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An integrated view of L-selectin and trophinin function in human embryo implantation

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Abstract

Determining molecular mechanisms of human embryo implantation is an extremely challenging task due to the limitation of materials and significant differences underlying this process among mammalian species. Recently, L-selectin and its ligand carbohydrate have been proposed as a system that mediates initial adhesion of human blastocysts to the uterine epithelia. We have also identified trophinin as a unique apical cell adhesion molecule potentially involved in the initial adhesion of trophectoderm of the human blastocyst to endometrial surface epithelia. In the mouse, the binding between ErbB4 on the blastocyst and heparin-binding epidermal growth factor-like growth factor on the endometrial surface enables the initial step of the blastocyst implantation. The evidence suggests that L-selectin and trophinin are included in human embryo implantation. This review summarizes findings relevant to the functions of L-selectin and trophinin in human embryo implantation, and proposes a model that reconciles these cell adhesion mechanisms.

Introduction

In the field of reproductive biology, there are many reports suggesting that cell surface complex carbohydrates play an important role in various processes including embryo implantation.^{1–5} For example, fucosylated oligosaccharide (blood group type 1H antigen) was proposed as a critical carbohydrate in mouse embryo implantation:⁶ in this hypothesis, H antigen expressed on endometrial surface epithelia is recognized by a presumptive carbohydrate-binding protein expressed on the surface of the mouse blastocyst.⁷ However, gene knockout mice deficient in fucosyl-transferases responsible for synthesis of fucosylated oligosaccharides showed no defects in reproduction including embryo implantation, suggesting that fucosylated carbohydrates do not play an essential role in these processes in the mouse.⁸

On the other hand, extensive studies on the mouse embryo implantation have established that trophectoderm cells of the mouse blastocyst express ErbB4, one of the ErbB-family epidermal growth factor (EGF) receptor proteins.^{9–12} When mouse blastocysts enter the uterine horn, the movement of each blastocyst is restricted in a narrow cavity of the endometrium. There, each blastocyst affects the maternal epithelia and induces membrane-bound form heparin-binding EGF-like growth factor (HB-EGF) locally in the epithelia surrounding the blastocyst.¹⁰ The blastocyst and maternal epithelia then adhere to each other through the binding between ErbB4 and HB-EGF.^{11,12}

ErbB4 is expressed on the trophectoderm cells of the human blastocyst,¹³ and HB-EGF is expressed in the human endometrial epithelia receptive to implantation.¹⁴ Therefore the ErbB4/HB-EGF system may play a role in human embryo implantation. However, due to significant differences in the process of embryo implantation among mammalian species¹⁵ and the difficulty in analyzing this process in humans, this hypothesis has not been vigorously tested in humans.

Independent from these studies, we have identified trophinin as an adhesion molecule mediating the initial step of blastocyst adhesion to endometrial epithelia in humans.¹⁶⁻²⁰ Trophinin mediates cell adhesion by homophilic trophinin-trophinin binding. Recently, L-selectin, a carbohydrate-binding protein, has been proposed to play a role in human embryo implantation.²¹ This review describes the potential roles of L-selectin, ErbB4/HB-EGF and trophinin in human embryo implantation, and proposes a new way of understanding these three cell adhesion systems in this intriguing process of human reproduction.

Characteristics of Human Endometrial Epithelial Cells

In general, apical cell surfaces of epithelial cells contain numerous microvilli, which are covered by a thick layer of carbohydrates called glycocalyx. Cell surface carbohydrates protect epithelial cells from proteolytic degradation and bacterial infection. Endometrial surfaces are covered by acidic carbohydrates carried by mucin called MUC1.^{4,22} When a mammalian blastocyst enters the uterine cavity, MUC1 carbohydrates are the first molecules a blastocyst encounters. The anti-adhesive function of MUC1 carbohydrates in the human endometrium is very important, given that a human blastocyst is notoriously adhesive.

