ORIGINAL ARTICLE: REPRODUCTIVE BIOLOGY

Human chorionic gonadotropin controls luteal vascular permeability via vascular endothelial growth factor by down-regulation of a cascade of adhesion proteins

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Objective: To study the functional interactions of junctional proteins acting as regulators of vascular permeability in the human corpus luteum. We investigated the role of vascular endothelial (VE)-cadherin, nectin 2, and claudin 5 as controllers of vascular endothelial cell permeability.
Design: Performing immunohistochemical dual staining, we colocalized the above-mentioned proteins in the human corpus luteum.
Setting: Not applicable.
Patient(s): Not applicable.
Intervention(s): Not applicable.
Main Outcome Measure(s): Using a granulosa-endothelial coculture system, we revealed that hCG-treatment down-regulates VE-cadherin, nectin 2, and claudin 5 in endothelial cells via vascular endothelial growth factor (VEGFA).
Result(s): Furthermore, the interaction of VE-cadherin, nectin 2, and claudin 5 was investigated by silencing these proteins that perform siRNA knockdown. Interestingly, knockdown of VE-cadherin and claudin 5 induced a decrease of the respective other protein. This down-regulation was associated with changed rates of vascular permeability: hCG induced a VEGFA-dependent down-regulation of VE-cadherin, nectin 2, and claudin 5, which increased the endothelial permeability in the coculture system. Furthermore, knockdown of VE-cadherin, nectin-2, and claudin 5 also resulted in a consecutive increase of endothelial permeability for each different protein.
Conclusion(s): These results demonstrate for the first time that VE-cadherin, nectin 2, and claudin 5 are involved in the regulation of vascular permeability in a mutually interacting manner, which indicates their prominent role for the functionality of the human corpus luteum. (Fertil Steril® 2013;■:■–■. ©2013 by American Society for Reproductive Medicine.)
Key Words: Tight junction, adherens junction, endothelial permeability, VEGFA

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The human corpus luteum is a transitory active endocrine gland consisting of different cell types including endothelial cells, granulosa lutein cells, and theca lutein cells. For uptake of nutrients to produce hormones that have to be secreted into the bloodstream, regulation of vascular permeability is a prerequisite for normal luteal function. Endothelial cells are responsible for controlling this vascular permeability. The space between endothelial cells is sealed by interendothelial molecules. Permeability is mediated by the strictly regulated opening and closing of cell-cell junctions (1–3). A decrease of intercellular junctions induces a gap between neighbored endothelial cells, thereby increasing vascular permeability. For

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that reason, any disturbance of junctional organization might result in dysregulated endothelial function leading to pathological conditions by modifying the regular structure of the vessel wall. Typical examples with clinical importance caused by increased vascular permeability are edema or ovarian hyperstimulation syndrome (OHSS), which is associated with assisted reproduction (4). It is characterized by increased capillary permeability leading to leakage of fluid from the vascular compartment (5). The characterization of the function of endothelial junctions may open new therapeutic implications for limiting tissue damage by influencing vascular permeability.

In the endothelium, paracellular permeability involves at least two different types of intercellular junctions: adherens junctions (AJ) and tight junctions (TJ). These are formed by different transmembrane proteins that promote homophilic cell-cell interactions and transfer of intracellular signals (6). Many reports support the concept that intercellular junctions are dynamically remodeled not only during embryogenesis but also in resting cells (7). Adhesive membrane proteins of AJ and TJ form adhesive complexes that act as zipper-like structures between interacting cells (8–11). Endothelial cells express tissue-specific transmembrane adhesion proteins: the AJ VE-(vascular endothelial)-cadherin and the TJ claudin 5 (1, 12). Knockdown of claudin 5 in mice is associated with a normal embryological development, but, due to a defective blood brain barrier function, the claudin 5–deficient mice die shortly after birth (12). In comparison, VE-cadherin–deficient mice experience severe lethal defects during developmental angiogenesis (13). This suggests a role for VE-cadherin going far beyond promoting only structural functions between endothelial cells.

