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Focal Adhesion Kinase (FAK) Involvement in Human Endometrial Remodeling During the Menstrual Cycle

Mahmoud Orazizadeh[*1], Iran Rashidi[2], Jamileh Saremi[3] and Mahmoud Latifi[4]

[1]*Cell and Molecular Research Centre* [2]*Dept. of Pathology and* [3]*Dept. of Anatomical Sciences, Faculty of Medicine, Ahwaz Jondishapoor University of Medical Sciences, Ahwaz;* [4]*Dept. of Statistics, Faculty of Health, Ahwaz Jondishapoor University of Medical Sciences, Ahwaz, Iran*

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ABSTRACT

Background: Endometrial remodeling occurs during each menstrual cycle in women. Reports have shown that, in a variety of cell types, processes such as proliferation, signaling complex formation and extra cellular matrix remodeling require a cytoplasmic tyrosine kinase, focal adhesion kinase (FAK). The present study has focused on the expression pattern of FAK in human endometrium during the menstrual cycle. The purpose of this study was to ascertain the probable function of FAK in menstrual cycle changes and the role of FAK in tissue repair and tissue remodeling *in vivo*. **Methods:** Formalin-fixed paraffin-embedded endometrial samples were obtained from 400 pre-menopausal, non-pregnant women, who underwent hysterectomy and biopsy for benign diseases. Forty six samples with no tissue abnormalities were studied and ABC staining method of immuno-histochemistry methods was applied. Positive staining of FAK by different cell types of human endometrium was scaled and compared with each other by using histologic score method. **Results:** All different cell types of endometrium showed various patterns of FAK expression in different stages of menstruation. FAK in glandular and luminal epithelial cells is up-regulated during the early proliferative (EP) to mid-secretory (MS) phases. FAK in stromal cells is up-regulated during the EP, early and MS phases in comparison to the late secretory (LS) phase. FAK expression in endothelial cells is up-regulated during the EP and MS phases in comparison to LS phase. This study showed that endometrial FAK expression is a phase-dependent manner during the menstrual cycle. **Conclusion:** It appears that up-regulation of FAK during the proliferative phases is responsible for endometrial regeneration and high expression of FAK in the EP and MS phases may associate with the implantation. Down-regulation of FAK during the LS phase may facilitate apoptosis in human endometrium. It seems that FAK as a key kinase plays a critical role in endometrial remodeling that it may regulate by steroid hormones. *Iran. Biomed. J. 13 (2): 95-101, 2009*

Keywords: FAK, Endometrial Remodeling, Steroid hormones, Menstrual cycle

INTRODUCTION

The human endometrium undergoes cyclic, hormonally dependent changes in proliferation, differentiation, sloughing and repair [1]. During the secretory phase of the menstrual cycle, the endometrium increases in thickness to approximately 5 mm in depth. By angiogenesis, spiral vessels extend to a point immediately below the epithelial membrane. These structural changes are controlled by an ovarian steroid hormone, estradiol. At the secretory phase, when progesterone reaches physiological levels, stromal edema occurs along with the infiltration of inflammatory cells such as large granular lymphocytes and macrophages. At the late secretory phase (LS), the fall in concentration of progesterone is followed by the onset of menstruation, in which the functional layer of endometrium is shed into the uterine lumen and bleeding occurs [2, 3].

Tissue regeneration is a complex cellular process, which includes the processes of inflammation, angiogenesis, extracellular matrix synthesis, re-epithelialization and collagen deposition [4]. Cell-to-cell communication is essential in many physiological processes. The main families of cell

*Corresponding Author; Mobile: (+98-916) 3059735; Fax: (+98-611) 333 7370; E-mail: M_ orazizadeh@yahoo.com

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surface receptors that mediate cell-cell or cell-extra cellular matrix interactions are integrins [5] which are heterodimeric transmembrane receptors. Although having no intrinsic enzymatic activity, integrins stimulate a number of intracellular signaling pathways, which are important to cell proliferation, survival and migration [6]. One of the central molecules in integrin mediated adhesion and signaling is focal adhesion kinase (FAK) [7].

