Traction force with extracellular matrix mediated by cytoskeleton had an effect on metastasis through SLC8A1 induced Wnt-β-catenin pathway in endometrial cancer

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Research Article

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Abstract

Endometrial cancer (EC) is a common malignant tumor of female reproductive system. It seriously affects women's health. The mechanical characteristics of EC have not been paid noticed. However, the mechanical characteristics of different types of EC are diverse. These characteristics will greatly influence the development and prognosis of cancer cells. In this study, we explored traction force in primary EC cells and EC cell lines for the first time. It was found that the traction force of primary EC cells or cell lines with metastatic capability was lower. Various clinicopathological features of EC patients are significantly associated with traction force. In addition, we also confirmed that SLC8A1 is the main molecule which had an effect on traction force. The stiffness of extracellular matrix (ECM) had an influence on its downstream cytoskeleton expression through SLC8A1, thus changing the traction force and metastatic capacity of EC cells. The mechanism behind the SLC8A1 may be related to Wnt-β-catenin pathway and ion-channel activity. SLC8A1 can significantly regulate the downstream cytoskeleton F-actin and promote EC cell metastasis. Therefore, in this study, we found that SLC8A1 is an important molecule that affects the mechanical characteristics of endometrial cancer. The molecular mechanism is through changing the cytoskeleton by regulating Wnt-β-catenin pathway.

Introduction

Endometrial cancer (EC) is one of the three major gynecological malignancies [1]. There is an increasing trend of younger generation in China [2]. In China, which is undergoing rapid socioeconomic transitions, the rates of EC have been increasing, and its onset shows a trend toward occurrence in younger women over the past decades [3]. Most patients are diagnosed with early-stage disease, which is largely curable with surgery, occasionally combined with adjuvant therapy [4]. However, outcomes in EC patients with advanced stage or metastasis are still far from satisfactory [5]. Therefore, it is more urgent to clarify the molecular mechanisms underlying the growth, metastasis, and recurrence of EC, which may foster research into potential targets for early diagnosis and gene therapy. In 2013, The Cancer Genome Atlas (TCGA) indicated that it is possible to conduct stratified treatment according to biomolecular typing [6]. However, the mortality rate of EC has not decreased, despite novel pathogenetic and molecular discoveries, and the high-cost of complex molecular sequencing technology is an obstacle to its application in developing countries. Hence, novel immunohistochemical markers need to be explored to solve this problem.

Traction force (TF) is a kind of endogenous mechanical forces in cells [7]. TF produced in cells and act on extracellular matrix (ECM) and extracellular tumor microenvironment through focal adhesion (FA), thereby regulating cell proliferation and migration [8]. The main method to measure the TFs of cells is to use the Traction Force Microscopy (TFM) [9]. TFM is not only a direct measurement of cell TF, but a measurement based on the deformation and reconstruction imposed by cells on their surrounding environment as well [10]. Research on malignant tumor TFM shows that TF plays a crucial role in the metastasis process of various malignant tumors, such as bladder cancer, breast cancer and ovarian cancer [11-13]. However,
the TF of cancer cells has tumor heterogeneity, and the TF produced by endometrial cancer remain to be unknown.

The SLC8A1, which encodes the Na⁺/Ca²⁺ exchanger, has a key role in calcium homeostasis. Our previous study suggested that as a mechanical-stimulus prognostic marker, SLC8A1 promoted the progression of EC cells. The mechanism of the biomechanics regulating progress of EC is through mediating the structure and expression of cytoskeleton. Down-regulation of the SLC8A1 lead to decreased calcium and consequently to suppressed apoptosis and increased tumor cell proliferation in penile carcinoma. What's more, SLC8A1 also play an important role in glioma through calcium flux induced by cannabidiol and TRPV4 activation in mitophagy initiation. However, the regulatory mechanisms of SLC8A1 and its surrounding mechanical force in EC have not yet been elucidated.

In the present study, we explored the mechanical characteristics in different cell lines and primary EC cells. The experiments indicated that the mechanical features are diverse in different metastatic cells. Further analyses suggested that SLC8A1 may change and promote the traction force of EC cells. The function and mechanism of SLC8A1 is validated in vitro and by sample sequencing. Our study is the first to explore mechanical force and to identify new therapeutic target for EC.

