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17β-Estradiol regulates cell proliferation, colony formation, migration, invasion and promotes apoptosis by upregulating miR-9 and thus degrades MALAT-1 in osteosarcoma cell MG-63 in an estrogen receptor-independent manner

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ABSTRACT

In bone, different concentration of estrogen leads to various of physiological processes in osteoblast, such as the proliferation, migration, and apoptosis in an estrogen receptor-dependent manner. But little was known about the estrogen effects on osteosarcoma (OS). In this study, OS cell MG-63 was treated with low (1 nM) or high (100 nM) dose of 17β-Estradiol (E2) with the presence or absence of estrogen receptor α (ERα), for evaluating the E2 effects on proliferation, migration, invasion, colony formation and apoptosis. Consistent with a previous study, high dose of E2 treatment dramatically downregulated expressing level of long non-coding RNA metastasis associated lung adenocarcinoma transcript 1 (MALAT-1). The observation of upregulation of miR-9 after a high dose of E2 treatment indicated the cause of MALAT-1 reduction. Downregulation of MALAT-1 promoted the combination of SFPQ/PTBP2 complex. It was also observed that the proliferation, migration, invasion, colony formation and apoptosis of OS cells were remarkably affected by high dose of E2 treatment, but not by low dose, in an ERα independent manner. Furthermore, the abolishment of the effects on these physiological processes caused by ectopic expression of miR-9 ASOs suggested the necessity of miR-9 in MALAT-1 regulation. Here we found that the high dose of E2 treatment upregulated miR-9 thus posttranscriptionally regulated MALAT-1 RNA level in OS cells, and then the downregulation of MALAT-1 inhibited cell proliferation, migration, invasion and epithelial–mesenchymal transition (EMT) processes in the E2-dose dependent and ER-independent ways.

1. Introduction

Osteosarcoma, which is considered as the most common human primary malignant bone tumor in minors, accounts for more than 60% of malignant bone tumors before 20 years old of life [1]. Growing regions on the bone take higher risk for OS. More and more evidences suggest OS essentially a differentiation disease. Osteoblast differentiation from mesenchymal stem cells was interrupted by genetic and epigenetic changes, and transformed to OS, which presents a high metastatic potential [2]. Although OS has been treated with surgical removal of the primary tumor, radio- and chemotherapy for several decades, patients still have very a poor prognosis because of its aggressiveness and metastases [3,4]. For understanding the mechanism of oncogenesis and oncotherapy, miRNAs have become a new research hotspot because of its tight association in most of physiological processes [5].

MicroRNA-9 (miR-9), which suppresses gene expression by interacting with the 3′ untranslated regions (UTRs) of target mRNAs, is ancient in animal evolution and indicates it's important roles. miR-9 is induced by several stress signals, including TLR2, TLR7/8, and LPS agonists. Report has found that miR-9 is not only highly expressed in human brain, but is also upregulated in breast cancer cells, and increases cell motility, invasiveness, and angiogenesis [6]. Recently, it has also been found to target to MALAT-1 for degradation, suggesting its potential regulatory role of several physiological processes through interacting with The MALAT-1.

MALAT-1, which is a 6.8 kb long nuclear-coded RNA transcript, is an evolutionarily highly conserved long non-coding RNA (lncRNA).

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Although it lacks open reading frames, MALAT-1 has been broadly expressed in normal human tissues, and especially overexpressed in various carcinomas, and thus be considered as an independent prognostic marker for survival in several carcinoma [7–9]. Both in normal tissue and tumor tissue, MALAT-1 participates in cell differentiation and development. Especially, in tumors, MALAT-1 could promote tumor growth and metastasis by competitively binding to SFPQ and then regulating the combination of PTBP2 and SFPQ complex [10,11]. These evidences suggest that MALAT-1 RNA regulates cell proliferation, migration and invasion through regulating the interaction between PTBP2 and SFPQ.

Estrogen is critical not only for breast development but also for healthy bone in women. In bone, estrogen stabilizes bone density and prevents from fracture by regulating apoptosis in osteoblast [12]. It has been reported that estrogen (17β-Estradiol, E2), in breast cancer, regulates proliferation, migration by post-transcriptionally regulation of MALAT-1 in a concentration-dependent way [13], indicates the potential regulatory activity of estrogen on OS.

In this study, the effects of high dose of E2 treatment on OS cells, despite the presence of ER or not, were identified. The downregulation of MALAT-1 caused by E2 treatment leaded us to identify the miR-9 expression profile, which targets directly to MALAT-1 RNA for degradation. Then regulation of downregulated MALAT-1 on combination of SFPQ/PTBP2 complex was tested. We also demonstrate that the effects of E2 treatment on cell proliferation, migration, invasion, colony formation and apoptosis. The results highlight the regulatory effects of the high dose of E2 treatment on OS through upregulating miR-9, which subsequently degraded MALAT-1, and support a novel physiological regulatory mechanism by estrogen in OS cells.

2. Materials and methods

2.1. Cell cultures

The Human OS cell line MG-63, which is ER null, is frozen in our lab. They were cultured in DMEM (Life Technology) supplemented with 10% FBS, 100-units/ml penicillin and 100 μg/ml streptomycin (Life Technology). Cells were kept at 37 °C in 5% CO_2 and passaged every 3–4 days.