FAK is a cytoplasmic tyrosine kinase that plays a key role in integrin-mediated signal transduction pathways and is localized in focal contacts [7, 8]. Upon activation by integrin-mediated cell adhesion, FAK associates with a number of SH2 domain-containing signaling molecules, including Src family kinases [9], p85 subunit of phosphoinositide-3 kinase [10], phospholipase C-γ [11] and Grb7 [12]. Interactions of FAK with these signaling molecules lead to a number of cell biological processes, including cell attachment, migration, invasion, proliferation and survival. These processes are important in cell, tissue and organ structuring, functioning and remodeling after injury [8]. In addition to its well-established role in mediating integrin signaling, FAK may participate in growth factor receptor-induced signal transduction [13].

In our knowledge, the role of FAK expression in endometrial remodeling *in vivo* has not been studied. Finding a relationship between FAK expression modulations and uterine repair and remodeling processes during menstrual cycle could be an acceptable option for the uterus dysfunctions and also in general for tissue engineering. The aim of the present study was to assess the FAK expression pattern during the human menstrual cycle in order to understand the role of FAK in tissue remodeling *in vivo*.

MATERIALS AND METHODS

Endometrial samples were obtained from 400 pre-menopausal, non-pregnant women (age range 29-48 years) who underwent hysterectomy for benign diseases (lieomyoma or prolapse) or biopsy without endometrial pathologies. This study was approved by the Ethics Committee of Research Affairs of Ahwaz Jundishapoor University of Medical Sciences, Ahwaz, Iran). All the women had a history of regular menstrual cycle. Specimens with endometrial abnormalities were excluded and 46 samples were studied. The specimens were dated according to the Noyes [14] standard histological criteria and were classified as early proliferative phase (EP, n = 9), mid proliferative phase (MP, n = 6), late proliferative phase (LP, n = 7), early secretory phase (ES, n = 8), mid-secretory phase (MS, n = 8) and LS phase (n =8).

Immuno-histochemistry for light microscopy. Formalin-fixed, Paraffin-embedded blocks of endometrial samples were sectioned at 5-µm intervals. The sections were heated at 50°C for 15 minutes. Deparaffinization in xylene and rehydration in graded ethanol were applied. Endogenous peroxidase activity was quenched by incubation of samples with 3% hydrogen peroxide in PBS for 25 minutes. Antigen retrieval was carried out by using citrate buffer (0.01 mol/L sodium citrate and pH 6.00) at 95°C-98°C for 15 minutes and then placed at room temperature for half an hour. Non-specific binding was blocked by 5% normal goat serum (Santa Cruz Biotechnology kit, USA) in PBS at room temperature for 60 minutes. The sections were incubated with primary antibody (mouse monoclonal antibody anti-FAK) (FAK, H-1) (Sc-1688, Santa Cruz Biotechnology, USA) at a 1:50 dilution in PBS at 4°C overnight. After three washes with PBS for 5 minutes each, sections were incubated with the secondary antibody (biotinylated goat anti-mouse antibody IgG) (Santan Cruz Biotechnology, USA) at room temperature for 30 minutes. Then, the sections were incubated with the avidin-biotin-peroxidase for 30 minutes. After washing, the sections were reacted with diaminobenzidine (Santa Cruz Biotechnology, USA) and counterstained with hematoxylin, then dehydrated, and mounted. In negative control, primary antibody was omitted and PBS was used instead. The positive control was colon carcinoma as recommended in antibody data sheet.

Assessment and scaling of positive immuno-staining. The intensity of FAK immuno-reactivity in endometrial tissue was evaluated semi-quantitatively as positively stained cells according to the following categories: 0, no staining; 1, weak but detectable; 2, moderate or distinct; 3, strong. For each tissue, a histologic score (HSCORE) value [15, 16] was derived by summing the percentages of cells that stained at each intensity category and multiplying that by the intensity of the staining, using the formula [HSCORE = P_i (i + l)]; where i represents the intensity scores and P_i is the corresponding percentage of the cells. In each slide, five different areas were evaluated under the microscope (×40