In the mouse, rat, and pig, MUC1 is down-regulated in the entire uterus prior to embryo implantation.²³⁻²⁷ Such observations are consistent with the idea that carbohydrates prevent cell adhesion and that down-regulation of MUC1 promotes endometrial receptivity by unmasking a cell adhesion molecule required for embryo implantation.²⁴⁻²⁷ However, in the human endometrium, MUC1 is up-regulated during the implantation period.²⁸⁻³⁰ This suggests that human endometrial surface epithelia extensively prevent the blastocyst adhesion, except for the precise spot where the blastocyst attaches. When human blastocysts were allowed to adhere to endometrial epithelial cells *in vitro*, MUC1 disappeared from endometrial epithelial cells located beneath and around the blastocyst, whereas MUC1 was found in endometrial epithelia a few millimeters away from the adhesion site.^{31,32} This finding strongly suggests that the human blastocyst down-regulates MUC1 in maternal epithelia.³³

The apical surface of the endometrial epithelium receptive for blastocyst implantation shows characteristic bleb-like structures, called pinopodes.^{34,35} Pinopodes are found in a variety of mammals. In humans, pinopodes appear for only 1–2 days during the receptive period.^{36,37} Blastocyst attachment has been shown to occur on top of pinopodes,^{38,39} which project above the microvilli and glycocalyx in the human endometrium.^{40,41} It has been suggested that a cell adhesion molecule required for blastocyst adhesion is located on the pinopodes.³ Cell adhesion molecules found in the pinopodes are HB-EGF¹⁴ and trophinin.⁴²

L-selectin and Its Ligand Carbohydrates

Selectins are a group of three carbohydrate-binding proteins – E-, P- and L-selectin – encoded in both the human and the mouse by three separate genes.^{43,44} E-selectin is expressed on the inflammatory endothelial surface, and P-selectin is expressed on the surface of activated platelets. L-selectin is constitutively expressed on lymphocytes, where it plays an essential role in lymphocyte homing.^{45,46} When lymphocytes enter the lymph node, they adhere to tall endothelial cells called high endothelial venules (HEV). This adhesion is mediated through L-selectin on lymphocytes and L-selectin ligand carbohydrates on HEV. Lymphocytes passing

the HEV are under sheer stress and therefore lymphocytes do not stop but 'roll' on the HEV.⁴⁶ The rolling allows lymphocytes receiving stimulation by cytokines concentrated on the endothelial surface, which activates a signal transduction pathway for integrin activation, leading into stronger adhesion of lymphocyte for extravasation.^{47,48}

Although previously it was thought that selectins were expressed only in the hematopoietic cells, L-selectin has been found in human blastocysts.²¹ Furthermore, L-selectin ligand oligosaccharides were detected by immunohistochemistry using MECA-79 and HECA-452 antibodies, whose epitope structures are closely related to the L-selectin ligand.^{49,50} MECA-79 and HECA-452 antigens were found widely on luminal and glandular endometrial epithelia in the human uterus.^{21,51,52} These antigens are up-regulated in the human endometrium during the period receptive to embryo implantation. These findings suggest that interactions between L-selectin on human blastocysts and oligosaccharide ligands on endometrial epithelia enable the initial adhesion of the human embryo for implantation.²¹ However, MECA-79 antigen is carried by MUC1,⁵³ of which the major function is to block embryo implantation.³² This suggests that L-selectin may allow weak adhesion to the uterine wall, which may affect both the blastocyst and endometrial epithelia for stronger adhesion.

Cross-talk between the Blastocyst and Maternal Cells Prior to Implantation

In the studies of mouse embryo implantation, it is well documented that the blastocyst and endometrial epithelial cells cross-talk prior to adhesion.^{12,54-56} When a mouse blastocyst is in the endometrial cavity, the blastocyst affects the endometrial epithelial cells surrounding the blastocyst.¹² For example, endometrial epithelial cells adjacent to the blastocyst start expressing membrane-bound form HB-EGF in response to an as yet unidentified embryonic factor.^{9,10} Trophectoderm cells of the mouse blastocyst express ErbB4.^{9,11} Thus, in the mouse, trophoctoderm and endometrial cells adhere to each other through the binding of ErbB4 and HB-EGF. Trophectoderm cells of the human blastocyst express ErbB4,¹³ and pinopodes in the endometrial surface epithelia express HB-EGF.¹⁴ Therefore, it is likely that the ErbB4/HB-EGF system is also involved in human embryo implantation.

Evidence for cross-talk between the human blastocyst and maternal epithelia has been suggested by a study of ectopic pregnancy.¹⁸ In humans, fallopian tubal pregnancy occurs at a relatively high frequency (0.25–1.4% of all pregnancies). However, this pathological condition does not occur either physiologically or experimentally in rodents.⁵⁷ The incidence of ectopic pregnancy in non-human primates is extremely rare,⁵⁸ suggesting that a mechanism of ectopic pregnancy is unique to humans.

