Downregulation of Dock1 and Elmo1 suppresses the migration and invasion of triple-negative breast cancer epithelial cells through the RhoA/Rac1 pathway

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Abstract. Dedicator of cytokinesis 1 (Dock1), a guanine nucleotide exchange factor, has been proven to facilitate cell survival, motility and proliferation via the activation of Ras-related C3 botulinum toxin substrate 1 (Rac1). Engagement of cell motility 1 (Elmo1) serves as a mammalian homolog of Ced-12, which has been evolutionarily conserved from worm to human. The present study aimed to investigate the roles and mechanisms of Dock1 and Elmo1 in the migration and invasion of triple-negative breast cancer (TNBC) epithelial cells. Cell Counting kit-8, cell migration and cell invasion assays were performed to assess cell viability, migration and invasion, respectively. A plate clone formation assay was performed to determine cell proliferation. Western blot analysis and reverse transcription-quantitative polymerase chain reaction (RT-qPCR) assays were used to evaluate mRNA and protein expression. The results revealed that the downregulation of Dock1 and Elmo1 inhibited cell viability, suppressed migration and invasion, and reduced Rac1 activity in MDA-MB-231 cells. Furthermore, downregulation of Dock1 and Elmo1 also attenuated the expression of migration-associated proteins and affected the Ras homolog gene family, member A (RhoA)/Rac1 pathway in MDA-MB-231 cells. In conclusion, the results of the present study suggested that the downregulation of Dock1 and Elmo1 suppresses the migration and invasion of TNBC epithelial cells through the RhoA/Rac1 pathway.

Introduction

Breast cancer, one of the most prevalent types of malignant tumor in females, severely impairs the health of females worldwide (1,2). Breast cancer is generally classified into five subtypes, including luminal type A, luminal type B, normal breast type, human epidermal growth factor receptor-2 (HER2) overexpression type and basal type (3). For clinical treatment and prognostic evaluation, it is conventional to assess the expression of three breast cancer markers, including estrogen receptor (ER), progesterone receptor (PR) and HER2, by immunohistochemistry, hence the concept of triple-negative breast cancer (TNBC) (4). TNBC refers to breast cancer with negative immunohistochemical results for ER, PR and HER2, and acts as a specific breast cancer subtype, which was proposed in recent years (5). Specific characteristics of TNBC include high invasiveness and metastasis, high recurrence and mortality rates, and a low survival rate. However, the exact molecular mechanisms of TNBC remain unclear.

Rho-family GTPases, a primary branch of the Ras superfamily of small GTPases, including RhoA and Ras-related C3 botulinum toxin substrate 1 (Rac1), are identified as crucial regulators of actin cytoskeleton dynamics and endothelial contractions (6). Deregulation of these Rho-GTPases may result in a disorder of the actin cytoskeleton and may further impact the integrity of the endothelial barrier. Previous studies have demonstrated that deregulated Rho-GTPases are associated with the development and progression of various types of tumor, including testicular cancer (7), colorectal tumors (8), gastric carcinoma (9) and breast cancer (10). Nevertheless, the precise role of these signaling mechanisms in the migration and invasion of TNBC cells is not well understood.

Dedicator of cytokinesis 1 (Dock1), a guanine nucleotide exchange factor, has been proven to facilitate cell survival, motility and proliferation via the activation of Rac1 (11,12). Engagement of cell motility 1 (Elmo1) serves as a mammalian homolog of Ced-12, which has been evolutionarily conserved from worm to human. Elmo1 itself has no intrinsic catalytic activity, but can regulate the activity of interacting proteins as a scaffold protein (13). Furthermore, Elmo1 also joins multiple cellular processes, including myoblast fusion, neurite outgrowth, phagocytosis of apoptotic cells and cell migration (14). Additionally, previous studies have demonstrated that by interacting with Dock1, Elmo1 acts as an element of a bipartite guanine nucleotide-exchange factor for Rac1 (15-20). Recently, it was revealed that the interaction between Dock1 and Elmo1 could modulate tumor metastasis, which is enhanced by the actin cytoskeleton in breast cancer (21). Therefore, Dock1

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and Elmo1 were selected as the objects of the present study, in order to determine their exact roles and mechanisms in the migration and invasion of TNBC epithelial cells.