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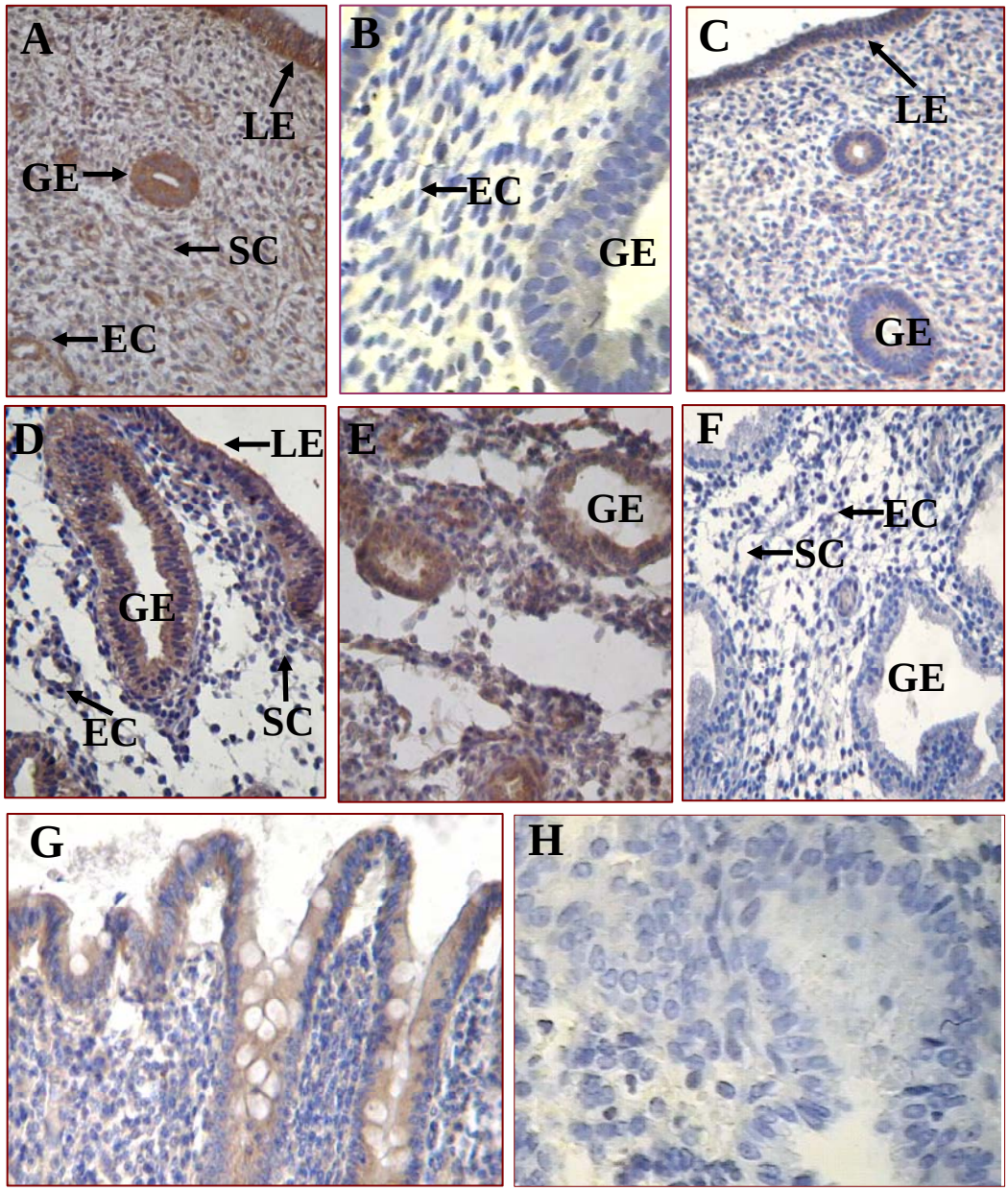


Fig. 1. Immuno-histochemical staining of human endometrium for FAK. Endometrial tissues at the early proliferative phase (**A**), mid proliferative phase (**B**), late proliferative phase (**C**) early secretory phase (**D**), mid-secretory phase (**E**), and late secretory phase (**F**). Positive control, a section from colon carcinoma **(G)**, and an endometrial section as negative control **(H)**. LE, luminal epithelium; GE, glandular epithelium; SC, stromal cell; EC, endothelial cell (magnification, ×400).

original magnification). The percentage of the cells for each intensity within these areas was determined at different times by two investigators, who were blinded to their evaluations, and the average scores were calculated.

