MicroRNA-661 Enhances TRAIL or STS Induced Osteosarcoma Cell Apoptosis by Modulating the Expression of Cytochrome c1

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Key Words
MicroRNA-661 • TRAIL • STS • Osteosarcoma • Cytochrome c1

Abstract
Aim: Osteosarcoma (OS) is an aggressive bone malignancy that affects rapidly growing bones and is associated with a poor prognosis. Our previous study showed that cytochrome c1 (CYC1), a subunit of the cytochrome bc1 complex (complex III) of the mitochondrial electron chain, is overexpressed in human OS tissues and cell lines and its silencing induces apoptosis in vitro and inhibits tumor growth in vivo. Here, we investigated the mechanism underlying the modulation of CYC1 expression in OS and its role in the resistance of OS to apoptosis. Methods: qRT-PCR, luciferase reporter assay, western blotting, flow cytometry, and animal experiments were performed in this study. Results: MicroRNA (miR)-661 was identified as a downregulated miRNA in OS tissues and cells and shown to directly target CYC1. Ectopically expressed miR-661 inhibited OS cell growth, promoted apoptosis, and reduced the activity of mitochondrial complex III. miR-661 overexpression enhanced TRAIL or STS induced apoptosis and promoted the release of cytochrome c into the cytosol, which induced caspase-9 activation, and these effects were abolished by a caspase-3 inhibitor. Overexpression of CYC1 rescued the effects of miR-661 on sensitizing OS cells to TRAIL or STS induced apoptosis, indicating that the antitumor effect of miR-661 is mediated by the downregulation of CYC1. In vivo, miR-661 overexpression sensitized tumors to TRAIL or STS induced apoptosis in a xenograft mouse model, and these effects were attenuated by co-expression of CYC1. Conclusion: Taken together, our results indicate that miR-661 plays a tumor suppressor role in OS mediated by the downregulation of CYC1, suggesting a potential mechanism underlying cell death resistance in OS.
Introduction

Osteosarcoma (OS), which affects rapidly growing bones, is the most common human primary malignant bone tumor among children and adolescents [1]. Despite significant advances in the treatment of OS, including surgery, multi-agent chemotherapy, and radiotherapy, the rate of relapse or distant metastasis remains high, with approximately 80% of patients developing metastatic disease after surgery [2]. The 5-year survival rate of patients with OS is 65%–75% in patients with localized disease and less than 30% in patients with metastatic disease [3], underscoring the need to identify biomarkers for early detection and therapeutic targets for the treatment of patients with OS.

MicroRNAs (miRNAs) are a class of small (22-nucleotide) noncoding RNA molecules that regulate gene expression by binding to the 3′ untranslated region (3′-UTR) of their target mRNAs, modulating mRNA stability and/or translation [4]. A single gene may contain binding sites for different miRNAs, and a single miRNA can regulate many different mRNAs, indicating a complex level of regulation. Deregulation of miRNAs is associated with several diseases including cancer, and tumor-associated miRNAs can function as tumor suppressors or oncogenes depending on whether they target oncogenes or tumor suppressor genes [5, 6]. Several miRNAs have been implicated in the development and progression of OS, including miR-143, miR-133a, and miR-376c. [7-9]. A recent microarray profiling study identified 177 miRNAs differentially expressed in osteosarcoma cell lines compared with normal bone, and the involvement of selected miRNAs was confirmed in clinical samples [10-12]. miR-661 was suggested to be involved in cancer, although its function is controversial. miR-661 was shown to induce epithelial to mesenchymal transition by targeting Nectin-1, a cell-cell adhesion protein, and lipid transferase StarD10 [13]; however, other reports showed that miR-661 inhibits cancer progression and is downregulated in correlation with cell invasiveness, and inhibits glioma cell proliferation and invasion by targeting hTERT [14, 15]. miR-661 targets the tumor suppressor p53 regulators Mdm2 and Mdm4 in breast cancer, and its positive or negative modulation of cancer aggressiveness was suggested to depend on p53 status [16]. However, the role of miR-661 in OS has not been described to date.