The present study analyzed the association between Dock1, Elmo1 and the migration and invasion of TNBC epithelial cells. Furthermore, the roles and mechanisms of Dock1 and Elmo1 were investigated, together with those of the RhoA/Rac1 pathway in the migration and invasion of TNBC epithelial cells.

Materials and methods

Cell culture. The human TNBC epithelial MDA-MB-231 cell line was obtained from the Type Culture Collection of the Chinese Academy of Sciences (Shanghai, China). Cells were maintained in Dulbecco's modified Eagle's medium (DMEM; Gibco; Thermo Fisher Scientific, Inc., Waltham, MA, USA) supplemented with 10% fetal bovine serum (FBS; Gibco; Thermo Fisher Scientific, Inc.), and then suspended in 5% CO₂ atmosphere at 37°C.

Cell transfection. Dock1 siRNA (targeting sequence: 5' GGCTCACCTTTGCTCTGCG-3'), Elmo1 siRNA (targeting sequence: 5'-CGCAGGAUGUAACUCUGACA-3') and unspecific scrambled siRNA (50 nM) (targeting sequence: 5'-ACGUGACACGUGUCCGAGAATT-3') vectors (Invitrogen; Thermo Fisher Scientific, Inc.) were transfected into MDA-MB-231 cells using Lipofectamine® 2000 (Invitrogen; Thermo Fisher Scientific, Inc.) at 37°C for 6 h. Subsequent experiments were performed 48 h after transfection.

Grouping. In the present study, there were four treatment groups, including the control group (MDA-MB-231 cells), NC group (MDA-MB-231 cells transfected with unspecific scrambled siRNA vector), si-Dock1 group (MDA-MB-231 cells transfected with Dock1 siRNA vector) and si-Elmo1 group (MDA-MB-231 cells transfected with Elmo1 siRNA vector).

Cell viability analysis. The viability of MDA-MB-231 cells was assessed by the Cell Counting kit-8 (CCK-8; Beyotime Institute of Biotechnology, Haimen, China). MDA-MB-231 cells (~6x10⁴ cells/ml) in the logarithmic phase were plated into the wells of 96-well plates, prior to being incubated in a 5% CO₂ atmosphere at 37°C for 12 h. Cells were then treated with one of the four aforementioned treatments. Subsequently, cells were maintained for 12, 24 or 48 h. Subsequently, 10 µl CCK reagent were added into each well and cells were main maintained for 12, 24 or 48 h. Subsequently, the protocols were identical for both assays. A 450 nm. Cell viability was evaluated by the percentage of live cells using Lipofectamine® 2000 (Invitrogen; Thermo Fisher Scientific, Inc.) at 37°C for 48 h. Subsequent experiments were performed 48 h after transfection.

