Original Article

MiR-490-3p promotes cell apoptosis and cell-cycle arrest in osteosarcoma via the modulation of CDCA8/ATF3 by targeting NUSAP1

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Background: *Micro RNA-490-3p* (*miR-490-3p*) is associated with a variety of malignancies. However, the role of *miR-490-3p* in osteosarcoma and its underlying mechanism are not yet fully understood. This study aimed to explore the role and the mechanism of *miR-490-3p* in osteosarcoma.

Methods: *MiR-490-3p* and nucleolar and spindle-associated protein 1 (NUSAP1) expression in osteosarcoma was detected using real-time quantitative polymerase chain reaction (RT-qPCR). Cell Counting Kit-8 (CCK-8), wound-healing, and transwell assays were used to detect cell proliferation, migration and invasion. Terminal deoxynucleotidyl transferase dUTP nick end labelling (TUNEL) and western blot were used to evaluate the cell apoptosis. Flow cytometry was used to assess the cell cycle. In addition, luciferase reporter assay was used to confirm the binding of *miR-490-3p* and NUSAP1. Western blot and RT-qPCR was used to examine cell division cycle associated 8 (CDCA8) and activating transcription factor 3 (ATF3) expression.

Results: *MiR-490-3p* expression was significantly decreased in the osteosarcoma cells. Following the transfection with *miR-490-3p* mimic, it was found that 143B cell proliferation, migration, and invasion were inhibited, while the cell apoptotic levels and cell-cycle arrest were promoted, accompanied with decreased B cell lymphoma protein-2 (Bcl-2) protein expression, and increased protein expressions of Bcl-2-associated X (Bax), cleaved caspase-3, and cleaved caspase-9. In addition, *miR-490-3p* was found to bind to NUSAP1, and negatively regulate NUSAP1 expression. NUSAP1 upregulation reversed the inhibitory effects of *miR-490-3p* overexpression on cell proliferation, migration, and invasion, and the promoting effects on cell apoptosis and cell-cycle arrest in osteosarcoma. Moreover, *miR-490-3p* was identified to mediate CDCA8/ATF3 by targeting NUSAP1.

Conclusions: *MiR-490-3p* upregulation inhibited cell proliferation and metastasis but promoted the cell apoptosis and cell-cycle arrest in osteosarcoma via the regulation of CDCA8/ATF3 by targeting NUSAP1. Thus, *miR-490-3p* might be a potential therapeutic target for the treatment of osteosarcoma

Keywords: *Micro RNA-490-3p* (*miR-490-3p*); nucleolar and spindle-associated protein 1 (NUSAP1); osteosarcoma; apoptosis; cell-cycle arrest

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Introduction

Osteosarcoma is the most common bone sarcoma in children and adolescents (1). Patients with osteosarcoma are prone to lung metastasis and death (2). Currently, the standard treatment is systemic chemotherapy combined with surgical resection (3). A previous study reported that advancements in limb salvage surgery and neoadjuvant chemotherapy result in an improved 5-year survival rate (of around 65–70%) in patients without metastasis (4). However, distant metastasis is found in about 20% of patients, which dramatically decreases the 5-year survival rate (to 15–30%) (5). The recurrent and metastatic osteosarcoma poses great challenges for cancer treatment (6). Thus, potential therapeutic targets for osteosarcoma improvement should be urgently identified and investigated.

MicroRNAs (miRNAs or miRs) regulate gene expression by mediating messenger RNA (mRNA) cleavage, translational repression, and destabilization (7). It is reported that miRNAs play pivotal roles in a variety of physiological processes, including cell proliferation, metastasis, apoptosis, and the cell cycle (8). In addition, miRNAs can form a complex combinatorial network to modulate tumor suppressor genes and oncogenes (9). *MiR-490-3p* has been shown to act as a tumor suppressor in colorectal cancer, breast cancer, and esophageal squamous cell carcinoma (10-12). In osteosarcoma tissues and cells, *miR-490-3p* expression is decreased compared with the neighboring normal tissues and hFOB1.19 cells (13). It is also reported that the reduction in *miR-490-3p* expression in osteosarcoma is associated with the development of distant metastasis (14). Besides, Dai *et al.* have evidenced that the overexpression of *miR-490-3p* can decrease the numbers of cell clones, migration and mobility in osteosarcoma cells (15). However, the role of *miR-490-3p* in osteosarcoma still needs further exploration.

According to ENCORI database (https://rnasysu.com/encori/), miR-490-3p can target nucleolar and spindle-associated protein 1 (NUSAP1). Combined with above findings, we speculated that *miR-490-3p* might participate in the proliferation, migration, invasion, apoptosis, and cell cycle in osteosarcoma cells via the interaction with NUSAP1. Our findings might provide novel insights into the development of therapeutic targets for osteosarcoma. We present this article in accordance with the MDAR reporting checklist (available at https://tp.amegroups.com/article/view/10.21037/tp-2024-529/rc).

Highlight box

Key findings

- *Micro RNA-490-3p* (*miR-490-3p*) might be a potential therapeutic target for the treatment of osteosarcoma.

What is known and what is new?

- *MiR-490-3p* overexpression inhibits the migration and invasion of osteosarcoma cells while inducing cell apoptosis and cell-cycle arrest.
- *MiR-490-3p* mediates cell division cycle associated 8 (CDCA8)/activating transcription factor 3 (ATF3) by targeting nucleolar and spindle-associated protein 1 (NUSAP1), and NUSAP1 overexpression reverses the effects of *miR-490-3p* overexpression on the malignant development of osteosarcoma cells.

What is the implication, and what should change now?

- *MiR-490-3p* might exert its anti-tumor property in osteosarcoma via the modulation of CDCA8/ATF3 by targeting NUSAP1.

