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Progesterone Regulates β-Catenin mRNA Levels in Human Endometrial Stromal Cells In Vitro

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Cadherin–catenin complexes mediate cell–cell interactions and may play a central role in intracellular signaling. To date, the factors capable of coordinately regulating cadherin and catenin expression levels within a mammalian cell remain poorly characterized. We have recently determined that progesterone is a key regulator of cadherin-11 mRNA and protein expression levels in cultured human endometrial stromal cells. As a first step in determining whether gonadal steroids are also capable of regulating stromal catenin expression, we have examined the ability of progestins, estrogens, and androgens to regulate β-catenin mRNA levels in these endometrial cell cultures. Here we report that progesterone, but not 17β-estradiol or dihydrotestosterone, increased β-catenin mRNA levels in cultured human endometrial stromal cells. The stimulatory effect of progesterone on the levels of the stromal β-catenin mRNA transcript could not be potentiated by 17β-estradiol. These studies not only demonstrate that gonadal steroids are capable of regulating β-catenin mRNA levels in human endometrial stromal cells, but may also give us useful insight into the cellular mechanisms by which gonadal steroids regulate the cyclic remodeling processes that occur in the human endometrium during each menstrual cycle.

Key Words: β-catenin; catenins; steroids; human; endometrium; stromal cells.

Introduction

The cadherins are a gene superfamily of integral membrane glycoproteins that mediate calcium-dependent cell adhesion in a homophilic manner *(1–3)*. The spatiotemporal expression of cadherin subtypes during tissue morphogenesis has been associated with such fundamental biological processes as cell sorting and aggregation, proliferation, and differentiation *(1,2,4)*. The ability of the cadherins to mediate cellular interactions and govern the developmental fate of cells is dependent on these cell adhesion molecules (CAMs) interacting with at least three cytoskeletal-associated proteins known as α-, β-, and γ-catenin *(5–7)*. The cytoplasmic domain of the cadherin interacts with either β- or γ-catenin in a mutually exclusive manner *(8,9)*. These two catenins, in turn, bind to α-catenin, which is responsible for anchoring the cadherins to the cytoskeleton either directly *(10)* or indirectly through interactions with the actin binding protein, α-actinin *(11)*. The catenins not only link the cadherins to the underlying actin cytoskeleton, but are believed to be involved in activating several intracellular signaling pathways *(12,13)*.

We have recently determined that the cadherin subtype, known as cadherin-11 (cad-11), is spatiotemporally expressed in the stroma of the human endometrium during the menstrual cycle *(14,15)*. In particular, cad-11 was first detected in the endometrial stroma during the secretory phase of the menstrual cycle when these cells are beginning to undergo decidualization in response to increasing levels of progesterone (P4). Maximum levels of cad-11 were detected in the decidua of early pregnancy. Similarly, β-catenin has been detected in the human endometrium *(16)* and been further localized to the endometrial stroma *(17)*. In view of these observations, it is tempting to speculate that cad-11 may play a central role in the steroid-mediated, differentiation of endometrial stromal cells into decidual cells by associating with β-catenin.

The factors capable of regulating cadherin and catenin expression within mammalian cells remain poorly characterized. We have recently demonstrated that progesterone (P4) is a key regulator of cad-11 mRNA and protein expression levels in human endometrial stromal cells in vitro *(15,18)*. In addition, the stimulatory effects of P4 on stromal cad-11 mRNA and protein expression is enhanced by 17β-estradiol (E2). As a first step in determining whether gonadal steroids coordinately regulate stromal cad-11 and β-catenin expression, we have examined the ability of E2

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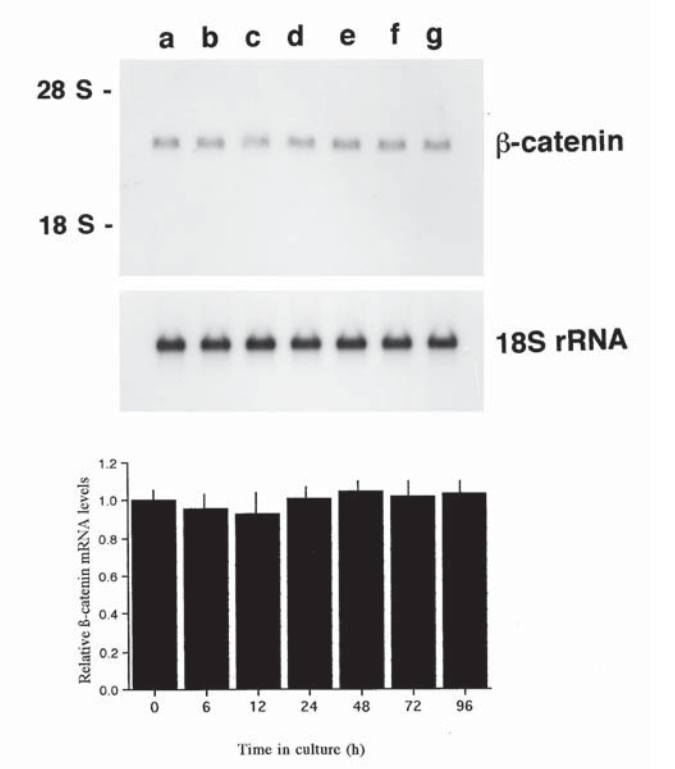