2.2. Plasmid constructions and generation of stable cells

The procedure of estrogen-receptor α (ERα) eukaryotic expression vector construction was described previously [13]. In brief, approximately 1788 base pairs (bp) coding sequence were PCR-amplified from cDNA reverse-transcripted by using MCF-7 total RNA. Primers are followed: P1, 5′-GCCGGCGCTAGCATGCCATG ACCCTCCACACCA-3′ (enzyme site: Nhe I) and P2, 5′-CCTTAACTTA AGCAGACCGTGGCAGGGAAACCC-3′ (enzyme site: Hind III). The correct sequences of amplified fragment were confirmed by DNA sequencing, double digested with Nhe I and Hind III, and cloned into pcDNA3.1 vector (Life Technology), carrying the neomycin resistance gene.

Transfection was performed using the Lipofectamine™ 2000 transfection reagent (Life Technology) following the manufacturer's instructions. Of establishing stable transfectants, MG-63 were transfected with either pcDNA3.1-ERα vector (MG-63-ER) or pcDNA3.1 vector (MG-63-vector). 24 h after transfection, 400 μg/ml G418 was added and maintained for 4 weeks. MiRNA antagomir™ (ASOs) (MG-63-ASOs) or scrambled control (SC) (MG-63-SC), which specifically blocks miR-9 bought from RIBOBIO company, was transfected and selected with 600 μg/ml G418 for 4 weeks.

2.3. Reverse transcription and quantitative real-time PCR (RT-qPCR)

Total RNA containing miRNA and mRNA was isolated from treated or untreated cells using Trizol Reagent (Life Technology), followed by integrity and purity verification using UV spectrophotometry and gel-electrophoresis on formaldehyde denaturation gel. For preventing cross-contamination, separate room for RNA extraction and RT-qPCR assay was required.

For evaluating the miR-9 expressing levels, quantification using the TaqMan microRNA assays was performed in two separated parts. In the RT part, cDNA was reverse transcripted from total RNA sample using specific miR-9 primers supplied in Taqman MicroRNA Assays kit (Applied Biosystems). In the qPCR part, Taqman Universal PCR Master Mix (Applied Biosystems) was employed. The qPCR results were normalized against an internal control U6 RNA, and then expressed as relative folds.

2.4. Cell proliferation

Cells (1 × 103) were seeded in 24-well plates overnight and incubated with certain concentration of E2 or ethanol (mock) for 24 h. The volume of ethanol added to the mock wells was the same as that added to the drug treated wells. After removal of the medium, the wells were washed three times with PBS, and the plates were frozen at −20 °C overnight before processing with CCK-8 Proliferation Assay Kit (Life Technology). Absorbance of OD450-620 was calculated.

2.5. Detection of apoptosis/caspase 3/7 activity

Cells (1.5 × 104) were seeded in 24-well plates overnight for attaching and incubated with medium, ethanol, E2 or etoposide (3 μM) for 24 h. Levels of caspase-3/7 activity were determined using the SensoLyte® Homogeneous AMC cASPASE-3/7 Assay Kit (Anaspec Inc). For detecting the splicing of Caspase-3, cells were lysed for extracting total protein and followed by Western Blot as described before.

2.6. Statistical analyses

Each experiment was performed at least three times, and all values in the paper are reported as means ± SD. In all experiments, comparisons between two groups were based on two-sided Student's t-test. P-values of <0.05 were considered statistically significant.

3. Results

3.1. E2 treatments caused decrease of the MALAT-1 RNA level in an ER-independent manner by upregulating miR-9, which targeted to MALAT-1

It has been reported that low concentration E2 (1 nM) treatment stimulated cell growth, and oppositely, high concentration E2 (100 nM) treatment largely inhibited cell proliferation, migration, invasion, and colony formation by decreasing MALAT-1 RNA level in breast [14]. Similarly, estrogen also has been found to regulate osteoblast cells' growth, even apoptosis [12,15]. This made us interested in studying the role of high concentration of E2 on OS. MG-63 was chosen for further study because it's an ER-null cell line. For finding out the role of ER in estrogen response, MG-63 stably expressing ER (MG-63-ER) or vector (MG-63-vector) was constructed and be treated with the range of E2, 1, 10, and 100 nM. Then MALAT-1, NEAT1, HN-1, GAPDH and β-actin mRNA were tested by qPCR separately. Results showed that, compared to Mock

