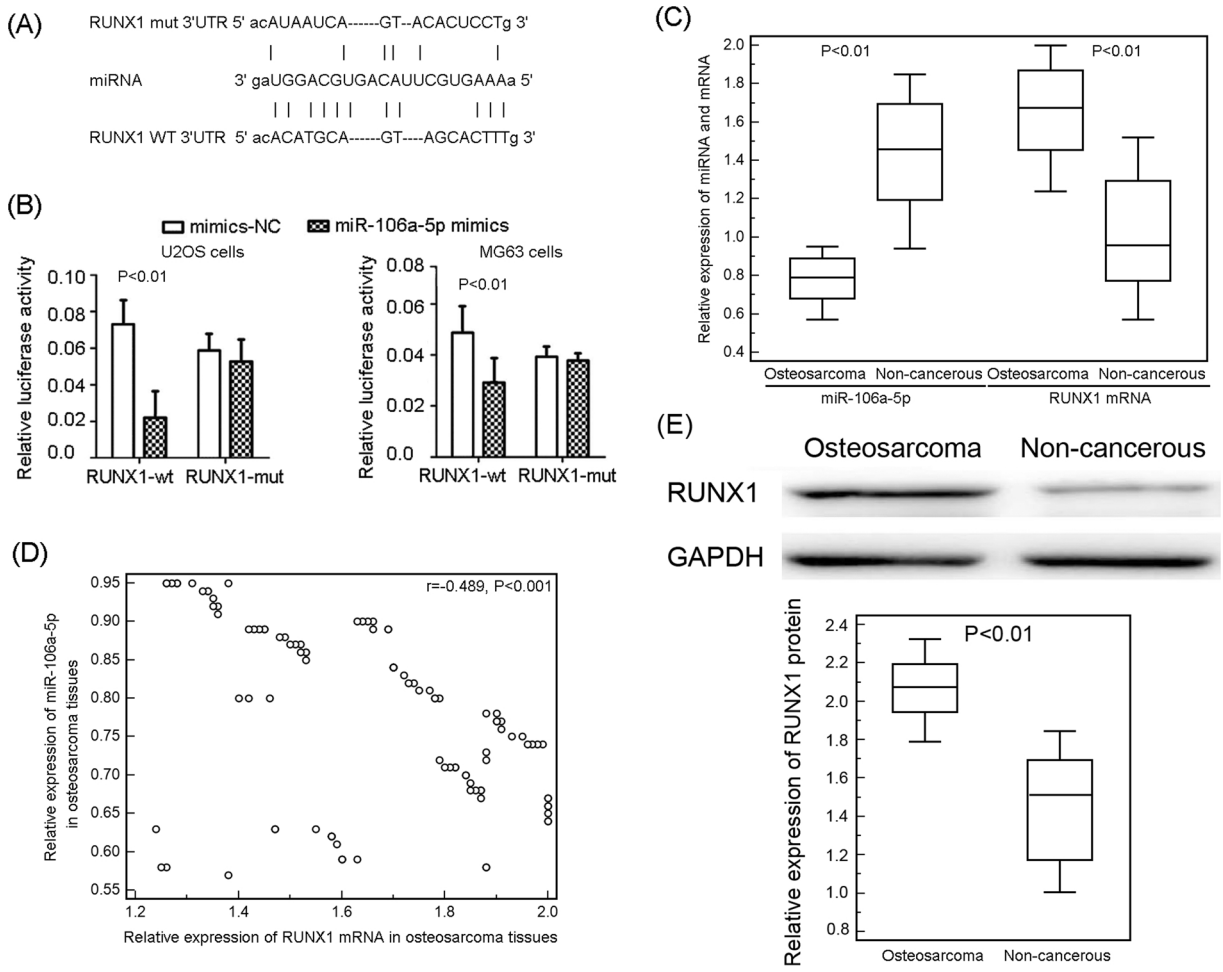

Fig. 1. RUNX1 is a direct target of miR-106a-5p in human osteosarcoma cells. (A) Schematic description of wild type (WT) and mutated 3′-UTR of RUNX1 mRNA. (B) Relative luciferase reporter activities in different groups based on U2OS and MG63 cells, respectively. (C) Relative expression levels of miR-106a-5p and RUNX1 mRNA in human osteosaroma and adjacent noncancerous tissues. (D) Correlation between miR-106a-5p expression and RUNX1 mRNA expression in human osteosarcoma tissues. (E) Relative expression levels of RUNX1 protein in human osteosaroma and adjacent noncancerous tissues.