Fig. 1. Autoradiograms of a Northern blot containing total RNA extracted from endometrial stromal cells cultured in the presence of vehicle (0.1% ethanol). The cells were harvested 0, 6, 12, 24, 48, 72, or 96 h after treatment (lanes a–g, respectively). The blot was probed for β-catenin (top panel) or 18S rRNA (bottom panel). The autoradiograms were scanned using a laser densitometer. The absorbance values obtained for the β-catenin mRNA transcript were then normalized to the values obtained for the 18S rRNA. The results derived from this analysis, as well as from two other studies (autoradiograms not shown), were standardized to the 0 h control and are represented (mean ± SEM; $n = 3$) in the bar graph (*$p \leq 0.05$).

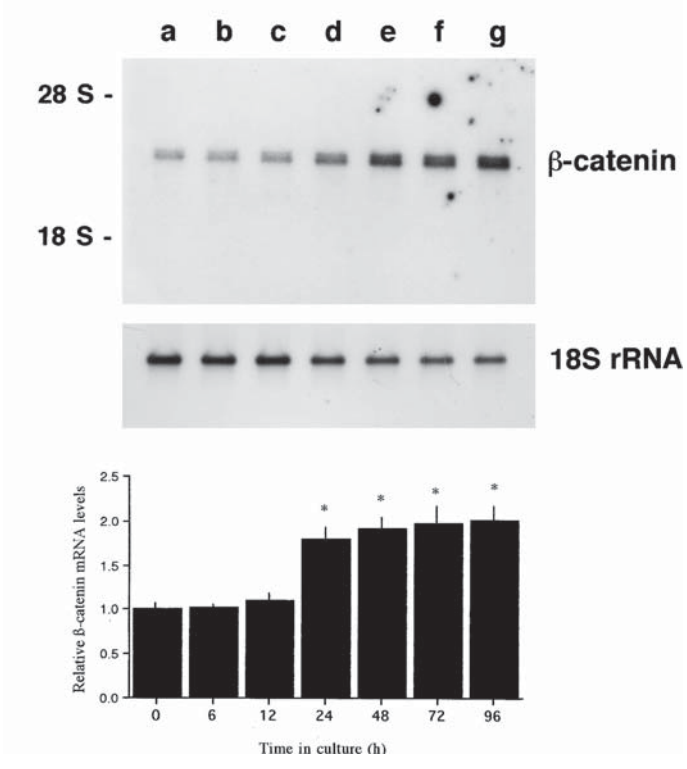

Fig. 2. Autoradiograms of a Northern blot containing total RNA extracted from endometrial stromal cells cultured in the presence of 1 μ*M* P4. The cells were harvested 0, 6, 12, 24, 48, 72, or 96 h after treatment (lanes a–g, respectively). *See* Fig. 1 for further methodological details.

and P4, alone or in combination, and the nonaromatizable androgen, dihydrotestosterone (DHT), to regulate β-catenin mRNA levels in these endometrial cell cultures.

Results

A single β-catenin mRNA transcript of 3.3 kb was detected in all of the total RNA extracts prepared from the cultured endometrial stromal cells (Fig. 1). The addition of vehicle to the culture medium had no significant effect on the levels of this stromal β-catenin mRNA transcript at any of the time-points examined in these studies (Fig. 1).

P4 caused a significant increase in stromal β-catenin mRNA levels after 24 h of culture in the presence of this steroid (Fig. 2). The levels of the β-catenin mRNA transcript remained elevated until the duration of these studies at 96 h. The effect of P4 on stromal β-catenin mRNA levels was also dose-dependent with maximal stimulation being observed at 1 μ*M* P4 (Fig. 3). There was no further increase in the levels of the stromal β-catenin mRNA transcript when the concentration of P4 was increased to 5 μ*M*.

E2 or DHT had no significant effect on stromal β-catenin mRNA levels at any of the time-points examined in these studies (data not shown).