**Material And Method**

**Tissue samples collection**

A total of 24 EC tissues and 20 normal endometrial tissues were obtained from patients who were undergoing complete or partial surgical resection at the Department of Obstetrics and Gynecology in Peking University People's Hospital (PKUPH) from January 2021 to January 2022. No patients had received preoperative chemotherapy, radiotherapy, and other related anti-tumor therapies. Samples were classified based on the International Federation of Obstetrics and Gynecology (FIGO2009) staging system. All diagnoses were confirmed by two expert gynecologic pathologists. Tissue specimens were kept at -80°C. The Ethics Committee of PKUPH approved this study. Each patient provided written informed consent.

**Cell culture and transfection**

Four EC cell lines (AN3CA, ishikawa, HEC1-A, and HEC-50B) were acquired from our lab storage. HEC-1A and HEC-50B cells were cultured in MYCOY'S5A medium, while AN3CA and ishikawa cells were cultured in DMEM/F12 medium. All the media were supplemented with antibiotics and 10% fetal calf serum (Gibco, Waltham, MA, USA). All cells were maintained in a humidified incubator at 37 °C in the presence of 5% CO2. The specific small interfering RNA (siRNA) for SLC8A1 and a negative control siRNA was obtained from GenePharm (Shanghai, China). Cells were transfected with Lipofectamine RNAiMAX (Thermo Fisher Scientific, USA) according to the manufacturer's instructions and harvested for further experiments after 48 hours.
Traction force microscopy

Cells were then allowed to settle on the surface and form monolayers over 48 hours prior to imaging. In this study, we identified monolayers as the interior of continuous multicellular regions providing full coverage of the optical field with high cell density and cell–cell contact. For imaging, cells were mounted on a confocal microscope (Leica TCS SP8 with a 10×0.4 NA objective). During imaging, cells were maintained at 37°C (stage heater, Cell MicroControls, VA) and 5% CO₂ (perfusing 100% humidity prebottled 5% CO₂ in synthetic air). Cells, nuclei, and fiduciary TFM bead displacements were simultaneously imaged using fluorescent and transmission microscopy over a several hour time course at time intervals of 10-20 min. Experimental images were corrected for lateral drift in ImageJ before being analyzed in the TFM workflow for the computation of displacements, traction stress, and monolayer contractile work.

Transwell

Transwell chambers (Corning, NY, USA) with a pore size of 8 µm were used to detect cell migration. Cell lines suspended with serum-free medium (2.5 × 10⁴ cells), and the bottom of the chamber contained the DMEM/F12 medium with 10% fetal bovine serum. After incubating for 24 hours, the cells that migrated and invaded from the upper to the bottom were fixed with 4% paraformaldehyde (Beyotime) for 30 minutes and stained with crystal violet. Migrated and invaded EC cells were counted under an inverted light microscope. The number of migrated or invaded cells was quantified by counting the number of cells from 10 random fields at ×100 magnification. Finally, the pictures were obtained under microscope. Three random fields were counted and cell numbers were calculated by Image J software.

Wound-Healing Scratch Assay

5 × 10⁶ target cells were transferred into 6-well plates and incubated at 37°C until 80-90% confluence. A 200 µL sterile plastic tip was used to create a wound line across the surface of the wells, and the suspended cells were removed with PBS. Cells were cultured in reduced serum MYCOY 5A or DMEM/F12 medium in a humidified 5% CO₂ incubator at 37°C for 48 h, and then images were taken with a phase-contrast microscope. Each assay was replicated three times.

