

Embryo-Endometrial Interaction

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Embryonic implantation, the process by which the human embryo orientates towards, attaches to and finally invades the underlying maternal endometrial tissue, requires a receptive endometrium, a functionally normal blastocyst and an adequate cross-communication between them. During apposition, human blastocysts find a location in which to implant, while they are guided to a specific area in the maternal endometrium. In the adhesion phase, which occurs 6 to 7 days after ovulation, within the so-called “implantation window”, direct contact occurs between the endometrial epithelium (EE) and the trophoctoderm (TE). Finally, in the invasion phase, the embryonic trophoblast traverses the basement membrane and passes the endometrial stroma and reaches the uterine vessels. Many molecules (hormones, cytokines, integrins, enzymes, etc) take part in the dialogue between the human blastocyst and the maternal endometrium to achieve implantation. Here, we present our published data on the embryonic regulation of endometrial epithelial molecules such as chemokine receptors and the leptin system. (*Chang Gung Med J* 2006;29:9-14)

Key words: embryo, endometrium.

Introduction

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endometrium to achieve implantation. Here, we present our published data on the embryonic regulation of endometrial epithelial molecules such as chemokine receptors and the leptin system.

Hormonal and Embryonic Regulation of Chemokine receptors CXCR1, CCR2, CCR5 and CXCR4 at the maternal-embryonic inter-phase

Chemokines (short for chemoattractant cytokines), a family of small polypeptides with molecular weight in the range of 8 to 12 kD, attract specific leukocyte subsets by binding to cell-surface receptors. In reproductive biology, these molecules have been implicated in crucial processes such as ovulation, menstruation, embryo implantation and parturition and in pathological processes such as preterm delivery, HIV infection, endometriosis and ovarian hyperstimulation syndrome.^(1,2) Accumulated evidence suggests that chemokines produced and

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incorporated by the endometrial epithelium and the human blastocyst is implicated in this molecular network. During implantation, leukocytes are recruited into the endometrium. The regulation of the uterine tissue during this process is thought to be orchestrated by uterine epithelial cells, which release an array of chemokines in a precise temporal pattern driven by ovarian steroids^(3,4) and, possibly, seminal factor.⁽⁵⁾ Chemokines act on a range of leukocyte subsets, which in turn release a number of proteases and other mediators that facilitate embryo invasion.⁽⁶⁾

Chemokines and their receptors are divided into two families based on structural and genetic considerations. All chemokines are structurally similar, having at least three beta-pleated sheets and a C-terminal-helix. In addition, most chemokines have at least four cysteines in conserved positions. In the CXC chemokine family (α -chemokines), the two cysteines nearest the N-termini of family members are separated by a single (and variable) amino acid whereas CC chemokines (β -chemokines) no amino acid is between the two cysteines.

Chemokine receptors belong to the superfamily of G-protein-coupled receptors (GPCRs). These receptors display seven sequences of 20-25 hydrophobic residues that form an α -helix and span the plasma membrane, an extracellular N-terminus, three extracellular loops, three intracellular domains and an intracellular C-terminal tail. These receptors transmit information to the cell about the presence of chemokine gradients in the extracellular environment. They are named depending on the structure of their ligand (CXC or CC). CXCR4 is expressed in neutrophils, monocytes, B and T lymphocytes, and its primary ligand is the stromal derived factor-1 (SDF-1). CCR5, a receptor of RANTES and macrophage inflammatory protein-1 (MIP-1) α and β , is expressed in monocytes, dendritic cells, activated T lymphocytes and NK cells. CCR2B, expressed in monocytes, basophils, dendritic cells, NK cells and activated T lymphocytes, is the main receptor for monocyte chemotactic proteins (MCP) 1, 2, 3 and 4. CXCR1, a receptor of interleukin 8 (IL-8) and granulocyte chemotactic protein-2 (GCP-2), is expressed mainly in neutrophils and dendritic cells. A specific molecular cross talk between embryo and endometrium has been reported to take place during the human implantation process.^(7,8) The endometrial epithelium is a key site where molecular interactions between

the embryo and endometrium seem to be initiated,^(9,10) and from which chemokines are produced and secreted.⁽¹¹⁾

