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Invited Review

Cell adhesion molecules in human embryo implantation

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Abstract: The process of human embryo implantation is mediated not only by evolutionarily conserved mechanisms, but also by a mechanism unique to humans. Evidence suggests that the cell adhesion molecules, L-selectin and trophinin, play a unique role in human embryo implantation. Here, we describe the dual roles of mucin carbohydrate ligand for L-selectin and trophinin protein and of the trophinin-associated proteins bystin and tastin. We then describe trophinin-mediated signal transduction in trophectoderm cells and endometrial epithelial cells. This review also covers cadherin and integrin in human embryo implantation.

Key words: embryonic implantation; gonadotrophin; apoptosis

人类胚胎植入过程中的细胞黏附分子

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摘　要：人类胚胎植入过程不仅受到在进化上保守的机制调节，而且也受到人类一种独有的机制调节。有证据显示，细胞黏附分子L-选择蛋白和trophinin在人类胚胎植入过程扮演独特的角色。在本文中，我们描述了L-选择素和trophinin的黏蛋白糖配体的双重作用，也描述了trophinin相关蛋白bystin和tastin的双重作用。我们随后描述了滋养外胚层细胞和子宫内膜上皮细胞中由trophinin调节的信号转导。本综述也涵盖了钙依粘连蛋白和整合素在人类胚胎植入过程中的作用。

关键词：胚胎植入；促性腺激素；凋亡
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1 Introduction

Embryo implantation is a unique form of mammalian reproduction. However, studies of embryo implantation in a variety of mammals have revealed that the process varies significantly among different mammalian species [1]. Mechanisms underlying human embryo implantation are considered unique to humans, an observation closely linked to the high incidence of ectopic pregnancy seen in humans but extremely rare in non-human primates and nonexistent in rodents [2]. Initial step of embryo implantation is feto-maternal interaction and cell adhesion of trophectoderm of blastocyst and endometrial luminal epithelial cells of uterus, at their respective apical cell surfaces. This occurs despite generally the non-adherent nature of apical cell surfaces of epithelial cells. Thus embryo implantation was characterized as cell biological paradox [3]. This minireview describes molecules involved in apical cell adhesion of trophectoderm and endometrial epithelia, focusing on the roles of mucins, L-selectin, trophinin, cadherin and integrin, in human embryo implantation.

2 Mucins

Apical cell surfaces of epithelia contain numerous microvilli, which are covered by thick layer of mucin car-

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bohydrate called the glycocalyx. The glycocalyx lubricates and hydrates cell surfaces as well as protects epithelial cells from microorganisms and degradative enzymes. In addition, mucins inhibit both cell-cell and cell-extracellular matrix interactions. The apical surface of human luminal and glandular uterine epithelia are covered by abundantly expressed MUC1 and other mucins [4, 5].

MUC1 is a type 1 membrane protein composed of a large N-terminal extracellular domain, a transmembrane domain, and a short C-terminal cytoplasmic domain [6] (Fig. 1*A*). The MUC1 cytoplasmic domain associates with signaling molecules including β-catenin and Grb2/Sos, suggesting a potential role for MUC1 in cell signaling [7]. Activation of receptor tyrosine kinase 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 [8, 9]. Thus, stimuli such as growth factors or cytokines may affect MUC1 stability, localization and phosphorylation directly or through activation of MUC-1 associated membrane proteins.

MUC1 expression in endometrial epithelial cells is regulated at the transcriptional level by steroid hormones and other factors [10]. In the mouse, rat, and pig, Muc1 is down-regulated in the entire uterus prior to embryo implantation [11], consistent with the idea that the glycocalyx made by mucins inhibits cell adhesion and therefore needs to be down-regulated 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 down-regulated at embryo implantation sites *in vivo* and *in vitro* [12]. In the human endometrium, MUC1 is significantly elevated in the early secretory phase or implantation window [13]. Although MUC1 has not been studied at the embryo implantation site in humans *in vivo*, *in vitro* implantation models indicate that MUC1 is down-regulated at the site of embryo attachment in humans as well [14]. This suggests that one or more factors expressed on or released from the to be implanted blastocyst triggers signals for down-regulation of MUC1 from the adjacent endometrial epithelia in humans. Although the major function of MUC1 in human endometrial epithelia before and during implantation is to prevent the blastocyst from adhering to endometrium to wrong place, carbohydrate moiety of MUC1 ex-