Bioinformatics analysis

Differentially expressed genes (DEGs) between EC and normal tissues were identified using Wilcoxon test after within-array replicate probes were replaced with their average via “limma” package in the R software (version 3.6.2). |Log₂ fold change (FC)| >1.0 and false discovery rate (FDR) adjusted to less than 0.05 were set as the cutoff criteria. Those significant DEGs are visualized using heatmaps and volcano plots via “pheatmap” package in the R software. Time-dependent receiver operating characteristic (ROC) curves were used to assess the accuracy of prognostic prediction models. The area under the ROC curve (AUC) >0.60 was considered an acceptable prediction, and an AUC >0.75 was recognized as an excellent predictive value. For survival analysis, patients were divided into low- and high-expression of SLC8A1
groups according to the median expression value, and then log-rank tests were used to analyze the survival data. Gene ontology (GO) \cite{17} and Kyoto Encyclopedia of Genes and Genomes (KEGG) \cite{18} pathway enrichment analyses were performed to explore the biological functions of the DEGs via the “clusterProfiler” R package. Adjusted P-value < 0.05 was set as the significance threshold, and the enrichment analysis result maps were presented by the “ggplot2” and “GOplot” R packages.

**Western blot**

Total protein extraction from endometrial tissue samples and EC cells was prepared as previously reported \cite{19}. After being quantified for protein concentration, protein was separated using SDS-PAGE electrophoresis. Fifty micrograms of protein was subjected to SDS-PAGE and transferred into NC membranes. The membranes were incubated with ImageJ software v.1.48 was used to measure the relative integrated density values (IDVs) based on GAPDH as an an the control.

**Immunofluorescence analysis**

For immunofluorescence, cells (2 × 105 cells per well) were seeded in the coverslip in a 6-well plate with a coverslip inside. After a 24 h culture, cells were then fixed in 4% paraformaldehyde at room temperature (RT) for 30 min and incubated in PBS with 0.1% Triton X-100 for 10 min on ice, and then blocked in 1% fetal bovine serum (FBS). Coverslips were moved to a piece of parafilm in a humid chamber, and 100μL anti-SLC8A1 (1:1000 dilution) was added on the coverslip for overnight incubation. Cells were then washed with 0.1% PBS, incubated with 200 μl rhodamine phalloidin of 100 nM for shielded from light 30 min, washed again, and treated with DAPI (1:5,000 dilution) for 10 min. Cells were immediately examined under the Leica SP8 Confocal Inverted Microscopy (Leica, Mannheim, Germany). ImageJ was used to analyze the gray values of immunostaining images. Briefly, images were normalized by the same parameter to subtract background staining. Then, gray values of the nucleus and cytoplasm of each cell were calculated separately. All experiments were repeated 3 times.

**Statistical analysis**

Data were presented as mean ± standard deviation (SD). Student’s t-test and one-way analysis of variance were used to analyze differences among groups. Means between the groups or within the groups were compared with the one-way ANOVA. The relationship between value of traction force and clinicopathologic parameters was analyzed using the χ2 test. Survival analysis was performed using the Kaplan–Meier method and log-rank test. Statistical analyses were performed using the SPSS 22.0 software program and GraphPad Prism 8.0 software (GraphPad Software, La Jolla, CA, USA). P values of <0.05 were considered to be significant.

**Results**

**Characteristics of primary cell from the enrolled patients**
The primary cells of tumor tissue from 24 patients with endometrial cancer were collected in this study, and their clinicopathological features are shown in **Table 1**. Among them, 91.67% (22 cases) were pathologically classified as endometrioid adenocarcinoma, 91.67% (22 cases) were positive for ER (estrogen receptor) expression, 70.83% (17 cases) were in the early stage (stage I) of endometrial cancer, and 29.17% (7 cases) were in the advanced stage (stage II-IV). The proportion of patients with pathological grading of G1, G2 and G3 was 20.83%, 45.83% and 33.33%, respectively. There were 8 patients with MELF infiltration (33.33%) and 4 patients with cervical infiltration (16.67%). In addition, 45.83% of the patients with LVSI positive, 8.33% of the patients with lymph node metastasis positive, and 16.67% of the patients with fallopian tubes and ovaries involved. The traction force difference between primary EC cells and normal endometrial cells was further measured and analyzed by single-cell traction test (**Figure 1A**). It was found that the traction force of primary EC cells in 24 cases was 79.25 ± 11.25Pa, and that of normal endometrial primary cells was 208.0 ± 28.15Pa, which was significantly higher than that of primary EC cells (**Figure 1B**). Area under ROC curve (AUC) analysis was used to evaluate accuracy of TF value in determining the nature of endometrium. The results showed that the AUC was 0.802, 95% CI was 0.670-0.933, \( p < 0.001 \) (**Figure 1C**). The results showed that the TF value of different primary cells could be used as a predictive tool for predicting EC. What's more, we also compare the TF value between patients with different metastatic features. The results indicated that primary metastatic cell performed lower value of TF (**Figure S1**). Taken together, these findings suggest that the higher the metastatic ability of EC cells, the lower the TF value, and TF value is a predictor for judging the invasion and metastasis for EC.