which were coated with Matrigel (3.9 μg/mL) on the upper surface of the polycarbonic membrane (diameter 6.5 mm, pore size 8 mm). Cells in the upper compartment of the chamber were suspended in serum-free medium, and the lower chamber contained medium supplemented with 20% fetal bovine serum. Following the incubation for 24 h, cells that passed through the matrigel membrane were fixed and stained with crystal violet and counted in 5 random microscopic fields.

2.6. Real-time quantitative PCR for miRNA and mRNA

Real-time quantitative PCR was performed to detect expression levels of miR-106a-5p and RUNX1 mRNA in 100 self-paired specimens of osteosarcoma and adjacent non-cancerous tissues, as well as human osteosarcoma cell lines. In brief, total RNA from tissues or cells was extracted using Trizol reagent (Thermo Fisher Scientific, Wilmington, DE, USA). Then, cDNA for miR-106a-5p or RUNX1 mRNA was synthesized using TaqMan. MicroRNA Reverse Transcription kit (Thermo Fisher Scientific) or High Capacity cDNA Reverse Transcription Kit (Thermo Fisher Scientific). RT-PCR reaction was performed using Universal SYBR Green PCR Kit (Takara, Dalian, China) and specific primers, and ran on an ABI 7500 Real-Time PCR system (Applied Biosystems, Foster City, CA, USA). The following primers were used: miR-106a-5p sense, 5′-GAT GCT CAA AAA GTG CTT ACA GTG CA-3′ and antisense, 5′-TAT GGT TGT TCT GCT CTC TGT CTC-3′; RNU6B sense, 5′-CGC TTC GGC AGC ACA TAT ACT A-3′ and antisense, 5′-CGC TTC ACG AAT TTG CGT GTC A-3′; RUNX1 sense, 5′-CCG GAA TTC ATG CGT ATC CCC GTA G-3′ and antisense, 5′-CCG CTC GAG GTA GGG CCT CCA CAC G-3′; GAPDH sense, 5′- GGA GCG AGA TCC CTC CAA AAT-3′ and antisense, 5′- GGC TGT TGT CAT ACT TCT CAG G-3′. RNU6B and GAPDH were employed as housekeeping genes to normalize the difference of miR-106a-5p and RUNX1 mRNA, respectively, in each sample. The delta-delta Ct method was employed to calculate the fold-change. For each sample, all experiments were done in triplicate.

2.7. Western blot assay

Western blot assay was performed to detect the expression levels of RUNX1 protein in 100 self-paired specimens of osteosarcoma and adjacent non-cancerous tissues, as well as human osteosarcoma cell lines. In brief, Tissues or cells were lysed 1% Nonidet P-40 lysis buffer for 48 h. Homogenates were extracted by centrifugation at 20,000 ÿg for 15 min at 4 . Protein concentration was calculated based on a BCA reagent curve (Pierce Biotechnology, Rockford, IL). Equal amounts of protein (15 μg) were loaded on 8 12% SDS-PAGE gel and then transferred to PVDF membranes (Bio-Rad), which were incubated overnight at 4 °C with primary antibodies. The following primary antibodies were used: anti-RUNX1 (dilution 1:1000, #ab92336, Abcam, San Francisco, USA) and anti-GAPDH (dilution 1:1000, #ab9485, Abcam, San Francisco, USA). The membranes were incubated with the corresponding peroxidase-linked secondary antibody for 1 h, and the specific protein bands were visualized using enhanced chemiluminescence Western Blotting Substrate (Thermo Fisher Scientific, Wilmington, DE,