Methods

Cell culture

Human osteosarcoma cell lines MG63, U2OS, and 143B were obtained from Cellverse Bioscience Technology Co., Ltd. (Shanghai, China), and human osteoblast cells hFOB1.19 were obtained from Ningbo Mingzhou Biotechnology Co., Ltd. (Ningbo, China). The cells were incubated in Dulbecco's Modified Eagle Medium (DMEM; Gibco; Thermo Fisher Scientific, Inc., Shanghai, China) supplemented with 10% fetal bovine serum (FBS; Gibco; Thermo Fisher Scientific, Inc.) at 37 ℃ in a humidified atmosphere containing 5% CO_2.

Cell transfection

MiR-490-3p mimic and the negative control (mimic-NC) were obtained from GenePharma Co., Ltd. (Shanghai, China). NUSAP1-specific pcDNA overexpression vector (Over-NUSAP1) and the empty vector (Over-NC) were obtained from Shanghai Integrated Biotech Solutions (Shanghai, China). Lipofectamine 2000 reagent (Invitrogen, Thermo Fisher Scientific, Inc.) was used to transfect these recombinants to the 143B cells according to the manufacturers' instructions. After 48 h, the 143B cells were collected for following experiments.

Cell Counting Kit-8 (CCK-8) assays

The 143B cells were inoculated into 96-well plates and then

incubated in DMEM (Gibco; Thermo Fisher Scientific, Inc.) with 10% FBS (Gibco; Thermo Fisher Scientific, Inc.) for 24, 48, and 72 h. CCK-8 solution (10 µL; Beyotime Institute of Biotechnology; Shanghai, China) was added to each well and the cells were incubated for an additional 2 h. A microplate reader (Thermo Fisher Scientific, Inc.) was used to detect the optical density at 450 nm.

Real-time quantitative polymerase chain reaction (RT-qPCR)

The RNA was extracted from the 143B cells using TRIzol reagent (Thermo Fisher Scientific, Inc.) and the quality was detected using a NanoDrop 3000 spectrophotometer according to the manufacturer's instructions. The total RNA was reverse transcribed into complementary DNA (cDNA) using a PrimeScript RT Master Mix (Takara Biotechnology Co., Ltd.) according to the manufacturer's instructions. The cDNA was amplified by qPCR using a SYBR green PCR Master Mix kit Takara, Toyobo, Japan). Relative gene expression level was assessed using the $2^{[-\text{Delta Delta C(T)}]}$ ($2^{-\Delta\Delta Ct}$) method (16). The primer sequences for qPCR were as follows: miR-490-3p (forward): 5'-CGGCGGTCAACCTGGAGGACTCC-3'; miR-490-3p (reverse): 5'-CCAGTGCAGGGTCC GAGGTAT-3'; U6 (forward): 5'-CTCGCTTCGGC AGCAGCACATATA-3'; U6 (reverse): 5'-AAATATGGAACGCTTCACGA-3'; NUSAP1 (forward): 5'-CTGACCAAGACTCCAGCCAGAA-3'; NUSAP1 (reverse): 5'-GAGTCTGCGTT GCCTCAGTTGT-3'; β-actin (forward): 5'-ACAGAGCC TCGCCTTTGC-3'; β-actin (reverse): 5'-AGGGTGA GGATGCCTCTCTT-3'.

Wound-healing assays

The 143B cells were inoculated into 6-well plates and incubated until 80–90% cell confluence was reached. The wounds were made in the cell monolayers using a white pipette tip. The cells were then incubated in serum-free medium for 24 h at 37 ℃. The wounds were observed using a light microscope (Olympus Corporation, Tokyo, Japan) at 0 and 24 h.

Transwell assays

The transwell chambers were pre-treated with 0.1 mL of Matrigel (Solarbio Technology Co., Ltd., Beijing, China). After different treatments, the harvested cells were suspended in serum-free DMEM. Subsequently, the 143B cells were added to the upper chamber of the transwell plates while medium with 10% serum was added to the lower chamber. After 48 h, the bottom of the chamber insert was subjected to methanol and 0.1% crystal violet staining (Beyotime Institute of Biotechnology, Shanghai, China). The invaded cells in five fields (selected at random) were calculated using a light microscope (Olympus Corporation, Tokyo, Japan).

Transferase dUTP nick end labelling (TUNEL) assays

The 143B cells were fixed with 4% paraformaldehyde and incubated with proteinase K according to the manufacturer's instructions. Then, the 143B cells were incubated with TUNEL working fluid (Beyotime Institute of Biotechnology, Shanghai, China) for 1 h at 37 ℃ in the dark according to the manufacturer's instructions, and then stained by 1 µg/mL 4',6-diamidino-2-phenylindole (DAPI; Beyotime Institute of Biotechnology). Finally, the labeled cells were visualized by fluorescence microscopy (Thermo Fisher Scientific, Inc.).

Flow cytometry

Briefly, the collected cells were inoculated in 24-well plates (3×10^5 cells/well) and incubated for 24 h. The cells were then centrifuged and the supernatant was discarded. After the fixation with 70% ethanol, the cells were exposed to 50 µL of ribonuclease A and 200 µL of propidium iodide (PI; Sigma-Aldrich, Shanghai, China), and incubated in the dark at room temperature for 20 min. FACSCaliber Flow Cytometer (BD Biosciences, USA) and Image J software (National Institute of Health, Bethesda, USA) were used to analyze cell-cycle distribution.

Luciferase reporter assays

The NUSAP1 wide type (WT) and the mutant type (MUT) reporter plasmids with *miR-490-3p* mimic or mimic-negative control (NC) binding sites were provided by GenePharm (Shanghai, China). The luciferase reporter vector and *miR-490-3p* or NC were transfected to the 143B cells. The dual luciferase reporter assay system (Promega Corporation, Beijing, China) was then used to examine luciferase activity.