Tumor necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL), a member of the TNF family, is a selective apoptosis inducer that has been studied as a target for cancer therapy because of its ability to kill tumor cells without significant cytotoxicity to normal cells [17]. TRAIL binds to the death receptors DR4 or DR5, inducing receptor trimerization and the recruitment of Fas-associated death domain protein (FADD), leading to the activation of caspase cascades and cell death [18, 19]. Staurosporine (STS), a protein kinase inhibitor, is an inducer of apoptosis in many cell types and has been shown to induce apoptosis via both caspase-dependent and -independent mechanisms [20].

Cytochrome C1 (CYC1), a heme-containing subunit of the cytochrome bc1 complex (or complex III) of the mitochondrial electron chain, mediates the transport of electrons from cytochrome B to cytochrome c (Cyctc) during oxidative phosphorylation [21]. The interaction between CYC1 and Cyctc is necessary for complex formation, its correct orientation and distance for electron transfer, and complex dissociation, and the disruption of this interaction results in the loss of oxidative phosphorylation and mitochondrial function [22, 23]. In a previous study from our group, we identified CYC1 as a potential biomarker for the early diagnosis of OS [24]. This was verified in a follow-up study that showed that CYC1 is overexpressed in human OS tissues and cell lines, and its silencing induces apoptosis in vitro and inhibits tumor growth in vivo [25]. We found that CYC1 silencing reduced complex III activity and promoted tumor necrosis factor-related apoptosis inducing ligand (TRAIL)-induced apoptosis via the mitochondrial pathway in OS cells, suggesting that CYC1 plays an important role in OS tumorigenesis. In the present study, we identified miR-661 as a dysregulated miRNA in OS and investigated its role in OS tumorigenesis via the modulation of the expression of its target CYC1 in vitro and in vivo.
Materials and Methods

Clinical specimens
Human OS and matched adjacent normal tissues (n = 30) were obtained from patients at Shanghai Tenth People’s Hospital affiliated with Tongji University. All patients provided written informed consent. The study protocol was approved by the Ethics Committee of Tongji University.

Cell culture and miRNA transfection
The human OS cell line 143B was provided by the Chinese Academy of Medical Sciences. Cells were grown in RPMI-1640 (Invitrogen, Carlsbad, CA, USA) supplemented with 10% fetal bovine serum (Gibco), 100 U/ml penicillin, and 100 μg/ml streptomycin. Cells were incubated at 37°C and 5% CO2 in a humidified incubator.

miRNAs were transfected at a working concentration of 100 nmol/l using Lipofectamine 2000 reagent (Invitrogen). The miR-661 mimic and a nonspecific miR control were purchased from GenePharma (Shanghai, China). Protein and RNA samples were extracted from subconfluent cells during the exponential phase of growth.

RNA isolation and quantitative real-time PCR
Total RNA was extracted using the Trizol reagent (Invitrogen) following the manufacturer’s instructions. cDNA was synthesized using PrimeScript RT reagent Kit with gDNA Eraser (Takara, Japan). Quantitative real-time PCR was performed using a SYBR-Green PCR kit (Takara) on a Step one plus System (Applied Biosystems).

The specific primers used for PCR are shown in Table 1. The relative mRNA expression was normalized to GAPDH. All reactions were performed in triplicate.

Protein extraction and western blot analysis
Protein was extracted from enriched fractions of mitochondria and the cytosol using isotonic mitochondrial buffer (MB: 210 mM mannitol/70 mM sucrose/1 mM EDTA/10 mM Hapes, pH 7.5) as described elsewhere [26]. Protein concentration was determined using a BCA protein assay kit (Keygen, China). Protein samples (50 μg) were separated by SDS-PAGE and transferred to nitrocellulose membranes (Millipore, USA). After incubation in 5% BSA/TBST for 1 h at room temperature to block non-specific binding, membranes were incubated with antibodies against CYC1 (Protein Tech Group, Chicago, IL, USA), PARP, cytochrome c, cytochrome c oxidase subunit 4 (COXIV), and β-actin (all from Santa Cruz Biotechnology, Santa Cruz, CA) in Tris-buffered saline at 4°C overnight. After washing, membranes were incubated with secondary antibody for 1 h at room temperature. Protein bands were visualized with the enhanced chemiluminescence system (Amersham, Arlington Heights, IL, USA). β-actin or COXIV was used as an internal control.