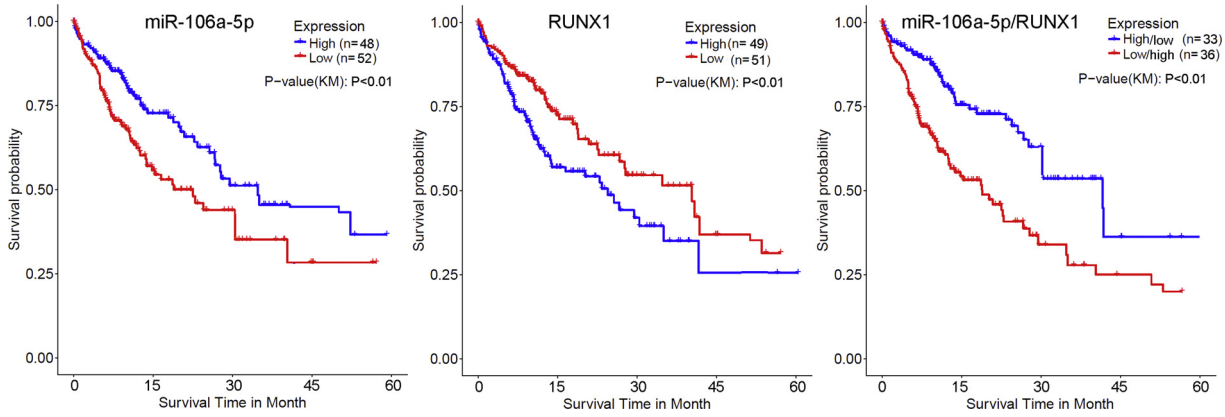

Fig. 2. Survival curves (Overall survival) for 100 osteosarcoma patients according to expression patterns of miR-106a-5p, RUNX1 and miR-106a-5p/RUNX1 in osteosarcoma tissues. Kaplan-Meier analysis showed that the osteosarcoma patients with low miR-106a-5p expression ($P < 0.01$), high RUNX1 expression ($P < 0.01$) and miR-106a-5p-low/RUNX1-high expression ($P < 0.01$) had shorter overall survival than those with the corresponding control clinicopathological characteristics.

USA).

2.8. Statistical analysis

Statistical analysis in the current study was performed using SPSS software (SPSS Standard version 12.0; SPSS Inc. Chicago, IL, USA). The experimental data were representative of three independent experiments. The differences in miR-106a-5p expression levels in osteosarcoma and adjacent normal esophageal tissues were analyzed by two-way analysis of variance (ANOVA). The relationships between miR-106a-5p expression and various clinicopathological data of patients with osteosarcoma were evaluated by the chi-square test. Kaplan-Meier survival curves were constructed with tumor locoregional progression, distant progression, and death as the end points. Univariate and multivariate analyses were performed using Cox's proportional hazard model to evaluate the correlation between miR-106a-5p expression and patients' prognosis. Differences were considered statistically significant when P was less than 0.05.

3. Results

3.1. RUNX1 is a direct target of miR-106a-5p in human osteosarcoma cells

According to miRTarBase which serves as a comprehensively annotated, experimentally validated miRNA-target interaction database (http://mirtarbase.mbc.nctu.edu.tw/php/search.php, Release 7.0) [18], RUNX1 is one of the experimentally validated targets of miR-106a-5p based on haematopoietic progenitor cells [20]. To verify whether RUNX1 was a direct target of miR-106a-5p in human osteosarcoma cells, the dual luciferase reporter assay was performed. As shown in Fig. 1A and B, the relative luciferase activities of the reporter gene in U2OS and MG63 cells co-transfected with psiCHECK2-RUNX1 WT 3′-UTR and miR-106a-5p mimics were significantly decreased by at least 30% compared with their control (both $P < 0.05$). In contrast, co-transfection of miR-106a-5p mimics or mimics-NC with psiCHECK2-RUNX1 mut 3′-UTR led to no luciferase intensity changes. The above data validated the miRNA/target 3′-UTR specificity.

