NME1 suppression promotes growth, adhesion and implantation of endometrial stromal cells via Akt and MAPK/Erk1/2 signal pathways in the endometriotic milieu

Ming-Qing Li†, Jun Shao†, Yu-Han Meng, Jie Mei, Ying Wang, Hui Li, Li Zhang, Kai-Kai Chang, Xiao-Qiu Wang, Xiao-Yong Zhu, and Da-Jin Li*

Laboratory for Reproductive Immunology, Hospital and Institute of Obstetrics and Gynecology, Fudan University Shanghai Medical College, No. 413, Zhaozhou Road, Shanghai 200011, China

*Correspondence address. Tel: +86-21-63457331; Fax: +86-21-63457331; E-mail: djli@shmu.edu.cn

Submitted on November 7, 2012; resubmitted on April 24, 2013; accepted on May 3, 2013

STUDY QUESTION: Is Nometastatic gene 23-H1 (NME1, also known as nm23-H1) involved in regulating the biological behavior of endometrial stromal cells (ESCs), and does it participate in the pathogenesis of endometriosis?

SUMMARY ANSWER: NME1 suppression induces ESC dysfunction in the endometriotic milieu.

WHAT IS KNOWN ALREADY: NME1 is a wide-spectrum tumor metastasis suppressor gene that plays an important role in suppressing the invasion and metastasis of tumor cells.

STUDY DESIGN, SIZE, DURATION: An in vitro investigation of the effect of NME1 on the proliferation, adhesion and invasion of eutopic ESCs from patients with endometriosis.

PARTICIPANTS/MATERIALS, SETTING, METHODS: Primary ESCs were prepared from 12 samples of ectopic endometrial tissue (6 peritoneal and 6 ovarian lesions), 18 samples of eutopic endometrial tissues (16 from women with ovarian and 2 from women with pelvic endometriomas) and 12 samples of normal endometrial tissue from women without endometriosis, after the tissues had been analyzed histologically. The growth, invasiveness and adhesion of ESCs were studied by the 5-bromo-2′-deoxyuridine cell proliferation assay and by the Matrigel invasion and adhesion assay. Additionally, the effects of NME1 on the activation or expression of related regulatory proteins were investigated by in-cell Western and flow cytometry assays.

MAIN RESULTS AND THE ROLE OF CHANCE: Expression of NME1 in ESCs derived from eutopic or ectopic endometrium from women with endometriosis is lower than in ESCs from women without endometriosis. Estrogen could down-regulate NME1 expression in ESCs. Silencing NME1 in ESCs promoted the expression of proliferating cell nuclear antigen (PCNA), the anti-apoptotic molecule, survivin, and the adhesion-related molecules, integrin β1 and integrin αvβ3. Silencing NME1 also stimulated ESC proliferation, adhesion and invasion but these effects were inhibited by MAPK/Erk and/or Akt blockers.

LIMITATIONS, REASONS FOR CAUTION: Further studies are needed to examine the regulatory mechanism of estrogen on NME1 expression of ESCs.

WIDER IMPLICATIONS OF THE FINDINGS: Abnormally low expression of NME1 in ESCs may be involved in the pathogenesis of endometriosis by up-regulating growth, adhesion and invasion of ESCs via activating the Akt and MAPK/Erk1/2 signal pathways.

STUDY FUNDING/COMPETING INTEREST(S): This work was supported by National Natural Science Foundation of China (NSFC) (31270969, 31101064 and 81270677) and Program for ZhouXue of Fudan University. None of the authors has any conflict of interest to declare.