The mRNA expression of these four chemokine receptors was analyzed throughout the natural cycle using quantitative fluorescence PCR (QF-PCR). CXCR1 and CCR5 receptors showed a progesterone dependent pattern in the early secretory phase (40 and 47-fold increase, respectively) that continued into the mid secretory phase (157 and 176 fold-increase and was maximal in the late secretory phase (628 and 560 fold-increase, respectively). Unlike the previous receptors studied, CXCR4 presented a more pronounced up-regulation in the mid luteal phase than in the early and late luteal phases (an increase of 9 fold versus increases of 0.5 and 5.7, respectively). Therefore, this receptor, which is located in the endometrial epithelium, is specifically up regulated during the implantation window.⁽¹²⁾

To study the "in vivo" hormonal regulation of chemokine receptors CXCR1, CCR2, CCR5 and CXCR4, endometrial biopsies were obtained in hormonal replacement therapy (HRT) cycles. Immunohistochemistry was carried out for protein localization.

On day 13 (n = 3), when patients were treated solely with estradiol, a very weak staining for CCR2B, CCR5 and CXCR4 was localized in the luminal and glandular epithelium and endothelial cells. During the pre-receptive and receptive periods (days 18 and 21 respectively), an increase of staining intensity for CXCR1 receptor was noted in the glandular compartment. A slight signal was observed in stromal cells. CCR5 receptor was also immunolocalized, mainly at the luminal epithelium but also in the stromal and perivascular cells, showing a slight increase compared to the non-receptive phase. CCR2B receptor showed a moderate increase of staining on day 18 and 21 in the luminal epithelium while no staining was observed in endothelial cells or stroma. CXCR4 receptor showed the same staining as CCR5, mainly expressed in the epithelium on days 18 and 21. Endothelial and stromal cells were also positive.⁽¹²⁾

The embryonic impact on immunolocalization and polarization of chemokine receptors CXCR1, CXCR4, CCR5 and CCR2B in cultured EEC was investigated using our apposition model for human implantation. When the blastocyst was absent,

Chemokine receptors CXCR1, CXCR4 and CCR5 produced a barely detectable staining in only a few cells at the EEC monolayer. However, when a human blastocyst was present, there was an increase in the number of stained cells for CXCR1, CXCR4, and CCR5 and polarization of these receptors in one of the cell poles of the endometrial epithelium.

An immunolocalization and polarization change in CCR2B receptor was not present in the EEC monolayer and this receptor was not up regulated by the presence of the human blastocyst. Finally, we have detected immunoreactive CCR2B and CCR5 receptors in the human blastocyst. CCR2B staining was localized mainly at the inner cell mass, whereas CCR5 staining can be visualized across the trophectoderm. In all cases (n = 3), CCR5 staining was more intense than that of CCR2B receptor, while the pellucide zone was not stained in any case. Immunoreactive CXCR4 and CXCR1 were not detected in human blastocysts when the same technique was used.

Relevance of leptin and leptin receptor in human endometrium

Obesity is a condition that is reaching epidemic proportions in the United States. The prevalence of obesity has doubled in the past decade and one of the pathological consequences is infertility, indicating a link between adipose tissue and the reproductive system.⁽¹³⁾ High BMI has been associated with low IVF pregnancy rates,⁽¹⁴⁾ suggesting the involvement of endometrial receptivity and implantation in these conditions.

Leptin is a 16 KDa non-glycosylated polypeptide of 146 amino acids discovered in 1994 by Zhang et al.⁽¹⁵⁾ It is initially thought to be secreted by adipose tissue. Its secretion is tightly linked to food consumption, energy balance and body weight. More recently, investigations have implicated leptin in the regulation of reproductive function.⁽¹³⁾