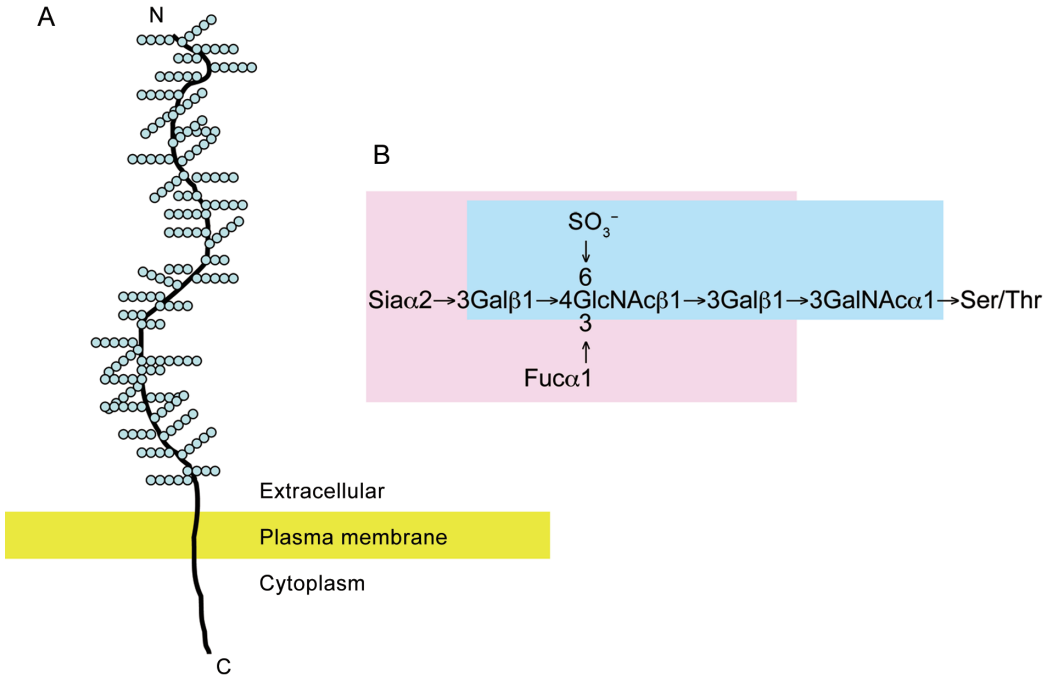

Fig. 1. Mucin and L-selectin ligand. MUC1, one of mucin glycoproteins, is a transmembrane protein, of which extracellular domain contains numerous carbohydrate chains (*A*). Some MUC1 carbohydrate in human endometrial epithelial cells contains sulfated and fucosylated oligosaccharide structure shown, which is specifically recognized by L-selectin (*B*).

pressed on the very spot for implantation expresses L-selectin ligand structure (Fig. 1*B*). As described below, L-selectin and L-selectin ligand play an important role in human blastocyst implantation.

3 L-selectin

Selectins are a group of carbohydrate binding proteins. In both human and mouse, three selectin genes exist and their products known as E-selectin, P-selectin, and L-selectin are expressed in hematopoietic cells; i. e., leukocytes and endothelial cells. E-selectin is expressed on the endothelial surface during inflammation, P-selectin is expressed on the activated platelet, and L-selectin is constitutively expressed on the lymphocytes [15–17].

Although previously it was thought that selectins are expressed only in the hematopoietic cells, L-selectin was found on the surface of human blastocysts [18]. Furthermore, L-selectin ligand oligosaccharides can be detected by antibodies as MECA79 and HECA452 antigens [19, 20] (Fig. 1*B*). These antigens were detected by immunohistochemistry on luminal and glandular endometrial epithelia in the human uterus [18, 21, 22]. MECA79 antigen is carried by MUC1 in human endometrial [23]. It has been suggested that interactions between L-selectin on human blastocysts and oligosaccharide ligands on endometrial epithelia enable an interaction of human embryo to endometrium for implantation [18] (Fig. 2).