Western blot

The proteins were extracted from the 143B cells using radioimmunoprecipitation (RIPA; Solarbio Technology Co., Ltd., Beijing, China) lysis buffer and the protein concentration was quantified using a bicinchoninic acid (BCA) protein assay kit (Thermo Fisher Scientific Inc.). Separated by 10% polyacrylamide gel electrophoresis, the proteins were transferred to polyvinylidene fluoride (PVDF; Thermo Fisher Scientific Inc.) membranes. The membranes were sealed with 10% non-fat milk, and then incubated with primary antibodies: Bcl-2 (cat. no. 12789-1-AP; 1:2,000; Proteintech, Wuhan, China), Bax (cat. no. 50599-2-Ig; 1:5,000; Proteintech), cleaved caspase-3 (cat. no. ABS132005; 1:500; Absin; Shanghai, China), cleaved caspase-9 (cat. no. ab202068; 1:2,000; Abcam, Shanghai, China), cell division cycle associated 8 (CDCA8) (cat. no. 12465-1-AP; 1:600; Proteintech), activating transcription factor 3 (ATF3) (cat. no. ab254268; 1:1,000; Abcam), NUSAP1 (cat. no. 12024-1-AP; 1:10,000; Proteintech), and β-actin (cat. no. GB11001; 1:2,000; Serivicebio, Wuhan, China) overnight at 4 ℃. On the next day, the membranes were then incubated with horseradish peroxidase-labeled secondary antibody (cat. no. BL003A; 1:4,000; Biosharp, Anhui, China). The protein bands were visualized using an enhanced chemiluminescence (ECL) detection system (Yeasen Biotech, Shanghai, China), and protein density was analyzed using ImageJ software (version 1.49; National Institute of Health).

Statistical analysis

All the experiments were repeated three times. The data were analyzed using GraphPad Prism software (version 5.0; GraphPad Software, Inc.; USA) and expressed as the mean ± standard deviation. One-way analysis of variance followed by the Bonferroni post-hoc test was used for multiple group comparisons, while the unpaired *t*-test was used for comparisons between two groups. $P<0.05$ indicated statistically significant.

Results

MiR-490-3p overexpression inhibits the migration and invasion of osteosarcoma cells

To investigate the role of *miR-490-3p* in osteosarcoma, *miR-490-3p* expression in osteosarcoma cells, and hFOB1.19 cells, was initially examined by RT-qPCR. As *Figure 1A* shows, *miR-490-3p* mRNA expression was significantly reduced in the osteosarcoma cells compared to the hFOB1.19 cells. Notably, the 143B cells had the lowest *miR-490-3p* expression; thus, we chose 143B cells for the subsequent studies. To investigate the effects of *miR-490-3p* overexpression on the proliferation of the 143B cells, *miR-490-3p* mimic was transfected into the 143B cells, and RT-qPCR was used to examine transfection efficiency (*Figure 1B*). The proliferation of the 143B cells was significantly decreased by *miR-490-3p* overexpression (*Figure 1C*). Additionally, *miR-490-3p* overexpression significantly decreased the migration and invasion of the 143B cells (*Figure 1D-1G*).

MiR-490-3p overexpression promoted the apoptosis and cell-cycle arrest of osteosarcoma cells

MiR-490-3p overexpression significantly increased the cell apoptosis rate in *miR-490-3p* mimic group compared with that of the mimic NC group (*Figure 2A,2B*). Similarly, *miR-490-3p* upregulation decreased Bcl-2 expression, but increased Bax, cleaved caspase-3 and cleaved caspase-9 expression in the 143B cells (*Figure 2C*). Moreover, as *Figure 2D,2E* show, *miR-490-3p* overexpression significantly increased the cell population in the gap 0/gap 1 (G0/G1) phase but decreased the cell population in the synthesis (S) phase.

MiR-490-3p binds to NUSAP1

The relationship between *miR-490-3p* and NUSAP1 in osteosarcoma was further investigated. As *Figure 3A* shows, *miR-490-3p* binds to NUSAP1. The luciferase reporter assay showed that *miR-490-3p* mimic significantly decreased the luciferase activity of NUSAP1-WT, but had no obvious effects on that of the mutant NUSAP1 reporter (*Figure 3B*). Additionally, *miR-490-3p* overexpression significantly decreased NUSAP1 expression in the 143B cells (*Figure 3C*).

NUSAP1 overexpression reverses the effects of miR-490-3p on the proliferation, migration and invasion of the 143B cells

To investigate the biological role of NUSAP1 in *miR-490-3p*-mediated 143B cells, NUSAP1 was overexpressed, and the transfection efficiency was examined (*Figure 4A*). As *Figure 4B* shows, NUSAP1 overexpression significantly