Moreover, we detected the expression levels of miR-106a-5p and RUNX1 mRNA in 100 self-pairs of osteosarcoma and adjacent non-cancerous tissues. As shown in Fig. 1C, miR-106a-5p expression was significantly downregulated (noncancerous *vs.* osteosarcoma: 1.44 ± 0.38 *vs.* 0.78 ± 0.32, $P < 0.01$), while RUNX1 mRNA expression was markedly upregulated in osteosarcoma tissues (noncancerous *vs.* osteosarcoma: 1.01 ± 0.23 *vs.* 1.65 ± 0.59, $P < 0.001$), compared with adjacent non-cancerous tissues. The spearman correlation analysis determined that miR-106a-5p expression was negatively correlated with RUNX1 mRNA expression in osteosarcoma tissues significantly ($r = -0.489$, $P < 0.001$, Fig. 1D). Consistently, the upregulation of RUNX1 protein was also confirmed by Western blot analysis (noncancerous *vs.* osteosarcoma: 1.51 ± 0.22 *vs.* 2.06 ± 0.28, $P < 0.001$, Fig. 1E).

3.2. miR-106a-5p downregulation and/or RUNX1 upregulation associates with aggressive clinicopathological features of osteosarcoma patients

The median values of miR-106a-5p (0.78) and RUNX1 (1.66) mRNA expression levels in osteosarcoma tissues detected by real-time quantitative PCR were used as cutoff points to divide all 100 osteosarcoma patients into low/high miR-106a-5p expression groups, and low/high RUNX1 expression groups, respectively. Of 100 osteosarcoma patients, 52 (52.00%), 48 (48.00%), 49 (49.00%) and 51 (51.00%) respectively belonged to miR-106a-5p-low, miR-106a-5p-high, RUNX1-low and RUNX1-high groups. Regarding to the combined expression, there were 15 (15.00%), 36 (36.00%), 33 (33.00%) and 16 (16.00%) osteosarcoma patients with high/high, low/high, high/low and low/low of miR-106a-5p and RUNX1 expression.

Table 1 summarized the associations of miR-106a-5p and/or RUNX1 expression with various clinicopathological features of osteosarcoma patients. miR-106a-5p-low, RUNX1-high and miR-106a-5p-low/RUNX1-high expression were more frequently occurred in osteosarcoma tissues with advanced Enneking stage and positive metastasis (all $P < 0.05$). However, there were no significant associations of miR-106a-5p expression with patients' age and gender, as well as tumor size (all $P > 0.05$).

3.3. miR-106a-5p downregulation and/or RUNX1 upregulation are independent unfavorable prognostic factors of osteosarcoma patients

Kaplan-Meier analysis showed that the osteosarcoma patients with low miR-106a-5p expression ($P < 0.01$), high RUNX1 expression ($P < 0.01$) and miR-106a-5p-low/RUNX1-high expression ($P < 0.01$) had shorter overall survival than those with the corresponding control clinicopathological characteristics (Fig. 2). In univariate analysis, advanced Enneking stage ($P = 0.01$), positive metastasis ($P = 0.02$), low miR-106a-5p expression ($P < 0.01$), high RUNX1 expression ($P < 0.01$) and miR-106a-5p-low/RUNX1-high expression ($P < 0.01$) were evaluated to correlate closely with poor overall survival (Table 2). No significant associations were found between patient survival and other clinicopathological features, including patients' age and gender, as well as tumor size (all $P > 0.05$, Table 2). Furthermore, the

Table 2
Univariate analysis on the impact of variables on prognosis in osteosarcoma patients.

Variable	Overall survival	
	HR (95% CI)	P
Gender		
Male *vs.* Female	1.22 (0.26–2.61)	0.28
Age (year)		
≤18 *vs.* > 18	1.35 (0.30–2.86)	0.25
Tumor size		
≤5 cm *vs.* > 5 cm	1.58 (0.38–3.32)	0.31
Enneking stage		
I-II *vs.* III-IV	6.02 (1.98–13.66)	**0.01**
Metastasis		
M0 *vs.* M1	5.22 (1.72–11.19)	**0.02**
miR-106a-5p expression		
High *vs.* Low	6.69 (2.02–15.16)	< **0.01**
RUNX1 expression		
High *vs.* Low	7.01 (2.18–16.08)	< **0.01**
miR-106a-5p/RUNX1 expression		
Low/high *vs.* others	8.16 (2.26–18.39)	< **0.01**

Note: "NS" refers to the difference among groups with no statistical significance.