L-selectin expressed on leukocytes interacts with their carbohydrate-ligands on the blood vessel endothelial cells. This interaction allows the rolling of leukocytes on vascular endothelium prior to their firm adhesion for extravasation [17, 24]. A parallel was made between the leukocyte rolling on vascular wall and the blastocyst apposition to the endometrial epithelium [18, 25]. Nonetheless, given the enormous difference in size between a human blastocyst (diameter, 115–265 μm) [26] and lymphocyte (diameter, 10 μm), it may be difficult for a blastocyst being immobilized to endometrial epithelia solely through L-selectin and L-selectin ligand, as the force of such interaction is weak [27]. It seems reasonable to speculate that a human blastocyst rolls over the glycocalyx of the endometrial epithelium through weak interactions with 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.

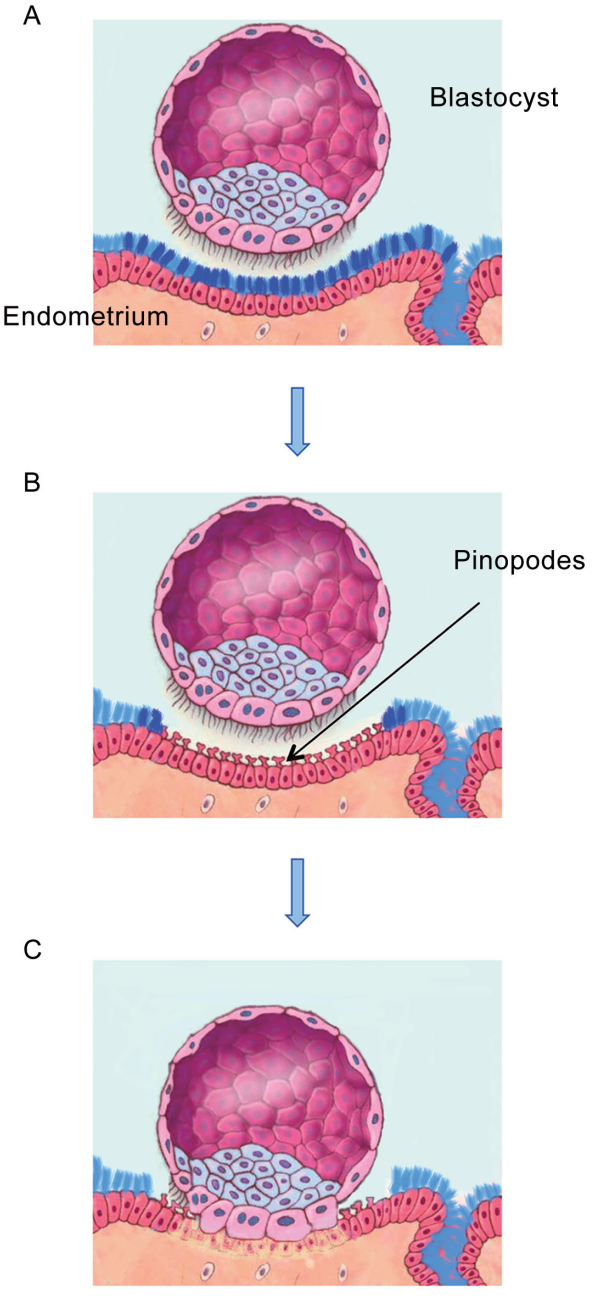

Fig. 2. Steps of human embryo implantation. *A*: A human blastocyst and endometrial epithelia covered by glycocalyx (shown by blue hairs) are shown. Some glycocalyx contains L-selectin ligand structure (dark blue). Blastocyst expressing L-selectin on its surface rolls over on glycocalyx with L-selectin-ligand structure. *B*: Endometrial epithelial cells underneath the blastocyst loose glycocalyx and develop pinopodes. Since both trophectoderm cells and pinopodes contain trophinin, this allows homophilic cell adhesion by trophinin-trophinin binding. *C*: Trophectoderm cells stimulated by trophinin-mediated cell adhesion invade maternal cells. By contrast, maternal epithelia undergo apoptosis and accept trophoblast invasion.