Immunohistochemistry shows that both embryonic and maternal cells at ectopic implantation sites express trophinin, a homophilic cell adhesion molecule.¹⁸ By contrast, maternal epithelia distant from the implantation site do not express trophinin (Fig. 1). This observation suggests the existence of an embryonic factor acting locally on maternal epithelia to induce trophinin expression. It is well known that the human blastocyst secretes human chorionic gonadotropin (hCG) at the time of implantation.^{59,60} When a fallopian tube explant was incubated with hCG, trophinin mRNA was elevated in fallopian tubal explants. Therefore hCG secreted from the blastocyst likely induces trophinin gene expression in adjacent maternal cells. It is also known that the human gene encoding beta subunit of hCG or CG β has diverged significantly from that of non-human primates.⁶¹ This evidence is consistent with the hypothesis that CG β and trophinin are uniquely involved in human embryo implantation.

Identification of Trophinin as a Unique Apical Cell Adhesion Molecule in Human Embryo Implantation

It is impossible to use human blastocyst in experiments. To analyze the molecular mechanism of human embryo implantation, we looked for an alternative system which allows us to apply molecular biology for studying human embryo implantation.^{16,62} Thus, we chose teratocarcinoma cell lines. Teratocarcinomas are tumors composed of undifferentiated stem cells and variously differentiated cell types.⁶³ Since stem cells in these tumors can differentiate into any cell types, those stem cells are considered pluripotent. Tumors containing such stem cells are called embryonal carcinomas (EC). Both human and mouse EC lines show characteristics of early embryonic cells.⁶³⁻⁶⁵ Mouse EC lines express stage specific embryonic antigen 1 (SSEA1), which is determined by fucosylated carbohydrate,⁶⁶ and are similar to cells included in the inner cell mass of the blastocyst.⁶⁷ By contrast, human EC lines are SSEA1-negative but express SSEA-3 and SSEA-4 antigens,⁶⁸ which are also determined by carbohydrate structures.⁶⁹ In mouse pre-implantation stage embryos, SSEA3/4 antigens are expressed at the 4–8 cell stage.⁶⁸ Mouse EC cells differentiate *in vitro* into parietal or visceral endoderm,⁷⁰ whereas human EC cell lines have the tendency to differentiate into trophoblastic cells.⁷¹ Trophoblastic cells derived from EC cells are assumed to mimic trophoblastic cells at the very early embryonic stage, such as those in the trophectoderm of the blastocyst.

HT-H is a human EC line isolated from a testicular teratocarcinoma tumor.⁷² HT-H cells spontaneously differentiate into syncytiotrophoblasts *in vitro* and secrete progesterone and hCG.⁶² Trophoblastic HT-H cells adhere and grow as a monolayer on tissue culture dishes. When HT-H cells are detached by trypsinization and added to human endometrial adenocarcinoma SNG-M⁷³ cells, they instantly adhere to SNG-M cells.¹⁶ HT-H cells also adhere to themselves but do not adhere to epithelial cells derived from other cell types, such as colon and lung. These observations suggest the existence of a trophoblast/endometrial cell-specific apical cell adhesion molecule in HT-H and SNG-M cells.

To identify this trophoblast-endometrial cell type specific apical adhesion molecule, we employed expression cDNA cloning. Based solely on the apical cell adhesion activity, we have identified trophinin.^{16,74} Trophinin is a trans-membrane protein with a cytoplasmic N-terminus. In trophoblastic cells, the trophinin cytoplasmic domain binds to a cytoplasmic protein, bystin.⁷⁵ Trophinin mediates homophilic cell adhesion through trophinin-trophinin binding. When trophoblastic cells expressing trophinin were overlayed on the apical surface of trophinin-expressing endometrial epithelia, both cell types adhered instantly with strong affinity. Trophinin protein was found in the trophectoderm cells in the monkey blastocyst, particularly in the cells at the embryonic pole where the blastocyst implantation takes place.¹⁶ A monoclonal antibody specific to human trophinin showed positive immunostaining in both trophoblast and maternal epithelia at embryo implantation sites in the human placenta.¹⁷