Cell proliferation assay
143B cells transfected with control, Lv-NC, or Lv-CYC1 shRNA were seeded in 96-well plates at 2×103/well and cultured at 37°C and 5% CO2 in a humidified incubator. At specific time points, medium was removed, and cells were incubated with MTT (Sigma, St. Louis, MO, USA) at a final concentration of 5 mg/ml for 4 h. The reaction was stopped by addition of 150 μl DMSO and the optical density at 570 nm was determined on a microplate reader (Molecular Devices, CA, USA).

Cell apoptosis assay
Cell apoptosis was determined using the ApoAlert Annexin V kit (Clontech, Mountain View, CA, USA) following the manufacturer’s instructions. The 143B and MG-63 cells transfected with control, Lv-NC, or Lv-CYC1 shRNA were seeded in six-well plates at a density of 2×104 cells per well and cultured for 24 h. Cells were

Table 1. Primers used for quantitative real-time PCR

<table>
<thead>
<tr>
<th>Gene</th>
<th>Primer</th>
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<tbody>
<tr>
<td>CYC1 (Cytochrome c)</td>
<td>5’-CTCCCTGCTCAGGCGTACT-3’ (forward)</td>
</tr>
<tr>
<td></td>
<td>5’-GGGTGGCCTGCTCAATCTTAAAACTCT-3’ (reverse)</td>
</tr>
<tr>
<td>GAPDH</td>
<td>5’-GCGAAATTCACGGCAAGGAG-3’ (forward)</td>
</tr>
<tr>
<td></td>
<td>5’-GCGCAGTACACTCCAGGAG-3’ (reverse)</td>
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subsequently incubated with TRAIL (1, 10, 50, 100 ng/ml) for 72 h. After treatment was completed, cells were harvested, stained with Annexin V-FITC and propidium iodide (PI), and analyzed on a FACscan flow cytometer (Beckton Dickinson, San Diego, CA).

**Complex III activity**

The 143B and MG-63 cells transfected with control, Lv-NC, or Lv-CYC1 shRNA were seeded in six-well plates at a density of 1 × 10^5 cells per well for 3 days. The activity of Complex III was measured by following the increase in absorbance due to the reduction of cytochrome c at 550 nm using the Complex III activity quantitative assay kit (Genmed, Shanghai, China) following the manufacturer’s instructions.

**Caspase 9 activity**

Caspase 9 activity was measured using a Caspase 9 activity assay kit (Beyotime, China) following the vendor’s protocol. The caspase activity assay is based on the ability of the active enzyme to cleave the chromophore from the caspase-9 substrate Ac-LEHD-pNA to p-nitroanilide (pNA) [27]. The release of pNA was measured at 405 nm in a microplate reader. Results are presented as the relative change in activity compared to the control.

**In vivo tumorigenesis**

Six-week-old male nu/nu nude mice were housed under pathogen-free conditions. 143B cells transfected with control, Lv-NC, or Lv-CYC1 shRNA were cultured to near confluence, harvested, and resuspended in PBS at a density of 1 × 10^7 cells/ml. Cells (0.1 ml) were then injected subcutaneously into the flanks of nude mice. Mice received a single intravenous injection of 10 mg/kg TRAIL (six in each treatment group) every three days for 6 weeks. Tumor size was measured every week using a caliper. The tumor volume was calculated using the formula 1/6 πab^2 (π = 3.14; a, long-axis diameter of the tumor; b, short-axis diameter of the tumor). Growth curves were plotted from the tumor volume (mean ± SD) in each treatment group. At the end of the 6-week treatment, the animals were sacrificed under anesthesia and tumors were immediately harvested, measured, and weighed.

**Tumor tissue analysis**

After the tumors (n = 6) were harvested, tumor tissues were immediately fixed in paraformaldehyde at room temperature for 48 h and embedded in paraffin. The nuclei with fragmented DNA were detected by the terminal deoxyribonucleotidyl transferase (TDT)-mediated dUTP-digoxigenin nick end labeling (TUNEL) assay using an in situ cell death detection kit (Roche Diagnostics, Mannheim, Germany) according to the manufacturer’s protocol.

**Statistical analysis**

Data are expressed as the mean ± SD. Multiple comparisons were made using one-way analysis of variance followed by Fisher’s tests. Differences with a p <0.05 were considered statistically significant.