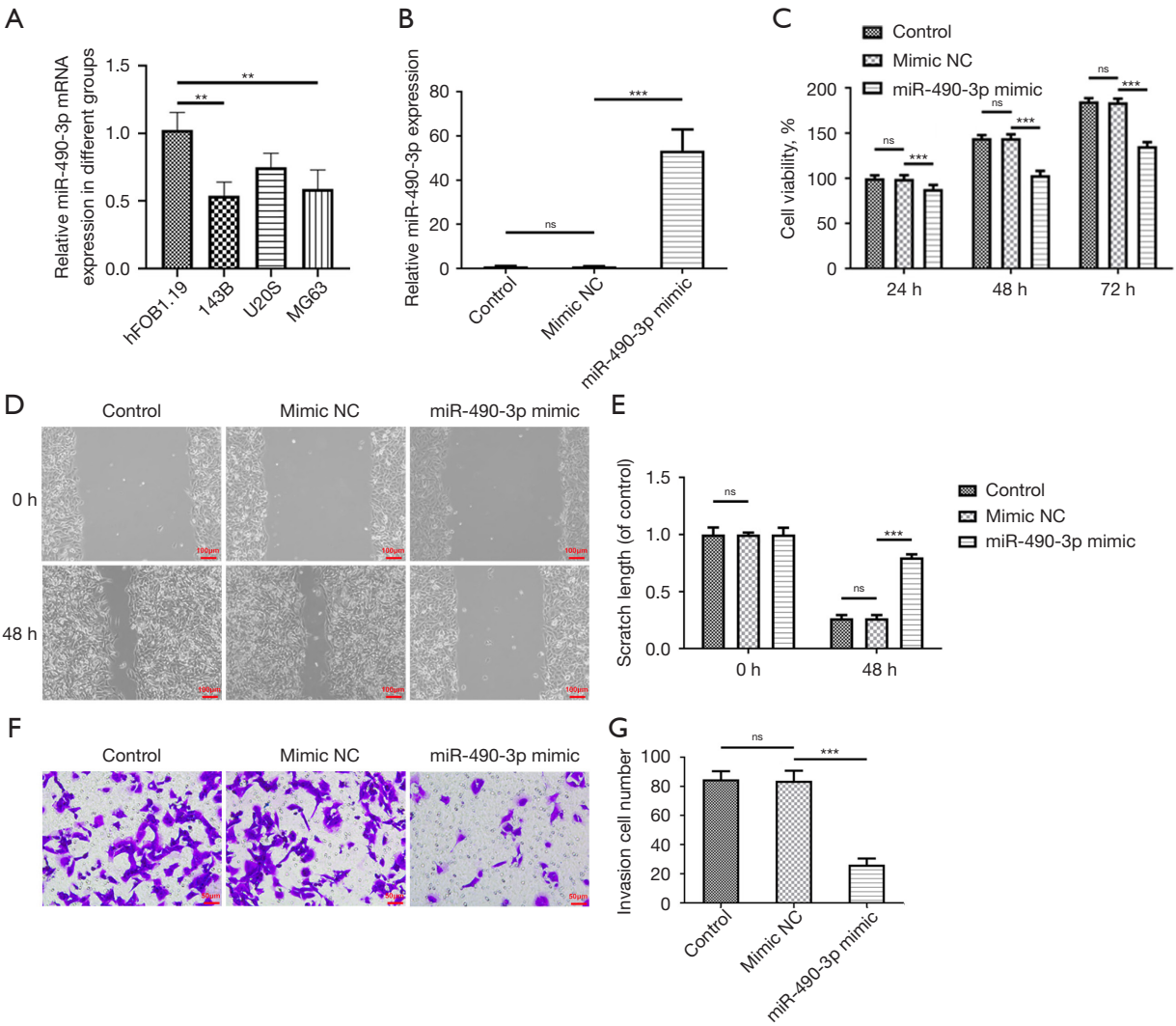

Figure 1 *MiR-490-3p* overexpression decreases the proliferation, migration and invasion of osteosarcoma cells. (A) RT-qPCR was used to analyze *miR-490-3p* expression in osteosarcoma and hFOB1.19 cell lines; (B) RT-qPCR was used to analyze *miR-490-3p* mRNA expression; (C) CCK-8 assays were used to analyze cell proliferation; (D,E) wound-healing assays were used to analyze cell migration. The cells were observed using a light microscope. (F,G) transwell assays were used to analyze cell invasion. The cells were observed using a light microscope. Cells were exposed to 0.1% crystal violet staining. **, P<0.01; ***, P<0.001. *miR-490-3p*, *micro RNA-490-3p*; RT-qPCR, real-time quantitative polymerase chain reaction; CCK-8, Cell Counting Kit-8; mRNA, messenger RNA; NC, negative control; ns, no significance.

increased the proliferation of the 143B cells. Moreover, the cell migration and invasion in *miR-490-3p* mimic + Over-NC group were found to be reversed by NUSAP1 overexpression (*Figure 4C-4F*).

NUSAP1 overexpression reverses the effects of miR-490-3p on apoptosis and cell-cycle arrest in 143B cells

We then examined whether NUSAP1 overexpression affected the cell apoptosis rate and cell-cycle arrest in *miR-490-3p*-mediated 143B cells. As *Figure 5A*,*5B* show, the cell apoptosis rate was significantly reduced by NUSAP1 overexpression. Additionally, NUSAP1 overexpression significantly reduced the cell population in the G0/G1 phase but increased the cell population in the G2 phase (*Figure 5C*,*5D*). Moreover, NUSAP1 upregulation increased Bcl-2 expression but decreased Bax, cleaved caspase-3, and cleaved caspase-9 expression in *miR-490-3p*-overexpressed