**Associations between clinicopathological features and TF**

To further investigated the associations between TF values and clinical features in EC patients, we compare the TF values in different clinicopathological characteristics (**Table 2**), including FIGO stage, MELF (Microcystic, elongated, and fragmented), histological type, ER (estrogen receptor), CI (cervical invasion), ovary involvement, tumor grade, and LVSI (lymph-vascular space invasion). As shown in **Figure 2**, there was no significant difference in primary cell traction in different histological types and ER expression. However, the traction force of primary cells in FIGO stage (stage I vs stage II-IV), MELF, CI, ovary involvement, grade, and LVSI were significantly different, and the traction force of primary cells with late stage, positive MEFL, positive CI, ovary involvement, higher grade, and positive LVSI were significantly lower than that of the control groups (all \( p < 0.05 \)). These results collectively indicated that poor clinical features showed lower TF values.

**High TF promotes the metastatic capability for EC cells**

In order to study the effect of cell traction on its invasion and metastasis ability, four representative EC cell lines, naming ishikawa, HEC-1-A, HEC-50B, and AN3CA were selected according to the tissue origin, pathological characteristics and pathological grading of EC cell lines. The results of single-cell traction test showed that HEC-1-A cells were taken as the control group. The migration ability of the four kinds of cells was first studied by scratch test, as shown in **Figure 3A**. It was found that the migration ability of
HEC-50B cells and AN3CA cells with low traction force was significantly stronger than that of Ishikawa cells and HEC-1A cells with higher traction force, and the migration ability of AN3CA cells with the lowest cell traction force was the strongest, which is statistically significant (p<0.05). Transwell invasion experiment is also used to investigate the invasive ability of EC cells, and the results are shown in Figure 3B. The invasive ability of HEC-50B cells and AN3CA cells is significantly stronger than that of Ishikawa cells and HEC-1A cells, which is also statistically significant. We then compare the traction force among the four cell lines. There is no significant difference in traction force between Ishikawa cells and HEC-1-A cells (57.43 ± 19.52Pa vs 103.9 ± 27.40Pa) (p>0.05). However, the traction force of HEC-50B cells (37.34 ± 9.933Pa) and AN3CA cells (17.97 ± 10.48Pa) was significantly lower than that of HEC-1-A cells. To further explore whether TF features were correlated with progesterone resistance, we investigated their TF values in progesterone sensitive and resistance groups. We found that the progesterone resistance could obviously increase the invasion and migration abilities of EC cells (Figure 3D-E). What's more, traction force measured by TFM also indicated that cells in progesterone resistance groups performed lower TF values (Figure 3F). Together, these data demonstrated that cell lines with higher metastatic ability had lower TF values.

**Bioinformatics analysis for SLC8A1 in EC**

Our previous study suggested that SLC8A1 played an essential role in mechanical-stimulus induced progression for EC patients. Therefore, we then explored whether the TF promote metastasis through SLC8A1 or not. We divided total patients into the low- and high-SLC8A1 group by its median threshold. We discovered that the high expression of SLC8A1 group possessed worse survival than the low expression group (Figure 4A, P=0.02). Then we investigated the association between SLC8A1 and different clinical variables. The results showed that EC patients, higher EC stage, and higher EC grade were prone to have a high expression of SLC8A1 (Figure 4B-D). What's more, expression of SLC8A1 also increased in positive lymph node metastasis, positive peritoneal cytology, dead status, and histological type of SEA (Figure S2A-D). We then selected DEGs between the low- and the high-SLC8A1 group with |Log FC|≥1 and FDR < 0.05. Finally, 445 DEGs were identified and these genes were plotted in heatmap and volcano (Figure 4E-F). As for functional analysis in Figure 4G, we found that most DEGs were enriched in regulation of ion transmembrane transport, calcium ion homeostasis, cellular calcium ion homeostasis, and regulation of membrane potential by GO analysis. KEGG pathway analysis showed that DEGs were mostly enriched in neuroactive ligand–receptor interaction, calcium signaling pathway, and Cell adhesion molecules (Figure 4H). In summary, these results indicated that SLC8A1 may act as a biomarker for the progression and survival for patients with EC.