The rolling may ensure for the blastocyst to settle in the proper spot in the uterus and in the correct orientation [28].

A microarray analysis of mouse blastocysts showed an elevation of L-selectin transcripts during the maturation stage, when the blastocysts are competent for implantation [29]. However, mutant mice deficient in the L-selectin gene show no defect in implantation [30], and mutant mice lacking fucosyltransferase and sulfotransferase required for synthesizing L-selectin ligand did not show a sign for reproduction failure [31–34]. Furthermore, the MECA79 antigen was not detected in mouse endometrial epithelial cells [18], suggesting that L-selectin plays a role in human, but not mouse, embryo implantation.

4 Trophinin

Trophinin was identified by expression cDNA cloning from cDNA library constructed from human embryonal carcinoma (EC) cell line HT-H [35, 36]. ECs are tumors composed of undifferentiated embryonic stem (ES) cells and variously differentiated cell types [37, 38]. Both human and mouse ECs show characteristics of early embryonic cells [37, 39, 40]. While undifferentiated mouse EC cells express SSEA1 (stage specific embryonic antigen 1) antigen as those ES cells of the blastocyst do [41], human EC cells express SSEA3 and SSEA4 antigens as cells earlier than those in blastocyst stage [42, 43]. Mouse EC cells have the tendency to differentiate into endoderm [44], whereas human EC cells have the tendency to differentiate into trophoblastic cells [40]. Trophoblastic EC cells are thought to represent those at early embryonic stage, as such in trophectoderm of the blastocyst.

HT-H cells spontaneously differentiate into syncytiotrophoblast-like cells *in vitro* and secrete trophoblast marker hCG [35]. 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-M cells, they instantly adhere to SNG-M cells [36]. 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 tro-