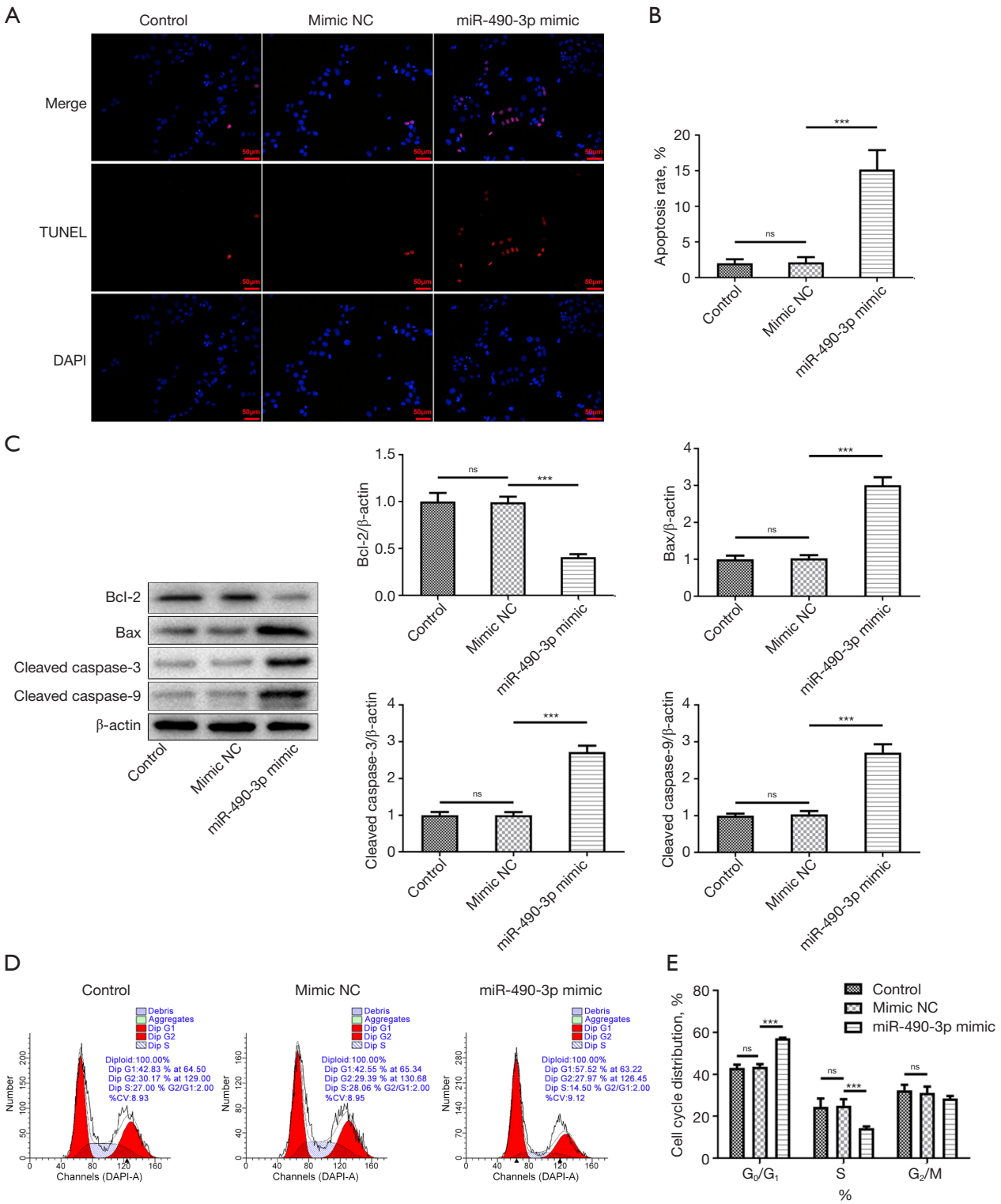

Figure 2 *MiR-490-3p* overexpression facilitates the cell apoptotic level and cycle arrest in osteosarcoma. (A,B) TUNEL was used to assess cell apoptosis and DAPI was used for staining; (C) Western blot was used to examine the proteins associated with apoptosis; (D,E) flow cytometry was used to examine the cell cycle. ***, P<0.001. *miR-490-3p*, *micro RNA-490-3p*; TUNEL, transferase dUTP nick end labelling; NC, negative control; DAPI, 4',6-diamidino-2-phenylindole; ns, no significance.

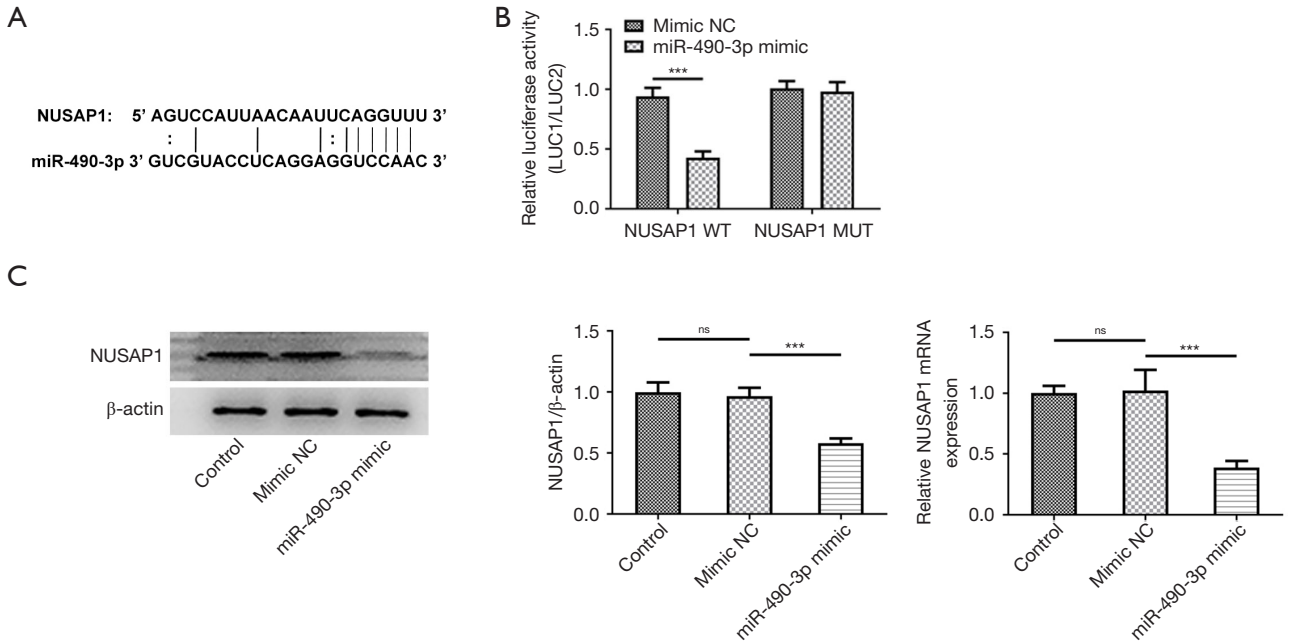

Figure 3 *MiR-490-3p* binds to NUSAP1. (A) The binding of *miR-490-3p* with NUSAP1; (B) luciferase reporter assays were used to confirm the luciferase activity; (C) Western blot and RT-qPCR were used to analyze NUSAP1 mRNA and protein expression. ***, P<0.001. *miR-490-3p*, *micro RNA-490-3p*; RT-qPCR, real-time quantitative polymerase chain reaction; mRNA, messenger RNA; NC, negative control; ns, no significance.