Statistical analysis. HSCORE level of immuno-histochemistry was normally distributed as tested by Kolmogorov-Smirnov test and were analyzed by ANOVA and post-hoc Tukey test. Statistical significance was defined as $P<0.05$.

RESULTS

Positive staining of FAK in endometrium during menstrual cycle. Expression of FAK was detected in the luminal and glandular epithelium, endothelial and stromal cells of the endometrium. The pattern of expression was different at various phases of menstrual cycle (Fig. 2-6). Clear and strong immuno -reactivity was detected in the colon carcinoma as positive control (Fig. 1G). Immuno-reactivity was not detected when the primary antibody was omitted from the negative control (Fig. 1H).

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In the EP phase, there was a strong staining in the luminal and glandular epithelium, endothelial and stromal cells (Figs. 1A and 6). In the MP phase FAK expression was strong in the luminal and glandular epithelium but moderate in endothelial and stromal cells (Figs. 1B and 6). In the late proliferative phase, FAK expression was strong in the luminal and glandular epithelium but moderate in endothelial and stromal cells (Figs. 1C and 6).

In the ES phase, there was a strong staining in the luminal epithelium, glandular epithelium and stromal cells but in the endothelial cells, FAK expression was moderate (Figs. 1D and 6). Moreover, during the MS phase, there was a strong staining in the luminal and glandular epithelium, endothelial and stromal cells (Figs. 1E and 6). LS phase showed a significant down-regulation of FAK expression in all cell types in comparison to other phases. (Figs. 1F and 6). Differences among proliferative and secretory phases and with each other were not significant. When various stages were compared, apart from LS phase, the other modifications were mild or moderate and no significant difference was observed.

Expression pattern of FAK in luminal epithelium during menstrual cycle. Positive staining of FAK was observed in luminal epithelium in different phases of menstrual cycle. Strong FAK expression was observed in the EP (P<0.001,), MP (P = 0.036), LP (P<0.001), ES (P = 0.001) and MS phases (P<0.001) compared with the LS phase. There was no significant difference between other phases (Fig. 2).

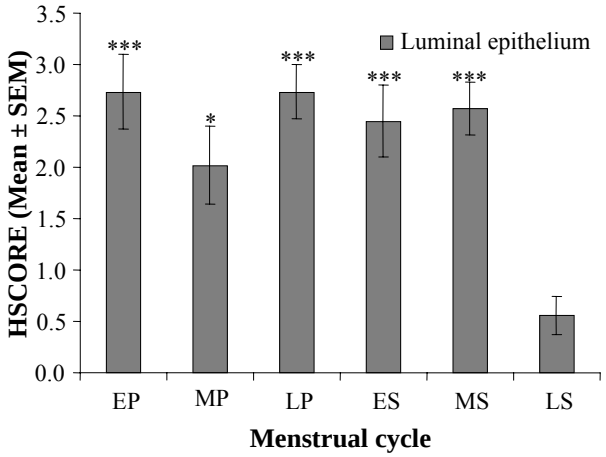


Fig. 2. Expression pattern of FAK in endometrial luminal epithelium. Focal adhesion kinase expression in endometrial luminal epithelium was significantly strong during the early proliferative to mid-secretory phases when different stages compared with late secretory (LS) phase. $^{*}P$<0.05; $^{***}P$<0.001.