Key words: NME1 / proliferation / adhesion / invasion / endometriosis

† These authors contributed equally to this work.
Introduction

Endometriosis characterized by the growth of endometrial tissue outside the uterine cavity is a very common benign gynecological disorder that affects 6–10% of the fertile women (Giudice and Kao, 2004). The pathogenesis of endometriosis still remains controversial despite extensive research. The most widely accepted theory is that the sloughed menstrual endometrial cells attach to the peritoneal serous membrane, and their subsequent proliferation and invasion into the underlying tissue results in endometriotic lesions (Sampson, 1925; Nisolle et al., 2000). Retrograde menstruation is, however, a physiologic process that takes place almost in all menstruation cycles, and an increasing body of evidence shows that the primary defect in endometriosis can be located in the eutopic endometrium. Different characteristics of the eutopic endometrium from women with endometriosis, such as aberrant production of cytokines (Akoum et al., 2000; Arici, 2002; Ulukus et al., 2006), growth, adhesion and angiogenic factors as well as specific cancer-related molecules (Li et al., 2011), are believed to contribute to the occurrence and continuation of this disease.

Endometriosis is an estrogen-dependent disease (Rizner, 2009). Our previous research has also demonstrated that 17β-estradiol can promote the invasion of endometrial stromal cells (ESCs) via suppressing CD82 expression and stimulating CCL2 secretion and CCR2 expression. Moreover, the enhanced interaction of CCL2-CCR2 recruited more macrophages into the ectopic milieu in a paracrine manner, which further promoted the viability, proliferation and invasiveness (Li et al., 2011, 2012a).

The Nometastatic gene 23 family includes two genes (NME1 and NME2, also known as nm23-H1 and nm23-H2). Their gene products have 92% amino acid sequence homology, and correspond to the primary structures of human nucleoside diphosphate (NDP) kinase A and B, respectively (Gilles et al., 1991). Based on tumor prognostic and transfecion studies, a correlation of increased NME1 expression to low metastatic potential of cancer cells has been established in several malignancies (Horak et al., 2007; Boissan et al., 2010; Lim et al., 2011). NME1 gene expression has an impact on the cell adhesion-related signaling pathway, through NDPK-mediated GTP synthesis, and significantly increases β-catenin, E-cadherin, and CD44 mRNA and protein expression to strengthen intercellular adhesion, which further inhibits tumor invasion and metastasis (Boissan et al., 2010). Previous research has established that NME1 is also expressed in human first-trimester trophoblasts and plays a significant part in the regulation of human trophoblast invasion through down-regulating Titin (also known as connectin, a protein that in humans is encoded by the TTN gene) expression (Xie et al., 2010) and thymic stromal lymphopoietin (TSLP) secretion (Wang et al., 2012). However, there are still questions about whether and how NME1 is involved in the origin and development of endometriosis through modulating the biological behavior of ESCs.

Therefore, the present study was undertaken to identify the expression of NME1 in ESCs from women with or without endometriosis and to analyze the regulatory effects of NME1 on the biological behaviors of ESCs.

Materials and Methods

Reagents

Monoclonal and polyclonal antibodies against NME1, actin, proliferating cell nuclear antigen (PCNA), mouse anti-human phosphorylated and rabbit anti-human unphosphorylated Erkl/2, P38, JNK and Akt were from Santa Cruz Biotechnology (Santa Cruz Biotechnology, TX, USA); 17beta-estradiol was from Sigma (Sigma-Aldrich Co. LLC, Shanghai, China); Marimastat and ARP 101 were from Tocsin (Tocsin Bioscience, Bristol, UK); Mouse anti-human MMP2/9 and TIMP1/2 antibodies, APC-conjugated anti-human CD29 (integrin β1) and PE-conjugated anti-human integrin α3β3 were from R&D Systems (Abingdon, UK); the polyclonal antibodies against survivin, U0126 and LY294002 were from Cell Signaling Technology (Beverly, MA, USA); second IRDye™ 700DX-conjugated affinity purified (red fluorescence) anti-mouse and IRDye™ 800DX-conjugated affinity purified (green fluorescence) anti-rabbit fluorescence antibodies were from Rockland, Inc. (Gibertville, PA, USA); and all other reagents were from Biolegend (San Diego, USA).