Finally, there was no significant difference between the β-catenin mRNA levels observed in endometrial stromal cells cultured in the presence of P4 plus varying doses of E2 and those detected in cells cultured in P4 alone (Fig. 4).

Discussion

A single β-catenin mRNA transcript of 3.3 kb was detected in all of the total RNA extracts prepared from the cultured endometrial stromal cells. This β-catenin mRNA transcript has been previously detected in stomach, colon, and breast carcinoma cells *(19–21)*.

In view of the direct correlation between catenin expression levels and the metastatic potential of carcinoma cells in vivo and in vitro *(22,23)*, previous studies have focused on the hormonal regulation of catenin mRNA and protein expression levels in epithelial cells. Fujimoto et al. *(24)* reported that P4 was not capable of increasing the levels of the mRNA transcripts encoding α- and β-catenin in Ishikawa cells, whereas E2 decreased the levels of these two mRNA transcripts in this endometrial adenocarcinoma cell line. The effects of E2 on α- and β-catenin mRNA levels were reversed by the addition of P4 to the culture medium. In contrast, P4 increased α-catenin mRNA levels

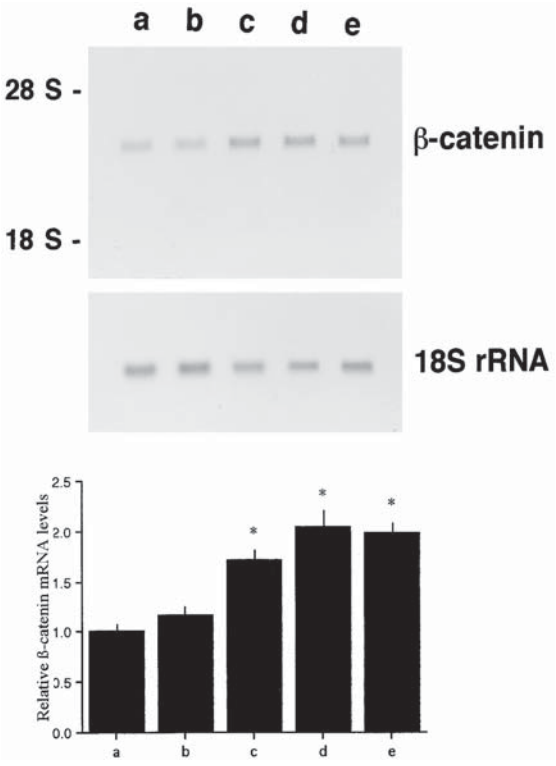

Fig 3. Autoradiograms of a Northern blot containing total RNA extracted from endometrial stromal cells cultured in the presence of vehicle (lane a) or 0.1, 0.5, 1, and 5 μ*M* P4 (lanes b–e, respectively) for 96 h. *See* Fig. 1 for further methodological details. The results for this and two other studies were standardized to the vehicle control and are represented (mean ± SEM; $n = 3$) in the bar graph (*$p \leq 0.05$).

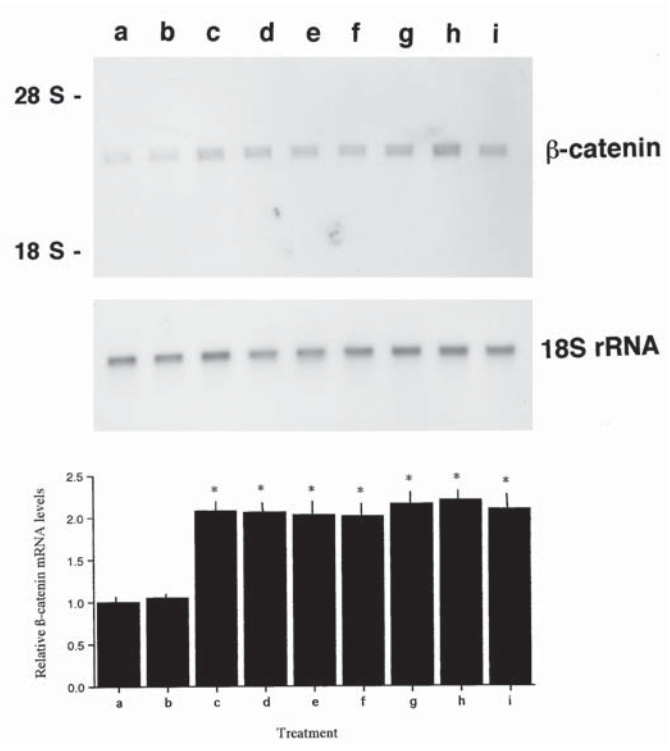

Fig 4. Autoradiograms of a Northern blot containing total RNA extracted from endometrial stromal cells cultured in the presence of vehicle (lane a), 30 n*M* E2 (lane b), 1 μ*M* P4 (lane c), or 1 μ*M* P4 plus 0.5, 1, 5, 10, 30, or 100 n*M* E2 for 96 h (lanes d–i, respectively). *See* Fig. 1 for further methodological details.