Leptin receptor is the product of the LEPR or OB-R gene and belongs to the class I superfamily of cytokine receptors. In humans and rodents, two major forms of leptin receptors (OB-R) are expressed. The short form (OB-RS) is detected in many organs and is considered to lack signaling capability,⁽¹⁶⁾ as it has a truncated intracellular domain.⁽¹⁷⁾ The long form (OB-RL), with a complete intracellular domain, predominates in the hypothala-

mus, anterior pituitary, and is also expressed in low amounts in peripheral tissues.⁽¹⁸⁾

An early observation indicated that ob/ob female mice (which lack functional leptin) and db/db mice (which lack functional leptin receptor) are characterized by obesity and sterility.⁽¹⁵⁾ Fertility in the ob/ob animals can be restored by exogenous administration of leptin but not by food restriction, indicating that leptin per se is required for normal reproductive functioning.⁽¹⁹⁾

Similar findings in congenital leptin deficiency⁽²⁰⁾ and leptin mutation⁽²¹⁾ have also been reported in humans. In keeping with its predominant role as a signal for starvation, leptin also seems to be important in mediating undernutrition-induced deficits in reproductive function. In starved mice, the lack of reproductive function coincides with the fall of plasma leptin level and several neuroendocrine changes. An exogenous leptin injection restores fertility in these mice.⁽²²⁾

Although the leptin system clearly influences reproduction, whether leptin exerts its effect, as an endocrine or paracrine mediator is yet to be resolved. A large body of data supports the notion that the reproductive actions of leptin involve a direct effect on the brain, specifically the hypothalamus. Expression of functional leptin receptors has also been detected in rodents⁽²³⁾ and humans,⁽²⁴⁾ and follicular and serum leptin production seems to be influenced by the ovarian functional state.⁽²⁵⁾ To the present date, the mechanism linking leptin, LH and estradiol levels has not been clearly established.

Several research groups have described the presence of the leptin receptor in the human endometrium. All groups affirm that the long form of leptin receptor (OB-RL) mRNA is detectable in the human endometrium. In addition, OB-RL has been detected by RT-PCR and Western Blot in cultured human endometrial epithelial cells (EEC).⁽²⁶⁾ Interestingly, *Kitawaki et al.* reported that OB-R mRNA expression peaked in the early secretory phase when semi-quantitative RT-PCR was employed⁽²⁷⁾ whereas others found that OB-RL was low during the early secretory phase and higher during the proliferative and late secretory phases.^(28,29) Leptin mRNA and protein have been also identified in secretory endometrium and in EEC.^(26,29) These findings suggest that the endometrium is a target tissue for circulating leptin and, in addition, is a site of local production.

In the embryonic context, leptin and STAT3 proteins have been immunolocalized in a polarized manner in mouse and human oocytes and in pre-implantation embryos. Both molecules were found in pre-implantation embryos, with differences in the allocation of blastomeres occurring after the first cell division (2-4 cell stage). A potential role of these proteins in early development has been suggested due to the fact that at the morula stage inner blastomeres contain little leptin/STAT3, while outer cells contain both leptin/STAT3 rich and poor cells. Using a coculture model, our group described, for the first time, that the concentration of immunoactive leptin secreted by human competent blastocysts was significantly higher than that secreted by arrested blastocysts cultured alone,⁽²⁶⁾ suggesting that this molecule may be a marker of embryonic vitality. In mouse, it has been described that leptin promotes the embryo development.⁽³⁰⁾ Moreover, it has been published that the leptin receptor is present throughout the early preimplantation development in human and mouse.^(29,30) Nevertheless, leptin is only detected at blastocyst stage.^(29,30) All these findings strongly suggest that this system takes part in the embryonic-endometrial dialogue during the adhesion phase of human embryonic implantation.

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胚胎與子宮內膜的相互作用

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胚胎著床的過程需要有可接受著床的子宮內膜、正常且有功能的囊胚且他們之間必須有適當的聯接。在著床的過程中，囊胚會找到適合的位置在子宮內膜著床。並且在著床時，有許多物質（比如荷爾蒙、激素、整合素及瘦素）扮演著囊胚與子宮內膜對話的因子。我們將介紹目前對於胚胎與子宮內膜調控機制的研究報告。(長庚醫誌 2006;29:9-14)

關鍵字：胚胎，子宮內膜。

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