ORIGINAL ARTICLE: REPRODUCTIVE SCIENCE

Increased expression of integrin-linked kinase during decidualization regulates the morphological transformation of endometrial stromal cells

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Objective: To study the impact of integrin-linked kinase (ILK) in endometrial stromal cells (ESCs) during decidualization.
Design: Laboratory study with the use of human endometrium.
Setting: University hospital.
Patient(s): Fertile reproductive-age women who had not received hormonal treatment for 3 months before tissue collection.
Intervention(s): Endometrium tissue collection, in vitro decidualization of isolated ESCs, and small interfering (si) RNA transfection.
Main Outcome Measure(s): Immunohistochemistry, ELISA, Western blot analysis, methylthiazolyl tetrazolium assay, and immunofluorescence staining.
Result(s): In vivo expression of ILK is significantly increased in distended-fusiform stromal cells of late secretory endometrium and in cobblestone-shaped decidual cells of early pregnancy. During in vitro decidualization for up to 8 days, confluent cultures of isolated ESCs consistently displayed increased ILK expression and morphologic transformation from fibroblast-like to polygonal cells. Subsequent ILK knockdown by siRNA transfection reversed this transformation, accompanied by decreased phosphorylation of glycogen synthase kinase (GSK) 3β and decreased viable cell numbers. Immunofluorescence staining of the decidualized ESCs demonstrated linkage of increased levels of ILK at the tips of the fan-shaped organization of actin stress fibers located in the submembranous area, which expanded the decidual cells into a typical polygonal appearance. Knock-down of ILK abrogated the polymerization and organization of actin fibers, which reverted the cells to their undecidualized morphology.
Conclusion(s): During human endometrial decidualization, ILK is essential for morphologic transformation of ESCs through organization of the actin cytoskeleton; it may also function through subsequent GSK3β signaling, which requires further studies. (Fertil Steril® 2016;■:■–■. ©2016 by American Society for Reproductive Medicine.)
Key Words: Integrin-linked kinase, decidualization, endometrium, actin

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In preparation for embryo implantation, the endometrium undergoes decidualization, a process that involves tissue remodeling, which includes molecular differentiation, morphologic transformation, and extracellular matrix (ECM) reorganization and integrin switching (1).

Integrins act as prototypic heterodimeric transmembrane receptors for ECM proteins to induce formation of focal adhesions (2). Integrin-linked kinase (ILK) is a key scaffold protein localized within focal adhesions where it acts as a central component of a heterotrimer (the ILK-PINCH-parvin complex) (3). Since its discovery, ILK has demonstrated an essential role in connecting the cytoplasmic tail of integrin β_1 and β_3 to the actin cytoskeleton and regulating actin polymerization (4).

ILK occupies a pivotal position in cell-matrix adhesion and can activate the transmembrane signals bidirectionally (5, 6). By down-regulating E-cadherin expression (7), ILK acts as a mediator of epithelial-to-mesenchymal transition, which plays important roles in embryo development and cancer progression (8–11). Corroboratively, aberrantly overexpressed or -activated ILK has been demonstrated in many types of human malignancies (3), and ILK-knockout mice die shortly after implantation owing to the defective epiblast polarization and abnormal F-actin accumulation (12). ILK can also behave as a multifunctional serine/threonine kinase to transmit its signal in a phosphatidylinositol 3-kinase–dependent manner (13, 14) through phosphorylating protein kinase B (Akt1), and/or glycogen synthase kinase-3β (GSK3β) pathways, which enable it to regulate such processes as cell proliferation, survival, migration, and invasion (15).