Table 3
Multivariate analysis on the impact of variables on overall survival in osteosarcoma patients.

Variable	Overall survival	
	HR (95% CI)	P
Enneking stage		
I-II *vs.* III-IV	5.66 (2.11–12.18)	**0.02**
Metastasis		
M0 *vs.* M1	3.52 (1.13–8.21)	**0.03**
miR-106a-5p expression		
High *vs.* Low	5.22 (2.03–11.02)	**0.02**
RUNX1 expression		
High *vs.* Low	5.59 (2.08–11.06)	**0.02**
miR-106a-5p/RUNX1 expression		
Low/high *vs.* others	6.03 (2.33–13.11)	**0.01**

parameters that were significant in univariate analysis were further examined in multivariate analysis. The results showed that miR-106a-5p expression and/or RUNX1 expression were identified as independent predictors of overall survival for osteosarcoma patients (all $P < 0.05$, Table 3).

3.4. miR-106a-5p suppresses cell proliferation and invasion of osteosarcoma cells by targeting RUNX1

The significant associations of miR-106a-5p-RUNX1 axis with aggressive progression and poor prognosis in osteosarcoma patients prompted us to investigate its functions and the underlying mechanisms in regulating osteosarcoma cell malignant phenotypes. As shown in Fig. 3, the cell models with the overexpression of miR-106a-5p, as well as the co-transfection of miR-106a-5p-mimics and RUNX1-expression vector were successfully constructed. Functionally, the enforced expression of miR-106a-5p markedly suppressed cell proliferation and cell invasion of both U2OS and MG63 cells (both $P < 0.05$, Figs. 4 and 5). In contrast, the trend of the suppressive role of miR-106a-5p mimics was significantly reversed by the co-transfection with RUNX1-expression vector (both $P < 0.05$, Figs. 4 and 5).

4. Discussion

miRNAs, via regulating its target genes, have become a kind of novel diagnostic and prognostic biomarkers, as well as promising therapeutic targets for human osteosarcoma. In the current study, our data based on a large clinical cohort determined the downregulation of miR-106a-5p in osteosarcoma tissues compared to the adjacent non-cancerous tissues, which was consistent with the previous study of He's group [17]. To investigate its underlying molecular mechanisms acting on osteosarcoma, we identified RUNX1 as the novel target gene of miR-106a-5p in this cancer by both database screening and dual luciferase reporter gene assay. The most important finding of the current study was that low miR-106a-5p, high RUNX1 and miR-106a-5p-low/RUNX1-high expression in osteosarcoma tissues were all significantly associated with advanced Enneking stage, positive metastasis and shorter overall survival. In addition, miR-106a-5p and RUNX1 expression, alone or in combination, were identified as independent prognostic factors for osteosarcoma patients' overall survival, implying the clinical potentials of this miRNA-mRNA axis in this malignancy. To the best of our knowledge, this is the first study to evaluate the prognostic value of miR-106a-5p alone or in combination with its target gene RUNX1 in osteosarcoma.

miR-106a-5p has been reported to either facilitate or suppress cancer development and progression by regulating different target genes. For example, Pan et al. [11] proved that miR-106a-5p inhibited the cell migration and invasion of renal cell carcinoma through directly binding to the 3′-UTR of the PAK5 mRNA and mediated a decrease in the protein expression of PAK5. He et al. [17] revealed that miR-106a-5p suppressed the cell proliferation, migration and invasion of osteosarcoma cells by targeting HMGA2. Zhi et al. [15] performed gain-of-function and loss-of-function studies to demonstrate that miR-106a-5p significantly inhibited astrocytoma cell proliferation and migration, as well as promoted cell apoptosis *in vitro* by targeting FASTK. In contrast, Li et al. [14] indicated that miR-106a-5p was involved in the invasive behavior of glioblastoma cells and by targeting adenomatosis polyposis coli protein and activating Wnt/β-catenin pathway. However, the target genes of miR-106a-5p in osteosarcoma have not been fully clarified.

