トピックス

Role of epithelial-mesenchymal transition in human adenomyosis

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Introduction

Adenomyosis is defined by the intramyometrial presence of gland and stroma derived from basalis endometrium surrounded by reactive hyperplastic or hypertrophic myometrium causing either focal or diffuse enlargement of the uterus [1, 2]. It preferentially affects parous women between the ages of 35 and 50 years. The differences between endometriosis and adenomyosis are origin of endometrium (functionalis or basalis) and anatomic site of the lesion (outside or inside the uterus) [2]. The exact physiopathology and pathogenesis of adenomyosis are still elusive. According to published literatures, there are different proposals for pathogenesis of adenomyosis, such as, de novo metaplasia of Müllerian remnants, invagination through vascular or lymphatic channel, and stress reaction at the endo-myometrial interface [1, 3–5]. However, the most widely accepted opinion is that adenomyosis develops as a down-growth and invagination of the basalis endometrium into the myometrium [1]. However, a possible mechanism of gland invagination from basalis endometrium deep into underlying myometrium is unknown. Recent reports demonstrated the role of EMT in adenomyosis [6, 7]. However most of them are confined to experiments in animal model or using endometrial cell line. Information on EMT using human endometrial cells in primary culture and tissues is lacking.

E-cadherin is a multigene family of transmembrane glycoproteins and normally acts as an "adhesive zipper" that means E-cadherin maintains tight cell-cell or cell-matrix contact for epithelial cells [8]. It has been demonstrated that epithelial cells, potentially invasive in human endometriosis, lack the expression of E-cadherin [9]. Studies with breast cancer, bladder cancer and hepatoma cells indicated that over expression of hepatocyte growth factor (HGF) may down-regulate cadherin-mediated cell-cell adhesion with consequent migration and invasion into mesenchymal cells [10]. Recent reports demonstrated that estrogen-induced epithelial-mesenchymal transition (EMT) of epithelial cells may contribute to the development of adenomyosis or may be involved in metastatic potential of cancer cells [11, 12]. As an estromedin and pleiotropic growth factor, we speculated that similar to estrogen, HGF might play a similar role in EMT and in the invasion of gland cells into myometriun in women with adenomyosis.

We demonstrated an inverse relationship between HGF and E-cadherin at the gene and protein level in cells and tissues derived from women with adenomyosis [13]. We also found that addition of HGF exhibits scattering effect on endometrial epithelial cells (EECs) with down-regulation of E-cadherin expression in EECs [13]. We investigated the possible ability of HGF to induce EMT in EECs and also in tissues derived from women with and without adenomyosis. In order to explore the possible mechanistic basis of gland invagination deep into the myometrium, we examined three cellular aspects of EMT in response to HGF such as cell-to-cell disruption, cell migration, and cell morphological change. We further investigated gene and protein expression of two transcriptional repressors of E-cadherin, Slug and Slain, in response to estrogen and HGF either alone or in combination.

Expression of E-cadherin and N-cadherin/ Vimentin in response to HGF

A dose-dependent cell separation/scattering effect of HGF on EECs and an inverse relationship between gene and protein expression of HGF and E-cadherin was observed and is described previously. In fact, a time-dependent increase in the mRNA expression of E -cadherin in EECs was found with maximum gene expression at 24 and 48 hrs (at pre-confluent to confluent cells) and this increasing expression of E-cadherin mRNA was decreased after treatment of pre-confluent EECs with HGF. In contrast, mRNA expression of mesenchymal cell marker, N-cadherin, was upregulated in response to HGF treatment. The inverse relationship between HGF and E-cadherin protein expression was significantly observed in the baslis endometria derived from women with adenomyosis. However, this inverse relationship between HGF and E-cadherin protein expression was lost in the functionalis/basalis endometria of control women and in the functionalis endometria derived from women with adenomyosis. Although no visible tissue expression of N-cadherin was found in endometrium derived from women with and without adenomyosis, protein expression of another mesenchymal cell marker, Vimentin, was significantly increased in the basalis endometria derived from women with adenomyosis.

Tissue concentration of HGF at endo-myometrial interface

We collected tissues from the site of endo-myomaterial interface after hysterectomy of control women, women with focal and diffuse adenomyosis. We homogenized these tissues using Polytron homogenizer (Kinematics, Luzern, Switzerland) and tissue level of HGF (pg/μg protein) was measured by ELISA. A significantly higher tissue concentration of HGF was found at the endo-myometrial interface derived from ipsilateral side of focal adenomyosis and anterior/posterior walls of diffuse adenomyosis when compared with similar tissues derived from control women or contralateral endometrium of focal adenomyosis.