**Validation of SLC8A1 expression and function in patients in PKUPH**

In order to verify the effect of SLC8A1 on the survival rate of EC and distribution of SLC8A1 in different clinicopathological types, we selected 24 EC patients from our hospital for verification. Survival analysis indicated that high expression of SLC8A1 is also associated with worse prognosis for EC patients (Figure 5A). What's more, SLC8A1 expression is higher in patients with high grade, positive LNM, positive
peritoneal cytology, and positive LVSI (Figure 5B-E). Functional analysis revealed that DEGs from high and low expression of SLC8A1 are enriched in regulation of membrane potential, regulation of Wnt signaling pathway, filopodium, and ion channel activity (Figure 5G). KEGG results suggested that these genes are enriched in Wnt signaling pathway, calcium signaling pathway, MAPK signaling pathway, and Hippo signaling pathway et al (Figure 5H). These findings illustrate that SLC8A1-related genes played an important role in the progression of EC, and Wnt signaling pathway may be induced by SLC8A1.

**SLC8A1 induces the metastasis in EC by mediating Wnt signaling pathway and TF**

In order to further study the effect of SLC8A1 on the function of EC cells, this study further investigated the expression of SLC8A1 in EC cell lines, including HEC-1-A, ishikawa, HEC-50B, and AN3CA. The results showed that the expression of SLC8A1 was the highest expression in ishikawa cell lines, with a more significantly different than other cell lines (Figure 6A). Thus, we chose ishikawa cell line to create knockdown and overexpression cell line, respectively. Next, we knockdown and overexpress SLC8A1 in ishikawa cells by transfecting small interferon plasmid. The transfection efficiency was shown in Figure 6B by western blot, and the results showed that the plasmid could significantly decrease or increase the expression of SLC8A1 compared with the control group. We further investigated whether SLC8A1 affected metastasis of EC cells. Transwell assay showed the invasive ability of ishikawa cells in SLC8A1 low expression group were significantly weakened compared with the control group, and its ability was enhanced in high expression group (Figure 6C). The migration speed of si-SLC8A1 in ishikawa cells was remarkably slower, and that of OE-SLC8A1 cells was significantly faster control group (Figure 6D). In order to study the regulatory mechanism of SLC8A1 on the mechanical stimulus of EC cells, we further explored the effect of SLC8A1 on the traction force in ishikawa by single-cell traction test. The results indicated that the traction force of endometrial cancer cells significantly decreased after overexpression of SLC8A1, and increased in the knockdown group of SLC8A1 (Figure 6E). Our previous study suggested that mechanical force and TF are closely related to cytoskeleton in cellular surface. Therefore, associations between structure or expression of cytoskeleton, F-actin, and expression of SLC8A1 are analyzed. Immunofluorescence results suggested that si-SLC8A1 and OE-SLC8A1 groups could obviously decrease or increase expression of F-actin (Figure 6F). According to enrichment of SLC8A1-associated DEGs, we investigate whether the downstream of Wnt-β-catenin pathway have an effect on F-actin. Western blot showed that the expression of Wnt, β-catenin, and F-actin decreased compared with si-SLC8A1 group. We also confirmed that overexpression of SLC8A1 is up-regulated, while its expression was significantly downregulated after adding the inhibitor of Wnt (Figure 6G). Our data strongly suggested that SLC8A1 could promote the progression and mechanical force, especially F-actin of EC through Wnt-β-catenin pathway.

**Discussion**

In this study, we investigated the biological function and clinical significance of mechanical force in the progression of EC through the regulation of SLC8A1 expression. The results of our studies provide evidence that TFs are significantly correlated with clinicopathological characteristics and metastatic
ability for EC. Primary cells or cell lines from EC with diverse invasive capability may induce deformation of cytoskeleton to change the traction force of EC cells through SLC8A1. SLC8A1 promoted EC migration through resulting in F-actin reorganization by Wnt-β-catenin signaling pathway. Specifically, we demonstrated that traction force in EC play a significant role during the progression of EC cells.