in T47D breast carcinoma cells *(25)*. Finally, retinoids that increased β-catenin protein stability in SKBR3 breast carcinoma cells had no significant effect on β-catenin mRNA levels in this cell line *(20)*. In our studies, we have determined that P4 is capable of regulating β-catenin mRNA levels in primary cultures of endometrial stromal cells. The stimulatory effects of P4 on stromal β-catenin mRNA levels appears to be specific for this gonadal steroid, since E2 and DHT had no significant effect on the levels of the β-catenin mRNA transcript present in these cell cultures. To our knowledge, this is the first demonstration that gonadal steroids are capable of regulating stromal β-catenin mRNA levels. Collectively, these observations suggest that the regulation of β-catenin mRNA levels is complex and that other factors, alone or in combination with the gonadal steroids, may be involved in differentially regulating β-catenin mRNA levels in human epithelial and stromal cells.

The P4-mediated increase in β-catenin mRNA levels in cultured human endometrial stromal cells correlates with the ability of this gonadal steroid to regulate cad-11 mRNA and protein expression levels in these primary cell cultures *(18)*. Taken together, these studies suggest that β-catenin and cad-11 mRNA levels are coordinately regulated in human endometrial stromal cells in vitro. In view of these observations, it is tempting to speculate that steroids exert their morphogenetic effects on the endometrium, at least in part, by virtue of their ability to regulate β-catenin and cad-11 expression. However, in contrast to the P4-mediated increase in stromal cad-11 mRNA levels, the stimulatory effects of P4 on the levels of the β-catenin mRNA transcript could not be potentiated by the addition of E2 to the culture medium. These observations suggest that P4 increases β-catenin and cad-11 mRNA levels in human endometrial stromal cells by different molecular mechanisms. To date, the mechanism(s) by which gonadal steroids regulate β-catenin and cad-11 mRNA and protein expression levels have not been defined.

The biological role(s) of the cad-11/β-catenin complex in cellular differentiation remain poorly understood. Colocalization of cad-11 and β-catenin mRNA transcripts in signet-ring cell carcinoma cells has led to the proposal that this cadherin/catenin complex may be involved in modulating the invasive capacity of these cells *(26)*. In addition, cad-11 has been shown to be coexpressed with β-catenin during the terminal differentiation of human osteoblasts in vitro *(27)*. In the human endometrium, β-catenin mRNA levels have been shown to increase during the secretory phase of the menstrual cycle when P4 is the predominant steroid *(16)*. Similarly, cad-11 is first detected in areas of early decidualization in the secretory endometrium

(14,15). The spatiotemporal expression of cad-11 and β-catenin in the human endometrium during the secretory phase of the menstrual cycle suggests that this cadherin/catenin complex may play a central role in the P4-mediated terminal differentiation of endometrial stromal cells into decidual cells. The ability of P4 to regulate β-catenin and cad-11 mRNA levels in human endometrial stromal cells in vitro suggests that these cells may provide a useful model system to define the role(s) of the cad-11/β-catenin complex in cellular differentiation.

In summary, we have demonstrated that P4, but not E2 or DHT, is capable of regulating β-catenin mRNA levels in cultured human endometrial stromal cells. These studies not only add to our understanding of the cell biology of β-catenin, but give us useful insight into the mechanism(s) by which gonadal steroids regulate the cyclic remodeling processes which occur in the human endometrium during each menstrual cycle. Future studies will define the role(s) of the cad-11/β-catenin complex in the decidualization of human endometrial stromal cells in vitro.

Materials and Methods

Tissues

Endometrial tissue biopsy specimens (n = 18) were obtained from women of reproductive age in accordance with a protocol for the use of human tissues approved by the Committee for Ethical Review of Research involving Human Subjects, University of British Columbia. All patients had normal menstrual cycles. The stage of the menstrual cycle was determined by the last menses and confirmed by histological evaluation according to the criteria of Noyes et al. *(28)*. Tissues used in this study were obtained during the midsecretory phase of the menstrual cycle.