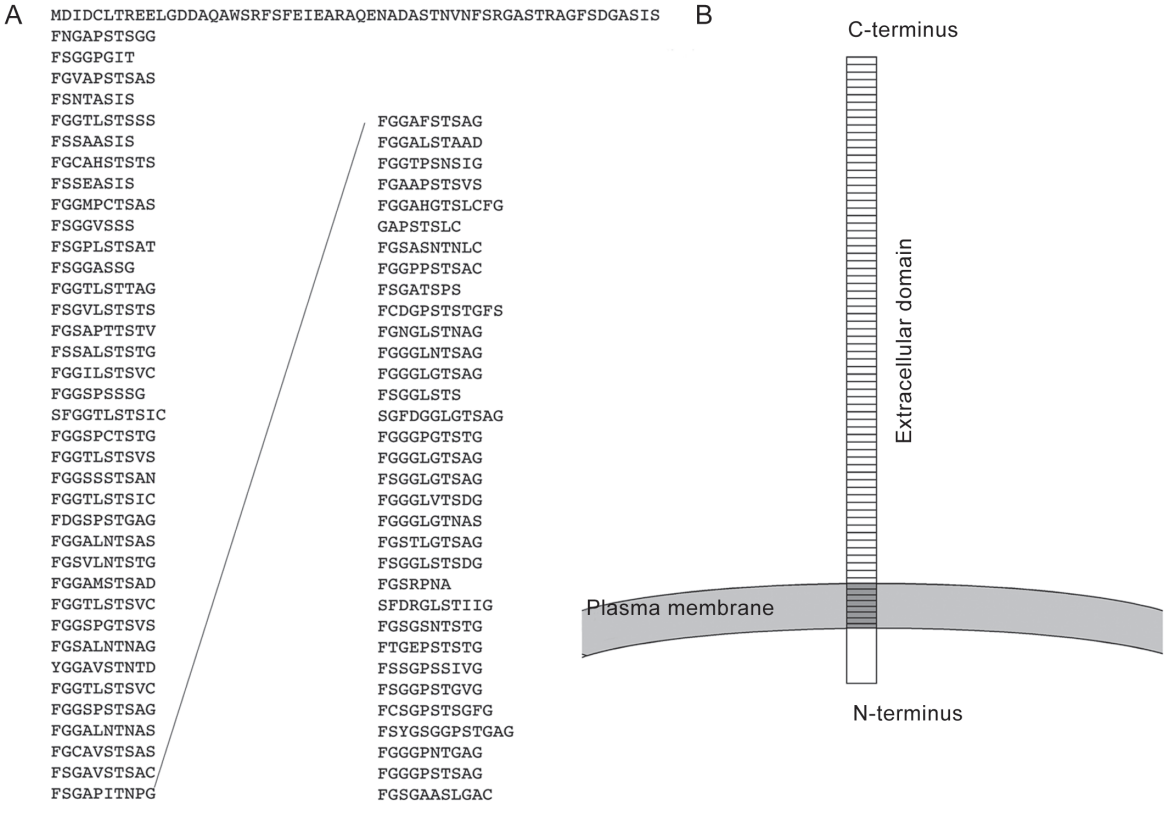

Fig. 3. Structure of human trophinin protein. *A*: Peptide sequence of human trophinin. Majority of the peptide is made of decapeptide repeats. *B*: Proposed topology of trophinin protein, with decapeptide repeats as outer cellular and N-terminal region in the cytoplasm.

phoblast/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 [36, 45]. Human trophinin is composed of hydrophilic N-terminal domain followed by the repeats (Fig. 3*A*). Although trophinin does not have a leader peptide characteristic of conventional plasma membrane proteins processed via the sorting pathway, experimental evidence indicates that trophinin is an intrinsic membrane protein [36, 46]. Although the existence of several hydrophobic domains in decapeptide repeats initially suggested that this protein traverses the lipid bilayer multiple times [36], a more plausible possibility is that the trophinin protein is a single transmembrane protein utilizing the first hydrophobic decapeptide repeats near the N-terminus to span the membrane (Fig. 3*B*). The remaining C-terminal decapeptide repeats may be extracellular.

Unlike many cell adhesion molecules requiring calcium for adhesion, adhesion by trophinin is independent of divalent cations [36]. Trophinins bind each other when they are presented *in trans* at the respective apical cell surface. Other well-characterized homophilic cell adhesion molecules, such as cadherins, also bind one another *in trans* at respective lateral surfaces [47]. A monoclonal antibody specific to human trophinin showed positive immunostaining in both trophoblast and maternal epithelia at embryo implantation sites in the human placenta [48].

In trophoblastic cells, the trophinin cytoplasmic domain binds to a cytoplasmic protein, bystin, which further binds to tastin and cytokeratin [45, 49]. When trophinin complexed with these cytoplasmic proteins in the cytoplasm, extracellular domain of trophinin can function as cell adhesion molecule [36, 45, 49].

In humans, trophinin gene is mapped to the short arm of X chromosome [50]. This region of X chromosome is closely linked to the evolution of mammal: genes encoded in this region in one placental mammals are likely located on the X chromosome in other mammals due to dosage compensation [51, 52]. Indeed, trophinin gene has been mapped to X chromosome in mouse [53], sheep [54], and bovine [55, 56]. Genes encoded in these region are autosomal in marsupials and monotremes [57], animals that do not undertake proper implantation.

During ectopic pregnancies, the condition unique to humans [58], trophinin was strongly expressed at the implantation site in both fetal and maternal cells: i. e., trophinin was expressed by the trophoblast in the chorionic villi and also by the maternal epithelia adjacent to the chorionic villi [2]. However, the epithelia at a slight distance (5 mm) from the implantation site showed no trophinin. Therefore, it appeared that expression of trophinin by maternal cells is induced by implanting embryo. One of the inducers may be human chorionic gonadotropin β-chain (CGβ), as transcription of the *TRO* gene in the fallopian tubal explant was induced by hCGβ [2]. Furthermore, CGβ together with IL-1β induced strong trophinin expression in human endometrial epithelial cells [59]. Interestingly, trophinin was found in the pinopodes [60, 61], tall protrusions presented above the glycocalyx, found in the implantation sites. Pinopodes containing trophinin were induced by CGβ and IL1β. [59] (Fig. 4). Cell adhesion molecules expressed on the surface of the pinopode should allow direct interaction of trophectoderm cells of blastocysts. Trophinin, which binds to each other with strong affinity is a good