Effect of HGF and estrogen in cell migration and morphological change of cells

Boyden's chamber assay indicated that HGF either alone or in combination with estrogen was able to significantly migrate EECs from upper chamber to lower chamber in a dose dependent (0, 50, 100ng/ml) fashion. An additive effect was observed in cell migration between HGF and estrogen. When EECs were pre-treated with anti-HGF antibody and ICI182720 (an ER antagonist), HGF and estrogen promoted cellular migration of EECs were significantly decreased.

In an attempt to examine changes in cell morphology of EECs, we found that HGF (100ng/ml) induced a sequential and time-dependent change in EECs morphology, from cobblestone-like appearance of EECs (at 0 hr) to a mesenchymal phenotype (elongated spindle -shaped cells) at 48 hr. A similar effect was also observed in response to estrogen either alone or in combination with HGF.

Effect of HGF and estrogen on transcriptional repressors of E-cadherin, Slug and Slain

Both basal and estrogen (E2) -stimulated and ER-positive Ishikawa cells displayed c-Met (HGF receptor) expression. Estrogen and HGF was able to significantly up-regulate mRNA expression of SLUG and SLAIN in a dose-dependent manner ($p<0.05$ vs. non-treated cells) and a synergistic effect was observed between E2 and HGF. The individual effect of E2 and HGF was more prominent in the over expression of SLUG gene comparing to SLAIN gene.

A similar pattern of E-cadherin and Slug immunoexpression was observed in both functional and basal endometria derived from contralateral side of focal adenomyosis. An overexpression of Slug was associated with a lower expression of E-cadherin at the basal endometrium (ipsilateral side) and a higher expression of both E-cadherin and Slug was observed at the functional endometria (ipsilateral side) derived from women with focal adenomyosis. Slain immunoreaction

was not observed in either endometria. The detail materials and methods and all these findings are already reported in our recent publication [13].

Discussion

We demonstrated here for the first time a possible involvement of HGF-induced epithelial-mesenchymal transition (EMT) in the development of human adenomyosis. Unlike all previous studies, we used isolated epithelial cells and endometrial tissues derived from women with both focal and diffuse adenomyosis for our current experimental evidence. We found an inverse relationship in the gene and protein expression between HGF and E-cadherin in EECs and in gland cells of basalis endometrium derived from women with both diffuse adenomyosis and ipsilateral side of focal adenomyosis. This means, a higher expression of HGF in tissues was associated with a lower expression of epithelial cell marker, E-cadherin. In contrast, an increased immunoexpression of mesenchymal cell marker, vimentin was observed.

We could find an apparent increase in the gene expression of mesenchymal cell marker, N-cadherin in EECs, but N-cadherin protein expression was lost[13]. This could be due to post-translational degradation of protein at tissue level. The increased tissue expression of HGF was coincided with significantly higher tissue concentrations of HGF in endometria derived from ipsilateral side of focal adenomyosis and anterior /posterior walls of endometria derived from women with diffuse adenomyosis. Interestingly, tissue level of HGF was remarkably low in the endometrium of control women and in the endometrium derived from lesion free contralateral side of focal adenomyosis.

According to published literatures, lower tissue expression of E-cadherin and increased tissue expression of Vimentin or N-cadherin dictates a transitional cascade from an epithelial cell phenotype to mesenchymal cell phenotype. In other words, a phenomenon of EMT that was reported to be involved in the metastatic invasion of cancer cells [10] might also work in the pathogenesis of adenomyosis. With this speculation in mind, we extended our experiments with two additional cellular aspects of EMT, cell migration and cell morphological change. We found that HGF was able to induce a time-dependent sequence of morphological change of EECs from cobblestone appearance of EECs to spindle-shaped cells. This HGF-induced EMT was further supported by HGF-induced cell migration as we confirmed here by Boyden's chamber assay. These findings give us further information that in addition to function as estromedin growth factor (regulated by E2) or multifunctional factor [14, 15] in endometriosis, HGF also retains potential capacity to induce EMT in EECs and in basal endometrium derived from women with adenomyosis.