**Results**

**Inverse correlation between miR-661 and CYC1 expression in osteosarcoma**

Based on our previous study showing that CYC1 is overexpressed in OS tissues and cell lines [25], we examined the potential mechanisms underlying the regulation of CYC1 expression in OS. Sequence analysis showed that the 3′-UTR of CYC1 contains a complementary site for the seed region of miR-661 (Fig. 1A). We therefore examined the expression of miR-661 in OS tissues in comparison with matched adjacent normal tissues, which showed a significant downregulation of miR-661 in tumor tissues, with a reduction in the levels of miR-661 of approximately 75% compared with the levels in normal tissues (Fig. 1B). Spearman’s correlation analysis showed a statistically significant inverse correlation between the mRNA levels of miR-661 and CYC1 in tumor tissues (r = -0.910; P < 0.001) (Fig. 1C), suggesting a potential role for miR-661 in OS.
CYC1 is a direct downstream target of miR-661

To determine whether CYC1 is a direct downstream target of miR-661, luciferase reporter vectors containing the wild-type (wt) or mutant (mut) miR-661 binding site on the 3′-UTR of CYC1 were transfected into HEK293T cells together with miR-661 mimics or anti-miR-661 and their respective controls. The results of luciferase assays showed that miR-661 significantly suppressed, whereas anti-miR-661 significantly increased the luciferase activity of the wt CYC1 3′-UTR but not that of the mut 3′-UTR (P < 0.01) (Fig. 2A). Western blot analysis of lysates from 143B OS cells transfected with miR-661 mimics or anti-miR-661 showed that miR-661 overexpression downregulated, whereas anti-miR-661 upregulated CYC1 (Fig. 2B). Taken together, these results suggest that CYC1 is a target of miR-661 in OS.

miR-661 inhibits growth and promotes apoptosis of OS cells

To further examine the role of miR-661 in OS, 143B cells overexpressing miR-661 through transfect with miR-661 mimics were assessed for cell proliferation and apoptosis. The successful overexpression of miR-661 is shown in Fig. 3A, which shows an approximately 2.5 upregulation of miR-661. Ectopic expression of miR-661 decreased cell viability compared with untransfected and miR controls, as determined by the MTT assay (Fig. 3B). Flow cytometry showed that miR-661 overexpression significantly increased the rate of apoptosis (Fig. 3C and D), increased the levels of cleaved PARP (Fig. 3E), and caused an approximately 50% inhibition of complex III activity in 143B cells (Fig. 3E). Taken together, these results suggested that miR-661 has an anti-tumor effect in OS.

miR-661 sensitizes OS cells to TRAIL and STS induced apoptosis

The involvement of miR-661 in OS was further investigated by examining the effect of miR-661 on TRAIL or STS induced apoptosis. Our results showed that miR-661
overexpression enhanced the effect of TRAIL or STS on inducing apoptosis at concentrations of TRAIL and STS above 10 ng/ml (Fig. 4A and B). Western blot analysis showed that PARP cleavage triggered by TRAIL or STS at 10 ng/ml was enhanced by ectopic expression of miR-661 (Fig. 4C and D). These results indicated that miR-661 potentiates TRAIL or STS induced caspase activation and apoptosis in OS cells.

miR-661 enhances TRAIL and STS-induced CytC release and caspase-9 activation in OS cells.

A key step in the mitochondrial-dependent apoptotic cascade is the release of CytC into the cytosol, where it binds with apoptotic protease-activating factor 1 (Apaf-1) leading to the formation of the apoptosome and caspase-9 activation, which cleaves caspase-3, the major enzyme mediating apoptosis [28]. We further examined the effect of miR-661 on TRAIL or STS induced apoptosis by measuring the levels of CytC and caspase-9 activity in 143B cells with or without miR-661 overexpression and treated with or without the caspase-3 inhibitor Z-DEVD-FMK (100 μM) for 72 h. In cells treated with 10 ng/ml TRAIL or STS for 72 h, miR-661 overexpression increased the levels of CytC in the cytosolic fraction with a concomitant decrease in mitochondrial levels, and this effect was abolished by treatment with Z-DEVD-FMK (Fig. 5A and B). Caspase-9 activity induced by TRAIL or STS was significantly increased by approximately 1.5-fold by ectopic expression of miR-661 (p<0.01), whereas Z-DEVD-FMK restored caspase-9 activity to control levels (Fig 5C and D).