143B cells (*Figure 5E*).

MiR-490-3p mediates CDCA8/ATF3 by targeting NUSAP1

As *Figure 6A* shows, NUSAP1 binds to CDCA8. The western blot results showed that *miR-490-3p* overexpression significantly decreased CDCA8 and ATF3 protein expression in the 143B cells in *miR-490-3p* mimic group compared with the mimic NC group, which was subsequently increased by NUSAP1 overexpression. The results indicated that *miR-490-3p* modulated 143B cell advancement via the modulation of CDCA8/ATF3 by targeting NUSAP1 (*Figure 6B*).

Discussion

Osteosarcoma cells are characterized by high drug resistance and metastasis, which are clinically considered as the leading cause of recurrence and distant metastasis in patients with osteosarcoma (17). Therefore, the identification of biomarkers in osteosarcoma cells is of great importance for diagnosis and the development of individualized treatment strategies (18). The results of the present study revealed that *miR-490-3p* overexpression inhibited the proliferation, migration, and invasion of osteosarcoma cells, but facilitated cell apoptosis and cell-cycle arrest through the modulation of CDCA8/ATF3 by targeting NUSAP1.

MiR-490-3p is aberrantly expressed in various cancers and has close relation with tumor development (19-21). In colon cancer, *miR-490-3p* alleviates the Warburg effect and inhibits cell proliferation (22). It is also reported that *miR-490-3p* expression is reduced in hepatocellular carcinoma and *miR-490-3p* overexpression can inhibit cell proliferation, invasion and metastasis (23). Intriguingly, Ma *et al.* identified osteosarcoma-related miRNAs by analyzing microarray datasets, and found that *miR-490-3p* expression was downregulated in osteosarcoma (24). Moreover, research has also shown that *miR-490-3p* expression is reduced in osteosarcoma cell lines, and *miR-490-3p* upregulation can inhibit osteosarcoma cell proliferation, induce G1 arrest, and facilitate cell apoptosis (25). Consistent with previous study, our data revealed that *miR-490-3p* was lowly expressed in osteosarcoma, and *miR-490-3p* upregulation could inhibit cell proliferation, migration, and invasion, while promoting the cell apoptotic level and cell-cycle arrest in osteosarcoma.

