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MELK as a potential target to control cell proliferation in triple-negative breast cancer MDA-MB-231 cells

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Abstract. Maternal embryonic leucine zipper kinase (MELK) is an important regulator in tumorigenesis of human breast cancer, and if silenced leads to programmed cell death in specific breast cancer cell lines, including MDA-MB-231 cells. In the present study, RNA interference, proliferation assay and semi-quantification of cell cycle relative proteins were performed to determine the effects of MELK in human breast cancer cells. Data demonstrated that the highest level of MELK protein in the MDA-MB-231 cell line among eight breast cancer cell lines. The sensitivity of MELK small interfering-RNA varied in different breast cancer cell lines, but MELK silencing resulted in marked suppression of proliferation of triple-negative breast cancer (TNBC) and non-TNBC cells. Specific silencing of MELK caused G2 arrest in TNBC MDA-MB-231 and HCC1143 cells, and G1 arrest in non-TNBC T47D and MCF7 cells. Notably, the knockdown of MELK did not induce apoptosis in HCC1143 cells, indicated by the lack of caspase-3 expression. In addition, in response to MELK silencing, cyclin B and cyclin D1 were downregulated in four breast cancer cell lines. Furthermore, the silencing of MELK resulted in the upregulation of p21, p27 and phosphorylated (p)-c-Jun N-terminal kinase (JNK) in HCC1143 TNBC cells, and downregulation of p21 and p-JNK in T47D non-TNBC cells. Additionally, MELK protein was markedly suppressed in non-TNBC cells in response to estrogen deprivation. The findings from the present study suggested that MELK may be a potential target in MDA-MB-231 cells, although genetic knockdown of MELK resulted in inhibitory effects on proliferation of TNBC and non-TNBC cells. MELK exert its effect on different breast cancer cells via arrest of different cell cycle phases and therefore mediated by different mediators, which may be involved in the crosstalk with MELK signaling and with the estrogen receptor signaling pathway.

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Introduction

As the most common malignancy in females with an increasing rate of morbidity, breast cancer is a heterogeneous disease with a high degree of diversity in histology, therapeutic response and treatment outcomes (1). Based on transcriptional profiling analysis, breast cancer is reproducibly identified as one of the major intrinsic subtypes, including normal breast-like, luminal A, luminal B, epidermal growth factor receptor-2 (HER2)/Neu-enriched and basal-like breast cancer (2,3).

Breast cancer can also be categorized on the basis of expression of the estrogen receptor (ER), progesterone receptor (PR) and human HER2 (4,5). Notably, basal-like breast cancer largely overlaps with tumors lacking ER/PR and HER2 expression. Triple-negative breast cancer (TNBC) is particularly 'stem-cell-like' as it adopts properties of stem cells, including self-renewal (6). Cancer stem cells (CSCs) have been considered as key contributors to the development and progression of malignant tumors, including initiation, sustenance, metastasis and recurrence, in addition to resistance to conventional chemotherapy. Therefore, simultaneous targeting of CSCs and non-CSCs holds great potential towards the development of more efficient therapeutic methodologies for each type of cancer (7).

Maternal embryonic leucine zipper kinase (MELK), also known as murine protein serine threonine kinase 38 (MPK38), is a member of AMPK/Snf1 family. MELK functions as a modulator of intercellular signaling, including the apoptosis signal-regulating kinase/Jun N-terminal kinase (JNK) pathway, p38 signaling (8) and NF-κB pathway (9), which affects various cellular and biological processes. Currently, MELK has been demonstrated as a key regulator in the malignancy and proliferation of CSCs, and therefore it is considered as an attractive molecular target to eliminate various CSCs (10-12). Previous data has documented that MELK is an important contributor in the tumorigenesis of human mammary epithelial cells. MELK is highly expressed in human breast cancer, and its overexpression is strongly associated with poor disease outcomes (13-20). In addition, MELK expression in breast cancer has a significant inverse correlation with the expression of luminal markers,