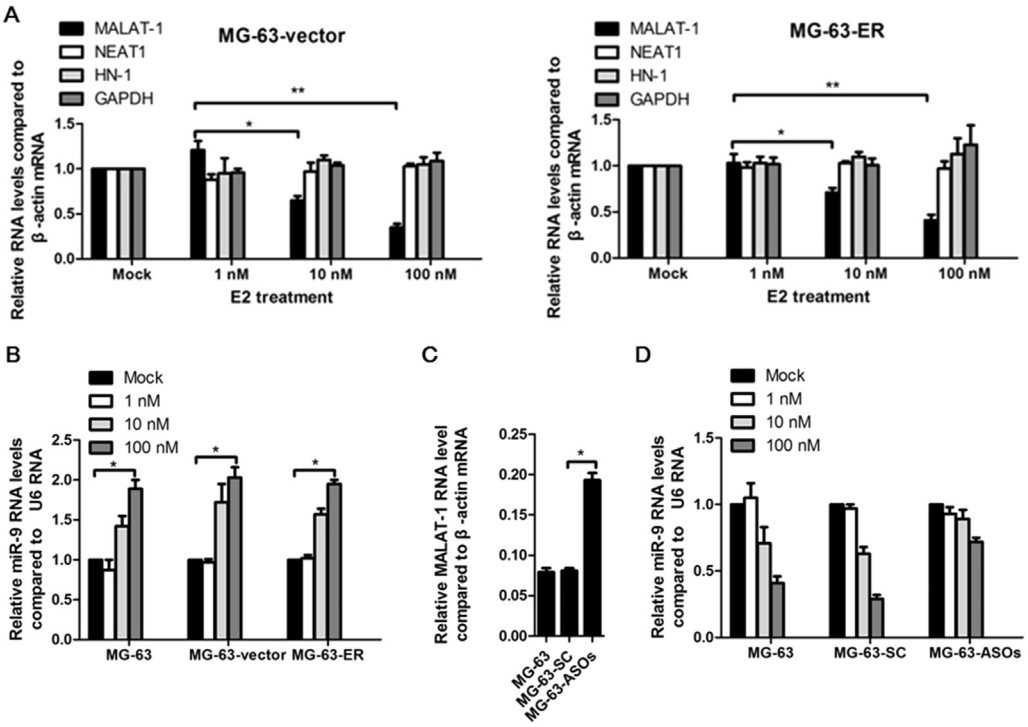

Fig. 1. 100 nM E2 treatment downregulated MALAT-1 RNA level by upregulating miR-9. (A) MG-63-vector and MG-63-ER were treated with the indicated dose of E2 for 24 h, and then be analyzed by RT-qPCR for detecting MALAT-1, NEAT1, HN-1, and GAPDH expressing levels. (B) Relative miR-9 expressing levels, compared with U6 RNA, were detected in MG-63, MG-63-vector, and MG-63-ER treated with indicated dose of E2 for 24 h. (C) MALAT-1 levels were detected by RT-qPCR after ectopic expression of miR-9 ASOs or scrambled control in MG-63. (D) MG-63 cells stably transfected with scrambled control or ASOs were treated with indicated dose of E2, and then be performed to RT-qPCR for detecting the MALAT-1 RNA levels. *$P < 0.05$, **$P < 0.01$.

and 1 nM, 10 and 100 nM E2 treatment decreased MALAT-1 RNA level in both MG-63-vector and ER, while showed no detectable effects on other RNAs, indicating the specific effect of E2 on MALAT-1 RNA level (Fig. 1A). Leucci et al. reported that miR-9 targeted directly to MALAT-1 RNA and downregulated its RNA level [16], and this made us further tested the miR-9 level after E2 treatment. Expectedly, high concentration E2 treatment upregulated miR-9 RNA level in both MG-63-vector and ER (Fig. 1B). Then miR-9 ASOs or scrambled control (SC) were transfected before E2 treatment (Fig. 1C), and abolished the effects of E2 on MALAT-1 RNA level, indicating the key role of upregulated miR-9 in regulating MALAT-1 RNA level (Fig. 1D).

3.2. Downregulation of MALAT-1 caused by E2 treatment promoted the binding of SFPQ to oncogene PTBP2

MALAT-1 competitively bound to SFPQ and released it from SFPQ/PTBP2 complex [17]. For figuring out whether the downregulation of MALAT-1 by E2 treatment effects SFPQ/PTBP2 complex, RIP assay, using the SFPQ antibody and MALAT-1 special primers was performed. Consistently, we found that MALAT-1 binding to SFPQ decreased in high dose E2 treated MG-63-vector and MG-63-ER (Fig. 2A). This indicated that with downregulation of MALAT-1 RNA level, the MALAT-1 binding to SFPQ decreased, suggesting that E2 treatment might affect the function of SFPQ. In order to understand the effects of E2 treatment on PTBP2/SFPQ complex which is regulated by MALAT-1 binding, we performed immunoprecipitation tests. The results showed that, compared with Mock or 1 nM E2 treated cells, both 100 nM E2 treated MG-63-vector and MG-63-ER cells had a stronger binding of PTBP2 to the SFPQ without changes on their protein levels, indicating that SFPQ-detached PTBP2 might be increased in the high dose E2 treated cells (Fig. 2B). These changes might affect proliferation, migration or invasion, because these physiological processes were tightly controlled by SFPQ/PTBP2 complex.

3.3. E2 treatment inhibited cell proliferation and colony formation in an ER-independent manner

Previous studies have shown that MALAT-1 plays key roles in cell proliferation, tumor formation, differentiation and development [18,19]. This made us wondering whether E2 treatment affects these physiological processes in OS. Firstly, PI staining followed by flow cytometry was performed to analyze the distribution of different cell phases. The results showed that high dose of E2 treatment arrest the cell cycle by accumulating G1 phase and decreasing G2/M phase (Fig. 2C). Unexpectedly, a remarkable increase in subG1 phase was observed, which indicated the increasing apoptosis (Fig. 2C). CCK-8 assay also showed that the 100 nM E2 treatment significantly inhibit proliferation (Fig. 2D). Meanwhile, we observed that E2 treatment caused a morphological change of cells to flatter, which indicates its effects on cell migration and invasion (data not shown). We further tested the effects of E2 treatment on colony formation, and consistent with proliferation result (Fig. 2D), high dose of E2 treatment also significantly inhibited colony formation of OS cells in soft agar in an ER-independent manner (Fig. 2E).