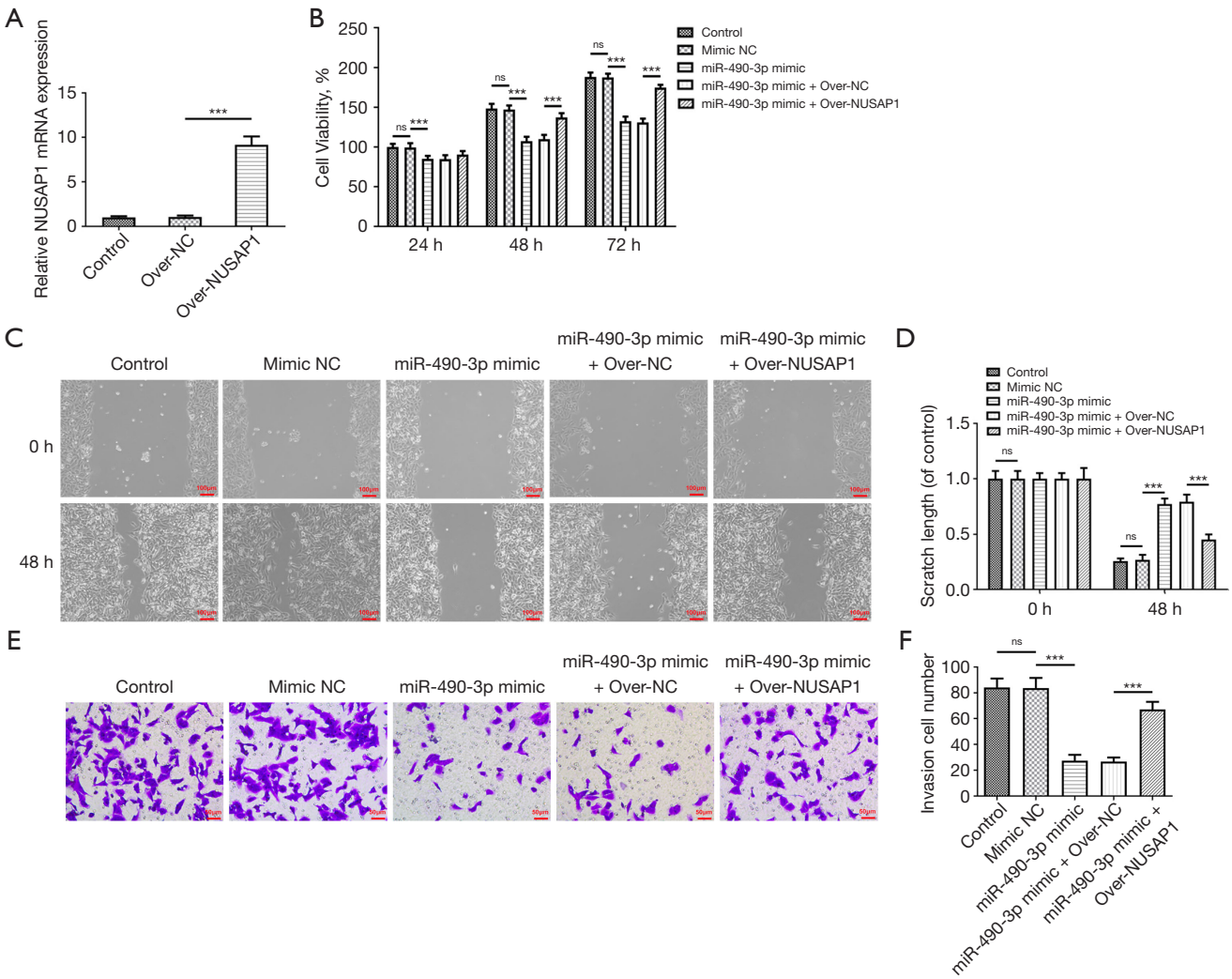

Figure 4 NUSAP1 overexpression reverses the effects of *miR-490-3p* on the proliferation, migration and invasion of osteosarcoma cells. (A) RT-qPCR was used to analyze NUSAP1 mRNA expression in 143B cells transfected with Over-NUSAP1; (B) CCK-8 assays were used to analyze cell proliferation; (C,D) wound-healing assays were used to analyze cell migration. The cells were observed using a light microscope. (E,F) transwell assays were used to analyze cell invasion. The cells were observed using a light microscope. Cells were exposed to 0.1% crystal violet staining; ***, P<0.001. *miR-490-3p*, *micro RNA-490-3p*; RT-qPCR, real-time quantitative polymerase chain reaction; CCK-8, Cell Counting Kit-8; mRNA, messenger RNA; NC, negative control; ns, no significance.

The Bcl-2 family proteins, Bcl-2 and Bax, play a crucial role in regulating apoptosis (26). Results of western blot showed that *miR-490-3p* overexpression decreased anti-apoptotic protein Bcl-2 expression while increasing the expressions of pro-apoptotic protein Bax and apoptosis-related protein cleaved caspase-3 and cleaved caspase-9, which further validated the promoting role of *miR-490-3p* overexpression in osteosarcoma cell apoptosis.

NUSAP1, an essential mitotic modulator, participates in spindle formation and stability, cytokinesis, and spindle assembly (27). NUSAP1 has been reported to be aberrantly expressed in several malignancies, including gastric cancer, hepatocellular carcinoma, and renal cell carcinoma (28-30). Zhang *et al.* revealed that hypoxia promotes the invasion of osteosarcoma cells through NUSAP1 elevation, and NUSAP1 depletion inhibits the cell migration and invasion (31). Additionally, in our previous study, we showed that NUSAP1 accelerates osteosarcoma cell proliferation and cell cycle progression via upregulating cell division cycle 20 homologue (CDC20) and Cyclin A2 (32).