including ER and PR (21). Therefore, MELK is aberrantly overexpressed in ER/PR$^-$ tumors compared with tumors with ER/PR$^+$ status (21). Indeed, MELK has been considered as an oncogenic kinase that is essential for mitotic progression in basal-like breast cancer cells (21). Specific targeting of MELK enables induction of programmed cell death of specific breast cancer cell lines, including TNBC MDA-MB-231, and BT-549, as indicated by cleaved PARP (poly ADP-ribose polymerase) (21). PARP, a 116 kDa nuclear polymerase, is a highly conserved nuclear enzyme implicated in DNA repair and in the apoptotic response of cells. This protein can be cleaved by numerous caspases *in vitro* and is one of the main cleavage targets of caspase-3 *in vivo*. The cleavage occurs between ASP214 and Gly 215, which separates PARP's N-terminal DNA binding domain (24 kDa) from its C-terminal catalytic domain (89 kDa). It has been demonstrated that cleavage of PARP facilitates cellular disassembly and inhibition of PARP cleavage attenuates apoptosis *in vitro* (22). Thus, MELK has promising potential as a molecular target in breast cancer therapy, and therefore it is warranted to extensive studies on the mechanisms involved.

The present study reports that MELK expression does not absolutely associate with ER expression. Although the knockdown of MELK may lead to marked inhibition in the proliferation of TNBC and non-TNBC cells, specific targeting of MELK did not result in apoptosis in TNBC or HCC1143 cells. MELK exerts its effect on TNBC and non-TNBC cells via inducing arrest at different phases of the cell cycle and by different mediators. The ER signaling pathway may participate in the regulation of MELK expression. When taking into consideration with previous data, MELK may be used as a specific target to control cell proliferation in MDA-MB-231 cells but not all TNBC cells.

Materials and methods

Cell lines, antibodies and reagents. Human mammary epithelial cell line MCF10A and different breast cancer cell lines (T47D, HCC712, MCF7, ZR75-1, MDA-MB-361, HCC1937, HCC1806 and MDA-MB-231) used in present study were obtained from the American Type Culture Collection (Manassas, VA, USA). DMEM/F12, RPMI 1640 and fetal bovine serum (FBS) were purchased from Thermo Fisher Scientific, Inc. (Waltham, MA, USA). Primary and secondary antibodies used for immunoblotting were purchased from Cell Signaling Technology, Inc. (Danvers, MA, USA). Other reagents including; EGF, insulin, hydrocortisone, antibiotics, 50 μg/ml gentamycin, pyruvate, 10 mM Hepes, 4.5 g/l glucose, 0.25% EDTA-containing trypsin, estradiol, dextran charcoal-stripped bovine serum, MTT reagent, propidium iodide and bovine serum albumin were products of Sigma-Aldrich (Merck KGaA, Darmstadt, Germany).

Cell culture. Human mammary epithelial cells, MCF10A were maintained in DMEM/F-12 supplemented with EGF (10 ng/ml), insulin (10 μg/ml), and hydrocortisone (0.5 μg/ml) in a humidified incubator with 5% CO_2 at 37˚C. All breast cancer cell lines (T47D, HCC712, MCF7, ZR75-1, MDA-MB-361, HCC1937, HCC1806 and MDA-MB-231) used in the present study were propagated in RPMI 1640 medium containing 10% FBS and antibiotics (penicillin and streptomycin) and supplements (50 μg/ml gentamycin, pyruvate, 10 μM Hepes and 4.5 g/l glucose) in a humidified 37˚C incubator containing 5% CO_2.

Estrogen deprivation treatment. The wild-type MCF7 and ZR75-1 cells were cultured in phenol red-free RPMI 1640 medium supplemented with 10% FBS and 1 nM estradiol (E2) in a 37˚C incubator for 1 week. For estradiol deprivation treatment, cancer cells were cultured in phenol-free RPMI medium in the absence of exogenous E2 and supplemented with 10% dextran charcoal-stripped bovine serum (DCC). The cells were trypsinized using 0.25% EDTA-containing trypsin at base line, 1-week post estradiol deprivation (short-term estradiol deprivation, STED) and at the point of resistance (long-term estradiol deprivation, LTED) (23).