CYC1 overexpression rescues the effect of miR-661

To determine whether the effects of miR-661 on OS cell apoptosis are mediated by its modulation of CYC1 expression, cells were co-transfected with miR-661 mimics and a
Overexpression of CYC1 rescued the effect of miR-661 on inhibiting osteosarcoma cell proliferation (Fig. 6A) and enhancing TRAIL induced apoptosis.

Fig. 4. miR-661 sensitizes OS cells to apoptotic induction by TRAIL and STS. 143B cells and 143B cells transfected with miR-control, or miR-661 were treated with TRAIL (1, 10, 50, 100 ng/ml) or STS for 72 h. (A and B) The percentage of apoptotic cells was determined by annexin V-FITC/PI double-staining and flow cytometry in cells treated with TRAIL (A) and STS (B). (C and D) The levels of PARP and cleaved PARP were analyzed by western blotting in cells treated with TRAIL (C) and STS (D). Data are expressed as the mean ± SD. **p < 0.01 compared with control.

Fig. 5. miR-661 enhances TRAIL and STS-induced cytochrome c release and caspase-9 activation in OS cells. 143B cells transfected with control, miR-control, or miR-661 were treated with 10 ng/ml TRAIL or STS for 72 h. Cell transfected with miR-661 were incubated with 10 ng/ml TRAIL in the presence of the caspase-3 inhibitor Z-DEVD-FMK (100 μM) for 72 h. (A and B) The levels of mitochondrial and cytosolic cytochrome c (Cytc) were analyzed by western blotting in cells treated with TRAIL (A) or STS (B). (C and D) Caspase-9 activity was analyzed in cells treated with TRAIL (C) or STS (D). Data are expressed as the mean ± SD. **p < 0.01 compared with control.

pcDNA3.1 vector expressing CYC1. Overexpression of CYC1 rescued the effect of miR-661 on inhibiting osteosarcoma cell proliferation (Fig. 6A) and enhancing TRAIL induced apoptosis (Fig. 6B).
Furthermore, CYC1 overexpression rescued the effects of miR-661 on increasing the levels of cleaved PARP (Fig. 6C), the miR-661 induced reduction in the levels of complex III (Fig. 6D), the release of Cytc (Fig. 6E), and the increase in the activity of caspase 9 (Fig. 6F). Taken together, these results indicated that miR-661 decreases cell viability and sensitizes OS cells to TRAIL or STS induced mitochondrial apoptosis by downregulating CYC1.

**miR-661 sensitizes OS tumors to TRAIL and STS induced apoptosis and growth inhibition via CYC1 in vivo**

To investigate the relevance of our *in vitro* findings to OS tumorigenesis *in vivo*, the growth of xenograft tumors derived from 143B cells transfected with control, miR-control or miR-661 was monitored. Consistent with our *in vitro* findings, tumors derived from miR-661-transfected 143B cells were smaller than those derived from control- or miR-con-transfected cells after 6 weeks of TRAIL treatment, and CYC1 overexpression partially restored tumor size (Fig. 7A). Treatment with 10 mg/kg TRAIL and STS every 3 days for 6 weeks only slightly inhibited the growth of tumors derived from control- or miR-con-transfected 143B cells, indicating that these tumors were resistant to TRAIL *in vivo*, whereas tumors derived from miR-661-transfected 143B cells were sensitive to TRAIL and STS, and this effect was attenuated by CYC1 overexpression (Fig. 7B and C). TUNEL staining showed that miR-661 significantly enhanced TRAIL and STS induced apoptosis, whereas CYC1 overexpression restored apoptosis to control levels (Fig. 7D).
OS is an aggressive malignant neoplasm that arises from transformed cells of mesenchymal origin, and its rapid progression makes it the leading cause of cancer death among adolescents [29]. Despite significant improvements in the survival rate of patients with OS in recent years with the introduction of wide tumor excision in combination with chemotherapy and radiotherapy, the rate of distant metastasis or local relapse after treatment remains high [30]. Chemoresistance in OS is mediated by several mechanisms, including decreased intracellular drug accumulation, drug inactivation, miRNA dysregulation, and evasion of apoptosis [31]. In a previous study, we showed that CYC1 is overexpressed in OS tumor tissues and cell lines and CYC1 knockdown sensitizes OS cells to TRAIL-induced apoptosis, reducing the activity of complex III and promoting caspase-9 activation [25]. In the present study, we identified miR-661 as a negative regulator of CYC1 expression and examined its role in the resistance of OS to apoptosis in vitro and in vivo.