It has been reported that expression of E-cadherin is negatively associated with the expression of a number of transcriptional repressors of E-cadherin such as Slug, Slain, SIP−1/Zeb−2, E12/E47 and Twist and their involvement in EMT [16−18]. Among them, Slug and Slain expressions are well described and they are found to interact with the proximal E-boxes of E-Cadherin promoter [16]. An inverse relationship between the expression of E-cadherin and Slug/Slain has been described in many different cell systems [16, 17)]. Bolos et al. [17] demonstrated that ectopic expression of Slug/Slain in epithelial cells caused EMT by the aid of migratory and invasive behaviors. A recent study [12] reported that estrogen regulates Snail and Slug in the down-regulation of E-cadherin and induces metastatic potential of ovarian cancer cells. As an estrogen growth factor, we found in our current study that similar to estrogen, HGF has the optimal capacity to up-regulate the gene expression of Slug/Slain and there was an additive effect between HGF and E 2 in further modulation of Slug/Slain expression in Ishikawa cells. We also found an inverse relationship in the protein expression between Slug and E-cadherin but not between Slain and E-cadherin. This effect was predominant in the basal endometrium derived from the ipsilateral side of focal adenomyosis. However, this inverse relationship between Slug and E-Cadherin was not observed in functional layer or in

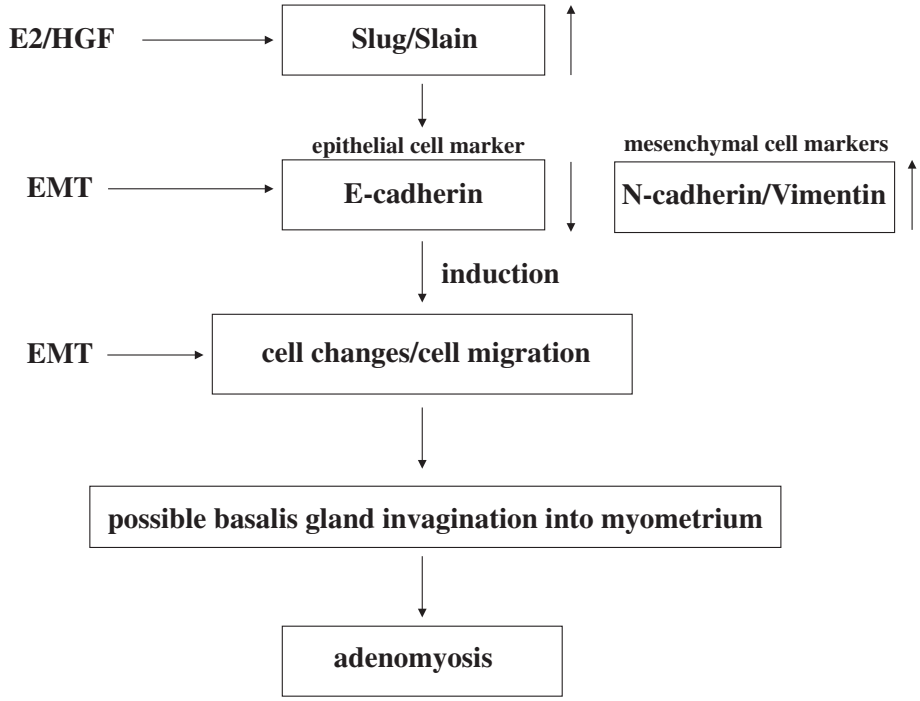

Figure legend

Figure 1 A diagrammatic representation of hepatocyte growth factor (HGF) -and estrogen (E2) -induced pathways in the occurrence of epithelial-mesenchymal transition (EMT) in adenomyosis. Up-regulation of SLUG and SNAIL, two transcriptional repressors of E-cadherin, in response to HGF and E2 is associated with decreased expression of E-cadherin (epithelial cell marker) and increased expression of N-cadherin/Vimentin (mesenchymal cell markers) causing disruption of tight cell-cell contact. These cellular events in endometrial cells and in intact tissues trigger morphological changes of endometrial epithelial cells (EECs) to a mesenchymal phenotype and induced increased cell migration, essential components of EMT that was induced by HGF and E2 either alone or in combination. All these events of EMT may be involved in the invagination of glandular epithelial cells from basalis endometrium into the myometrium and finally result in the development of adenomyosis.

similar tissues derived from contralateral side of focal adenomyosis.

Collectively, we found higher tissue levels of HGF in endometrium, HGF-mediated up-regulation of Slug/Slain with consequent down-regulation of E-cadherin, HGF-induced morphological change to a fibroblast-like phenotype, a shift from epithelial marker expression to mesenchymal marker expression and HGF-induced increased cell migration, All these biological events in response to HGF support in favor of EMT in adenomyosis[13]. A diagrammatic representation of different cascades of EMT involved in the development of human adenomyosis is shown in Figure 1. We conclude that similar to estrogen, HGF may be involved in gland invagination deep into myometrium by inducing EMT at the endo-myometrial interface in adenomyosis and this effect was augmented by the local estrogen. Estrogen-induced EMT has found to be abrogated after treatment with raloxifene, a selective estrogen receptor modulator [12]. Possible changes in HGF-induced cellular cascade of EMT in response to estrogen suppressing agents can be the future target of investigation. Further studies are needed to strengthen our current findings.

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