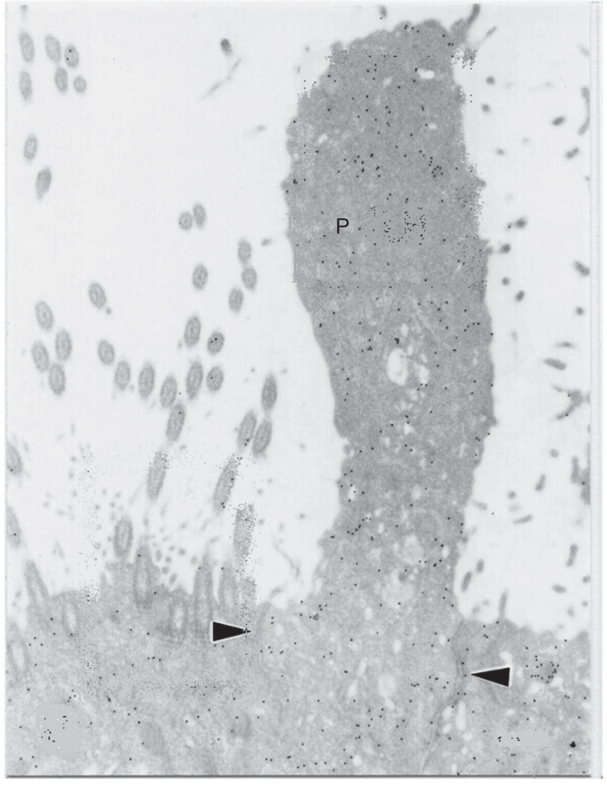

Fig. 4. Immunoelectron micrograph of pinopode developed by human endometrial epithelial cell treated by hCG. A pinopode (P) and the neighboring ciliated area with the lateral junctional complex (arrowheads) stained for trophinin by gold particles. Modified from Sugihara *et al* [59].

candidate for this function.

It is also possible that the strong cell adhesion in embryo implantation requires multiple adhesion machineries. In the mouse, ErbB4 and HB-EGF play the major role in the initial adhesion in embryo implantation [62]. Expression pattern of ErbB4 in human trophectoderm cells and HB-EGF in human endometrial epithelial cells support the involvement of these molecules in human embryo implantation [63, 64]. While, gene knockout mouse experiments for trophinin [53] and L-selectin [30] and its ligand carbohydrates [32, 33, 65–68] indicate that neither trophinin nor L-selectin plays an essential role in embryo implantation in the mouse, HB-EGF gene knockout mouse showed a failure in embryo implantation [69]. The evidence collectively suggests that functions of L-selectin and trophinin are acquired uniquely to human embryo implantation as additional mechanisms to ErbB4/HB-EGF. An integrated view of L-selectin and trophinin has been proposed [70] (Fig. 2*A*, *B*).

Morphological observations of human embryo implantation sites indicate that trophectoderm cells of the blastocyst adhered to the uterus proliferate and invade, whereas trophectoderm not in contact with the uterine epithelium remains a monolayer [48, 71, 72]. This finding suggests that the initial adhesion triggers activation of cells in trophectoderm for proliferation and invasion. By contrast, epithelial cells in contact with the blastocyst underwent apoptosis and disappeared [73–75] (Fig. 2*C*). It is known that trophoblastic cells express FAS ligand (FASL) and endometrial epithelial cells express FAS [76]. Therefore, trophectoderm adhesion to endometria epithelia may induce apoptosis by FAS/FASL pathway.

Trophinin-mediated adhesion on the cell surface of trophoblastic cells triggers EGF-mediated cell activation [46, 77, 78], whereas it triggers an apoptotic signal in maternal cells [79, 80] (Fig. 5). Therefore, trophinin is a dual signaling molecule: in embryonic cells it promotes proliferation and invasion, while in maternal cells it promotes cell death in order to accept invading embryo.