The RUNX transcription factor family function as significant regulators in controlling cancer progression [21]. Together with RUNX2 and RUNX3, RUNX1 belongs to this family and plays a crucial role in the cell lineage determination, development of normal hematopoiesis and even stem cell proliferation [22]. Accumulating studies have indicated the involvement of RUNX1 in multiple human cancers by activating or suppressing the transcription of essential regulators of growth, differentiation and survival [23]. RUNX1 may function either as an oncogene or a tumor suppressor in different cancers depending on its regulation of downstream genes. For example, Cheng et al. [24] provided evidence regarding the role of RUNX1 as an oncogene through the inhibition of miR-93 in pancreatic cancer. In contrast, Ramsey et al. [25] determined the loss of RUNX1 as driver of lung adenocarcinoma aggression, potentially through deregulation of the E2F1 pathway. Hong et al. [26] revealed that the low RUNX1 expression was associated with poor prognosis in patients with breast cancer. In the current study, we identified a novel interaction of miR-106a-5p and RUNX1 in osteosarcoma. Mechanistically, miR-106a-5p was demonstrated to interact with the 3′UTR binding site of RUNX1 mRNA and negatively regulates it. Following the confirmation of the tumor suppressive role of miR-106a-5p, our findings revealed that the enforced expression of RUNX1 reversed the partial function of miR-106a-5p *in vitro*.

In conclusion, our findings show the dysregulation of miR-106a-5p-RUNX1 axis in human osteosarcoma tissues and suggest its crucial roles in cancer progression and patients' prognosis. More interestingly, miR-106a-5p may function as a tumor suppressor in osteosarcoma cells via regulating its target gene RUNX1.

Author's contributions

Prof. Guobiao Pan conceived and designed the study; Dr. kun Chen performed the experiments and wrote the manuscript; Prof. Guobiao

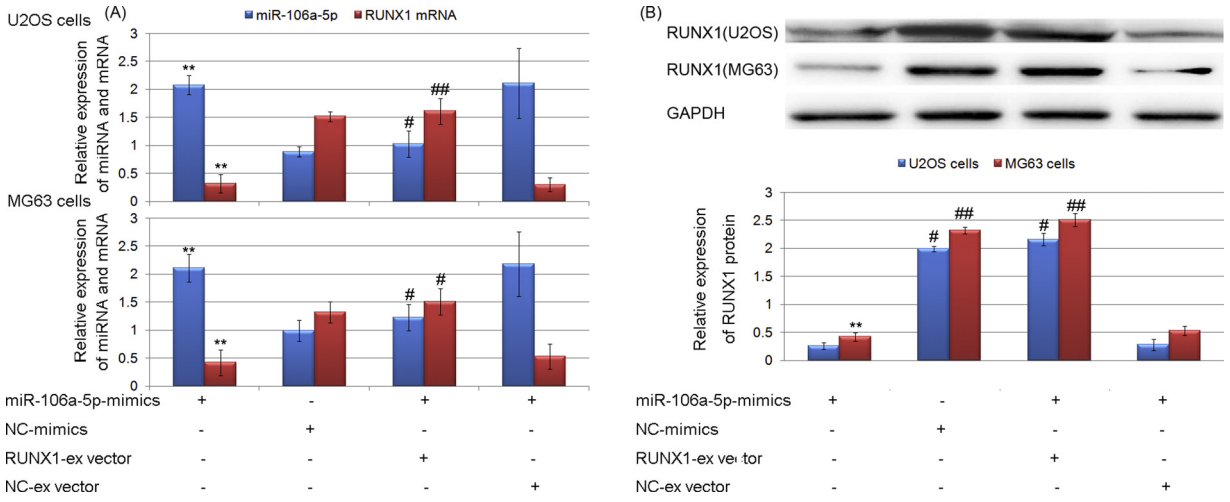

Fig. 3. Relative expression levels of miR-106a-5p and RUNX1 mRNA/protein in osteosarcoma cells (U2OS and MG63) transfected with miR-106a-5p mimics and/or RUNX1 expression vector. (A) Relative expression levels of miR-106a-5p and RUNX1 mRNA in U2OS and MG63 cells transfected with miR-106a-5p mimics and/or RUNX1 expression vector. (B) Relative expression levels of RUNX1 protein in U2OS and MG63 cells transfected with miR-106a-5p mimics and/or RUNX1 expression vector. **P < 0.01, compared to group transfected with NC mimics. #P < 0.05, ##P < 0.01, compared to group co-transfected with miR-106a-5p-mimics and NC expression vector.