3.4. 100 nM E2 treatment promoted apoptosis in OS cells

The observation of the previous result (Fig. 2C) indicated the potential effects of E2 treatment on promoting apoptosis. Interestingly, it also has been reported that E2 regulates apoptosis in Osteoblasts [12]. Both the evidences led us to determine whether or

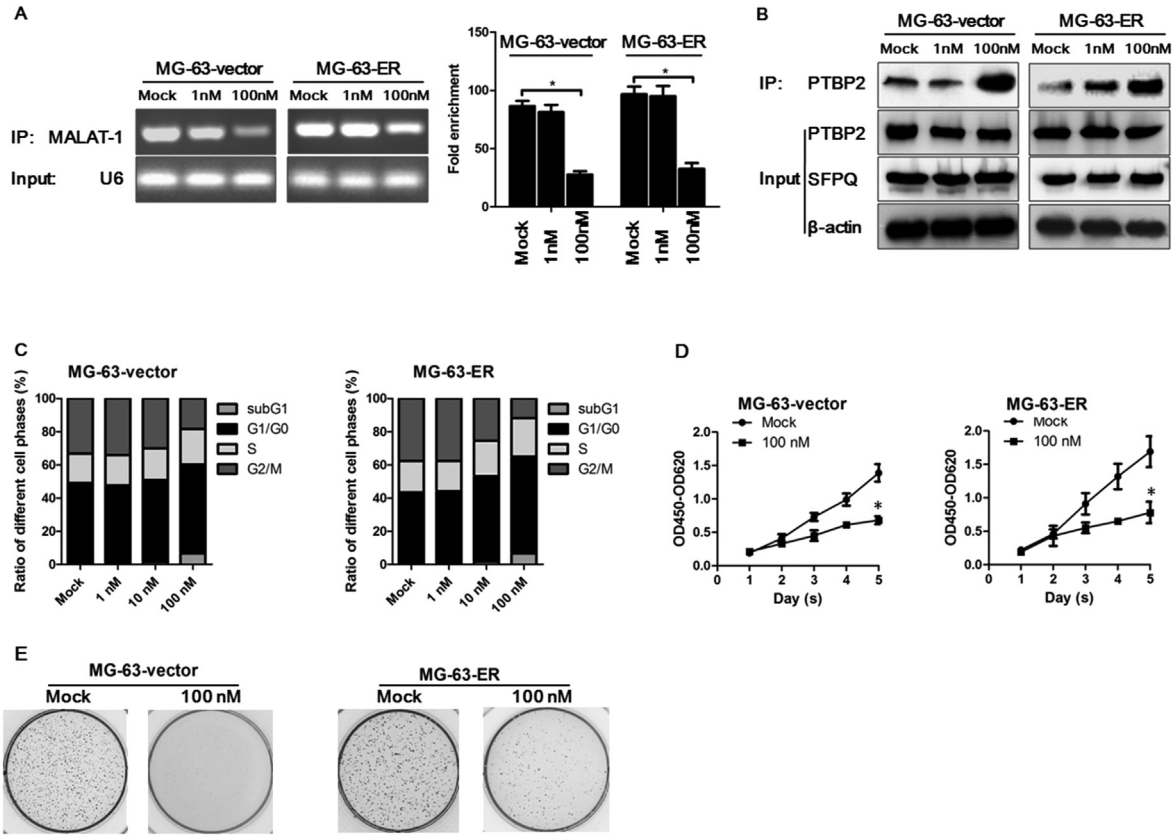

Fig. 2. Downregulation of MALAT-1 caused by E2 treatment promoted combination of SFPQ/PTBP2 complex and effected cell cycle processes, proliferation, and colony formation. (A) MG-63-vector and MG-63-ER were treated with indicated dose of E2 for 24 h, and then be lysed for RIP. IP products were reverse-transcripted for semiquantitative PCR (left) and qPCR (right). (B) The cells described above were also co-immunoprecipitation with anti-PTBP2 antibody. Input and IP products were immunoblotted by indicated antibodies. (C) Cells were treated with indicated E2 for 24 h, stained by PI and analyzed by flow cytometry. (D) Treated cells were counted every 24 h, until 5 days. Data are presented as the mean ± SEM of the three separate experiments. (E) 2 × 103 Treated cells were seeded in 6-well plates containing semisolid medium (n = 3) and grew for 4 weeks before colonies were visualized and microscopically. *P < 0.05.

not E2 treatment affected caspase-3/7, which is universally increased during apoptosis [20,21], activity in MG-63 cells treated with agents which promote apoptosis. Cells were pretreated for 24 h with 100 nM E2 or mock and then exposed to 3 μM Etoposide for 24 h, caspase-3/7 activity in cellular extracts was assayed. The results showed that, compared to other treatments, 100 nM E2 treatment significantly stimulated caspase-3/7 activity in both MG-63-vector and MG-63-ER (Fig. 3A). Testing of Caspase-3's inactive form (32 kDa), and active form (17 kDa) showed that E2 pretreatment significantly increased Caspase-3's active form after Etoposide treatment (Fig. 3B). We further tested the apoptotic rate of these cells by PI staining. Consistent with previous result, E2 treatment increased apoptotic rate caused by Etoposide in both MG-63-vector and MG-63-ER (Fig. 3C).

