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Signal Transduction Upstream and Downstream of Trophinin in Human Embryo Implantation

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Introduction

The processes associated with mammalian embryo implantation differ substantially among mammalian species (1). Studies in mouse established that the initial adhesion is mediated by an epidermal growth factor family receptor, ErbB4, expressed on the trophectoderm surface and a membrane-bound ligand, heparin-binding epidermal growth factor-like factor (HB-EGF), on the endometrial epithelium (2, 3). This process in humans contains evolutionarily conserved elements as well as elements uniquely developed in this species. An example of this is the function of trophinin, a homophilic cell adhesion molecule expressed on both trophectoderm cells and endometrial epithelial cells. Although mouse genome encodes trophinin, which is similar to human trophinin, their expression patterns and functions appear different in the two species. Trophinin mediate blastocyst adhesion in humans but not in mice. Since we discovered trophinin in 1995 (4-6), considerable progress has been made in the study of human embryo implantation (7-10). In this mini review, we describe events upstream and downstream of trophinin-mediated cell adhesion, focusing on the signal transduction pathways unique to human embryo implantation.

A glycocalyx covers the maternal epithelia

In general, apical cell surfaces of epithelia contain microvilli, which are covered by thick layer of mucin carbohydrate called the glycocalyx. The glycocalyx lubricates and hydrates cell surfaces as well as protecting epithelial cells from microorganisms and degradative enzymes. In addition, mucins inhibit both cell-cell and cell-extracellular matrix interactions. MUC1 and other mucins are abundantly expressed at the apical surface of luminal and glandular uterine epithelia (11, 12).

MUC1 is a type I membrane protein composed of a large N-terminal extracellular domain, a transmembrane domain, and a short C-terminal cytoplasmic domain (13) (Fig. 1). The MUC1 cytoplasmic tail associates with â-catenin, as well as with other signaling molecules (e.g., Grb2/Sos), suggesting a potential role for MUC1 in cell signaling (14). Activation of ErbB1 by EGF induces tyrosine phosphorylation of the MUC1 cytoplasmic tail and activation of ERK1/2. Direct interactions between the MUC1 ectodomain and a carbohydrate-binding protein may also trigger signaling reactions (15, 16). Thus, stimuli such as growth factors or cytokines may affect MUC1 stability, localization at the cell surface, or phosphorylation state directly or through activation of their receptors, and MUC1 has the potential to function as a receptor either alone or in cooperation with other signal transducing proteins.

MUC1 expression in endometrial epithelial

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cells is regulated at the transcriptional level by steroid hormones and other factors (17). In the mouse, rat, and pig, Muc1 is downregulated in the entire uterus prior to embryo implantation (18), consistent with the idea that MUC1 inhibits cell adhesion and therefore needs to be downregulated before blastocysts can adhere to the uterine epithelium. In the rabbit, although Muc1 expression in the entire uterus is elevated during the peri-implantation period, Muc1 is downregulated at embryo implantation sites *in vivo* and *in vitro* (19). In the human endometrium, MUC1 is significantly elevated in the early secretory phase or implantation window (20). Although MUC1 has not been studied at the embryo implantation site in humans *in vivo*, *in vitro* implantation models indicate that MUC1 is lost at the site of embryo attachment in humans as well (21). This suggests that one or more factors expressed on or released from the blastocyst surface trigger MUC1 loss from the adjacent endometrial epithelia in rabbits and humans. These observations led to the hypothesis that an endogenous protease, called "sheddase," removes MUC1 from the endometrial epithelial surface (14, 22, 23). On the other hand, it is possible that an unknown factor secreted from the blastocyst binds to adjacent endometrial cells and downregulates MUC1 gene expression at the transcriptional level through signal transduction.