Tissue collection

All tissue samples were obtained with informed consent in accordance with the requirements of the Research Ethics Committee in Hospital of Obstetrics and Gynecology, Fudan University Shanghai Medical College. Samples of endometriotic peritoneal lesions (n = 6) and ovarian lesions (n = 6) were obtained from women aged 27–44 years undergoing laparoscopy for pain or other benign indications. Eutopic endometrial tissue was obtained from fertile women (aged 22–48 years) with ovarian (n = 16) and pelvic (n = 2) endometriomas, or without (n = 12) endometriosis (controls). None of the women had received hormonal medication in the 3 months prior to the surgical procedure. All the samples were confirmed histologically according to established criteria. The tissues used for immunohistochemistry were collected during both the proliferative and secretory phase of the cycle, but for all other experiments, the samples were obtained only in the proliferative phase of the cycle.

Immunohistochemistry

Paraffin sections (5 μm) of the endometriotic (n = 12) and eutopic endometrial (n = 12) tissues from women without endometriosis (ovarian and pelvic endometriomas) and endometrium from healthy controls (n = 12) in the proliferative or secretory phase of the cycle were dehydrated in graded ethanol and incubated with hydrogen peroxide in 1% bovine serum albumin in Tris-buffered saline (TBS) to block endogenous peroxidase. The samples were then incubated with mouse anti-human NME1 monoclonal antibody (25 μg/ml) or mouse IgG isotype antibody overnight at 4°C. After washing three times with TBS, the sections were overlaid with peroxidase-conjugated goat anti-mouse IgG antibody (SP-9002; Golden Bridge International, Inc., Beijing, China) and the reaction was developed with 3,3’-diaminobenzidine (DAB); the sections counterstained with hematoxylin. The experiments were repeated five times.

Cell isolation and culture

The endometrial tissues (from women with ovarian and pelvic endometriomas, and healthy controls) and endometriotic ovarian lesion were collected under sterile conditions and transported to the laboratory on ice in DMEM (Dulbecco’s modified Eagle’s medium)/F-12 (Gibco, USA) with 10% fetal calf serum (FCS; HyClone, Logan, UT, USA). The ESCs were isolated according to the previous methods (Li et al., 2011, 2012a). Immunocytochemistry showed > 95% vimentin-positive and cytokeratin-negative ESCs.

Quantitative real-time PCR

The NME1 mRNA levels in normal ESCs from healthy controls (n = 6) and ESCs from eutopic (n = 6) and ectopic (n = 6) sites in women with endometriosis in the proliferative phase of the cycle were evaluated by performing
quantitative real-time PCR. The primer sequences were synthesized by TaKaRa Biotechnology Co., Ltd (Tokyo, Japan): NME1: sense, 5′-CAG GAA CCA TGG CCA ACT GTG-3′; antisense, 5′-CGG ATG GTC CCA GGC TTG G-3′. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH): sense, 5′-GGG GAG CCA AAA GGG TCA TCA TCT-3′; antisense, 5′-GAG GGG CCA TCC ACA GTC TTC T-3′. The relative gene expression for individual cDNA samples was determined by calculating ΔCt values (ΔCt) by subtraction of the Ct value for GAPDH from the Ct value for NME1. The relative fold expression of NME1 was determined compared with the control. The experiments were carried out in triplicate.

In-cell Western analysis

According to the description by Egorina et al. (2006) and our previous procedure (Li et al., 2011, 2012b), we used a newly set up assay called in-cell Western to determine the in-cell protein level of interest. The procedure was as follows: normal ESCs (n = 6) from healthy controls or eutopic or eutopic ESCs from women with endometriosis (n = 6) in the proliferative phase of the cycle were seeded at a density of 2 × 10^4 cells/well in 96-well plates. The cells were incubated with 0–10 nM 17beta-estradiol for 48 h; then we evaluated the expression of NME1 in the ESCs by the in-cell Western. In a similar way siRNA-transfected eutopic ESCs were seeded in 96-well plates (2 × 10^4 cells/well), and incubated with or without 30 μM-U0126, 50 μM-LY294002, 30 nM-Marimastat (broad spectrum MMP inhibitor), 5 nM-ARP 101 (selective inhibitor of MMP-2; invasion assay only), 1 μg/ml anti-integrin β1, 1 μg/ml anti-integrin αvβ3-neutralizing antibody for adhesion assay only) for another 24 h, with vehicle as control. Then the proliferation, invasion and adhesion of ESCs were measured with 5-bromo-2′-deoxyuridine (BrdU) cell proliferation assay kits (Millipore, MA, USA), Matrigel invasion assay and adhesion assay kits (Cell Biolabs; San Diego, CA, USA), respectively, according to the manufacturer’s instruction or our previous procedure (Li et al., 2011, 2012b; Mei et al., 2012).