5 E-cadherin

E-cadherin is located in the adherens junctions on the lateral side of the plasma membrane of epithelial cells [69,70]. Ultrastructure of human embryo implantation revealed a formation of adherent junction, desmosome-like structure, between originally the apical cell surface of trophectoderm cells and endometrial epithelial cells [81]. When trophoblastic HT-H cells were added

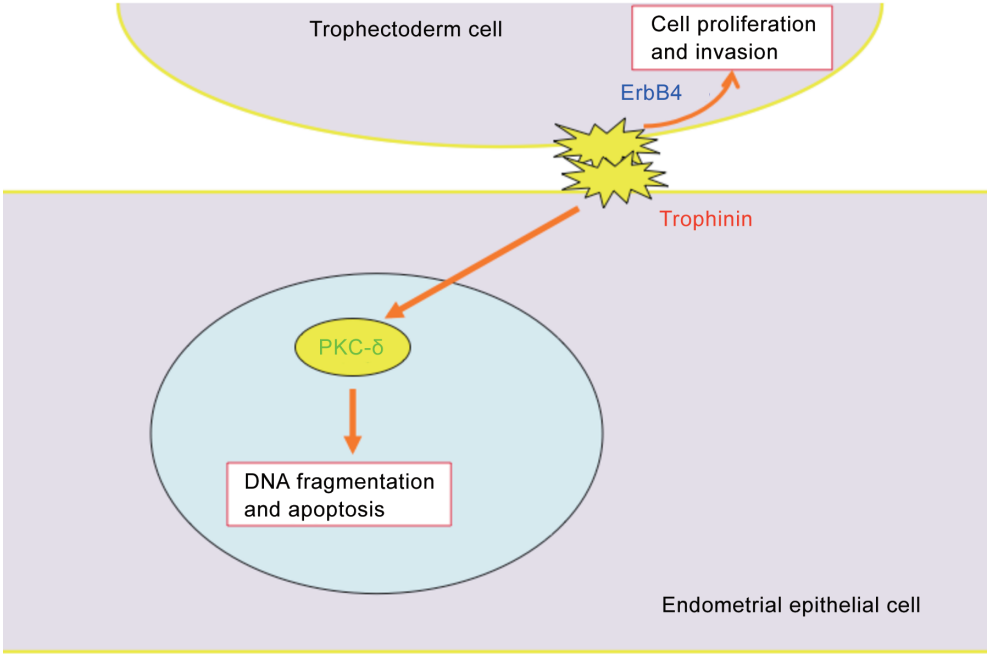

Fig. 5. Signals triggered by trophinin-mediated cell adhesion in trophectoderm cells and endometrial epithelial cells. In trophoblastic cells, ErbB4 (receptor tyrosine kinase) is arrested by bystin/trophinin complex. When trophinin-mediated cell adhesion takes place, ErbB4 is released from bystin. This allows ErbB4 to be activated by phosphorylation. In endometrial epithelial cells, trophinin-mediated cell adhesion releases PKCδ from trophinin. PKCδ is then translocated to the nucleus, where it activates caspase 3 for apoptosis.

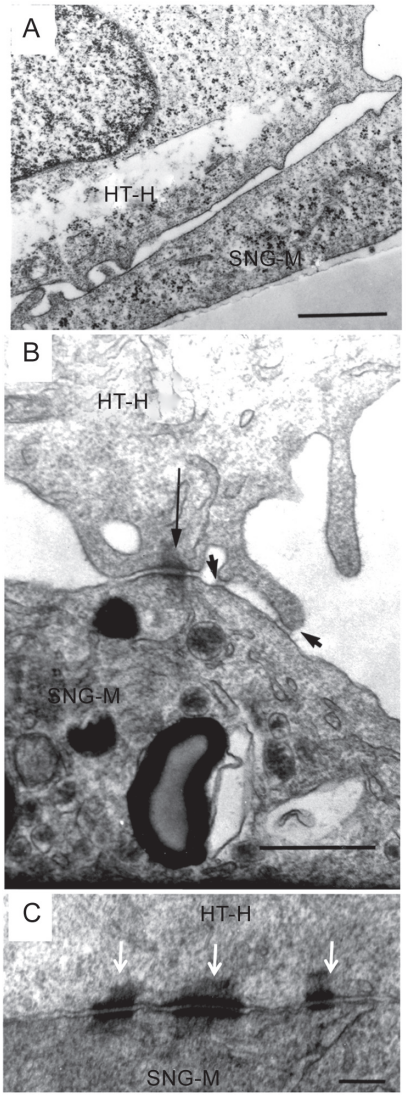

Fig. 6. Adherent junctions formed between trophectoderm cells and endometrial epithelial cells. Electron micrographs of HT-H cultured on SNG-M for 6 hours (*A*, scale bar, 1 μm), for 20 hours (*B*, scale bar, 1 μm) and 7 days (*C*, scale bar, 0.5 μm). Note that adherent junctions and desmosomes are developed between these two cell types at originally the apical cell surfaces (arrows). Modified from Fukuda *et al*.[36] and Aoki *et al*[107].