In a variety of mammals, the apical surface of the endometrial epithelium that is receptive for blastocyst implantation shows characteristic bleb-like structures, called pinopodes (24, 25). Pinopodes extend beyond the glycocalyx layer. Therefore, cell adhesion molecules presented by pinopodes, such as trophinin, can directly interact with adhesion molecules expressed on the trophectoderm surface, including trophinin (26). Blastocyst attachment occurs on the tops of pinopodes (25, 27). Trophoblastic cells also contain carbohydrates on their cell surfaces, but they do not have a glycocalyx or mucins (14). An *in vitro* experiment using primary cultured human endometrial epithelial cells showed that the b-subunit of human chorionic gonadotropin (CGb) and interleuken-1b (IL-1b) induced trophinin expression by endometrial epithelial cells in the pinopodes (26).

L-selectin and its ligand carbohydrates

L-selectin is a carbohydrate-binding protein expressed on the surfaces of lymphocytes in a wide variety of mammals (28). L-selectin gene knockout mice show a clear defect in lymphocyte homing to lymph nodes (29). Recently, L-selectin was found on the surface of human blastocysts, and L-selectin ligand oligosaccharides were detected in the endometrium by immunohistochemistry using antibodies called MECA79 and HECA452, whose epitope structures are closely related to the L-selectin ligand (30) (Fig. 1). These carbohydrate antigens are upregulated in human endometrium during the period when it is receptive to embryo implantation (31). These findings suggest that interactions between L-selectin on human blastocysts and carbohydrate ligands on the endometrial epithelium enable the initial adhesion of human embryos for implantation (30).

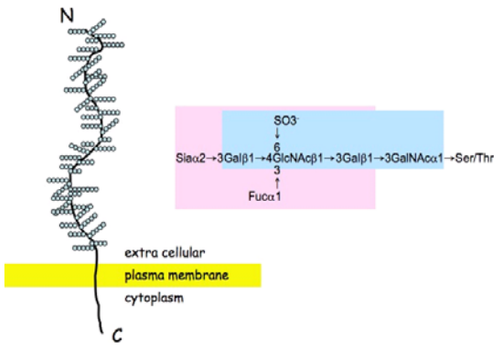

Fig. 1 : MUC1 and structure of L-selectin ligand carbohydrate. MUC1 is a highly glycosylated protein with numerous O-glycans attached to Ser/Thr-rich extracellular domain. L-selectin recognizes sulfated and fucosylated O-glycan structure (pink) attached on MUC1 in human endometrial epithelia (54). Part of this structure (blue) is recognized specifically by MECA 79 antibody (55). MECA 79 antigen is found in human, but not in mouse, endometrial epithelia (30).

A microarray analysis of mouse blastocysts showed an elevation of L-selectin transcripts during the maturation stage, when the blastocysts are competent for implantation (32). However, mutant mice deficient in the L-selectin gene show no defect in implantation, indicating that L-selectin is not essential for embryo implantation in the mouse. Furthermore, the MECA79 antigen was not detected in mouse endometrial epithelial cells (30), suggesting that L-selectin plays a role in human, but not mouse, embryo implantation.

A large force is required to physically immobilize a free-floating cell to a flat surface. Given the enormous difference in size between a human blastocyst (diameter, 115~265 mm (33)) and lymphocyte (diameter, 10 mm), it is difficult to imagine that a blastocyst could be immobilized to endometrial epithelia solely through L-selectin, given the somewhat weak selectin–carbohydrate interactions (34). It seems reasonable to speculate that a human blastocyst rolls over the glycocalyx of the endometrial epithelium through weak interactions with L-selectin, similar to the movement of lymphocytes over endothelial cells that is mediated by L-selectin. L-selectin-mediated rolling may allow cross-talk between the blastocyst and maternal epithelia, leading to stronger cell adhesion by direct binding between the components embedded in the plasma membranes on the fetal and maternal sides.

Trophinin

Trophinin is a homophilic cell adhesion molecule: trophinin expressed on the apical cell surface of trophectoderm cells binds to trophinin expressed on the apical cell surface of endometrial epithelial cells (4). The trophinin protein is made up of an N-terminal cytoplasmic domain followed by decapeptide repeats, which include a membrane-spanning domain. However, how the trophinin protein is integrated to the lipid bilayer has not yet been established.