Flow cytometry was performed to analyze the expression of integrin α1β1, α2β1, α4β1, α5β1, α6β1 and αvβ3 on ESCs. Samples were analyzed in a FACS Calibur flow cytometer (Becton Dickinson, USA) by using the Cellquest software (Becton Dickinson). Statistical analysis was conducted by using isotype-matched controls.

Figure 1  The expression of NME1 is decreased in the eutopic and ectopic ESCs from women with endometriosis. (A) Representative photomicrographs to show the expression of NME1 (brown staining) in eutopic endometrium from a healthy woman (normal) and a woman with endometriosis in the proliferative and secretory phases of the cycle and in ectopic endometrium in the proliferative phase. Slides are counterstained with hematoxylin. Original magnification: × 200. (B) Quantitative real-time PCR of NME1 mRNA (C) in-cell Western measurements of NME1 protein. (B) and (C) compare ESCs from women without endometriosis (n = 6), with ESCs from eutopic (n = 6) or ectopic (n = 6) sites in women with endometriosis. Data are mean ± SD, **P < 0.01 compared with normal.
**Statistics**

All values are shown as mean ± SD. Data were analyzed using one-way analysis of variance and the least significant difference (equal variances assumed), or Tamhane’s test (equal variances not assumed) for post hoc multiple comparisons. Calculations were done with Statistical Package for the Social Sciences software version 11.5. Differences were considered as statistically significant at \( P < 0.05 \).

**Results**

The expression of NME1 is decreased in the eutopic and ectopic ESCs from women with endometriosis

To investigate the possible regulation of NME1 on the biological behavior of ESCs, we first compared the expression level of NME1 in the endometrium with or without endometriosis. As depicted in Fig. 1, both epithelial and stromal cells from the eutopic endometrium and endometriotic tissues were weakly stained for NME1, in contrast to stronger staining in normal endometrium. In parallel, we isolated normal ESCs from healthy controls and eutopic and ectopic ESCs from women with endometriosis, and found that both mRNA and protein levels of NME1 were higher in the healthy ESCs than that in the eutopic and ectopic ESCs (Fig. 1B and C). However, there was no difference in NME1 expression between the eutopic and ectopic ESCs (Fig. 1B and C).

Estrogen down-regulates NME1 expression in ESCs

It is thought that endometriosis is an estrogen-driven disease. Therefore, we next evaluated the modulation of estrogen on the NME1 expression in ESCs. Data are presented in Fig. 2 that 17-beta estrogen could down-regulate the NME1 expression in all the three kinds of ESCs at a concentration of \( 10^{-8} \) M (Fig. 2).

NME1 down-regulates PCNA and survivin, and limits the proliferation of ESCs through Akt and MAPK/Erk1/2 signaling pathways

The phosphatidylinositol 3-kinase (PI3K)/Akt and MAPK signaling pathways are involved in regulation of cell growth and invasion. To further clarify the action of tumor metastasis suppressor gene NME1 on ESC biological behavior, we studied the effect of silencing NME1 expression in eutopic ESCs from women with endometriosis (Fig. 3A), on proliferation as measured by the BrdU proliferation assay and on the phosphorylation level of the Akt and MAPK signal pathways by in-cell Western. As shown in Fig. 3, NME1 obviously restricted the proliferation (Fig. 3B) and inhibited Erk1/2 and Akt signaling (Fig. 3C), but did not influence the phosphorylation level of P38 and JNK (Fig. 3C). On the other hand, silencing NME1 strikingly increased the expression of the survival-related molecules PCNA (Bischoff and Simpson, 2004) and survivin (Ueda et al., 2002) (Fig. 3D), and the increase in these molecules and in cell proliferation were abrogated by LY294002 or U0126 (Fig. 3D and E).

