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Dual-specificity phosphatase 6 predicts the sensitivity of progestin therapy for atypical endometrial hyperplasia

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HIGHLIGHTS

- Dusp6 expression was higher in progestin-sensitive endometrial precancerous lesions.
- Dusp6 expression was increased by progestin in progestin-sensitive patients.
- Dusp6 expression enhanced progestin-induced growth-inhibition in Ishikawa cells.

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ABSTRACT

Objective. We previously found that Dual-specificity phosphatase 6 (Dusp6) over-expression enhanced the growth-promoting effect of estrogen in endometrial adenocarcinoma cells. The aim of this study was to explore the correlation of Dusp6 expression with progestin sensitivity in atypical endometrial hyperplasia (AEH) and earlier endometrial carcinomas (EC).

Methods. Using immunohistochemistry study, we analyzed the expression of Dusp6 protein in AEH.

Results. We found that progestin treatment was effective in 89% of AEH and 50% of EC. Before treatment, Dusp6 expression was significantly higher in progestin-sensitive AEH groups compared with progestin-resistant groups. After treatment, Dusp6 expression was significantly upregulated in progestin-sensitive groups, but not in progestin-resistant groups. Moreover, a high-dose of Dusp6 transfection significantly enhanced progestin-induced growth-inhibition in Ishikawa cells.

Conclusions. Dusp6 could be a predicting marker for deciding the effectiveness of progestin therapy in AEH.

Introduction

Endometrial carcinoma is one of the most common gynecologic malignancies and the increased number of patients with endometrial carcinoma has been noted in recent years [1,2]. For younger patients with endometrial carcinoma who desire to preserve their fertility, fertility-sparing management is increasingly considered as an option for early-stage diseases. Progestin therapy is a well known effective treatment for early-stage of endometrial carcinoma [3] with a response rate of about 57–75% [4–6], compared to 20–40% in advanced or recurrent cases [7]. Secondary progestin therapy may be used for treatment in select circumstances of relapse after primary conservative treatment [8].

For patients with precancerous lesions or localized disease of early stages treated by surgery and/or following radiotherapy, complete remission and long-term survival are achievable. For patients resistant to hormone therapy, failed primary progestin treatment may lead to concerns about disease progression. Many patients are initially responsive to progestin therapy. It has been reported that progestin-induced apoptosis occurs during the early period of treatment for endometrial hyperplasia [10]. However, the emergence of resistance is a major clinical problem [4]. When progestin-resistant cancer cell subtypes arise, progestin therapy can enhance cell proliferation and invasiveness in these subtypes [9]. Moreover, patients resistant to progestin treatment may present with invasion of myometrium, isthmus and/or lymph-nodes during surgery [11]. Thus, response to hormone therapy is an important factor for prognosis and survival. However, it is not yet clear how to assess a responder from a non-responder, and to distinguish the responder to hormonal therapy is significant for the treatment of earlier endometrial carcinomas and precancerous lesions.

Finding molecular markers for disease monitoring during treatment becomes more and more important for the individualization of therapy. The expression of hormone receptors e.g. progesterone receptor (PR) is a prognostic factor for successful hormone therapy [12–14]. Reduced

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expression of PRs has been noted in endometrial tumors compared with normal endometrium and the development of the disease is correlated with aberrant expression of PRs [15]. However, association of hormonal receptor status with outcome cannot fully explain the mechanism of progesterone resistance.

Recent studies have found that series growth factors signaling, including transforming growth factor-α (TGF-α) and epidermal growth factor (EGF) [16–18], are involved in the development of progesterone resistance. In previous in vitro studies, we found that Dual-specificity phosphatase 6 (Dusp6), a negative feedback mechanism of fibroblast growth factors (FGFs)/mitogen-activated protein kinase (MAPK)/extracellular signal-regulated kinases 1/2 (ERK1/2) signaling, is upregulated in endometrial adenocarcinoma and the over-expression of Dusp6 significantly enhanced the growth-promoting effect of estrogen in endometrial cancer cells. The aim of the current study was to explore the correlation of Dusp6 expression with the effectiveness of progesterone therapy in earlier endometrial carcinomas and precancerous lesions.

Materials and methods

Tissue collection and immunohistochemistry analysis

Twenty-seven women presenting with abnormal vaginal bleeding underwent diagnostic curettage (Fig. 1). Pathological examination showed atypical endometrial hyperplasia (AEH) (n = 19) and earlier endometrial carcinomas (EC) (n = 8). Medroxyprogesterone acetate (MPA, 250 mg/day, orally) was given to the patients after diagnostic curettage. 3 months later, diagnostic curettage was performed on the patients again and the biopsy specimens were pathologically examined. The expressions of Dusp6 in pre and post-treated AEH biopsies were determined using immunohistochemistry analysis. The study has been approved by the institutional review board of Shandong Provincial Hospital Affiliated to Shandong University and written informed consent was obtained from all participants. Diagnosis was confirmed by histological examination performed by more than 2 experienced pathologists with expertise in gynecologic malignancy.

