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Original contribution

Epithelial to mesenchymal transition in early stage endometrioid endometrial carcinoma☆,☆☆

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Summary Epithelial to mesenchymal transition is thought to be implicated in tumor invasion and metastasis. To investigate its role in myometrial invasion, samples from 42 stage I (confined to the corpus) endometrioid endometrial carcinomas were analyzed. All E-cadherin repressors (*SNAI1*, *SNAI2* (*SLUG*), *ZEB1*, *HMGA2*, and *TWIST1*) had a higher expression in endometrioid endometrial carcinomas than in normal endometrium ($P < .0001$), whereas *CDH1* (E-cadherin gene) tended to be lower. In comparison with nonmyoinvasive (stage IA) tumors, those with deep myometrial invasion (stage IC) had increased messenger RNA expression of *SLUG*, *ZEB1*, and *HMGA2* ($P < .001$). Furthermore, samples from the myoinvasive front of deeply invasive tumors had higher levels of *SLUG, ZEB1*, and *HMGA2* than the corresponding superficial samples. Immunohistochemical analysis of these cases revealed that the decrease in E-cadherin was concordant with an increase in Snail and Twist protein expression. Trying to induce epithelial to mesenchymal transition in endometrioid endometrial carcinomas, we initially produced persistent activation of this pathway in Ishikawa cells. The cell line was infected with lentiviruses carrying the V600E mutation of BRAF, inducing loss of β-catenin, E-cadherin, and cytokeratin and increase in vimentin and Snail. These changes were mediated by ERK1/2 phosphorylation, which was also increased at the myoinvasive front. Furthermore, MEK1/2 inhibitor UO126 reversed the mesenchymal phenotype. Our findings suggest that epithelial to mesenchymal transition regulators are implicated in myometrial invasion of endometrioid endometrial carcinoma and may be potential therapeutic targets through the MAPK/ERK pathway.

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1. Introduction

Endometrial carcinoma is the most common malignant gynecologic tumor in the Western world. Approximately 80% of cases are estrogen-dependent, well- to moderately differentiated endometrioid endometrial carcinoma (EEC) that are usually confined to the uterine corpus at diagnosis (stage I), and thus, most are surgically curable. In contrast, the estrogen-independent non-EECs account for only 10% to 15% of cases and are poorly differentiated tumors that frequently invade the myometrium, extend beyond the uterus at the time of hysterectomy, and are associated with poor outcome.

Although EEC and non-EEC follow different pathogenetic pathways, loss of epithelial markers such as E-cadherin is associated with adverse prognosis in both tumor types [1-3].

E-cadherin provides a physical bond between epithelial cells both normal and neoplastic, and its function is essential for integrity of epithelia. Thus, down-regulation of E-cadherin is the key target of epithelial to mesenchymal transition (EMT) modulators [4-6]. Because of its crucial role in EMT, E-cadherin requires a tight control. Various pathways, such as Ras/MAPK, PI3K/Akt, and transforming growth factor β, participate in EMT regulation [7-9], and all share a common end point: the activation of a series of transcription factors that directly repress E-cadherin. The main transcription factors are Snail, Slug, ZEB1, ZEB2, HMGA2, and Twist. Indeed, Snail has been identified as a powerful E-cadherin inhibitor both in normal and pathologic conditions [10-12]. Although some authors have proposed a hierarchy in the function of these transcription factors [13], others have suggested that coexpression of ZEB1 and Snail is the most relevant EMT mechanism [6,14,15].

Recently, it has been shown that Snail protein expression increases and correlates inversely with E-cadherin immunoreactivity both in primary and metastatic endometrial carcinomas [16,17]. Furthermore, high Twist expression has also been shown in invasive endometrial carcinoma and affects patient survival [18].

Although EMT has mainly been related to metastatic spread, we have investigated stage I EEC in an attempt to elucidate the role of EMT in myometrial invasion. To provide further insight into the role of E-cadherin repressors, we analyzed the expression profile of Snail, Slug, ZEB1, Twist, and HMGA2 in a series of 42 stage I EECs.

Because we wanted to explore the potential reversibility of EMT in EEC, we created an in vitro scenario with Ishikawa cells. It has been reported that Ras downstream signaling is required for EMT in many different cell systems [19-22]. Even if *BRAF* mutation is exceedingly rare in EEC, its V600 missense mutation induces a persistent activation of the MAPK/ERK pathway that allows tumor cells to undergo EMT [21,22]. We used this scenario to mimic an EMT in vitro as a model for new therapies based on the regulation of E-cadherin.

2. Material and methods

2.1. Patient and tissue specimens

A total of 42 patients with EEC (mean age, 59.2 years; range, 26-81 years) who underwent hysterectomy at the Gynecology Department of Hospital de la Santa Creu i Sant Pau, Barcelona, from January 1998 to February 2007 were enrolled in the study. Using the surgical staging system of the International Federation of Gynecology and Obstetrics, all 42 tumors were classified as stage I (confined to the uterine corpus), and substages were IA (tumor limited to endometrium), 11 tumors; IB (tumor invades less than one half of myometrium), 7 tumors; and IC (tumor invades one half or more of myometrium), 24 tumors. In deeply myoinvasive tumors, areas from the most superficial and deep zones were selected separately. On histologic examination, 14 tumors were classified as grade 1 (G1); 13, grade 2 (G2); and 18, grade 3 (G3). Tumor samples were collected at the time of surgery after written informed consent and approval by the Hospital Ethics Committee. As control, we used 10 samples of normal endometrium of age-paired women obtained from hysterectomy specimens for leiomyomas or prolapse.

2.2. RNA extraction, reverse transcription, and quantitative real-time polymerase chain reaction

Total RNA extraction and reverse transcription have been described elsewhere [15]. Briefly, 1 μg of total RNA was converted into complementary DNA (cDNA) by the first-strand cDNA synthesis using the AMV reverse transcriptase (Roche, Mannheim, Germany) with random hexamers. All the cDNA samples were eluted in a final volume of 120 μL. Quantitative real-time polymerase chain reaction was performed using TaqMan technology on an ABI7300 detection system (Applied Biosystems, Carlsbad, CA). The reaction was performed with 10-μL TaqMan Universal PCR Master Mix, No AmpErase UNG 2X, 1 μL 20X TaqMan probes, and 5 μL of cDNA diluted in RNase-free water adjusted to 20-μL volume reaction. The thermal cycler conditions were UNG activation 2 minutes at 50°C, AmpliTaq activation 95°C for 10 minutes, denaturation 95°C for 15 seconds, and annealing/extension 60°C for 1 minute (repeat 40 times). Each reaction was performed in triplicate from different cDNA dilutions. Cycle threshold values were analyzed with Quantitative Relative software using comparative cycle threshold ($\Delta\Delta$Ct) method as described by the manufacturer. The amount of target was obtained by normalizing to an endogenous reference gene (*ABL-1*). Results are presented as a relative messenger RNA (mRNA) amount compared with control normal pooled samples. The sequence of oligonucleotides and TaqMan probes used that were obtained using the Assay-on-demand Gene (Applied Biosystems) were *SNAI1* (Hs00195591_m1),