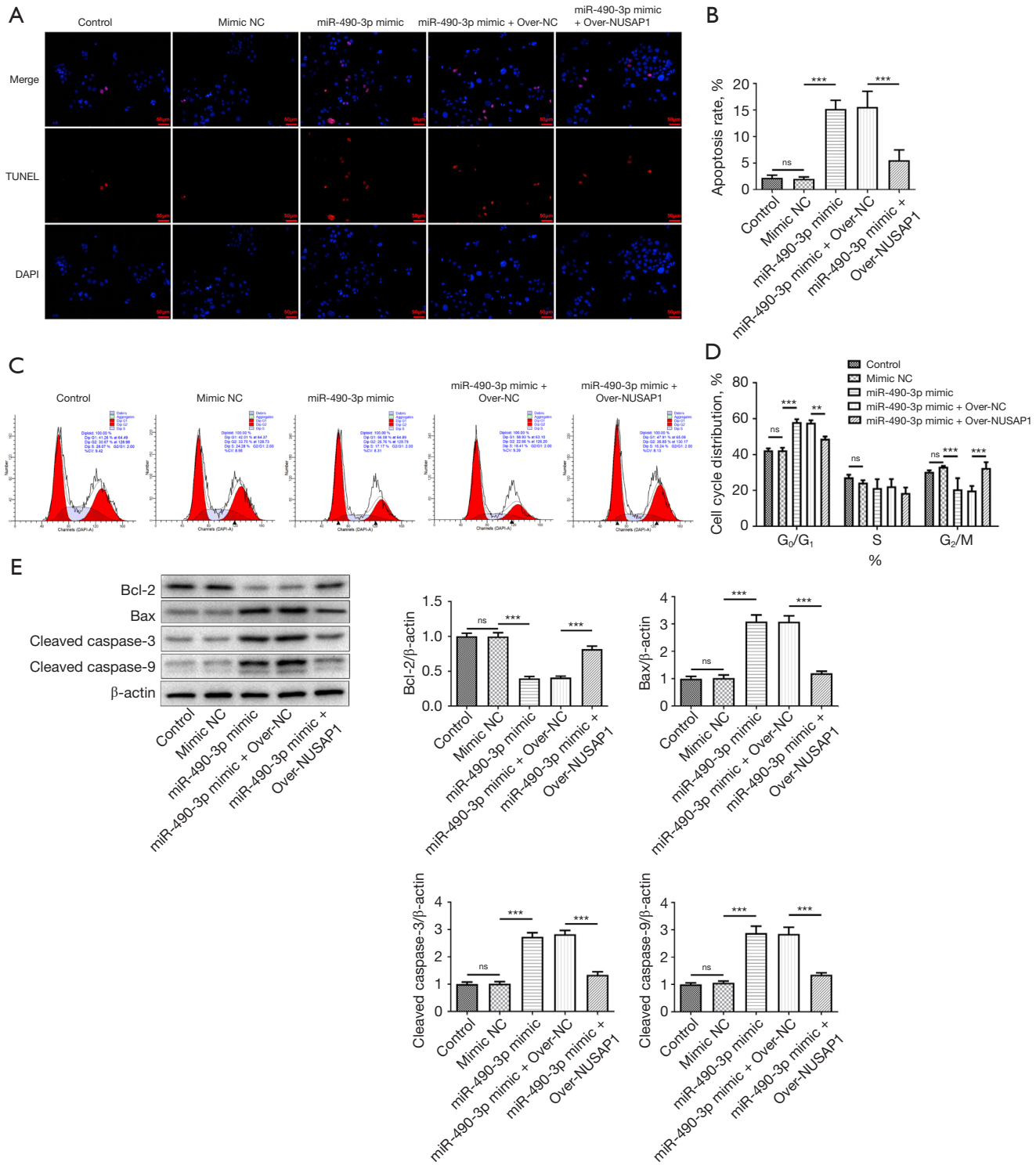

Figure 5 NUSAP1 overexpression reverses the effects of *miR-490-3p* on 143B cell apoptosis and cell-cycle arrest. (A,B) TUNEL was used to examine cell apoptosis and DAPI was used for staining; (C,D) flow cytometry was used to examine cell cycle; (E) Western blot was used to examine apoptosis-related proteins. **, P<0.01, ***, P<0.001. *miR-490-3p*, *micro RNA-490-3p*; TUNEL, transferase dUTP nick end labelling; NC, negative control; DAPI, 4',6-diamidino-2-phenylindole; ns, no significance.

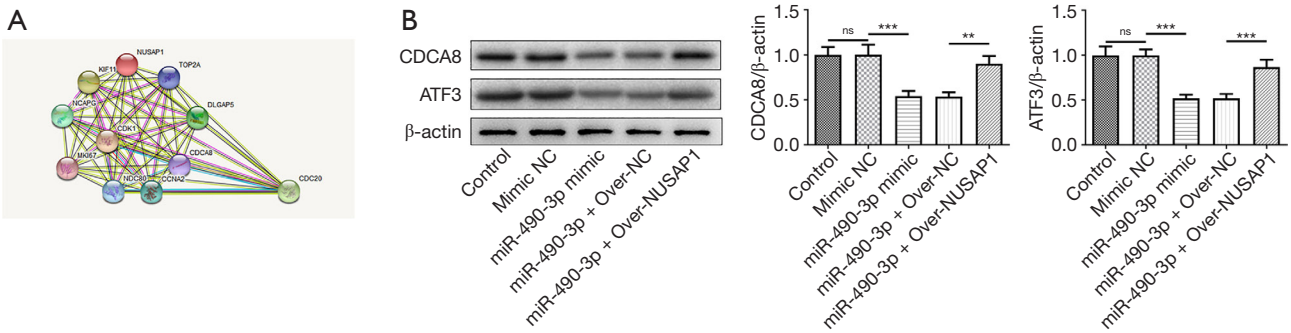

Figure 6 *MiR-490-3p* mediates CDCA8/ATF3 by targeting NUSAP1. (A) According to the STRING database, NUSAP1 binds to CDCA8; (B) western blot was used to detect the protein expression of the CDCA8 and ATF3. **, P<0.01; ***, P<0.001. *miR-490-3p*, *micro RNA-490-3p*; STRING, Search Tool for the Retrieval of Interacting Genes/Proteins; NC, negative control; ns, no significance.

Through bioinformatics analysis, it was found that *miR-490-3p* can bind to NUSAP1. Our data also showed that *miR-490-3p* overexpression could reduce NUSAP1 activity and negatively regulate NUSAP1 protein expression. To further explore the reaction mechanism of *miR-490-3p* associated with NUSAP1 in malignant osteosarcoma cell phenotypes, *miR-490-3p* mimic and Over-NUSAP1 were co-transfected into 143B cells and above functional experiments were conducted again. The results showed that NUSAP1 overexpression reversed the inhibitory effects of *miR-490-3p* overexpression on the proliferation, migration, and invasion and the promoting effects on the apoptosis and cell-cycle arrest in 143B cells, indicating that *miR-490-3p* upregulation might exert its anti-tumor property in osteosarcoma via the interaction with NUSAP1.

Disrupted cell-cycle regulation is a typical characteristic of tumor advancement (33). Numerous studies have shown that CDCA8 expression is increased in various tumors, and is necessary for tumor cell growth and progression (34,35). Jeon *et al.* showed that targeting CDCA8 inhibits hepatocellular carcinoma via the restoration of the ATF tumor suppressor (36). Li *et al* revealed that CDCA8 expression is highly expressed in osteosarcoma tissues and CDCA8 significantly promotes the proliferation of osteosarcoma cells *in vitro* (37). Of note, according to the Search Tool for the Retrieval of Interacting Genes/Proteins (STRING) database (https://ngdc.cncb.ac.cn/databasecommons/database/id/62), NUSAP1 can bind to CDCA8. Our data found that the decreased expression of CDCA8 and ATF3 in the *miR-490-3p*-overexpressed 143B cells was increased by NUSAP1 overexpression, which suggests that *miR-490-3p* mediates CDCA8/ATF3 by targeting NUSAP1.

Conclusions

This study showed that *miR-490-3p* upregulation suppressed cell proliferation, migration, and invasion in osteosarcoma, and promoted cell apoptosis and cycle arrest via the modulation CDCA8/ATF3 by targeting NUSAP1. Our findings revealed the mechanism by which *miR-490-3p* facilitates the progression of osteosarcoma and indicated that *miR-490-3p* could serve as a prospective target for the treatment of osteosarcoma. However, the present study also has some limitations. For instance, we didn't validate our findings in animal models, which will be the research focus of our future studies.

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Footnote

Reporting Checklist: The authors have completed the MDAR reporting checklist. Available at https://tp.amegroups.com/article/view/10.21037/tp-2024-529/rc

Data Sharing Statement: Available at https://tp.amegroups.com/article/view/10.21037/tp-2024-529/dss

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Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://tp.amegroups.com/article/view/10.21037/tp-2024-529/coif). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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