3.5. E2 treatment blocks MG-63 cell migration by inhibiting epithelial-to-mesenchymal transition (EMT)

It is reported that the downregulation of MALAT-1 RNA results in inhibition of EMT in lung cancer and bladder cancer [22], indicating the possible role of E2 treatment in EMT inhibition. To assess this hypothesis, the expression of EMT marker genes was measured by qRT-PCR in E2-treated MG-63-SC or MG-63-ASOs. As expected, 100 nM E2 treatment caused the downregulation of the early transcriptional mesenchymal markers Snai1, Snai2, and N-cadherin, upregulation of E-cadherin, an epithelial marker and suppressor of tumor cell invasion and metastasis (Fig. 4A). Notably, expression of ASOs of miR-9 in MG-63 abolished the effects of E2 treatment on these genes' transcription level, suggested that the miR-9 is critical in EMT regulation by E2 treatment (Fig. 4B). We further tested whether inhibition of EMT causes a decrease in tumor cell motility and invasive capacity by an in vitro mechanical wound healing assay. As showed in Fig. 4C, compared with untreated or 1 nM E2 treated MG-63 cells, 100 nM E2 treated MG-63 cells showed a significant delay in the healing process. Consistently, ASOs overexpression in MG-63 abolished the detectable delay. In keeping with these results, time-lapse observation suggested that 100 nM E2 treated cells at the leading edge of the wound display a decreasing pro-migratory phenotype when compared to mock or 1 nM treated cells, as indicated by the reduction in the number of cell membrane ruffles (Fig. 4D). Taken together, these results support the understanding that 100 nM E2 treatment, but not 1 nM E2 treatment, affects the motility of MG-63 cells by upregulated miR-9.

4. Discussion

After comparison of expressing profiles of non-small cell lung cancer (NSCLC) patients with metastatic tumors to those who have no metastatic tumors, lncRNA MALAT-1 which is widely expressed in normal human tissues, was found remarkably overexpressed [18,19]. It is also being found to be upregulated in a variety of human cancers, including prostate, breast, colon, liver and uterus. Recent studies have suggested MALAT-1 in the regulation of cell growth, cell cycle progression, migration and invasion, due to its upregulation in

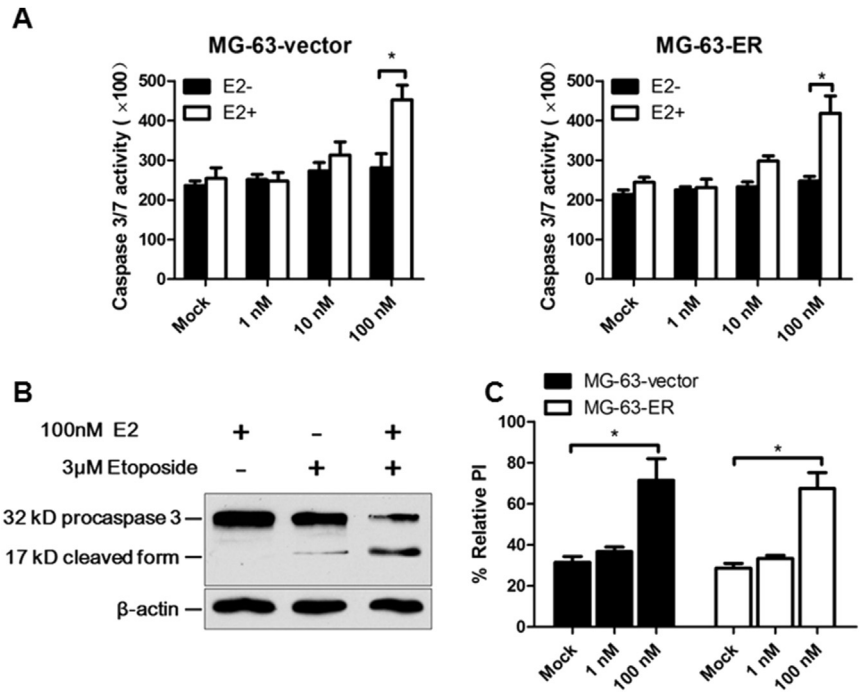

Fig. 3. E2 treatment promoted etoposide-induced apoptosis. (A) MG-63 cells stably transfected with vector or ER were pre-treated with indicated dose of E2 for 24 h, and then incubated with 3 μM etoposide for 24 h. The caspase 3/7 activity was detected. (B) The cells described in (A) were also detected by performing western blot using anti-caspase 3 antibody for detecting the activated form. (C) The cells described in (A) were further stained with PI and be performed on flow cytometry for detecting the apoptotic rate. *P < 0.05.

cancers. Tano et al. found that, in lung adenocarcinoma cells, MALAT-1 knockdown using RNA interference reduced the in vitro migration and invasion by regulating the migration-related genes [23]. MALAT-1 RNA is reported to be targeted by miR-9 for degradation, indicating a novel direct regulatory link between them and potential regulatory role of miR-9 on cell physiological processes, such as cell proliferation, migration and invasion [16].