Small-interfering RNA (siRNA) treatment. For knockdown experiments, breast cancer cell lines (HIM3, HCC1806, MDA-MB-231, HCC1143, BT549, HCC1937, SKBR3, T47D, MCF7 and HCC712) and human mammary epithelial cell MCF10 were transiently transfected with 200 pmol oligo siRNA using Lipofectamine® RNAiMAX (Invitrogen; Thermo Fisher Scientific, Inc.) according to the manufacturer's protocol. The siRNA targeting MELK (siMELK, 5'-GACAUC CUAUCUAGCUGCA-3') and scrambled negative control (5'-GUGGGCAACAUUCUUCGAATT-3') were purchased from Sigma-Aldrich (Merck KGaA, Darmstadt, Germany). Subsequent experimentation was conducted 3 days following transfection.

Cell proliferation assay. The cells treated with siMELK or negative control (50 nM) were seeded at a density of $1x10^4$ cells/well in 96-well plates. Cell proliferation was quantified by MTT reduction (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl-tetrazolium bromide). Formazan salt was dissolved in acid isopropanol, and absorbance was assessed at 570 and 630 nm on a microplate reader. The results are expressed as increases in absorbance (570-630 nm). The experiments were performed at least three times in triplicate.

Immunoblotting. The cells were lysed at 4˚C for 15 min with RIPA buffer (25 mM Tris, pH 7.4, 150 mM NaCl, 1% Nonidet p-40, 0.5% sodium deoxycholate, and 0.1% sodium dodecyl sulfate) containing protease cocktail inhibitors (Roche Applied Science, Penzberg, Germany) and phosphatase inhibitor cocktail (Thermo Fisher Scientific, Inc.). Protein quantification was performed using a BCA Protein assay (Pierce Biotechnology; Thermo Fisher Scientific, Inc.), and aliquots of 20 μg total protein was resolved on SDS-PAGE (12% gel) and transferred onto a nitrocellulose membrane. Subsequently, the membrane was blocked with 5% non-fat milk (dissolved in TBST; incubation for 1 h at room temperature) and was then incubated with the following primary antibodies: Anti human β-actin (monoclonal; cat. no. 3700; 1:1,000), anti human MELK (polyclonal; cat. no. 2274; 1:1,000), anti human p-JNK (monoclonal; cat. no. 9255; 1:2,000), anti human p-p38 (polyclonal; cat. no. 4511; 1:1,000), anti human p-ERK1/2 (polyclonal; cat. no. 4370; 1:2,000), anti human p27 (polyclonal; cat. no. 3686; 1:1,000), anti human p21 (polyclonal; cat. no. 2947; 1:1,000), anti human p53 (monoclonal; cat. no. 2524;

1:1,000), anti human cyclin B (monoclonal; cat. no. 4135; 1:2,000), anti human p-cdc2 (polyclonal; cat. no. 9111; 1:1,000), anti human cyclin A (monoclonal; cat. no. 4656; 1:2,000), anti human cyclin E (polyclonal; cat. no. 20808; 1:1,000), anti human cyclin D1 (polyclonal; cat. no. 2978; 1:1,000), anti human CDK2 (polyclonal; cat. no. 2546; 1:1,000), anti human caspase-3 (polyclonal; cat. no. 9662; 1:1,000) overnight at 4˚C. After washing with TBST, the membrane was incubated with horseradish peroxidase-conjugated secondary antibodies including: Anti-mouse immunoglobulin G (cat. no. 7076; 1:1,000) and anti-rabbit immunoglobulin G (cat. no. 7074; 1:1,000) at room temperature for 2 h. The target signals were visualized and semi-quantified as the ratio of target protein relative to β-actin.

Cell cycle analysis. The cells were trypsinized and repeatedly pipetted into single-cell suspension. Following centrifugation at 600 x g for 5 min at 4˚C, the cells were fixed by adding 70% ethanol (-20˚C) in drops while vortexing. Subsequently, the cells were stained with 50 μg/ml propidium iodide solution containing 50 μg/ml DNase-free RNase A and 0.5% bovine serum albumin (BSA). Following incubation for 30 min, the cells were washed and resuspended in 0.5% BSA. Cell cycle analysis was performed on a LSRFortessa (BD Biosciences, San Jose, CA, USA) at the DFCI flow cytometry core facility. Single cells were gated by plotting FL3-A to FL3-H in order to exclude cell debris and doublets. A minimum of $1x10^4$ single cells was collected from each sample.