The role of ILK in reproductive sciences has been underexplored. In patients with preeclampsia, the expression of ILK and the number of endothelial progenitor cells are diminished (16). A recent study observed increased ILK expression in endometrial stromal cells (ESCs) of women with endometriosis and suggested a correlation with the increased migration and invasion functions of these cells in such patients (17). A separate study observed that ILK is highly expressed on the plasma membranes of extravillous trophoblast cells and that ILK may enhance the migration of chorionic villi (15). However, no study has focused on the potential roles of ILK in endometrium, although three integrins, $\alpha1\beta1$, $\alpha4\beta1$, and $\alpha v\beta3$, are coexpressed during the window of implantation (18–20), which suggests the possible involvement of ILK in decidualization and implantation. In view of the well documented occurrence of morphologic changes of ESCs during decidualization, we hypothesized that ILK expression could contribute to specific decidualization-related cellular functions and performed experiments to test this hypothesis.

MATERIALS AND METHODS

The study protocols were reviewed and approved by the Human Investigation Review Board of Yale University and Linkou Chang Gung Memorial Hospital, Taiwan. Written informed consent was obtained from every patients before surgery and tissue collection.

Patients and Tissue Specimens

Endometrial tissues were obtained from surgical specimens of fertile women with regular menstrual cycles who underwent laparoscopy or hysterectomy (21, 22) for benign gynecologic conditions, without endometriosis, adenomyosis, or hyperplastic endometrial disease, and who did not receive hormonal medication in the preceding 3 months. For immunohistochemical staining (n = 5 for each menstrual phase; total n = 30; mean age 40.4 years, range 23–48), the date of the menstrual cycle was classified as early proliferative (days 1–5), midproliferative (days 6–10), late proliferative (days 11–14), early secretory (days 15–18), midsecretory (days 19–23), and late secretory (days 24–28) phases, according to menstrual history and confirmed by means of endometrial histology with the use of the criteria of Noyes et al. (23). Decidual tissues (n = 5 for immunohistochemistry; n = 3 for decidual cell culture) were collected from voluntary terminations of normal pregnancies during the first trimester.

Endometrial tissue for ESC isolation and long-term culture (n = 23) was also obtained from surgical specimens of fertile women with the use of the same criteria as mentioned above, and placed in Hank balanced salt solution (HBSS; Sigma-Aldrich). Despite isolation from different menstrual cycle stages, study showed that ESCs by passage 2 did not differ in the expression of cycle-specific genes and were naïve (or without memory) in their response in the experimental treatment protocols (24).

Immunohistochemistry

Formalin-fixed paraffin-embedded specimens were cut into 5-μm sections. After deparaffinization, antigen retrieval was performed by boiling in citrate buffer (10 mmol/L, pH 6.0) for 15 minutes, and endogenous peroxidase was blocked by means of immersion in 3% hydrogen peroxide (in 50% methanol:50% distilled water solution) for 12 minutes. Slides were then incubated in a humidified chamber with 5% normal horse serum (Vector Laboratories) in Tris-buffered saline solution (TBS; 0.05 mol Tris [pH 7.4], 0.85% saline) for 30 minutes at room temperature. After drainage of excess serum, sections were incubated with a 1:6,000 dilution (0.33 μg/mL) of mouse monoclonal anti–human ILK antibody (Sigma) overnight at 4°C in a humidified chamber. Nonimmune (normal) mouse IgG1 was used at the same concentration of the primary antibody for isotype control samples. With the use of the horse anti–mouse biotinylated secondary antibody (Vector Laboratories), the antigen-antibody complex was detected by means of the avidin-biotin-peroxidase complex (Vectastain ABC kit; Vector Laboratories) and 3,3′-diaminobenzidine tetrahydrochloride (Sigma-Aldrich) with light hematoxylin counterstaining. Images were collected at room temperature with the use of an Axioplan 2 Imaging (Carl Zeiss) microscope with an Axiocam (Zeiss) camera, and Axiovison (release 4.6; Zeiss) software.

Immunoreactivity was evaluated by means of H-score in an area of containing ~100 cells (×400), with the use of the formula: H-score = ΣP_i (intensity score), where the intensity score is graded as 0 for none, 1 for weak but detectable, 2 for moderate or distinct staining, or 3 for strong staining, and P_i is the corresponding percentage (range 0–100) of the positively stained cells in each category and therefore yielding a value ranging from 0 to 300 (25). The final average scores