The increase in ESC invasiveness induced by NME1 suppression is independent of MMPs and TIMPs

Next we investigated the effect of silencing NME1 on the invasiveness of ESCs and the expression of metalloproteinase 2/9 (MMP2/9) and the tissue inhibitor of metalloproteinase 1/2 (TIMP1/2). NME1 knockdown significantly increased the invasiveness of cells (Fig. 4A). Both U0126 and LY294002 could reverse this effect (Fig. 4C), and modulate the protein level of MMP1/2 and TIMP1/2 (Fig. 4B). However, silencing NME1 in ESCs failed to change the expression of MMP2/9 and TIMP1/TIMP2 (Fig. 4B). Moreover, neither a broad spectrum MMP inhibitor (Marimastat) nor a selective inhibitor of MMP-2 (ARP 101) abrogated the increase in invasiveness of ESCs induced by NME1 silence, although these inhibitors significantly down-regulated the invasiveness of ESCs (Fig. 4D).
Figure 3  NME1 down-regulates PCNA and survivin, and limits the proliferation of ectopic ESCs through the Akt and MAPK/Erk1/2 signaling pathways. (A) NME1 expression in eutopic ESCs from women with endometriosis (n = 6) transfected for 72 h with an irrelevant siRNA, no siRNA (control) and with siRNA designed to silence NME1 as measured by in-cell Western analysis (NME1 (red); actin (green)). (B) The effect of silencing NME1 on the proliferation of ESCs as measured by the 5-bromo-2′-deoxyuridine (BrdU) assay (n = 6). (C) The phosphorylation level of Akt, p38, JNK, Erk1/2 in siRNA-transfected eutopic ESCs from women with endometriosis (n = 6) after 72 h. Here Phospho-Akt, phospho-p38, phospho-JNK and phospho-Erk1/2 are depicted in red; Akt, p38, JNK and Erk1/2 are depicted in green. (D and E) Then in-cell Western and BrdU proliferation assays were used to detect the expression of PCNA and survivin (D), and the proliferation (E) of NME1-silenced eutopic ESCs from women with endometriosis (n = 6) treated with U0126 (MAPK/ERK1/2 inhibitor) or LY294002 (Akt inhibitor). Here PCNA, actin for survivin group is depicted in red; survivin, actin for PCNA group is depicted in green. Here blank: transfection without siRNA; Ctrl: the non-targeting siRNA oligonucleotides; si-NME1: NME1 is silenced. These pictures are representatives of three individual experiments. *P < 0.05, **P < 0.01 compared with the negative control. #P < 0.05, ##P < 0.01 compared with NME1 silence.
Figure 4 The increase in ESC invasiveness induced by silencing NME1 is independent of matrix metalloproteinases (MMPs) and tissue inhibitors of metalloproteinases (TIMPs). (A) Invasiveness of siRNA-transfected ESCs from eutopic endometrium from women with endometriosis and non-transfected controls measured with the Matrigel invasion assay (mean ± SD, n = 6). The photomicrographs show representative fields of Matrigel invaded by control and siRNA-transfected ESCs. Original magnification: ×200. (B) In-cell Western analyses to show the effect of silencing NME1 on the expression of MMP2, MMP9, TIMP1 and TIMP2, and the effects of U0126 and LY294002 (mean ± SD, n = 3). In the pictures of representative in-cell Western analyses MMP2, MMP9, TIMP1 and TIMP2 stain red and actin green. (C and D) The effects of U0126 and LY294002 (C), and Marimastat (a broad spectrum MMP inhibitor) or ARP 101 (a selective inhibitor of MMP-2) (D) on the invasiveness of NME1 silenced and control ESCs from eutopic endometrium from women with endometriosis (mean ± SD, n = 6). Statistical significance, *p < 0.05, **p < 0.01 compared with the negative control. ###p < 0.01 compared with NME1 silenced. *p < 0.05 compared with the negative control plus U0126 or LY294002 treatment (C), plus Marimastat or ARP 101 treatment (D).
NME1 silence-mediated up-regulation of cell adhesion-related molecules integrin β1 and integrin αvβ3 on ESCs is dependent on different signaling pathway

Silencing NME1 in ESCs enhanced their adhesion to Collagen IV and Fibronectin (Fig. 5A), but did not change their adhesion to Fibrinogen, Lamin I and Collagen I (Fig. 5A).