Statistical analysis. The data are presented as the mean ± standard deviation, and analyzed using SPSS 17.0 (SPSS, Chicago, IL, USA). Two-tailed unpaired Student's t-test was used to analyze difference between two groups. $P<0.05$ was considered to indicate a statistically significant difference.

Results

High MELK protein expression is observed in MDA-MB-231 cells. To validate the expression patterns of MELK protein, human breast epithelial MCF10A cell line and eight breast cancer lines, including ER^+ and ER^- breast cancer cell lines, were subjected to semi-quantification analysis. MELK was hardly detectable in human breast epithelial MCF10A cells, but aberrantly overexpressed in ER^+ and ER^- human breast cancer cells (Fig. 1).

Treatment with MELK siRNA suppresses the proliferation of TNBC and non-TNBC cells. Following the confirmation of MELK overexpression in breast cancer cell lines, MELK siRNA and scrambled siRNA (as a negative control) was used to treat human breast epithelial MCF10A cells and several ER^+ and ER^- human breast cancer cell lines. Following 5 days of incubation, cell proliferation was analyzed. As indicated in Fig. 2, the silencing of *MELK* resulted in a small decrease in the proliferation rate of human breast epithelial cells MCF10A, which is as hypothesized as MELK expression was barely detectable in this cell line. By contrast, the knockdown of *MELK* in breast cancer cell lines (duration of incubation, 5 days) resulted in marked decreases in proliferation compared

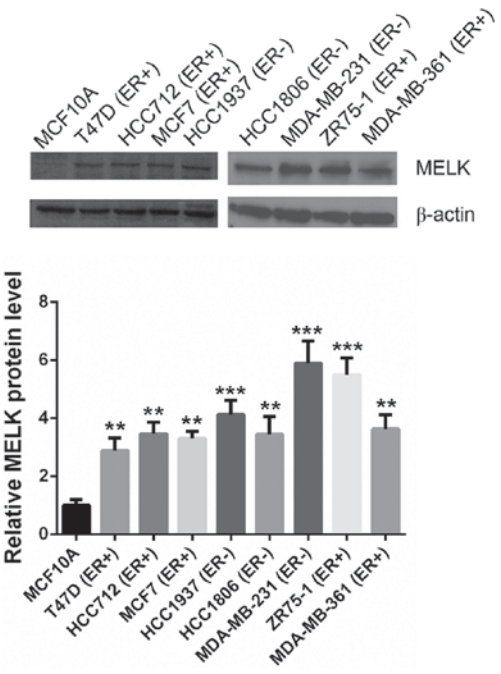


Figure 1. Expression of MELK protein in human mammary epithelial cells and eight breast cancer cell lines. ER, estrogen receptor; MELK, maternal embryonic leucine zipper kinase. Data is presented as the mean ± SD. $^{**}P<0.01$, $^{***}P<0.001$.

with the cells that were transfected with negative control. Notably, the biggest decrease in proliferation was observed in the siRNA-transfected HCC1143 TNBC cells. By contrast, there was only a small decrease in proliferation in siRNA-transfected HCC712 cells, which are $ER^+/PR^+/HER^-$. Furthermore, specific targeting of *MELK* results in inhibitory effects on MDA-MB-231 cells, which was comparable to the effects observed on MCF-7 and T47D cells.

Treatment with MELK siRNA induces G2 arrest in TNBC cell line and G1 arrest in non-TNBC cell line. In order to determine whether MELK affects the proliferation of breast cancer cells, which may be attributable to its regulation of the cell cycle, two TNBCs lines (MDA-MB-231 and HCC1143) and two non-TNBC breast cancer lines (T47D and MCF7) were analyzed for cell cycle distribution. Compared with the data from MDA-MB-231 cells that were treated with scrambled siRNA, specific silencing of *MELK* in MDA-MB-231 cells for 4 days produced a decrease in the number of cells in the G1 phase, and markedly increased the number of cells in the G2 phase (Fig. 3). Notably, the number of si-MELK-treated HCC1143 cells in the S phase was markedly increased compared with scrambled siRNA treated cells. Additionally, non-TNBC T47D cells that were untreated or treated with scrambled siRNA were predominantly distributed in the G1 phase, and a small proportion of the cells were distributed in the G2 phase. Specific silencing of *MELK* for 3 days resulted in a decreased number of cells in the G2 phase, and the number of cells in the G1 phase increased. In TNBC MCF7 cells, specific silencing of *MELK* for 4 days led to an increase in the proportion of cells in the G1 phase, accompanied with a marked decrease in cells in the S and G2 phases compared with untreated cells and negative siRNA-transfected with (Fig. 3).