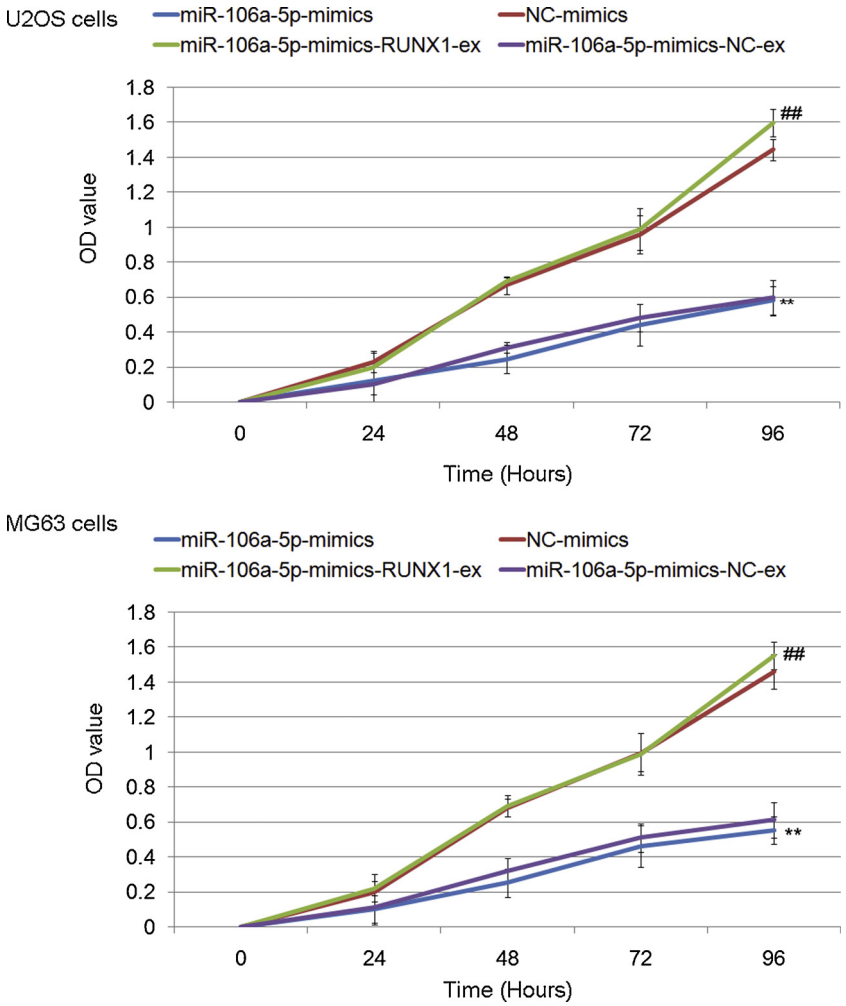

Fig. 4. Roles of miR-106a-5p-RUNX1 axis in regulating cell proliferation of osteosarcoma cells (U2OS and MG63) transfected with miR-106a-5p mimics and/or RUNX1 expression vector. **P < 0.01, compared to group transfected with NC mimics. #P < 0.05, ##P < 0.01, compared to group co-transfected with miR-106a-5p-mimics and NC expression vector.