to endometrial epithelial SNG-M cells, adherent junctions were developed between these cell types [36, 45] (Fig. 6) Because adherent junctions and desmosomes are characteristically formed at the lateral junctions between two epithelial cells [82, 83], such observations suggested that these epithelial cells changed their polarity after initial apical cell adhesion.

Intracellular calcium concentrations affect epithelial cell adhesiveness and polarity by triggering redistribution of cell adhesion molecules [84]. *In vitro* experiments on cultured endometrial adenocarcinoma Ishikawa cells demonstrated that a transient rise in intracellular calcium, triggered by calcitonin, suppresses E-cadherin expression at cellular contact sites [85]. Interestingly, calcitonin, a potential regulator and biomarker of endometrial receptivity [86, 87], is induced by progesterone in the human endometrial epithelium specifically during the mid-secretory phase of the menstrual cycle [88]. Progesterone could regulate E-cadherin, probably via endometrial calcitonin induction leading to increased intracellular calcium. Thus, it is possible that E-cadherin expression at the lateral cell surface is required to maintain the polarity of endometrial epithelial cells, whereas E-cadherin may be down-regulated to enable epithelial cells dissociation to accept blastocyst invasion. The up-regulation of E-cadherin and catenin in the epithelial cells of peri-implantation uteri and the down-regulation of cadherin, catenin and calcium ion in invasive trophoblast appear to be associated with embryo-uterine interactions during early pregnancy [89].

6 Integrins

Integrins are a family of heterodimeric transmembrane glycoproteins, formed by the association of two, noncovalently linked α and β subunits, and are expressed on the basal cell surface to adhere to extracellular matrix through tripeptide arginine-glycine-aspartic acid (RGD) sequence [90, 91]. These subunits contain extracellular, transmembrane and cytoplasmic domains. The extracellular domain enables integrins to function as a receptor to extracellular matrix. The cytoplasmic domain interacts with the cytoskeleton and other cytoplasmic proteins.

In human endometrium, expression pattern of integrins is correlated to fertility and implantation [92–94]. While the majority of the integrins are constitutively expressed throughout the entire menstrual cycle, some integrins exhibit expression patterns dependent on hormonal cycle, and integrins whose expression is increased in the mid-luteal phase were proposed as markers for the frame of the window of implantation [95, 96]. αvβ3 integrin as well as its ligand osteopontin was detected by immunohistochemistry on the endometrial luminal epithelial surface, which may interact with the trophoblast [97]. The cycle-specific expression pattern of endometrial integrin is suggestive of hormonal regulation. Indeed, αvβ3 integrin expression is orchestrated in the human endometrium both by positive and negative

factors [98]. Both estrogen and progesterone are thought to act as paracrine stromal factors to induce epithelial β3 integrin expression that serves as the rate-limiting step in αvβ3 formation [99]. In addition, signalling through αvβ3 has been reported to be important to maintain a balance between cell proliferation and apoptosis, along with the modulation of inflammatory responses of decidual cells [100]. Although αvβ3 was found in the pinopodes, later studies in infertile women revealed this marker serves poorly for dating the receptive phase for implantation [101, 102].

7 Perspective

Analysis of human embryo implantation at molecular level is a challenging task, as this process includes a mechanism unique to humans. Availability of large number of human embryos from *in vitro* fertilization and human ES cell lines allowed gene microarray analyses, which are providing unprecedented amount of data [103, 104]. As human ES cells differentiate into trophoblastic cells *in vitro* [105, 106], analysis of embryo implantation can be achieved using ES cell-derived *in vitro* culture system in the future.

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