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Decay-accelerating factor promotes endometrial cells proliferation and motility under ovarian hormone stimulation

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ABSTRACT

The intent of the study was to explore the elevating expression of decay-accelerating factor(DAF) exerts influence on biological behaviors of endometrial stromal cells except in classical immunology on the basis of bioinformatic statistics and clinical miscarriages findings suggesting its potential role in the establishment of endometrial receptivity. We confirmed that DAF locates on the cellular surface of endometrial epithelium and stroma. By using plasmid transfection to down-regulate DAF expression in primary endometrial stromal cells (ESCs), we discovered that DAF expression in ESCs increases in response to estradiol and progesterone stimulation in dose- and time-dependent manners; moreover, tamoxifen and RU486 stimulations to block estrogen receptors(ERs) and progesterone receptors(PRs) respectively result in reduced DAF mRNA and protein, and it is more obvious to block PRs. Meanwhile, knocked-down DAF in ESCs weakens the proliferation, migration and invasion of endometrial cells. Cell cycle analysis showed knocked-down DAF accumulates cells in S phase and diminishes cells in G0/G1 phase, which substantiates DAF mediates endometrial cells proliferation. In conclusion, DAF is a potential molecule involving in endometrial cellular proliferation and motility to verify up-expressed DAF during the WOI may facilitate endometrial physiobiological behavior changes, which shed light on DAF function and potential role in the endometrial receptivity establishment.

1. Introduction

The endometrium, lining along the uterine cavity, is a subtle hormone-responsive mucosa undergoing a cyclic proliferation and differentiation to support embryo apposition, attachment, and embedment. The transition of the endometrium from proliferative phase to secretory phase, culminating a defined period called the window of implantation (WOI, occurring from postovulatory day 6–10 in human [1]), during when the endometrium becomes receptivity orchestrated directly and indirectly by female hormone estradiol and progesterone [2]. The sex steroid hormones are also responsive for the meticulous paracrine and autocrine growth factors, cytokines, and immunomodulators to trigger the establishment of endometrial receptivity [3]. A milestone finding by Wilcox et al. in 1999 [1] noted euploid embryo implanting beyond the normal endometrial receptivity period had greater chance for implantation failure. However, little is known about the precise and specific stromal and epithelial expression and localization of critical factors and their variations in human endometrium during the WOI.

Our group employed bioinformatics tools (GeneSifter and Ingenuity Pathway Analysis) to dig out 148 highly potential biomarkers for the endometrial receptivity, one of which is decay-accelerating factor(DAF) [4]. Additionally, it was found by Anahi et al. in 2008 [5] that DAF expression was significantly increased throughout the WOI, and it was stained in both glandular and stromal compartment in human endometrial biopsies from cycle day 21 and 24. These findings suggest that DAF acts a critical role in establishment of the endometrial receptivity; however, no further and detailed research on DAF in this area.

DAF, also called CD55, was firstly detected on the erythrocyte surface to regulate complement system activation in 1969 [6], and it is a glycophosphatidylinositol (GPI)-anchored IV type transmembrane protein with four spheric short consensus repeat region (SCR). Besides the membrane bound isoform, a soluble isoform of DAF is detected in plasma, tears, saliva, urine, and synovial fluids [7]. As a classic complement regulator protein, DAF binds and consequently accelerates the decay of C3 convertases in all three complement activation pathway, thereby down-streaming complement system activation [8]. Recently, DAF draws greater attention in human tumorigenesis and progression

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Table 1
shDNA template sequence for plasmid transfection.

	shDNA template sequence	product size (bp)
shRNA/NC	Fw:5'-CACCGTTCTCCGAACGTGTCACGTTTCAAGAGAACGTGACACGTTCGGAGAATTTTTTG-3' Rv:5'-GATCCAAAAAAATTCTCCGAACGTGTCACGTTCTCTTGAAACGTGACACGTTCGGAGAAC-3'	55
DAF shRNA	Fw:5'-CACCGGCATATTATTTGGTGCAACCTTCAAGAGAGGTTGCACCAAATAATATGCCTTTTTTG-3' Rv:5'-GATCCAAAAAAGGCATATTATTTGGTGCAACCTCTCTTGAAGGTTGCACCAAATAATATGCC-3'	58

[9,10]. It is already uncovered DAF exists in all mammalians carcinomas like colorectal cancer, gastric cancer, thyroid cancer and etc. [11–13], even in metastases of colorectal carcinoma [14]. In gastric and colorectal tumor, the higher expression of DAF has greater chance to invade and migrate [15,16]. DAF decreases cell adhesion of T-lymphocytes to U-937 human leukemic cells, so we wonder DAF may play a role in invasive tumor growth and formation of metastases by mediating decreased cell adhesion [17]. Simultaneously, CD97, a ligand of DAF [18], is a protein in correlation with not only the stage of malignancy and increased dedifferentiation of carcinoma but also their possibility of growth, migration and lymph node metastases [19,20]. Obviously, these findings expand our understanding on DAF potential role in tumor cell proliferation, migration, and invasion beyond immunological area.