The molecular mechanism of MALAT-1 regulation on mobility is largely unknown. Previous studies identified that MALAT-1 regulates the splicing processes of a family of genes while others indicated a mechanism of transcriptional regulator [24,25]. Conversely, study strongly indicates that MALAT-1 has no major impact on alternative splicing but transcriptionally regulates a set of genes associated with cancer metastasis. MALAT-1 could also affect the interaction of SFPQ and PTBP2 protein by binding to SFPQ [17]. SFPQ contains one DNA-binding domain and two RNA-binding domains, and combines with Proto-oncogene PTBP2 to inhibit cancer cells' growth [26]. MALAT-1 could bind to SFPQ, thus disassociating SFPQ/PTBP2 complex. Subsequently, released PTBP2 from SFPQ/PTBP2 complex promoted cell proliferation and migration, indicating the regulatory role of SFPQ on MALAT-1 activity. However, since only a few genes have a proven association with metastasis without a significant enrichment, MALAT-1 could also have effects on other important physiological processes.

Estrogen does not only play roles in breast development and regulating proliferation of breast cells, but also arrests cell proliferation and induces apoptosis in breast tumor cell. Zhao et al. reported that, in breast tumor cells, the high dose of E2 treatment regulates several cell physiological processes by downregulating MALAT-1 RNA level, without knowing the molecular mechanism of this regulation on MALAT-1 [13]. Studies also indicate that physiologic and pharmacologic estrogen exposure direct effects on bone-forming osteoblasts and promotes a resistance of osteoblasts to the processes of apoptosis. By exposing to estrogen, caspase-3 activity is inhibited acutely as well as the transcriptionally suppression of a battery of apoptosis-promoting genes. The effects of estrogen on osteoblast suggest its potential effects on osteosarcoma. The effects of estrogen in breast and breast cancer cells in an ER-independent manner also raise the question that whether the effects of estrogen on osteosarcoma are depending on the expression of ER or not. According to these findings, we hypothesized that E2 may play regulatory role on osteosarcoma cells.

In this study, we found that E2 treatment had a significant downregulation of MALAT-1 RNA level in vitro, through upregulating miR-9 expression which targets directly to MALAT-1 RNA for degradation. The regulatory mechanism between miR-9 and MALAT-1 caused by E2 treatment is a potential explanation for the effects of E2 treatment in breast tumor cells [13]. The downregulated MALAT-1 by overexpressed miR-9 induced by E2 exposure significantly decreased osteosarcoma cell MG-63 growth, migration, invasion and apoptosis. This growth suppressive effect is associated with an increase in G1-phase population and a decrease of the S-phase followed. Similar effects on cellular proliferation rate and cell cycle distribution after E2 treatment was also observed in MG-63 ectopically expressing ER, indicating the effects of E2 treatment are ER-independent. Mechanically, it is observed that downregulated MALAT-1 by E2 treatment decreased its binding to SFPQ, and promoted the combination of SFPQ/PTBP2 complex. This may explain the E2 regulation on cell proliferation, migration, invasion and apoptosis.

In summary, this study demonstrates that a novel pathway, involving transcriptional upregulation of miR-9, which directly targets to MALAT-1 for degradation, plays an important role in regulating the combination of SFPQ/PTBP2 complex and thus affects proliferation, migration, invasion and apoptosis in osteosarcoma. Moreover, the effects of E2 treatment on osteosarcoma are independent on estrogen receptor α. These findings therefore identify E2 as an important factor for MALAT-1 mediated osteosarcoma cell migration, invasion, metastasis and potentially apoptosis, and provide important evidence for further development of therapeutic method using E2.