In both human and mouse, the trophinin gene is on the X chromosome (35, 36). It produces several splicing variants encoding MAGE family proteins, designated magphinins (37, 38). Translation of the trophinin protein initiates from an alternative site downstream of those for magphinins (Fig. 2). In human, there is no overlap in peptide sequence between magphinin

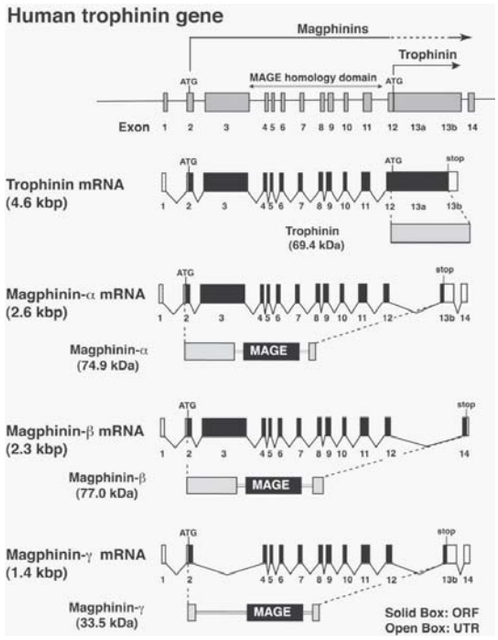

Fig. 2 : Human trophinin gene (TRO) and transcripts for magphinins and trophinin (38). Note that trophinin protein (blue) is produced by an alternative translation initiation site downstream of those for magphinins.

and trophinin (38). Therefore human trophinin gene produces two distinct proteins.

Although trophinin is a homophilic adhesion molecule, and human trophoblastic HT-H cells adhere to each other through trophinin-trophinin binding (4), we do not see self-aggregation of human blastocysts. This evidence suggests that trophinin expressed on the trophectoderm cell surface is not readily active for adhesion. We speculate that trophinin expressed on the surface of trophectoderm cells is silent until the blastocyst receives a signal from the endometrial epithelial cells. In lymphocytes, L-selectin–mediated rolling results in activation of integrin through cytokines

accumulated on the surface of endothelial cells (39, 40). It is an interesting possibility that L-selectin-mediated rolling triggers the signal transduction for trophinin activation, or the human blastocyst is stimulated by a factor concentrated in the glycan layer of the endometrium.

Trophinin and human chorionic gonadotropin (hCG)

Trophinin expression in the mouse uterus is independent of the blastocyst (41), whereas trophinin expression in human is dependent on the implanting embryo (26, 42). An analysis of trophinin in ectopic pregnancy suggested that fallopian tubal epithelia express trophininin in response to the implanting blastocyst, because immunohistochemistry of fallopian tubes showed the trophinin protein only in the cells adjacent to the implantation site (42). Furthermore, hCG induces trophinin expression in human endometrial epithelial cells in primary culture (26).

Anti-trophinin immunohistochemistry of human endometrial biopsies shows remarkably strong immunostaining of endometrial epithelial cells within a limited (100 (m) area of the endometrial luminal epithelium, but such strong staining is extremely rare in endometrial biopsies (4, 26). The pattern of immunopositivity suggests that the trophinin-positive specimens contain embryo implantation sites, where the blastocyst was in close apposition to the endometrium and induced trophinin locally in the endometrial luminal epithelia (Fig. 3). Future studies should address the molecular basis of trophinin expression by human endometrial epithelial cells.