Potential involvement of trophinin in human embryo implantation has been strengthened by recent findings relevant to signaling triggered by trophinin-mediated cell adhesion.^{19,20} When HB-EGF binds to ErbB4 on trophinin-expressing human trophoblastic cells, ErbB4 is not activated because ErbB4 is arrested by bystin and trophinin.¹⁹ When trophinin-mediated cell adhesion occurs, bystin is released from trophinin, allowing the activation of ErbB4 protein tyrosine kinase and promoting proliferation and increased motility of trophoblastic cells. Thus trophinin-mediated cell adhesion acts as a molecular switch for trophoblast activation, promoting invasion and proliferation.^{19,20} This mechanism explains why the trophectoderm cells of the human blastocyst adhering to the uterine epithelia grow and invade, while those at non-adhesive sites remain silent.⁷⁶

L-selectin and Trophinin: Mechanisms Unique to Human Embryo Implantation

As noted, human endometrial epithelial cells in the early secretory phase express high levels of MUC1. MUC1 carries numerous short carbohydrate chains, and sometimes functions as an L-selectin ligand. Since human blastocysts express L-selectin, the blastocyst may interact with the L-selectin ligand carried by MUC1 and roll on the endometrial epithelia (Fig. 2a). During rolling, hCG and other factors secreted from the human blastocyst may signal the endometrial epithelia to induce trophinin in the pinopodes⁴² (Fig. 2b). Given that MUC1 is down-regulated at the blastocyst attachment site^{31,32} and the pinopodes represent an adhesive endometrial site for implantation, direct adhesion between trophoblast cells and endometrial epithelial cells may be mediated by a molecule other than L-selectin.

Since pinopodes are tall protrusions and present above the glycocalyx, cell adhesion molecules expressed on the surface of the pinopode should allow direct interaction of trophoblast cells of the blastocysts and endometrial surface epithelia. Unlike the mouse blastocyst adhering in the narrowly enclosed uterine cavity, a human blastocyst adheres to pinopodes presented on the flat endometrial surface. Under such conditions, the adhesion of the human blastocyst to the pinopodes can only be achieved by an adhesion machinery or machineries. Trophinin, which exhibits an extremely high affinity is a strong candidate for this function (Fig. 2c). It is also possible that the strong cell adhesion requires two adhesion machineries: ErbB4/HB-EGF and trophinin.

Gene knockout mouse experiments for trophinin⁷⁷ and L-selectin and its ligand carbohydrates⁷⁸⁻⁸⁴ indicate that neither trophinin nor L-selectin plays an essential role in embryo implantation in the mouse. The HB-EGF gene knockout mouse showed a failure in embryo implantation.⁸⁵ The evidence collectively suggests that L-selectin and trophinin functions are acquired uniquely to human embryo implantation as additional mechanisms to ErbB4/HB-EGF. More work is needed for further defining the mechanisms underlying human embryo implantation.

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Figure 1. Trophinin in ectopic pregnancy

(a) Hematoxylin-eosin stained specimen of ectopic pregnancy in the fallopian tube. The implantation site is marked by arrowheads. Chorionic villi are marked as CV. Scale bar = 2 mm. (b) High magnification of inset area 'J' in (a), stained for trophinin. (c) High magnification of inset area 'K' in (a), stained for trophinin. (b) and (c) are shown at the same magnification. Scale bar = 100 μ m. Note that trophinin is expressed in the maternal tubal epithelia located close to the implantation site (b). By contrast, trophinin is not found in the maternal epithelia distant from the implantation site (c).

Figure 2. Proposed role of L-selectin and trophinin in human embryo implantation

(a) A human blastocyst entering the uterine cavity is prevented from adhering to endometrial surface epithelia by heavily glycosylated MUC1, except for epithelia that express L-selectin ligand (T). The human blastocyst expresses L-selectin (L), and 'rolls' on the endometrial surface covered by glycocalyx (cell surface layer shown by grey color). (b) The blastocyst weakly interacts on the glycocalyx. During this interaction, human chorionic gonadotropin (hCG) secreted from the blastocyst acts locally on endometrial surface epithelia to induce trophinin expression in the endometrial epithelial cells. (c) Trophinin expressed by endometrial epithelia is enriched in the pinopodes, the structure extended above the glycocalyx.⁴² MUC1, which is the major component of endometrial glycocalyx and carries L-selectin ligand, is down-

regulated from the endometrial surface epithelia underneath the blastocyst,³¹ allowing direct contact of blastocyst trophectoderm cells and pinopodes. Trophectoderm cells and pinopodes then bind strongly together by trophinin-trophinin binding.