In recent years, tumor biomechanics has been the focus in many studies. The study of tumor biomechanics indicated that cancer cells play specific mechanical characteristics in the process of tumor growth, metastasis, invasion, and adhesion [20]. The adhesion force between tissue cells and their environment is crucial in all multicellular organisms. Integrins transmit force between the intracellular cytoskeleton and the extracellular matrix [21]. Some study suggested that mechanical stimulus induced malignant behavior of tumor cells by regulating cytoskeletal signal pathway [22]. Adhesion forces between cells and ECM are correlated with vinculin levels, and increase of the vinculin limits the probability of cell detachment and, therefore, cell motility and migration [23]. Stimulation generated by the stiff substrates from ECM triggered the F-actin skeleton, resulting in viscosity decrease and migration of cancer cells [24]. Leader cells also generate high protrusive forces and overcome extracellular matrix (ECM) resistance at the leading edge. Single-cell sequencing find that CDH3 controls leader cell protrusion dynamics through local production of integrin/focal adhesion function [25]. Researchers also found that the remodeling and reorganization of cytoskeleton and cell matrix can change the traction force, thus regulating cell movement behavior in breast cancer [26]. In our study, association between the traction force and metastasis was first recognized. Furthermore, we prove that SLC8A1 act as the key gene during the process of cytoskeleton formation and deformation, therefore promote invasion and metastasis in EC cells.

Nowadays, bioinformatics analysis is widely used to explore the key genes of pathogenesis and prognosis of various cancers [27]. Nomogram model is increasingly used to predict the prognosis of cancer with different clinicopathological features [28]. Our previous study identified MMP12 as a potential biomarker for prognosis and potential target for clinical treatment with integrated bioinformatics analyses and in vitro validation [29]. The underlying mechanism is through gene methylation and gap junction. We focused on the analysis of gene expression related to mechanical stimulation and recognized SLC8A1 as the potential target for prognosis of EC. SLC8A1 belongs to the solute carrier family [30]. It encodes glucose transporter, which is related to abnormal metabolism during cancer cell invasion [31]. SLC8A1 is not only related to mechanical stimulation, but also plays an important role in the prognosis of EC caused by calcium channels [32]. SLC8A1 regulates Na+/Ca2+exchange and participates in regulating the migration of tumor cells through TRPV4 pathway [16]. This conclusion is consistent with the previous study of our research group. The previous study clarified that TRPV4 as a pressure-sensitive calcium channel can promote the metastasis of endometrial cancer cells [19], and also participates in promoting the development of endometrial tissue and cell heterogeneity [33]. SLC8A1 is involved in molecular signature with diagnostic and prognostic significance related to OS and DFS in oral squamous
The fusion of SLC8A1 and its downstream intergenic region ALK had an excellent response with ceritinib treatment and promising relapse-free survival [35, 36].

The results of survival analysis for SLC8A1 showed that there were significant differences in survival rate between patients with different expression of SLC8A1, and patients with low expression group had higher survival rate. This conclusion was verified in the gene expression of 24 patients with EC in our center. Further functional experiments revealed that increased expression of SLC8A1 would reduce the cell traction and enhance the ability of cell migration or invasion, which indicated that SLC8A1 could promote the metastasis of EC cells. Previous studies suggested that SLC8A1 participated in the regulation of cytoskeletal protein as a mechanical stimulus-related gene, and F-actin was the main component of cytoskeletal protein, and participated in the invasion and metastasis of malignant tumor cells as a direct regulatory gene [37]. Our study also indicates that SLC8A1 is involved in promoting the metastasis of EC by regulating F-actin as a downstream molecule by Wnt-β-catenin signaling pathway.