Downstream of trophinin-mediated adhesion

Morphological studies of human embryo implantation in vivo suggest that the initial adhesion of the trophectoderm to the apical cell surface of the uterine epithelium triggers a series of signal transduction cascades that activate the trophoblast (43–45); once activated,

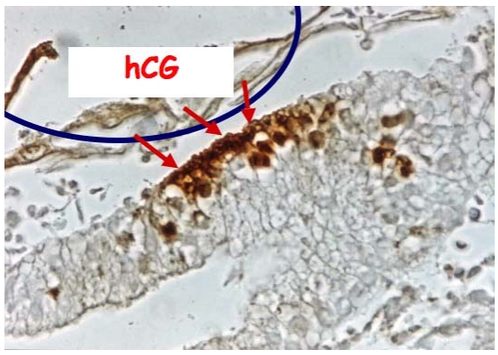

Fig. 3 : Induction of trophinin in endometrial epithelia by hCG secreted from the trophectoderm of a human blastocyst. Modified from Sugihara et al. (26).

previously silent trophectoderm cells lose their epithelial polarity, mobilize for invasion, and grow to multinucleated cells by rapid protein synthesis followed by cell division.

We investigated the mechanism underlying this adhesion-triggered trophoblast activation using trophoblastic HT-H cells as a model (7, 46). In HT-H cells, the cytoplasmic domain of trophinin directly binds to bystin (47, 48), which in turn binds the cytoplasmic domain of ErbB4, a receptor tyrosine kinase. When HB-EGF binds to ErbB4, ErbB4 is not phosphorylated because trophinin arrests ErbB4 in the cytoplasm. However, when trophinin-mediated cell adhesion occurs, bystin is released from trophinin, allowing activation of ErbB4 (Fig. 4). Thus trophinin-mediated cell adhesion acts as a molecular switch for trophoblast activation (7, 46). Apical cell adhesion through trophinin thus explains how silent trophectoderm cells become aggressive trophoblasts.

Endometrial epithelia function as a barrier against toxic intruders such as infectious agents. The embryo needs to overcome this epithelial barrier for successful implantation. Morphological studies of embryo implantation sites in rodents and primates have shown signs of apoptosis in endometrial epithelial cells at the implantation site (49–52). Models of human embryo implantation suggest that trophoblastic

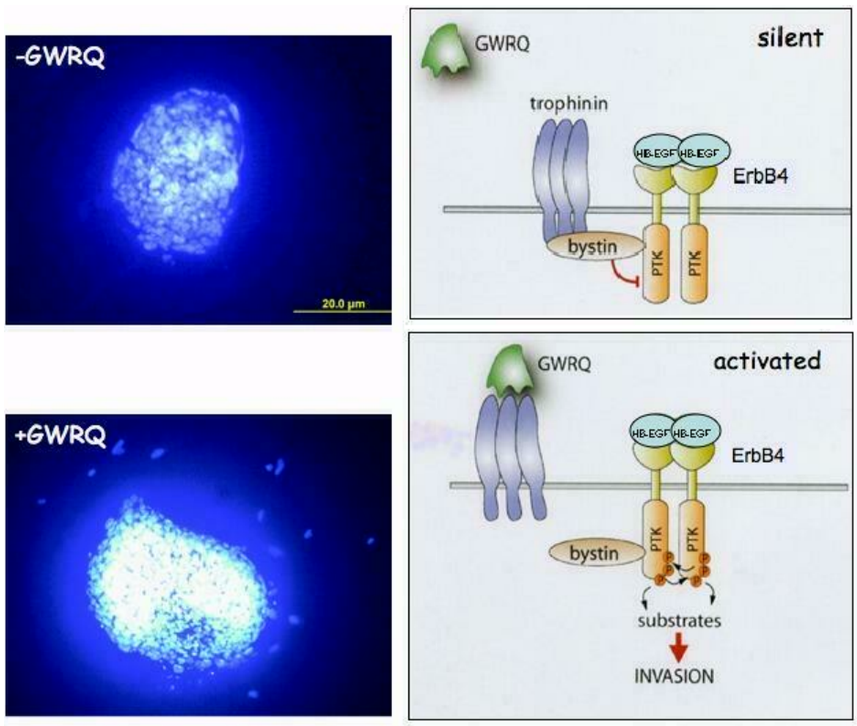

Fig. 4 : Trophinin as a molecular switch for trophectoderm activation (46). In silent trophectoderm cells (left), trophinin arrests ErbB4 through bystin. Under this condition, ErbB4 is inactive for protein kinase. On the other hand, upon trophinin-mediated cell adhesion, bystin dissociates from trophinin and ErbB4 is activated. GWRQ peptide can mimic trophinin-mediated cell adhsion. Modified from Sugihara et al. (46).