Western blot analysis. The protein of cells was extracted using radioimmunoprecipitation assay buffer (Beyotime Institute of Biotechnology). The bicinchoninic acid method was applied to assess the concentration of protein. Protein lysates (35 µg) of cultured MDA-MB-231 cells were separated by 12% SDS-PAGE. Subsequently, the separated products were transferred to polyvinylidene difluoride membranes (EMD Millipore, Billerica, MA, USA). TBST containing 5% skim milk was used out to block the membranes at 37°C for 60 min. Western blotting was performed using the following specific antibodies: Rabbit anti-human anti-Dock1 (dilution, 1:1,000; catalog no. ab97325; Abcam, Cambridge, UK), rabbit anti-human anti-Elmo1 (dilution, 1:10,000; catalog no. ab174298; Abcam), rabbit anti-human anti-FAK (dilution, 1:1,000; catalog no. ab40794; Abcam), rabbit anti-human anti-Talin (dilution, 1:1,000; catalog no. ab71333; Abcam), rabbit anti-human anti-Vinculin (dilution, 1:10,000; catalog no. ab129002; Abcam), rabbit anti-human anti-RhoA (dilution, 1:5,000; catalog no. ab187027; Abcam), rabbit anti-human anti-Rac1 (dilution, 1:1,000; catalog no. ab155938; Abcam) and rabbit anti-human anti-actin (dilution, 1:5,000; catalog no. ab179467; Abcam). A horseradish peroxidase-conjugated secondary antibody (goat anti-mouse IgG-HP; catalog no. sc-2005; dilution, 1:6,000; donkey anti-goat IgG-HP; cat. no. sc-2020; dilution, 1:7,000; Santa Cruz Biotechnology, Inc., Dallas, TX, USA) was added, and the membranes were incubated at room temperature for 1 h. Enhanced chemiluminescent reagents (EMD Millipore, Billerica, MA, USA) were added and an enhanced chemiluminescence system (GE Healthcare, Chicago, IL, USA) was used for evaluating the results. The blots were visualized by Gel documentation.
the migration ability of MDA-MB-231 cells. siRNA vectors targeting Dock1 and Elmo1, si-Dock1 and si-Elmo1, were constructed in the present study. The knockdown efficiency was ~45 and 70% in MDA-MB-231 cells, following stable transfection with si-Dock1 and si-Elmo1, respectively (Fig. 1A). Additionally, the western blot analysis results indicated that following transfection with si-Dock1, the expression levels of Dock1 and Elmo1 proteins in MDA-MB-231 cells were significantly reduced (P<0.01; Fig. 1B). Similarly, following transfection with si-Elmo1, the expression levels of Dock1 and Elmo1 proteins in MDA-MB-231 cells were significantly downregulated (P<0.05; Fig. 1B). Therefore, it was confirmed that interfering with the Dock1 and Elmo1 genes could simultaneously impact the expression levels of Dock1 and Elmo1 in MDA-MB-231 cells, which indicated that there is an interaction between Dock1 and Elmo1. Therefore, the CCK-8 assay was performed to measure the viability of MDA-MB-231 cells in the aforementioned treatment groups. The results demonstrate that, compared with the control and NC groups, the viability of MDA-MB-231 cells transfected with si-Dock1 and si-Elmo1, for 24 and 48 h, was significantly reduced (P<0.05; Fig. 2A).

Subsequently, the proliferation of MDA-MB-231 cells in the four treatment groups was assessed. Compared with the NC group, significant decreases in proliferation capacity were observed in MDA-MB-231 cells transfected with si-Dock1 and si-Elmo1 (P<0.05; Fig. 2B). Taken together, these results suggested that the downregulation of Dock1 and Elmo1 inhibited the viability of MDA-MB-231 cells.

Downregulation of Dock1 and Elmo1 suppresses the migration and invasion ability of MDA-MB-231 cells. Downregulation of Dock1 and Elmo1 reduces the expression levels of migration-associated proteins in MDA-MB-231 cells. In the present study, the associated migration mechanisms and Rac1 activity in MDA-MB-231 cells were also assessed. The results revealed that Rac1 activity in MDA-MB-231 cells was significantly reduced by transfecting with si-Dock1 and si-Elmo1 (P<0.01; Fig. 4A). Additionally, the expression levels of migration-associated proteins, including focal adhesion kinase (FAK), Talin, and Vinculin, in MDA-MB-231 cells were measured. The RT-qPCR results demonstrated that Dock1 and Elmo1 silencing significantly decreased the expression levels of FAK, Talin and Vinculin in MDA-MB-231 cells (P<0.05; Fig. 4B). Furthermore, western blotting results also revealed similar trends in the levels of migration-associated proteins in MDA-MB-231 cells from each treatment group (Fig. 4C). Based on these results, it was concluded that the downregulation of Dock1 and Elmo1 decreased the migration and invasion capacities of MDA-MB-231 cells.