Figure 5 Mediation of the up-regulation of cell adhesion-related molecules integrin β1 and integrin αvβ3 induced by silencing NME1 in ESCs depends on different signaling pathway. (A) The ability of NME1-silenced ESCs to adhere to various extracellular matrices (mean ± SD, n = 6). (B and C) Flow cytometry analysis of the expression of integrin α1β1, α2β1, α4β1, α5β1, α6β1 (B), or β1 and αvβ3 (C) on siRNA-transfected ESCs from eutopic endometrium from women with endometriosis incubated with or without 30 μM-U0126 or 50 μM-LY294002 (mean ± SD n = 6). Statistical significance, *p < 0.05, **p < 0.01 compared with the negative control. ***p < 0.01 compared with NME1 silence. Δp < 0.05, ΔΔp < 0.01 compared with non-targeting siRNA transfection plus U0126 or LY294002 treatment (B and C).

Integrins work alongside other proteins such as cadherins, immunoglobulin superfamily cell adhesion molecules, selectins and syndecans to mediate cell–cell and cell–matrix interaction and communication. Integrins bind cell surface and cell-extracellular matrix (ECM) components such as fibronectin, vitronectin, collagen and laminin. To investigate whether integrins and other signaling molecules were involved in the NME1-elicited adhesion regulation on ESCs, flow cytometry for integrins, E-Cadherin, CD62L, CD62E and CD44 on NME1-silenced ESCs was performed. Interestingly, silencing NME1 could promote the expression of integrin α1β1, α2β1, α4β1, α5β1 and αvβ3 on ESCs (Fig. SB and C and Supplementary data, Figs S1 and S2), whereas this...
had no effect on the expression of α6β1 (Fig. 5B and Supplementary data, Fig. S1), E-Cadherin, CD62L, CD62E and CD44 (Supplementary data, Fig. S3). Addition of U0126 to the NME1-silenced ESCs abolished the effect of NME1 on the expression of integrin α1β1 and α2β1. Similarly to U0126, LY294002 could neutralize the increase of integrin α4β1 and α5β1 induced by NME1 silence (Fig. 5B and Supplementary data, Fig. S1). Both U0126 and LY294002 decreased the expression of integrin α3β3, but did not influence the expression of integrin α1β3 induced by NME1 (Fig. 5C and Supplementary data, Fig. S2).

Moreover, not only U0126 but also LY294002 could completely abolish the enhancement of the adhesion of ESCs to Collagen IV and Fibronectin induced by silencing NME1 (Fig. 6A and B). Different from integrin β1-neutralizing antibody, blocking integrin αβ3 only reversed the NME1 silence-mediated elevation of adhesion to Fibronectin but not to Collagen IV (Fig. 6A and B).

Collectively, these data have demonstrated that silencing NME1 leads to the increase of integrins and the ability of ESC adhesion to Collagen IV and Fibronectin, and these effects could be partly abrogated by Akt and/or MAPK/Erk1/2 inhibitors.

## Discussion

Endometriosis results from increased cellular proliferation, adhesion and invasion of the retrograde endometrium in response to appropriate stimuli. These differences between the biological phenotype of the eutopic endometrium from women with endometriosis, and that of women without endometriosis may contribute to the survival and ectopic implantation of the regurgitated endometrial cells into the peritoneal cavity and thus to the development of endometriosis.