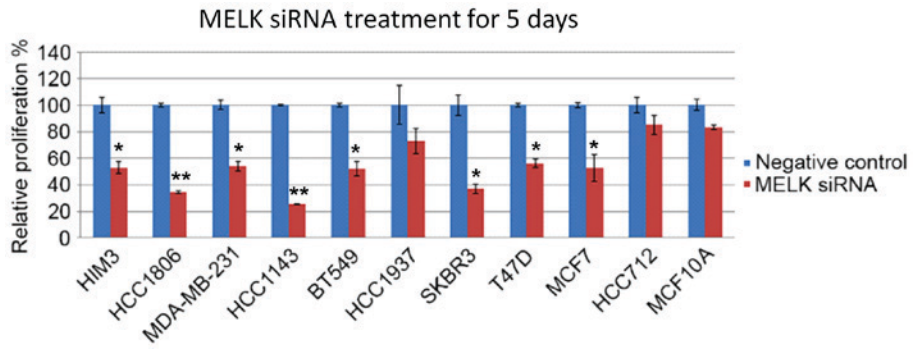


Figure 2. Effects of MELK siRNA treatment on the proliferation of human mammary epithelial cells and various TNBC and non-TNBC cell lines. Human mammary epithelial cells are MCF10. TNBC cell lines are as follows: HIM3, HCC1806, MDA-MB-231, HCC-1143, BT-549. Non-TNBC cell lines are as follows: HCC1937, SKBR3, T47D, MCF7A HCC712. Data is presented as the mean ± standard deviation. *P<0.05, **P<0.01 compared with negative siRNA treatment controls. MELK, maternal embryonic leucine zipper kinase; TNBC, triple-negative breast cancer; HIM, human in mouse.

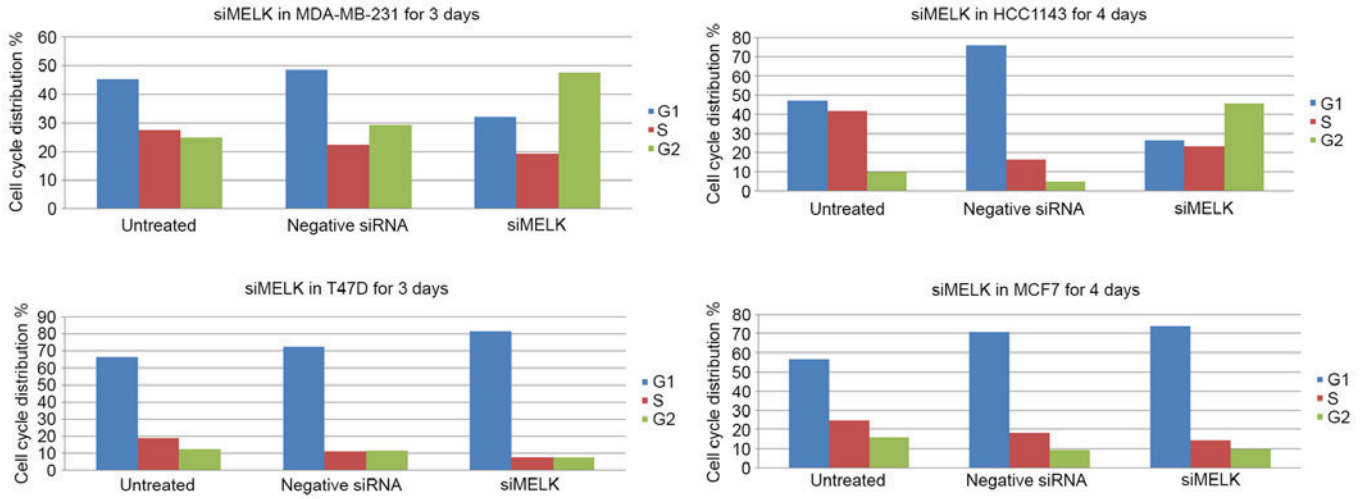


Figure 3. Cell cycle analysis of TNBC and non-TNBC cells that were treated with MELK siRNA. MELK, maternal embryonic leucine zipper kinase; siRNA, small-interfering RNA; TNBC, triple-negative breast cancer.