Downregulation of Dock1 and Elmo1 reduced Rac1 activity and the expression of migration-associated proteins in MDA-MB-231 cells. In the present study, the associated migration mechanisms and Rac1 activity in MDA-MB-231 cells were also assessed. The results revealed that Rac1 activity in MDA-MB-231 cells was significantly reduced by transfecting with si-Dock1 and si-Elmo1 (P<0.01; Fig. 4A). Additionally, the expression levels of migration-associated proteins, including focal adhesion kinase (FAK), Talin, and Vinculin, in MDA-MB-231 cells were measured. The RT-qPCR results demonstrated that Dock1 and Elmo1 silencing significantly decreased the expression levels of FAK, Talin and Vinculin in MDA-MB-231 cells (P<0.05; Fig. 4B). Furthermore, western blotting results also revealed similar trends in the levels of migration-associated proteins in MDA-MB-231 cells from each treatment group (Fig. 4C). Based on these results, it was concluded that the downregulation of Dock1 and Elmo1 decreased Rac1 activity and reduced the migration and invasion abilities of MDA-MB-231 cells by downregulating the expression of FAK, Talin and Vinculin.

Downregulation of Dock1 and Elmo1 affects the RhoA/Rac1 pathway. Finally, the expression of RhoA GTPase, total RhoA, Rac1 GTPase and total Rac1 was evaluated in MDA-MB-231 cells from all four treatment groups. Western blotting results indicated that the expression level of RhoA GTPase in MDA-MB-231 cells was significantly upregulated by transfecting with si-Dock1
and si-Elmo1 (P<0.01; Fig. 5A). Additionally, it was also revealed that the downregulation of Dock1 and Elmo1 significantly reduced the expression level of Rac1 GTPase in MDA-MB-231 cells (Fig. 5B; P<0.001). However, there was no significant difference in the total RhoA and total Rac1 expression in MDA-MB-231 cells from all treatment groups (Fig. 5A and B). Therefore, it was concluded that the downregulation of Dock1 and Elmo1 affected the RhoA/Rac1 pathway in MDA-MB-231 cells.

**Discussion**

A previous study has demonstrated that Dock1 and Elmo1 are highly expressed in invasive cancer cells, accelerating cancer progression (23). In addition, an increasing volume of evidence has revealed that the silencing of Dock1 and Elmo1 can impede tumor development, including cell proliferation, invasion and migration (17,24,25). To the best of our knowledge, the roles of Dock1 and Elmo1 in the prevention and treatment of TNBC have not yet been studied. In the present study, plasmids cloned with si-Dock1 and si-Elmo1 were prepared. The knockdown efficiency was ~45% and 70% in the MDA-MB-231 cells, following stable transfection with si-Dock1 and si-Elmo1, according to the RT-qPCR and western blot analysis data. Additionally, an interaction between Dock1 and Elmo1 was identified, which was consistent with the results of previous studies (15-20). Therefore, the present study further assessed the influences of Dock1 and Elmo1 on cell proliferation. The results indicated that the downregulation of Dock1 and Elmo1 significantly inhibited the proliferation of MDA-MB-231 cells.

Cell motility is important for the majority of pathological and physiological processes, including early embryonic development, wound repair, and tumor cell migration and invasion (26). Previous studies have demonstrated that Dock1 and Elmo1 serve crucial roles in the migration and invasion of various types of tumor cells (23,27-29). Therefore, the effects of Dock1 and Elmo1 on the migration and invasion capacities of MDA-MB-231 cells were assessed. The results indicated that the downregulation of Dock1 and Elmo1 could significantly decrease the migration and invasion abilities of MDA-MB-231 cells. Furthermore, by using the Transwell chamber assay, it was demonstrated that Dock1 and Elmo1 synergistically promote the Rac-dependent cell migration process (24).
Figure 3. Downregulation of Dock1 and Elmo1 suppresses the migration and invasion abilities of MDA-MB-231 cells. (A) Migration and (B) invasion assays were performed to evaluate the migration and invasion abilities of MDA-MB-231 cells, MDA-MB-231 cells transfected with an empty vector, MDA-MB-231 cells transfected with si-Dock1 and MDA-MB-231 cells transfected with si-Elmo1. *P<0.05, **P<0.01 and ***P<0.001 vs. NC. Dock1, dedicator of cytokinesis 1; Elmo1, engulfment and cell motility 1; si, small interfering RNA; NC, negative control.