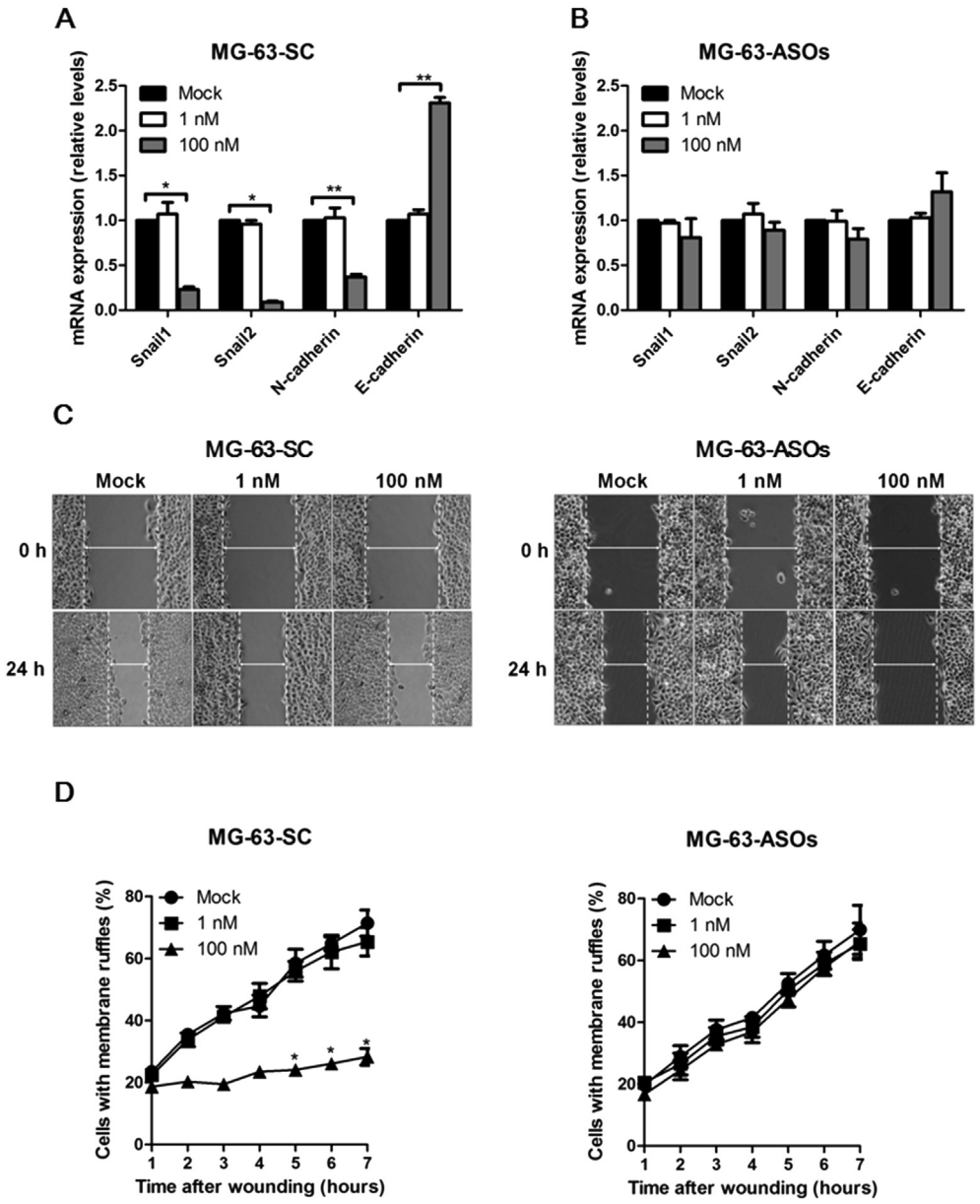

Fig. 4. E2 treatment inhibited EMT, thus inhibiting migration and invasion in vitro. MG-63 cells stably transfected with vector (A) or ER (B) were treated with indicated dose of E2 for 24 h. RT-qPCR was performed to detect the EMT-related genes' expression, including Snail1, Snail2, N-cadherin and E-cadherin. The effect of E2 treatment on the cells described in was detected by scratch assay (C) and transwell assay (D) at the indicated time point. *P < 0.05.

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Transparency document

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References

[1] O. Ma, W.W. Cai, L. Zender, T. Davaram, J. Shen, A.J. Herron, S.W. Lowe, T.K. Man, C.C. Lau, L.A. Donehower, MMP13, Birc2 (cIAP1), and Birc3 (cIAP2), amplified on chromosome 9, collaborate with p53 deficiency in mouse osteosarcoma progression, Cancer Res. (2009) 2559–2567.

[2] C.R. Walkley, R. Qudsi, V.G. Sankaran, J.A. Perry, M. Gostissa, S.I. Roth, S.J. Rodda, E. Snay, P. Dunning, F.H. Fahey, F.W. Alt, A.P. McMahon, S.H. Orkin, Conditional mouse osteosarcoma, dependent on p53 loss and potentiated by loss of Rb, mimics the human disease, Genes. Dev. (2008) 1662–1676.

[3] A.J. Chou, D.S. Geller, R. Gorlick, Therapy for osteosarcoma: where do we go from here? Paediatr. Drugs (2008) 315–327.

[4] K. O'Day, R. Gorlick, Novel therapeutic agents for osteosarcoma, Expert Rev. Anticancer Ther. (2009) 511–523.

[5] W. Zhang, J.E. Dahlberg, W. Tam, MicroRNAs in tumorigenesis: a primer, Am. J. Pathol. (2007) 728–738.

[6] L. Ma, J. Young, H. Prabhala, E. Pan, P. Mestdagh, D. Muth, J. Teruya-Feldstein, F. Reinhardt, T.T. Onder, S. Valastyan, F. Westermann, F. Speleman, J. Vandesompele, R.A. Weinberg, miR-9, a MYC/MYCN-activated mcroRNA, regulates E-cadherin and cancer metastasis, Nat. Cell. Biol. (2010) 247–256.

[7] R. Lin, S. Maeda, C. Liu, M. Karin, T.S. Edgington, A large noncoding RNA is a marker for murine hepatocellular carcinomas and a spectrum of human carcinomas, Oncogene (2007) 851–858.