cells do induce apoptosis in the surrounding endometrial epithelial cells (49, 51). Therefore, trophoblast-induced apoptosis in the endometrial epithelial cell is an important step in embryo implantation. However, the signaling mechanisms leading to apoptosis during interactions between the trophectoderm and endometrial epithelium remain elusive.

Apoptosis is a cell death process that removes surplus or damaged cells in mammals and can be induced by a variety of stimuli that include anti-cancer drugs, deprivation of growth factors, and death factors. Apoptosis is initiated upon binding of FasL to Fas, which activates the caspase cascades (53). In experiment using BeWo human trophoblast spheroids adhered to RL95-2 human endometrial epithelial cell monolayers, BeWo spheroids rapidly attached to endometrial monolayers and then progressively expanded, with marked dislodgment of endometrial cells adjacent to the spreading trophoblast cells (50). Apoptosis was detected in the endometrial cells being adhered by the trophoblast spheroid. Experiments in which a single human blastocyst was placed on the human endometrial epithelial cells showed that in the apposition phase, the presence of a blastocyst rescues endometrial cells from the apoptotic pathway (49). However, when the human or monkey blastocyst adhere to the endometrial epithelial monolayer, it induces a paracrine apoptotic reaction (49, 52). Fas ligand (Fas-L) was present at the embryonic trophoectoderm. Fas was localized at the apical cell surface of endometrial epithelial cells. Therefore, it is likely that when FasL expressed

on trophectoderm cells binds to Fas on endometrial epithelial cells, the epithelial cells undergo apoptosis, breaching the epithelial barrier (Fig. 5). The immediate consequence is that the trophectoderm comes in direct contact with the basement membrane and, therefore, stromal invasion can proceed. Although we do not know presently where the trophinin-mediated cell adhesion is placed in the apoptosis cascade, it is possible that trophinin plays a role in regulating apoptosis in endometrial epithelial cells.

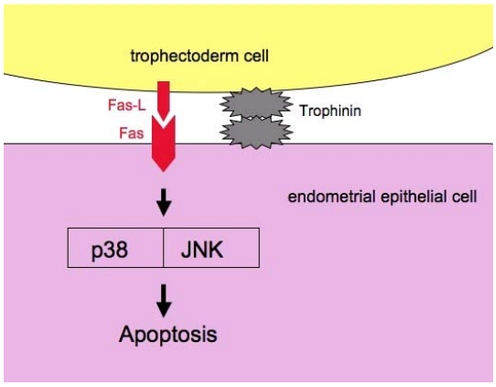

Fig. 5 : Apoptosis of endometrial epithelial cells caused by human embryo implantation. Fas The Fas/Fas-L death system may be an important mechanism to cross the epithelial barrier to establish embryo implantation. Fas-L was found on the surface of embryonic trophectoderm, and Fas was localized at the apical cell surface of endometrial epithelial cells (50, 51). Modified from Hsu et al. (51).

Perspectives

Because each mammal produces a limited number of offspring, nature protects each mammalian embryo with extra care. This includes strict quality control of embryos, and a high incidence of implantation failure is natural selection of the best embryo. In addition to the ErbB4/HB-EGF mechanism found in the mouse, L-selectin and trophinin have been added to the human implantation process, probably as safeguard. We therefore propose that human endometrial epithelial cells regulate the invasion of trophoblast by a mechanism that is unique to humans.

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