Figure 4. Downregulation of Dock1 and Elmo1 reduces Rac1 activity and the expression of migration-associated proteins in MDA-MB-231 cells. (A) A Rac activity assay was performed to evaluate the Rac1 activity of MDA-MB-231 cells. (B) Reverse transcription-quantitative polymerase chain reaction and (C) western blot analysis assays were performed to measure the expression levels of FAK, Talin and Vinculin in MDA-MB-231 cells, MDA-MB-231 cells transfected with an empty vector, MDA-MB-231 cells transfected with si-Dock1 and MDA-MB-231 cells transfected with si-Elmo1. *P<0.05, **P<0.01 and ***P<0.001 vs. NC. Dock1, dedicator of cytokinesis 1; Elmo1, engulfment and cell motility 1; Rac1, Ras-related C3 botulinum toxin substrate 1; FAK, focal adhesion kinase; si, small interfering RNA; NC, negative control.
Additionally, the present study also evaluated the Rac1 activity of MDA-MB-231 cells transfected with si-Dock1 and si-Elmo1. The results revealed that the downregulation of Dock1 and Elmo1 significantly reduced the proliferation ability and Rac1 activity of MDA-MB-231 cells. Furthermore, the impact of Dock1 and Elmo1 silencing on the migration and invasion of MDA-MB-231 cells was investigated in the present study. Based on previous reports, the expression levels in MDA-MB-231 cells of several migration-associated proteins, including FAK, Talin and Vinculin, were assessed (30-32). The data revealed that the downregulation of Dock1 and Elmo1 markedly reduced the expression levels of FAK, Talin and Vinculin in MDA-MB-231 cells. Therefore, it was confirmed that the downregulation of Dock1 and Elmo1 may decrease the migration and invasion abilities of MDA-MB-231 cells by regulating FAK, Talin and Vinculin expression.

Previous studies have demonstrated that the RhoA/Rac1 pathway is involved in the migration and invasion of tumor cells (33-36). In the migration and invasion of breast cancer cells, the RhoA/Rac1 pathway also serves vital functions (36-39). Nonetheless, in TNBC epithelial cells, the role of this pivotal, migration-associated signaling pathway remains unclear. Therefore, the present study measured the expression levels of RhoA GTPase, total RhoA, Rac1 GTPase and total Rac1 in MDA-MB-231 cells from each treatment group, and revealed that the downregulation of Dock1 and Elmo1 markedly enhanced the expression of RhoA GTPase, while reducing the expression level of Rac1 GTPase. Additionally, there was no significant difference in the total RhoA and Rac1 expression between MDA-MB-231 cells from each treatment group. Therefore, these results confirmed that the downregulation of Dock1 and Elmo1 affected the RhoA/Rac1 pathway in MDA-MB-231 cells.

Taken together, the results of the present study demonstrated that the downregulation of Dock1 and Elmo1 decreased the migration and invasion abilities of MDA-MB-231 cells by affecting the RhoA/Rac1 pathway. These results provided novel insights into the pathogenesis of TNBC, giving rise to novel possibilities for TNBC therapy.

In summary, the present study demonstrates that the downregulation of Dock1 and Elmo1 decreased the migration and invasion abilities of TNBC epithelial cells through the RhoA/Rac1 pathway. These results offer crucial insight into the mechanisms of Dock1 and Elmo1 in TNBC epithelial cells. The observed effects of Dock1 and Elmo1 on the migration and invasion of TNBC epithelial cells suggested that Dock1 and Elmo1 may be promising targets for TNBC therapies.

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Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Authors’ contributions

YL was responsible for the design of the experiment. SW and YZ were responsible for processing data. YL was a major contributor in writing the manuscript. All authors confirmed the final manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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