Wnt/β-catenin target genes regulate cell proliferation and apoptosis, thereby mediating initiation and progression in many cancers [38]. Emerging studies have shown that Wnt/β-catenin signaling pathway plays an important role in EMT and tumor progression and β-catenin accumulation is closely associated with HCC progression and poor prognosis [39]. β-catenin destruction complex and β-catenin degradation regulated by the ubiquitin-proteasome pathway are the core mechanisms regulating intracellular β-catenin levels [40]. The upregulation of Wnt is also commonly observed during progression of breast tumor and confirms that tumor cells are dependent on this pathway Wnt/β-catenin induction prevents apoptosis that is of importance for mediating drug resistance [41]. One study found that curcumin induced cytoskeletal remodeling through inhibition of Wnt/β-catenin signaling pathway, and the addition of curcumin also inhibited the EMT process [42]. Evidence showed that dysregulation of Wnt signaling contributes to the progression of endometrial carcinoma. Chung reported that Müllerian inhibiting substance (MIS) functions as a tumor suppressor, potentially by regulating Wnt- and cytoskeleton-related signaling pathways that could contribute to endometrial carcinogenesis and progression [43]. Our previous study revealed that TRPV4 in deeply involved in the metastasis by mediating cytoskeleton, especially F-actin and paxillin. The downstream pathway is mainly correlated with RhoA-ROCK-confilin [19]. In combination with these two studies, we speculated that SLC8A1 and TRPV4 participate in the regulation of cytoskeleton in the process of metastasis for EC together. Meanwhile, both of the two molecules also change the traction force and mechanical force characteristics of cellular surface. However, the interaction and mechanism between the two genes needs to be further explored.

In summary, this is the first study to confirm that traction force is one of the key characteristics involved in the progression and drug resistance of EC. Based on our research, it may act mainly by affecting the mechanical environment in tumor cells and regulating the expression of SLC8A1. Our data demonstrated that high expression level of SLC8A1 could be considered as a standard biomarker to evaluate the metastatic capability in EC patients. The mechanism is mainly through regulating cytoskeleton and Wnt/
β-catenin signaling pathway. Our results provide novel theoretical evidence for metastasis and new targets for treatment in EC.

Declarations

Funding

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Author Contributions

Xingchen Li is responsible for conceptualization; Xin Xu are responsible for methodology. Jingyi Zhou is responsible for software; Xin Xu is responsible for formal analysis; Jingyi Zhou is responsible for investigation; Xin Xu and Xingchen Li are responsible for data curation; Xingchen Li is responsible for writing the original draft; Jianliu Wang and Xingchen Li are responsible for funding acquisition. Jianliu Wang and Xingchen Li is responsible for writing-review & editing the article. All authors read and approved the final manuscript.

Data availability statement

The data underlying this article are available in the article and in its online supplementary material.

Conflicts of interest

The authors declare that they have no competing interests.

References


**Tables**

**Table 1** Characteristics of patients in EC and control groups

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<th>Category</th>
<th>EC</th>
<th>NC</th>
<th>(p)-value</th>
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<tr>
<td>Total</td>
<td>24</td>
<td>20</td>
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<tr>
<td>Age (year)</td>
<td>59.54±9.179</td>
<td>38.95±12.79</td>
<td>&lt;0.0001</td>
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<tr>
<td>Height (cm)</td>
<td>160.4±5.635</td>
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<td>Weight kg</td>
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<td>60.47±11.31</td>
<td>&lt;0.05</td>
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<tr>
<td>BMI kg/cm²</td>
<td>26.91±12.66</td>
<td>23.08±3.737</td>
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<td>TF Pa</td>
<td>59.25±11.25</td>
<td>148.0±28.15</td>
<td>&lt;0.05</td>
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</table>

Footnote: EC, endometrial cancer; NC, normal control; BMI, body mass index; TF, traction force