[8] F. Guo, Y. Li, Y. Liu, J. Wang, Y. Li, G. Li, Inhibition of metastasis-associated lung adenocarcinoma transcript 1 in CaSki human cervical cancer cells suppresses

cell proliferation and invasion, Acta Biochem. Biophys. Sin. – Shanghai (2010) 224–229.
[9] L.H. Schmidt, T. Spieker, S. Koschmieder, S. Schaffers, J. Humberg, D. Jungen, E. Bulk, A. Hascher, D. Wittmer, A. Marra, L. Hillejan, K. Wiebe, W.E. Berdel, R. Wiewrodt, C. Muller-Tidow, The long noncoding MALAT-1 RNA indicates a poor prognosis in non-small cell lung cancer and induces migration and tumor growth, J. Thorac. Oncol. (2011) 1984–1992.
[10] J.G. Patton, E.B. Porro, J. Galceran, P. Tempst, B. Nadal-Ginard, Cloning and characterization of PSF, a novel pre-mRNA splicing factor, Genes. Dev. (1993) 393–406.
[11] M. Meissner, T. Dechat, C. Gerner, R. Grimm, R. Foisner, G. Sauermann, Differential nuclear localization and nuclear matrix association of the splicing factors PSF and PTB, J. Cell. Biochem. (2000) 559–566.
[12] G.B. Peter, V.G. Ken, L.R. Renée, G.C. Brian, Estrogen regulation of apoptosis in osteoblasts, Physiol. Behav. (2010) 181–185.
[13] Z.Y. Zhao, C.J. Chen, Y. Liu, C.F. Wu, 17β-Estradiol treatment inhibits breast cell proliferation, migration and invasion by decreasing MALAT-1 RNA level, Biochem. Biophys. Res. Commun. (2014) 1803–1808.
[14] T. Peritz, F. Zeng, T.J. Kannanayakal, K. Kilk, E. Eiríksdóttir, U. Langel, J. Eberwine, Immunoprecipitation of mRNA-protein complexes, Nat. Protoc. (2006) 577–580.
[15] K.M. Waters, D.J. Richard, B.L. Riggs, S. Khosla, J.A. Katzenellenbogen, B.S. Katzenellenbogen, J. Moore, T.C. Spelsberg, Estrogen regulation of human osteoblast function is determined by the stage of differentiation and the estrogen receptor isoform, J. Cell. Biochem. (2001) 448–462.
[16] L. Eleonora, P. Francesca, W. Johannes, H. Kim, L. Morten, P. Bo, K. Sakari, H.L. Anders, microRNA-9 targets the long non-coding RNA MALAT-1 for degradation in the nucleus, Sci. Rep. (2013) 2535–2540.
[17] Q. Ji, L. Zhang, X. Liu, L. Zhou, W. Wang, Z. Han, H. Sui, Y. Tang, Y. Wang, N. Liu, J. Ren, F. Hou, Q. Li, Long non-coding RNA MALAT1 promotes tumour growth and metastasis in colorectal cancer through binding to SFPQ and releasing oncogene PTBP2 from SFPQ/PTBP2 complex, Br. J. Cancer (2014) 736–748.
[18] P. Ji, S. Diederichs, W. Wang, S. Böing, R. Metzger, P.M. Schneider, N. Tidow, B. Brandt, H. Buerger, E. Bulk, W.E. Berdel, H. Serve, C. Müller-Tidow, MALAT-1, a novel noncoding RNA, and thymosin beta4 predict metastasis and survival in early-stage non-small lung cancer, Oncogene (2003) 8031–8041.
[19] J.N. Hutchinson, A.W. Ensminger, C.M. Clemson, C.R. Lynch, J.B. Lawrence, A. Chess, A screen for nuclear transcripts identifies two linked noncoding RNAs associated with SC35 splicing domains, BMC Genomics (2007) 8–39.
[20] K.M. Debatin, P.H. Krammer, Death receptors in chemotherapy and cancer, Oncogene (2004) 2950–2966.
[21] J.G. Walsh, S.P. Cullen, C. Sheridan, A.U. Lüthi, C. Gerner, S. J. Martin, Executioner caspase-3 and caspase-7 are functionally distinct proteases, Proc. Natl. Acad. Sci. U. S. A. (2008) 12815–12819.
[22] L. Ying, Q. Chen, Y. Wang, Z. Zhou, Y. Huang, F. Qiu, Upregulated MALAT-1 contributes to bladder cancer cell migration by inducing epithelial-to-mesenchymal transition, Mol. Biosyst. (2012) 2289–2294.
[23] K. Tano, R. Mizuno, T. Okada, R. Rakwal, J. Shibato, Y. Masuo, K. Ijiri, N. Akimitsu, MALAT-1 enhances cell motility of lung adenocarcinoma cells by the expression of motility-related genes, FEBS Lett. (2010) 4575–4580.
[24] L. Yang, C. Lin, W. Liu, J. Zhang, K.A. Ohgi, J.D. Grinstein, P.C. Dorrestein, M.G. Rosenfeld, ncRNA- and Pc2 methylation-dependent gene relocation between nuclear structures mediates gene activation programs, Cell (2011) 773–788.
[25] B. Zhang, G. Arun, Y.S. Mao, Z. Lazer, G. Hung, G. Bhattacharjee, X. Xiao, C.J. Booth, J. Wu, C. Zhang, D.L. Spector, The lncRNA Malat1 is dispensable for mouse development but its transcription plays a cis-regulatory role in the adult, Cell. Rep. (2012) 111–123.
[26] X. He, M. Pool, K.M. Darcy, S.B. Lim, N. Auersperg, J.S. Coon, W.T. Beck, Knockdown of polypyrimidine tract-binding protein suppresses ovarian tumor cell growth and invasiveness in vitro, Oncogene (2007) 4961–4968.