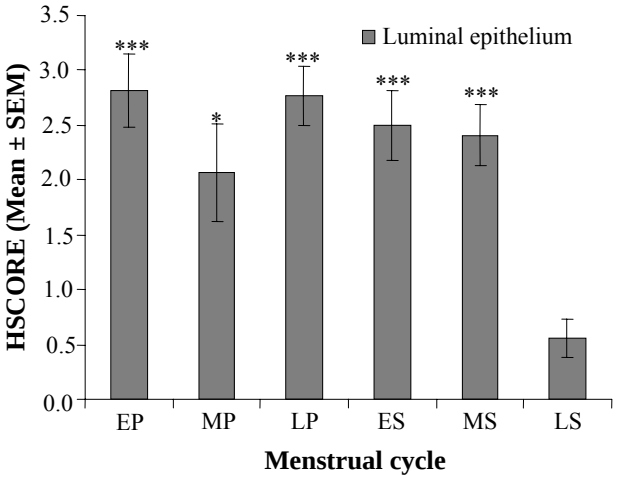


Fig. 3. Expression pattern of FAK in endometrial glandular epithelium. FAK expression in endometrial glandular epithelium was significantly strong during the early proliferative to mid-secretory phases when different stages compared with late secretory phase. $^{*}P$<0.05; $^{***}P$<0.001.

Expression pattern of FAK in glandular epithelium during menstrual cycle. Positive staining of FAK was observed in glandular epithelium in different phases of menstrual cycle. Strong FAK expression was observed during the EP (P<0.001), MP (P = 0.026), LP (P<0.001), ES (P = 0.001) and MS phases (P = 0.001) when compared with the LS phase. There was no significant difference between other phases (Fig. 3).

Expression pattern of FAK in stromal cells during menstrual cycle. In stromal cells, FAK expression was up-regulated in the EP (P = 0.006) ES (P = 0.020) and MS (P = 0.007) phases in comparison to the LS phase. There was no significant difference between other phases (Fig. 4).

Expression pattern of FAK in endothelial cells during menstrual cycle. FAK expression in endothelial cells was up-regulated in the EP (P = 0.031) and MS (P = 0.018) phases in comparison to LS phase. There was no significant difference between other phases (Fig. 5).

DISCUSSION

In the present study, we have shown that FAK expression changes temporally and spatially, *in vivo*, throughout the menstrual cycle and that estradiol and progesterone may play a role in regulating these changes. Estrogen typically stimulates cell proliferation by activating genes that promote cell cycle progression, such as cyclin D1 and c-myc [17].

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FAK signaling pathways regulate cell survival and cell cycle progression [18-20]. FAK regulates cyclin D1 expression upon cell adhesion, most likely through activation of the extracellular signal-regulated kinase signalling pathway [21]. Strong expression of FAK in the proliferative phase suggests that FAK expression is likely to be related to estrogen levels that reach a peak at this phase.

Enhanced expression of FAK during the EP phase may relate to the rapid capillary growth and endometrial regeneration. High expression of FAK in the ES and MS phases may associate with the preparing of endometrium for implantation. Significant down-regulation of FAK during the LS phase may relate to inhibition of cell survival and facilitate apoptosis in human endometrium and an important signal for happening of menstruation.

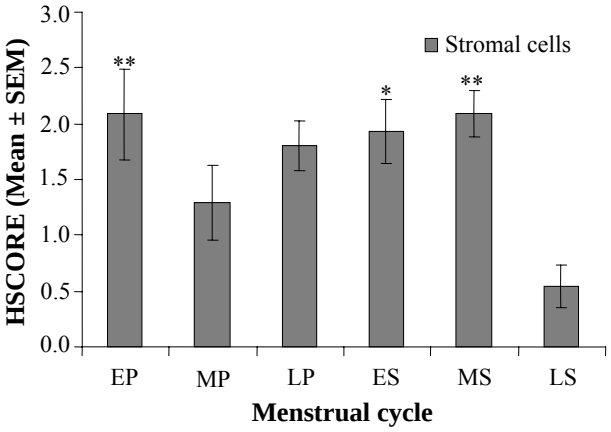


Fig. 4. Expression pattern of FAK in endometrial stromal cells. FAK expression in stromal cells was very strong during the early proliferative, and mid-secretory phases in comparison to the late secretory phase. $^{*}P<0.05$; $^{**}P<0.01$.

It appears that FAK as a key kinase plays a critical role in endometrial remodeling that it may be regulated by steroid hormones.

Previous studies have shown that inhibition of FAK by antisense techniques results in the onset of apoptosis [22] and has clearly confirmed a protective role for FAK in apoptosis [23]. In hence, down-regulation of FAK expression at the LS phase is likely to be related to the fall in concentration of steroid hormones.