Cell Preparation and Culture

The endometrial stromal cells were separated from the glandular epithelium by enzymatic digestion and mechanical dissociation using a protocol modified from that reported by Shiokawa et al. *(29)*. Briefly, the endometrial biopsy specimens were minced and subjected to 0.1% collagenase (type IA, Sigma, St. Louis, MO) and 0.1% hyaluronidase (type I-S, Sigma) digestion in a shaking water bath at 37°C for 1 h. The cell digest was then passed through a nylon sieve (38 μm). The isolated glands were retained on the sieve, and the eluate containing the stromal cells collected in a 50-mL tube. The stromal cells were pelleted by centrifugation at 800*g* for 10 min at room temperature. The cell pellet was washed once in phenol red-free Dulbecco's Modified Eagle's medium (DMEM) containing 10% charcoal-stripped fetal bovine serum (FBS) before being resuspended and plated in phenol red-free DMEM containing 25 m*M* glucose, 25 m*M* HEPES, 2 m*M* L-glutamine, antibiotics (100 U/mL penicillin, 100 μg/mL streptomycin, and 2.5 μg/mL fungizone), and supplemented with 10% charcoal-stripped FBS. The culture medium was replaced 30 min after plating in order to reduce epithelial cell contamination. The purity of the cell cultures was determined by immunocytochemical staining for vimentin, cytokeratin, muscle actin, and factor VIII (data not shown). These cellular markers have been previously used to determine the purity of human endometrial stromal cell cultures *(30)*. As defined by these criteria, the endometrial stromal cell cultures used in these studies contained <1% of muscle, epithelial, and vascular cells.

Hormone Treatments

The stromal cells (passage 2) were grown to confluence, washed with PBS, and cultured in phenol red-free DMEM supplemented with 10% charcoal-stripped FBS under the following conditions.

To determine the effects of P4 on β-catenin mRNA levels in human endometrial stromal cells, cultures were exposed to the vehicle (0.1% ethanol) or a fixed concentration of P4 (1 μ*M*) for 0–96 h. Endometrial stromal cells were also cultured in the presence of vehicle or increasing doses of P4 (0.1–5 μ*M*) for 96 h before being harvested for Northern blot analysis.

The ability of other gonadal steroids to regulate stromal β-catenin mRNA levels was determined by culturing the cells in the presence of E2 (30 n*M*) or DHT (0.1 μ*M*) for 0–96 h before being harvested for Northern blot analysis.

Finally, since a combination of E2 and P4 is required for maximal cad-11 mRNA levels in endometrial stromal cells *(18)*, we examined whether different concentrations of E2 could potentiate the P4-mediated increase in stromal β-catenin mRNA levels. The stromal cell cultures were cultured in the presence of vehicle (0.1% ethanol), E2 (30 n*M*), P4 (1 μ*M*), or P4 (1 μ*M*) plus varying doses of E2 (0.5–100 n*M*) for 96 h before being harvested for Northern blot analysis.

The concentrations of hormones used in these experiments were selected on the basis of previous studies *(30–32)*. In all of these studies, the culture medium was changed every 24 h.

Northern Blot Analysis

Total RNA was prepared from the cultured stromal cells by the phenol-chloroform method of Chomczynski and Sacchi *(33)*. The RNA species were resolved by electrophoresis in 1% agarose gels containing 3.7% formaldehyde. Approximately 20 μg of total RNA were loaded per lane. The fractionated RNA species were then transferred onto charged nylon membranes.

The Northern blots were hybridized with a radiolabeled cDNA probe specific for human β-catenin (kind gift from S. W. Byers, Georgetown University, Washington, DC) according to the methods of MacCalman and Blaschuk *(34)*. The blots were then washed twice with 2X SSPE (20X SSPE consists of 0.2 *M* sodium phosphate monobasic,

pH 7.4 containing 25 m*M* EDTA and 3 *M* NaCl) at room temperature, twice with 2X SSPE containing 1% SDS at 55°C, and twice with 0.2X SSPE at room temperature. The blots were subjected to autoradiography in order to detect the hybridization of the radiolabeled probe to the β-catenin mRNA species. To standardize the amounts of total RNA in each lane, the blots were then probed with a radiolabeled synthetic oligonucleotide specific for 18S rRNA as described by MacCalman et al. *(35)*. The blots were again subjected to autoradiography to detect the hybridization of the radiolabeled probe to the 18S rRNA. The autoradiograms were then scanned with an LKB laser densitometer (LKB, Rockville, MD). The absorbance values obtained for the β-catenin mRNA transcript were normalized relative to the corresponding 18S rRNA absorbance value.

Statistical Analysis

The results are presented as the mean relative absorbance (±SE) for three independent experiments. Statistical differences between time-points and treatments were assessed by the analysis of variance (ANOVA). Significant differences between the means were determined using the least significance test. Differences were considered to be significant for $p \leq 0.05$.

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