Treatment with MELK siRNA induces differential expression patterns in cell cycle-regulatory proteins. To investigate the molecular mechanisms of MELK on the proliferation of breast cancer cells, the levels of cell cycle-regulatory proteins were assessed in TNBC cells and non-TNBC cells. It was demonstrated that specific silencing of *MELK* resulted in marked decrease in the levels of MELK protein in HCC1143 cells. Accompanied with this inhibition, specific targeting of *MELK* by siRNA for 2 days resulted in the inhibition of cyclin B, cyclin D1 and phosphorylated (p)-cdc 2 expression, as well as the promotion of p-c-JNK, p27 and p21 in HCC1143 cells. Furthermore, detectable changes in p-p38, p53, and CDK2 expression were not observed in HCC1143 cells. Notably, caspase-3 was not detected in cells that were treated with siRNA-MELK or scrambled siRNA in HCC1143 cells. The increase in p27 and p-JNK expression as a consequence of *MELK* knockdown was also revealed in TNBC MDA-MB-231 cells, compared with scrambled siRNA. Additionally, a significant decrease in cyclin B expression was revealed in MDA-MB-231 cells as a result of specific silencing of *MELK*. As for non-TNBC MCF7 and T47D cells, specific silencing targeting *MELK* for 2 days resulted in marked decrease in cyclin D1, cyclin B, p-cdc2, p-extracellular signal-regulated kinase (ERK) 1/2, p-JNK and p21. There was no significant difference in p27 expression following the silencing of *MELK* (Fig. 4).

MELK protein is downregulated in response to estrogen deprivation in ER^{+} MCF-7 and ZR75-1 cells. To investigate the role of estrogen on MELK expression, ER^{+} breast cancer lines, MCF7 and ZR75-1 underwent estradiol deprivation treatment. In the presence of E2, there was a low expression of MELK in ER^{+} breast cancer MCF7 and ZR75-1 cells. MELK expression was significantly suppressed in breast cancer cell lines that were cultured in STED medium compared with untreated cells, and hardly detectable in the LTED medium (P<0.01; Fig. 5).

Discussion

Despite marked advances in breast cancer therapy, TNBCs remain a challenge in the clinic, attributable to stem cell-like characteristics and a relative unresponsiveness to targeted therapies. MELK is a key regulator of malignancy and proliferation of CSCs and an oncogenic kinase that is essential for mitotic progression in basal-like breast cancer cells (21). A previous study has documented that targeting MELK resulted in cell death of TNBC MDA-MB-231 cells (24). However,