Angiogenesis in the human uterus is required to support the reconstruction of endometrium after the menstrual period and to provide a vascularized, receptive endometrium for implantation and placentation three weeks later [24]. It has been suggested that the initial wave of capillary proliferation is under the control of steroid hormones

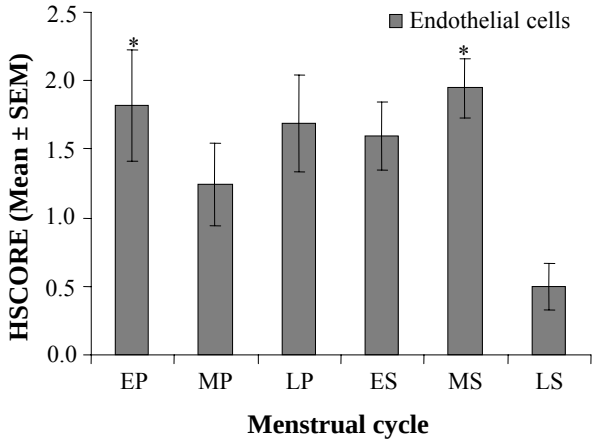


Fig. 5. Expression pattern of FAK in endothelial cells of endometrium. FAK expression in endothelial cells was significantly up-regulated during the early proliferative and mid-secretory phases in comparison to late secretory phase. $^{*}P<0.05$.

[25-27]. Several studies suggest a potential role of FAK in angiogenesis [13, 28]. Deletion of the FAK gene in mice results in embryonic lethality with major defects in embryonic vasculogenesis and angiogenesis [29]. We found a cyclic variation of FAK expression in endothelial cells of human endometrium, with significantly up-regulation during the EP and MS phases. The increase in FAK expression in the early proliferative endometrium may responsible for elongation of the existing vessels and is essential for tissue reconstruction in this phase of the cycle. In the MS phase, the high expression of FAK in the endometrium may be necessary for both elongation and coiling of the spiral arterioles.

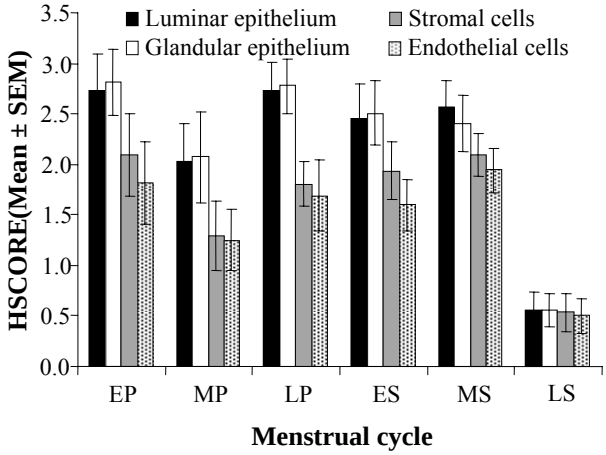


Fig. 6. Comparison of semi-quantitative immuno-istochemical staining pattern (mean HSCORE ± SEM) of FAK in different cell types of normal human endometrial tissue throughout the menstrual cycle. Late secretory phase showed significant down-regulation of FAK expression in all cell types in comparison to other phases.

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Recently, Strange *et al.* [30] have shown that in mammary epithelium during the rat oestrous cycle, FAK protein levels and phosphorylation varied with stage of the cycle. Additionally, Mu *et al.* [31] have shown a positive correlation between serum 17ß-estradiol (E_2) levels and FAK expression in proliferative endometrium and the ratio of serum E_2 to progesterone (P) at the secretory phase. They suggested that E_2 and P exert by contrast regulation of FAK expression in human endometrium at the secretory phase. Although the function of FAK in normal human endometrium is not properly known, the variations of FAK expression during the menstrual cycle suggest a possible role of FAK in endometrial remodeling.

Our results showed that human endometrial FAK expression pattern is a phase-dependent manner during the menstrual cycle. These findings suggest that there may be a relationship between FAK expression and tissue repair or remodeling. This kinase could be a good candidate for therapeutic goals and also in general for tissue engineering.

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