The experiment was performed as we did before [19]. In brief, fresh tissue was washed with phosphate buffered saline (PBS) and fixed. After dehydration and paraffin-embedding, samples were cut and mounted onto glass slides. Sections were deparaffinated, rehydrated and incubated with 3% H_2O_2. Then antigen retrieval was performed and the sections were blocked and incubated overnight with rabbit anti-human Dusp6 primary antibody (diluted 1:100 in PBS, Abcam) in a wet chamber at 4 °C. HRP-conjugated goat anti-rabbit IgG was used as second antibody. Sections incubated with non-immune serum instead of primary antibody were used as negative control. The experiments were repeated in duplicate.

The immunohistochemical score was evaluated as we did before [19]. Two sections per sample were evaluated blind for immunostaining without any knowledge of the clinical or pathologic data.

Cell cultures and treatments

In the in vitro study, Ishikawa cells (a high-differentiated endometrial adenocarcinoma cell line) was used in the study. The cells were maintained in DMEM supplemented with 5% charcoal-stripped fetal bovine serum (FBS), 100 μ/ml of penicillin and 100 μ/ml of streptomycin at 37 °C in a humidified environment with 5% CO_2 in air. Pre and post-transfected cells were treated with MPA. MPA (Sigma) was dissolved in 100% ethanol and the ethanol concentrations were not higher than 0.1% in culture medium. 1 μmol/L was used in cell treatments. Mock-treatment with an identical volume of ethanol was used as a control.

Constructs and generation of stably transfected cell clones

In in vitro study, Ishikawa cell was transfected with Dusp6 plasmid or empty vector as described previously [20]. The pcDNA3.1-Dusp6-V5 plasmid was kindly provided by Dr. Nelly Pitteloud and Dr. Yisrael Sidis (Centre Hospitalier Universitaire Vaudois, Switzerland). Ishikawa cells were plated on 24-well plate and maintained in DMEM containing 10% FBS. Transfections were performed using FuGENE6 transfection reagent (Roche) according to the manufacturer's protocol. In briefly, cells were transfected with different amounts of pcDNA3.1-Dusp6-V5 plasmid (75 ng, 50 ng, 25 ng) or empty vector (EV) (75 ng, 50 ng, 25 ng). 24 h after transfection, the cells were placed under Geneticin (G418-sulfate, at 600 mg/L, Gibco, Invitrogen) selection for 20 days. Individual colonies were removed by trypsinization and expanded. The G418-resistant clones were maintained in medium containing 300 mg/L of Geneticin.

Cell number counting experiments

Stably transfected cells (5×10^3 per well) were plated on a 6-well plate in DMEM containing 10% FBS and allowed to attach overnight. Then the culture medium was replaced with medium containing 5% charcoal-stripped FBS and 300 mg/L of Geneticin and incubated at 37 °C overnight. Then, MPA or DMSO was added into medium and the cells were cultured for 5 days without any disturbance. After incubation, the medium was removed and the cells were collected by trypsinization

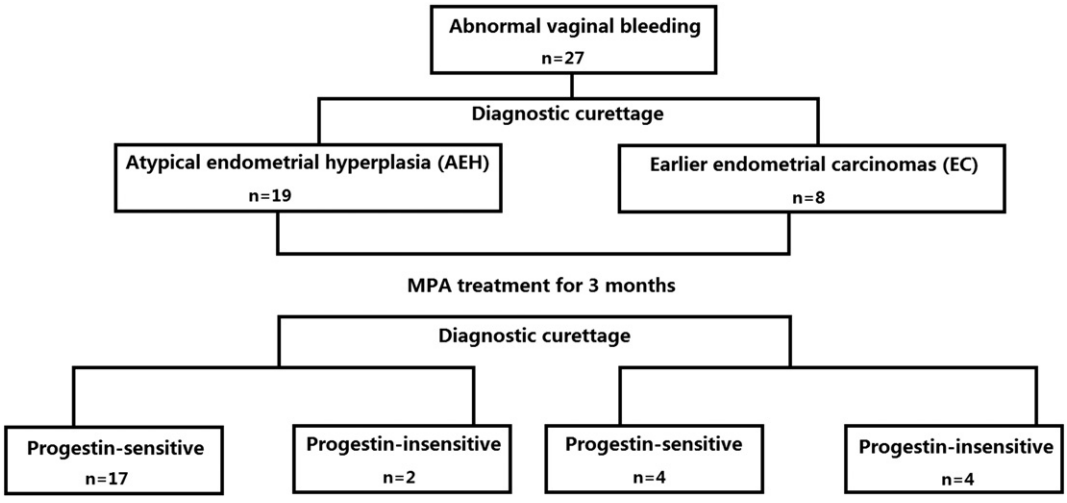

Fig. 1. The information of the participants.