The key finding of the present study is that down-regulation of NME1 can promote the proliferation, adhesion and invasion of ESCs by activating MAPK/Erk1/2 and Akt signaling pathways. In addition, as shown in Fig. 7, we have found that these effects are dependent on the elevated expression of downstream molecules, such as PCNA, survivin and integrin β1 via activating Akt and MAPK/ERK1/2 signaling pathways and integrin αβ3 through an unknown signal, which leads to enhanced survival, adhesion and invasion of ESCs in the endometriotic lesions.

**Figure 7**  Schematic roles of NME1 in regulating the biological behavior of ESCs. The ESCs in which NME1 is suppressed owing to inherent defects or the effect of estrogen regurgitate into the peritoneal cavity, which up-regulates the expression of PCNA, survivin and integrin β1 via activating Akt and MAPK/ERK1/2 signaling pathways and integrin αβ3 through an unknown signal, which leads to enhanced survival, adhesion and invasion of ESCs in the endometriotic lesions. 
signaling pathways, such as Akt, MAPK/Erk1/2 and other unknown signal pathways.

Moreover, integrins regulate various cellular functions including motility, migration, death, metastasis and proliferation (Hynes, 1992), and they are also related to the progression of uterine adenomyosis (Klemmt et al., 2007). Integrin β1 signal is involved in stimulating CCL2 secretion and CCR2 expression of ESCs (Li et al., 2011), which may further promote proliferation, invasion and implantation of ESCs (Li et al., 2012a). Therefore, it can be proposed that increased integrin β1 induced by NME1-deficient ESCs may contribute to growth, survival and invasion in endometriotic lesions.

Accumulating evidence suggests that NME1 silence promotes cellular scattering, motility and ECM invasion of cancer cells by up-regulating several MMPs, including MMP9 and MMP2, and increasing TIMPs through the RAS-RAF-MEK-Erk-ELK1 pathway, PI3K-Akt-NF-kB/AP-1 pathway and RAS-RAC-MKK3/6-p38 pathway, and promotes VEGF expression, finally weakening the metastatic ability of tumor cells (Ohba et al., 2003; Che et al., 2006; Horak et al., 2007; Boissan et al., 2010). However, interestingly, our data demonstrated that NME1 knockdown can activate the MAPK/Erk1/2 and Akt signals, but not the P38 and JNK signals, and further enhance the invasion mediated by survivin (Li et al., 2012a) and integrins, but not MMP2/9 and TIMP1/2, which echoed the previous results (Xie et al., 2010). These effects of NME1 are different from those of tumor cells that are dependent on MMP2 and MMP9.

In conclusion, our findings suggest that the down-regulation of NME1 by estrogen is partly responsible for the up-regulation of ESC survival, growth, adhesion and invasion in vitro by MAPK/Erk and Akt-integrins and other signaling pathways to a certain extent.

Supplementary data
Supplementary data are available at http://humrep.oxfordjournals.org/.

Authors’ roles
M.-Q.L. and J.S. conducted all experiments and prepared the figures and the manuscript. Y.-H.M, J.M., Y.W., H.L., L.Z. and K.-K.C. assisted with the flow cytometry assay, cell adhesion assay, immunohistochemistry and in-cell Western analysis, respectively. X.-Y.Z. and X.-Q.W. examined patients, obtained specimens and generated clinical data. D.-J.L. initiated and supervised the project and edited the manuscript. All the authors were involved in writing the manuscript.

Funding
This study was supported by National Natural Science Foundation of China (NSFC) (31270969) to D.-J.L.; NSFC 31101064 to M.-Q.L.; Program for ZhuoXue of Fudan University to M.-Q.L. and NSFC 81270677 to X.-Y.Z.

Conflict of interest
None declared.

References
Kim SH, Kim J. Reduction of invasion in human fibrosarcoma cells by ribosomal protein S3 in conjunction with Nm23-H1 and ERK. Biochim Biophys Acta 2006; 1763: 823–832.
Li MQ, Luo XZ, Meng YH, Mei J, Zou XY, Jia LP, Li DJ. CXCL8 enhances proliferation and growth and reduces apoptosis in endometrial stromal