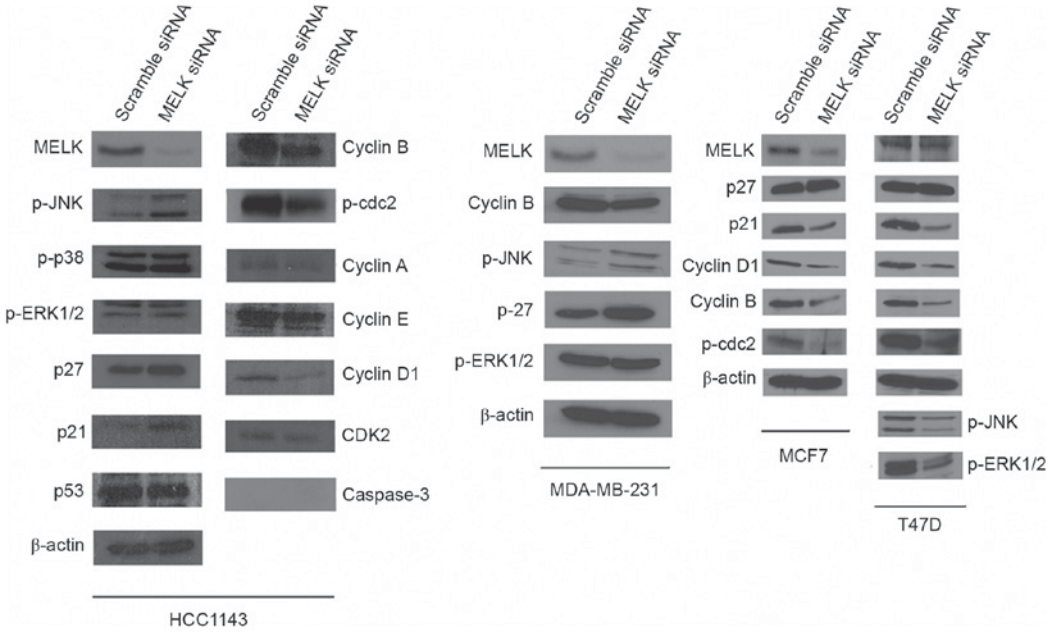


Figure 4. Expression of cell cycle-regulatory proteins in TNBC and non-TNBC cell lines that were treated with MELK siRNA. CDK2, cyclin-dependent kinase 2; ERK, extracellular signal-regulated kinase; JNK, c-Jun N-terminal kinase; MELK, maternal embryonic leucine zipper kinase; p-, phosphorylated; siRNA, small-interfering RNA; TNBC, triple-negative breast cancer.

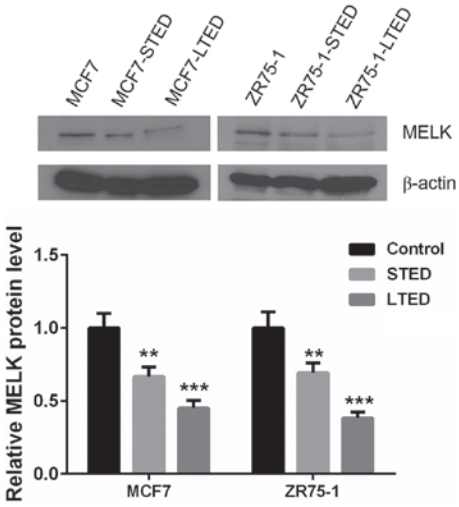


Figure 5. Estrogen deprivation caused marked increases in MELK protein in non-TNBC cells. Data is presented as the mean ± standard deviation. **P<0.01, ***P<0.001 compared with non-treated cells. MELK, maternal embryonic leucine zipper kinase; TNBC, triple-negative breast cancer; STED, short-term estrogen deprivation; LTED: long-term estrogen deprivation.

the molecular mech TNBC anism underlying this effect is still unknown (24).

In the present study, it was observed that MELK protein was aberrantly expressed in 8 ER^- and ER^+ breast cancer cell lines. MELK is highly expressed in MDA-MB-231 cells revealed in the present study may provide new data that MELK could be used a specific target to eliminate MDA-MB-231 cells. Furthermore, it was indicated in the present study that the silencing of MELK induced a marked decrease in the proliferation of MDA-MB-231 cells, which was in accordance with the findings in a previous study where the loss of MELK promoted programmed cell death of MDA-MB-231 cells (21). It was further observed that the biggest decrease in proliferation was observed in the siRNA-transfected HCC1143 TNBC cells. In addition, inhibition in the viability of non-TNBC T47D and MCF7 cells was also observed, which was comparable to the effects observed in MDA-MB-231 cells. Therefore, the four cell lines (HCC1143, T47D, MCF7 and MDA-MB-231) were employed for subsequent analysis.

In order to identify whether MELK is a cell cycle regulator, which participates in mediating inhibitory effects on breast cancer cells, the aforementioned four cell lines were employed for cell cycle analysis. The results revealed that specific targeting of MELK caused G2 arrest in TNBC lines (HCC1143 and MDA-MB-231), and G1 arrest in non-TNBC lines (T47D and MCF7), suggesting that different molecules mediate the specific targeting MELK on the proliferation of TNBC and non-TNBC cells. It is notable that specific targeting of MELK resulted in weak downregulation in the level of MELK protein in TNBC HCC1143, non-TNBC T47D and MCF7 cells. The sensitivity to specific siRNA targeting MELK in various breast cancer cell lines should be investigated individually (21). Protein quantification revealed that caspase-3 was undetectable in HCC1143 cells treated with MELK siRNA or scrambled siRNA, suggesting that genetic knockdown of MELK did not promote apoptosis of HCC1143 cells. In addition, cyclin B and cyclin D1 in TNBC and non-TNBC cells were markedly downregulated in response to silencing of MELK. Additionally, silencing of MELK in HCC1143 TNBC cells resulted in upregulation of p27 and p21, and downregulation of p21 in non-TNBC cells (MCF7 and T47D). Notably, p-JNK was upregulated in TNBC cells and downregulated in non-TNBC T47D cells as a consequence of silencing of MELK. In response to silencing of MELK, the level of p-ERK1/2 protein was decreased in T47D cells.