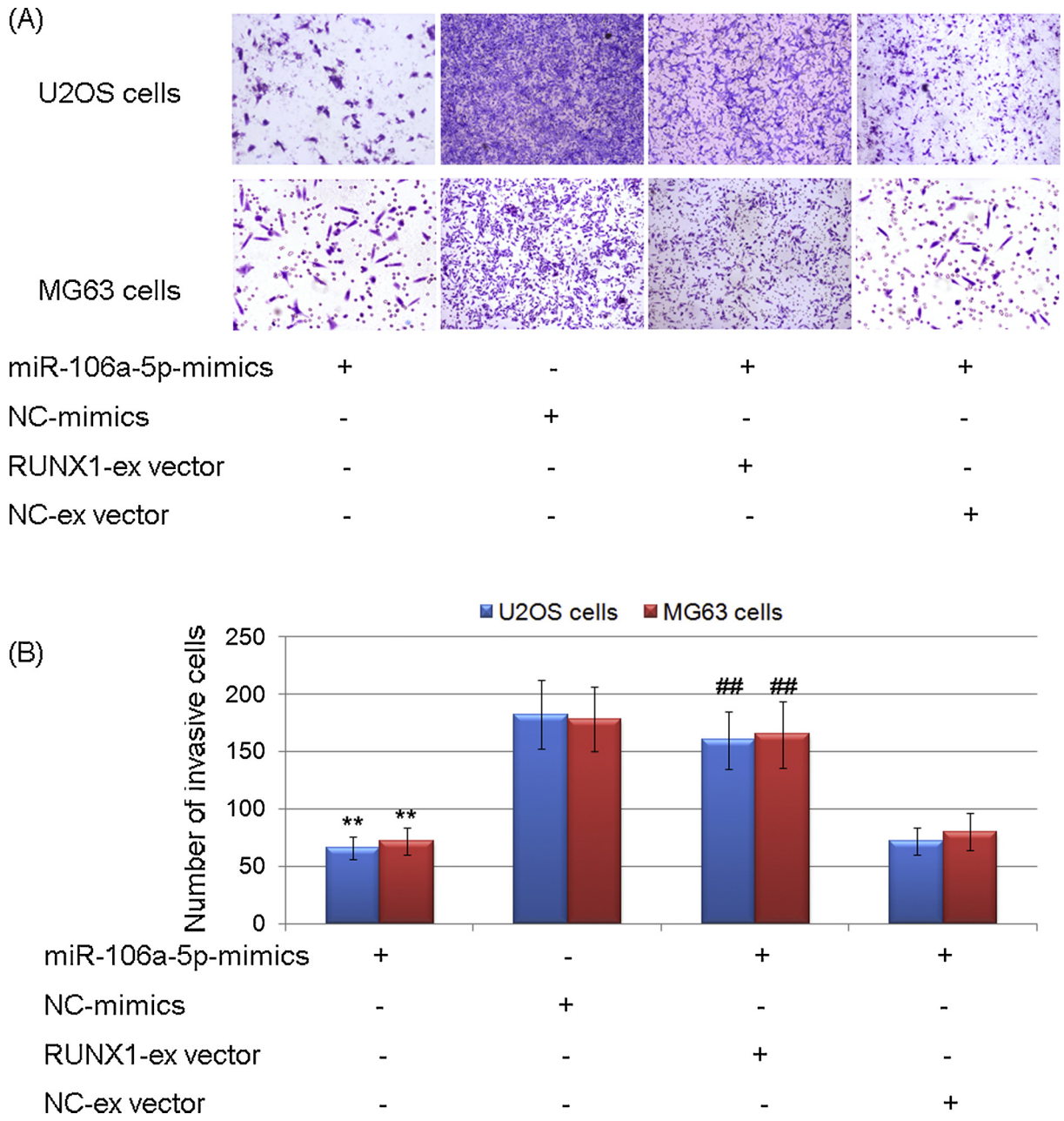

Fig. 5. Roles of miR-106a-5p-RUNX1 axis in regulating cell invasion of osteosarcoma cells (U2OS and MG63) transfected with miR-106a-5p mimics and/or RUNX1 expression vector. (A) Representative images of transwell assay in different groups based on both U2OS and MG63 cells. The magnification was 10. (B) The number of invasive cells in different groups based on both U2OS and MG63 cells. $^{**}P < 0.01$, compared to group transfected with NC mimics. $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, compared to group co-transfected with miR-106a-5p-mimics and NC expression vector.

Pan analyzed and interpreted the data. All the authors approved the final version of the manuscript.

Declaration of Competing Interest

The authors declare that there are no conflicts of interest.

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