For the junctional proteins VE-cadherin and claudin 5, paracrine regulatory mechanisms have been shown in the human corpus luteum. We demonstrated recently that claudin 5 and VE-cadherin were expressed in the luteal vasculature and that the expression of both proteins was decreased after in vivo treatment with hCG (14). This effect may be secondary to VEGFA as hCG induces *VEGFA* expression in luteal cells in vitro (15, 16) and in vivo (17). Indeed, VEGFA has been shown to be able to enhance vascular permeability (18, 19) and to influence endothelial AJs (20). Furthermore, inhibition of VEGFA after luteal angiogenesis is complete in a primate model in vivo and suppresses P levels, emphasizing the importance of permeability changes in the corpus luteum (21, 22). In addition, it has been shown in an in vitro corpus luteum model that down-regulation of claudin 5 in endothelial cells is associated with increased vascular permeability (23), demonstrating the importance of this junctional protein for regulation of luteal permeability.

Going beyond the regulatory role of claudin 5 itself for luteal permeability, there are data supporting the hypothesis of a VE-cadherin-dependent expression of claudin 5 in endothelial cells (24). It has been shown that VE-cadherin-mediated adhesion is the prerequisite for claudin 5 expression. VE-cadherin prevents the nuclear accumulation of repressors of claudin 5 expression, the transcriptional regulators FoxO1 and ß-Catenin (24): VE-cadherin activates the PI-3 kinase/AKT pathway, subsequently causing phosphorylation of the forkhead repressor transcription factor Fox01 resulting in its cytosolic localization. Thus, Fox01 is enabled to bind to its nuclear DNA target and to inhibit claudin 5 gene expression. However, on the other hand, in the absence of VE-cadherin, nonphosphorylated Fox01 accumulates in the nuclear compartment of the cell, leading to the repression of claudin 5 expression.

In addition to VE-cadherin and claudin 5 interactions, the nectin-afadin system has recently been described as a novel modulator of AJ and TJ (25), acting as a cooperator system of AJ and TJ regulation (26, 27). The nectins comprise a family consisting of at least four members (nectin 1–4; [28–33]). Nectins are associated with the actin cytoskeleton through afadin, an F-actin-binding protein (34–36) forming homophilic and heterophilic *trans*-dimers (26). Thereby, they function as cell-cell adhesion molecules and regulate the initial step of cell-cell junction formation of both AJs and TJs in endothelial cells. In this context, we focused on nectin 2, since it has been recently described to be expressed in the corpus luteum.

While in different in vivo and in vitro models functional interaction of junctional proteins including nectins for regulation of vascular permeability has been demonstrated, such regulatory mechanisms are largely unknown with respect to the human corpus luteum. Here we address the question of whether or not VE-cadherin, nectin 2, and claudin 5 interact in the corpus luteum. Thus, we first colocalized the proteins of interest in the human midluteal corpus luteum. We hypothesized that these proteins interact in a way such that changes in one protein influence the expression of the others, which results in changed rates of vascular permeability. We therefore went on to study the interaction of VE-cadherin, nectin 2, and claudin 5 in a coculture system of granulosa and endothelial cells after hCG stimulation and VEGFA inhibition as well as the principal effects of knockdown of those junctional proteins on each other and on changes of endothelial permeability.

MATERIALS AND METHODS

Tissue Collection and Preparation

Corpora lutea from patients undergoing hysterectomy for benign reasons were enucleated during early midluteal phase as described in detail elsewhere (37, 38). The date of the preovulatory LH surge was determined by measuring LH concentrations in serial early morning urine samples: LH + 6 to LH + 10 = midluteal phase. The tissue was fixed in 4% paraformaldehyde for 24 hours. In all cases, morphological dating of the luteal phase endometrium was used to confirm the luteal-phase classification.

The embedded tissue was sectioned (5 μm), and tissue sections were placed onto BDH SuperFrost slides (BDH, Merck & Co., Inc.). The sections were used for each protein (VE-cadherin, nectin 2, and claudin 5) and the corresponding CD 31 staining. Tissue sections were dewaxed in xylene, rehydrated in descending concentrations of ethanol, and washed in distilled water.

The study was approved by the Southeast of Scotland Medical Research Ethics Committee, and informed consent was obtained from all patients before tissue collection.