The effects of MELK silencing are mediated through substrates. According to previous data, MELK is able to

phosphorylate CDC25B on Ser323 *in vitro* (25), which is a critical 14-3-3 binding site (26). The 14-3-3 binding to the Ser323 site on CDC25B, blocks access of the substrate, cyclin/cdk, to the catalytic site of the enzyme and therefore directly inhibit the activity of CDC25B, which initially results in arrest at G2 (27). Additionally, MELK phosphorylates zinc finger-like protein 9 (ZPR9) and promotes its nuclear localization (28), therefore ZPR9 interacts with and increases the transcriptional activity of Myb-like protein 2 (29). This has been shown to promote DNA replication and transcriptional activation of genes, including cyclin B1, which is essential for G2/M phase progression (30,31).

In addition to substrates, molecules regulating MELK expression should be also considered. As demonstrated in the present study, estrogen deprivation led to a marked suppression in MELK protein expression in non-TNBC MCF7 and ZR75-1 cells, indicating that estrogen may be required to maintain MELK expression. It has been well documented that the ER signaling pathway crosstalks with the phosphatidylinositol 3-kinase/protein kinase B/mechanistic target of rapamycin (PI3K/Akt/mTOR) signaling pathway. Furthermore, protein kinase B (PKB)/Akt is able to phosphorylate Forkhead box protein O1 (FOXO) transcription factors and create docking sites for 14-3-3 (32). The binding of 14-3-3 to FOXO excludes FOXO from the nucleus, therefore the transcriptional activity of FOX is inhibited. By contrast, c-Jun N-terminal kinases (JNKs) and MST1 are activated by stimuli, which results in the phosphorylation of FOXOs at two different sites. The phosphorylation by JNK or MST1 promotes the nuclear localization of FOXO despite phosphorylation by Akt, thus various genes are targeted, including p27 and p21 (32,33).

It has been documented that FOXOs have a major role in G1 arrest by upregulating cell cycle inhibitors (p21 and p27), and the consequent attenuation of cell cycle promotes CDKs. Furthermore, the activation of FOXO3 is sufficient to elevate p27 mRNA and protein levels and to induce apoptosis (32-34). The data from a previous study on glioma stem cells indicated that JNK signaling regulates MELK expression and forms a complex with oncoprotein c-JUN in glioma stem cells (35), suggesting that JNK may have a dual role in regulating FOXOs and MELK expression. However, the hypothesis described here should be further validated by future studies.

In conclusion, MELK expression does not absolutely associate with ER expression in breast cancer tissues. Although the sensitivity of MELK for specific siRNA varies in TNBC and non-TNBC cells, the genetic knockdown of *MELK* resulted in a marked decrease in the proliferation of TNBC and non-TNBC cells. The silencing of MELK did not result in apoptosis in HCC1143 cells, which is indicated by the lack of caspase-3 expression. The specific targeting of MELK on TNBC and non-TNBC cells induces cell cycle arrest and different stages, for example, induces G2 arrest in TNBC cell lines and G1 arrest in non-TNBC cell lines, due to causing a decrease in cyclin B1 and an increase in p27 and p-JNK in TNBC cell lines; and a decrease in p21, cyclin B1, cyclin D1 and p-cdc2 in non-TNBC cell lines. In addition to ER66, other ER isoforms may participate in the regulation of MELK expression (36). The expression of JNK and p27 varied in response to silencing of MELK, therefore it is warranted to further investigate the role of JNK and p27 in mediating the inhibitory effect of MELK siRNA. Taken together, the results from the present study provide evidence that MELK may have potential as a specific target in MDA-MB-231 cells.

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