**Table 2** Clinicopathological features for 24 EC patients
<table>
<thead>
<tr>
<th>Variables</th>
<th>N %</th>
<th>Traction Force Pa mean±SD</th>
<th>p-value</th>
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<td>Histological type</td>
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<td>EEC</td>
<td>22(91.67)</td>
<td>61.22±57.23</td>
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<td>Other types</td>
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<td>Negative</td>
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<td>57.71±56.15</td>
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<td>5(20.83)</td>
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<td>G2</td>
<td>11 (45.83)</td>
<td>56.27±27.48</td>
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<td>G3</td>
<td>8(33.33)</td>
<td>35.15±13.80</td>
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<td>FIGO stage</td>
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<tr>
<td>I</td>
<td>17(70.83)</td>
<td>71.24±61.62</td>
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<td>II-IV</td>
<td>7(29.17)</td>
<td>30.54±11.05</td>
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<td>Negative</td>
<td>16(66.67)</td>
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<td>Positive</td>
<td>8(33.33)</td>
<td>74.25±26.74</td>
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<td>LVSI</td>
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<td>13(54.17)</td>
<td>58.89±70.22</td>
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<tr>
<td>Positive</td>
<td>11(45.83)</td>
<td>59.93±32.44</td>
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<tr>
<td>CI</td>
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<tr>
<td>Negative</td>
<td>20 83.33</td>
<td>56.12±28.04</td>
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<tr>
<td>Positive</td>
<td>4(16.67)</td>
<td>31.73±10.87</td>
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<tr>
<td>LNM</td>
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<td></td>
<td>0.4427</td>
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<tr>
<td>Negative</td>
<td>22 91.67</td>
<td>61.56±57.06</td>
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<tr>
<td>Positive</td>
<td>2  8.33</td>
<td>35.20±7.396</td>
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<tr>
<td>Involving ovary and/or fallopian tube</td>
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<td>0.0388</td>
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<tr>
<td>Negative</td>
<td>20 83.33</td>
<td>73.97±62.61</td>
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Footnote: EEC, endometroid endometrial carcinoma; ER, estrogen receptor; G, grade; FIGO, International Federation of Gynecology and Obstetrics; MELF, microcystic, elongated, fragmented; LVSI, lymph-vascular space invasion; CI, cervical invasion; LNM, lymph node metastasis

**Figures**

**Figure 1**

Traction force of EC patients and normal control. (A) Heatmap of TFM in normal control and EC patients. (B) Barplot of the TF values. (C) ROC curve of predictive accuracy between TF and EC. EC, endometrial cancer; NC, normal control; TFM, Traction Force Microscopy
Figure 2

Associations between TF and different clinicopathological characteristics. (A) FIGO stage. (B) MELF. (C) Histological type. (D) ER. (E) CI. (F) Ovary involvement. (G) Tumor grade. (H) LVSI. EEC, endometroid endometrial carcinoma; ER, estrogen receptor; G, grade; TF, traction force; FIGO, International Federation of Gynecology and Obstetrics; MELF, microcystic, elongated, fragmented; LVSI, lymph-vascular space invasion; CI, cervical invasion; LNM, lymph node metastasis.
Figure 3

Figure 4

Bioinformatics analysis of SLC8A1 with EC patients in TCGA database.

(A) Survival curve for patients with low and high expression of SLC8A1. (B-D) Expression of SLC8A1 in different tissue types, FIGO stage, and tumor grade. (E) DEGs between low and high expression of SLC8A1 groups shown by heatmap. (F) Volcano plot of DEGs. (G-H) GO and KEGG analysis of DEGs.

DEGs, differentially expressed genes; GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes.
Figure 5

Validation of SLC8A1 function in PKUPH.

(A) Survival analysis between low and high expression of SLC8A1 in PKUPH.

(B-E) Expression of SLC8A1 in different tumor grade, lymph node metastasis, peritoneal cytology, and LVSI.

(F) Expression of SLC8A1 in different stiffness of extracellular matrix.

(G-H) GO and KEGG analysis of DEGs of low and high expression of SLC8A1 subgroups.
Figure 6

Function and downstream pathway for SLC8A1. (A) Protein levels of SLC8A1 in four EC cell lines. (B) The efficacy of SLC8A1 knockdown and overexpression in ishikawa cells measured through Western blotting. (C-D) The invasion and metastasis ability of ishikawa in different expression of SLC8A1. (E) Heatmap of TFM in ishikawa with different expression of SLC8A1. (F) expression of cytoskeleton (F-actin) in different SLC8A1 groups. (G) Effects of SLC8A1 on the Wnt-β-catenin signaling pathway measured by western blot. * p < 0.05, ** p < 0.01, *** p < 0.001

Supplementary Files

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• FigureS1.jpg
• FigureS2.jpg