Alterations in the expression of miRNAs play a role in several cancers, and many miRNAs have been implicated in the tumorigenesis and invasiveness of OS. A miRNA expression profiling study that screened 762 miRNAs in two OS cell lines and human OS tissue samples identified 22 differentially expressed miRNAs in OS, of which miR-135b, miR-150, miR-542-5p, and miR-652 were confirmed to be dysregulated in tumors [32]. Maire et al. screened 723 human miRNAs in seven OS cell lines and identified 38 differentially expressed miRNAs [33]. These authors showed that some of the predicted gene targets of the differentially expressed miRNAs are involved in signaling pathways known to play a role in OS, such as Notch, RAS/p21, MAPK, Wnt, and the Jun/FOS pathways, confirming that the regulation of gene expression by miRNAs plays a role in OS. miR-1, miR-133b, and miR-378 were shown to be downregulated in tumors compared with non-tumor tissues, and ectopic expression of miR-1 and miR-133b in OS cells reduced cell proliferation, invasion and cell motility [34]. The involvement of miRNAs in the sensitivity of OS tumors to chemotherapy was demonstrated in a study that analyzed miRNA signatures capable of discriminating responders from non-
responders to ifosfamide treatment. In that study, miR-92a, miR-99b, miR-132, miR-193a-5p, and miR-422a were identified as diagnostic markers to predict the sensitivity of tumors to ifosfamide [35]. Cai et al. showed that miR-15a and miR-16-1 induce apoptosis and cell cycle arrest in OS by modulating the expression of cyclin D1 [36]. In the present study, we showed that miR-661 is downregulated in OS tissues and its ectopic expression inhibits growth and induces apoptosis in OS cells. We identified CYC1 as a target of miR-661, and showed that their expression is negatively correlated in OS cells. Our data suggest that miR-661 plays a tumor suppressor role in OS mediated by the downregulation of CYC1.

CYC1 downregulation was previously shown to sensitize OS cells to apoptosis via the mitochondrial-dependent apoptosis pathway possibly through the destabilization of the interaction between CYC1 and Cytc, leading to the release of Cytc and the activation of caspase 3 [25]. The release of Cytc from mitochondria into the cytosol is a critical event leading to apoptosis [28]. Intrinsic or acquired resistance to apoptosis is one of the hallmarks of cancer, and apoptosis resistance contributes to treatment failure in many tumors, underscoring the need to improve our understanding of the mechanisms by which tumor cells evade apoptotic cell death to identify molecular targets for therapeutic intervention. In the presence of sublethal stress signals, cancer cells mount an anti-apoptotic response that enables evasion from cell death and ensures survival [37]. The interference with apoptosis can occur at different points in the signaling cascade. In the mitochondrial pathway, apoptosis can be regulated at the level of mitochondria by the modulation of the redox status of Cytc, which determines its ability to induce caspase activation, or by the expression of Bcl-2 family proteins, which play a role in the regulation of Cytc release [38, 39]. At the postmitochondrial level, apoptosis is regulated by modulating the expression level or activity of Apaf via post-translational modifications [40]. In the present study, we showed that miR-661 sensitized OS cells to TRAIL and STS induced apoptosis, enhancing the release of Cytc into the cytosol, and caspase-9 activity. These effects were abolished by a caspase-3 inhibitor, confirming that the effects of miR-661 were mediated by the mitochondrial pathway of apoptosis. CYC1 overexpression rescued the effect of miR-661, restoring cell viability, suppressing miR-661 induced apoptosis, and inhibiting the miR-661 enhanced release of Cytc into the cytosol in response to apoptosis induction. These results were confirmed in an in vivo xenograft tumor model, which showed that the resistance of tumors to TRAIL-induced apoptosis was attenuated by miR-661 overexpression and this effect was partially reversed by CYC1 overexpression. Taken together with our previous results showing that CYC1 downregulation sensitizes OS to apoptosis, the present data provide a mechanism by which the modulation of CYC1 by miR-661 could be involved in the tumorigenesis of OS. The downregulation of miR-661 in OS tumors could result in the upregulation of CYC1 and increased resistance of OS cells to apoptosis. These results elucidate a potential mechanism underlying the resistance of OS to therapy, and suggest potential novel targets and strategies for the treatment of OS.

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Disclosure Statement

The authors declare that they have no conflict of interest.

References

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