Considering the roles of DAF in carcinoma, it gives us a hint on its potential effect on female reproductive system where little is known and done. With similarity with tumor cell biological characteristics, it is believed that establishment of endometrial receptivity consists of endometrial cell proliferation, transition, migration, and apoptosis to be conducive to embryo penetration, but research of DAF effects on endometrial cellular biological behaviors is limited. Therefore, we analyzed the endometrial cellular DAF protein and mRNA levels under estradiol, progesterone stimulation, while we used plasmid transfection to down-regulate DAF expression in endometrial stromal cells (ESCs) to explore cellular DAF function on endometrial physiological changes like cell cycle, proliferation, and migration, which is beneficial to our first glimpse on the reason of its increased expression during the WOI.

2. Materials and methods

2.1. Tissue collection and human endometrial stromal cells culture

Normal endometrial tissues were collected from 10 fertile women (< 45 years old) who had undergone total hysterectomy because of leiomyoma after their written informed consent and Obstetrics and Gynecology Hospital, Fudan University ethics committee approval (Kyy2017–26). None of the patients had received any hormonal therapy within 3 months before the operation.

Stromal cells were isolated after endometrium rinsed and minced into small pieces under sterile condition followed by digestion in DMEM/F12(1:1) (Gibico; Invitrogen, CA) culture medium containing type IV collagenase (0.25%; Sigma, St Louis, USA), DNase I (15 IU/ml; Takara Shuzo, Tokyo, Japan) for 90 min at 37 °C with agitation. The dissociated cells were filtered through sterile cell strainer assembled with 100 μm and 40 μm pore size (Corning, New York, USA) to separate endometrial epithelial cells (EECs) from ESCs. ESCs passed through the two strainers and were resuspended in DMEM/F12 (1:1) supplemented with 10% fetal bovine serum (FBS) and penicillin (100 U/ml) and streptomycin (100 U/ml) at 37 °C in humidified atmosphere with 5% CO_2. The purity of the ESCs was confirmed by positive cellular staining for vimentin (1:500, Guge biological company, China) and cytokeratin (1:500, Guge biological company, China). Culture medium was changed every other day.

2.2. Immuno-histochemistry

The endometrial samples were collected under sterile conditions and fixed in 4% paraformaldehyde to embed in paraffin blocks, then the tissues blocks were cut into 4-μm sections and mounted on polylysine-coated slides, dewaxed and rehydrated. The dewaxed and rehydrated slides were boiled in 0.1 mol/L citric acid after incubation in 3% hydrogen peroxide for 15 min at room temperature. After 10 min washing in phosphate buffered saline (PBS), the slides were incubated with 10% normal goat serum (dilute in PBS) for 30 min; then incubated with the primary antibody: monoclonal rat anti-DAF antibody (1:2000, Abcam, USA) overnight at 4 °C. The negative control was incubated with a rabbit polyclonal IgG at the same dilution (1:2000, Abcam, USA) in the humidified box. After rinsing in PBS, the slides were incubated with biotinylated secondary antibody(1:1000, Guge, Wuhan, China) for 1 h at room temperature. Subsequently, these sections were immersed in PBS and incubated with 0.1% diaminobenizidine tetrahydrochloride for 3 min followed by washing in running diluted water and counterstaining with haematoxylin (Sigma, USA). The yellowish-brown staining particles were recognized as positive, and representative fields were captured at 200 and 400 magnification with an Olympus microscope (Olympus Corp., Tokyo, Japan). The images were processed by ImagePro Plus 6.0 software.

2.3. shRNA plasmid transfection

To generate stable down-expressed DAF ESCs, short hairpin RNA (shRNA) specifically targeting human DAF and scrambled control shRNA were purchased from Genepharma (Shanghai, China). The plasmid vector was pGpU6/GFP/Neo and the DNA sequences are reported in Table 1. shRNA DAF plasmids were transfected into approximately 1×10^6/ml ESCs plated on a 6-well plate after 48 h growing in medium containing 10% FBS without antibiotics by using Lipofectamine3000 (ThermoFisher, USA) according to the manufacturer's protocols, and stable shRNA expressing colonies were selected by 1 μg/ml puromycin (Sigma-Aldrich, St. Louis, MO). The efficiency of transfection was confirmed and counted GFP stained cells by immunofluorescent microscope (Olympus, Japan). After continuous culture and replacement of the culture medium, stable DAF down-expressing ESCs cell line and scramble (NC) cells were obtained for verification of DAF protein expression.

2.4. Hormone stimulation protocol

For the hormone stimulation test, 1×10^6/ml ESCs were plated on a 6-well plate and grown for 24 h in normal medium containing 10% FBS without antibiotics. Then, the cells were cultured in serum-free medium for 24 h to prepare it for the next stimulation experiment.

Stimulation was performed using various concentrations of estradiol alone (E_2; at 0 and 10^{-9}–10^{-7} mol/L; Sigma), various concentrations of progesterone (P_4; at 0 and 10^{-9}–10^{-6} mol/L; Sigma, USA), E_2 combined with P_4 (10^{-8} mol/L E_2 with 10^{-7} mol/L P_4; Sigma, USA), and tamoxifen (at 10^{-8} mol/L; Sigma, USA) and RU486 (at 10^{-8} mol/L; Sigma, USA) or with control and 0.01% Dimethyl sulfoxide (DMSO) in DMEM/F12 culture medium containing 10% charcoal-stripped FBS (Bioind, Shanghai, China). After 24 h, 48 h, and 72 h, cells were