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REVIEW

Adhesion molecules in endometrial epithelium: tissue integrity and embryo implantation

Harmeet Singh and John D. Aplin

Maternal and Fetal Health Research Group, University of Manchester, Manchester, UK

Abstract

Cell adhesion in endometrial epithelium is regulated to maintain the continuity and protectiveness of the luminal covering cell layer while permitting interstitial implantation of the embryo during a restricted period of about 4 days. Many apparently normal embryos fail to implant, and epithelial-embryo adhesion remains a poorly understood phenomenon. After menstruation, epithelial regeneration occurs by epiboly from the basal residues of glands, an activity that requires migration on extracellular matrix as well as cell–cell cohesion. Here we review current knowledge of adhesion molecules in the epithelium.

Key words adhesion; embryo; endometrium; epithelium; implantation.

Introduction

Implantation in human involves initial weak interaction between the blastocyst and endometrial surface followed by stable adhesion and transient disruption and resealing of the epithelium as the embryo positions interstitially. The molecular mechanisms are incompletely understood. A few studies have analysed adhesion molecule expression on trophectoderm in the human blastocyst (Campbell et al. 1995a,b; Bloor et al. 2002; Fujiwara et al. 2002, 2003; Genbacev et al. 2003) but given the scarcity of embryos for research, information is slow to accumulate. Implantation requires maternal receptivity, a property vested in the epithelium (Aplin, 2000, 2006; Aplin & Kimber, 2004), and identification of molecular candidates in the epithelium has enabled strong hypotheses to be formulated regarding attachment mechanisms. Adhesion molecules in the epithelium are in addition required to mediate adhesion to the underlying basement membrane or adjacent epithelial cells. Cell surface components are relatively poorly represented in transcriptomic libraries (Reese et al. 2001; Yoon et al. 2004; Aplin, 2006), although other informatics-related approaches may be informative (Aplin & Singh, 2008). Most existing data arise from conventional immunolocalization studies, some of which have been extended using *in vitro* models of implantation to examine function. We here review molecular families that have been identified in endometrial epithelium and evidence pertaining to their function.

Correspondence
J. D. Aplin, Room 74, Research Floor, St Mary's Hospital, Manchester M13 0JH, UK. T: +44 161 276 6487; E: john.aplin@manchester.ac.uk

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Cadherins

E-cadherin and calcium

Members of the cadherin superfamily are transmembrane glycoproteins that share sequence repeats of about 110 amino acids in the ectodomain. They mediate cell–cell interaction by calcium-dependent homotypic or heterotypic binding (Stemmler, 2008). Several subgroups have been defined: the classical (type I) and closely related type II cadherins, desmosomal cadherins, and protocadherins. The transmembrane domain links the extracellular repeats to a shorter cytoplasmic domain, which interacts non-covalently with p120 catenin and β-catenin. β-Catenin in turn binds α-catenin, which can link the complex to the actin cytoskeleton both directly through interaction with actin filaments and indirectly through the actin-binding proteins vinculin, zonula occludens-1 (ZO-1), α-actinin and afadin (Kaplan et al. 2001; Hartsock & Nelson, 2008; Stemmler, 2008).

Deletion of catenin binding sites results in the loss of cellular re-organization and adhesive function, showing that catenins mediate activity of the cadherins (Rosales et al. 1995). Loss of cadherin–catenin complex formation due to the expression of truncated β-catenin correlates with the loss of lateral adhesion in epithelial cells (Oyama et al. 1994). Expression of full length β-catenin restores both complex formation and cell adhesion (Kawanishi et al. 1995).

The E-cadherin-null mouse shows defective pre-implantation embryo development and failure to implant (Larue et al. 1994; Riethmacher et al. 1995). β-Catenin is expressed by the mouse blastocyst at cell–cell borders. In endometrium, E-cadherin is located at the lateral epithelial plasma membrane and is likely to be critical for the

establishment and maintenance of adherens junctions (Gumbiner, 1996; Huber et al. 1996; Poncelet et al. 2002). Other cadherins present include type 1 N-cadherin and P-cadherin, and the type 2 cadherin-6 (K-cadherin) (van der Linden et al. 1995; Getsios et al. 1998; MacCalman et al. 1998; Dai et al. 2002; Tsuchiya et al. 2006).

In vitro experiments using Ishikawa (well-differentiated endometrial carcinoma) cells have demonstrated that a transient rise in intracellular calcium, triggered by calcitonin, down-regulates E-cadherin at cellular contact sites and activates tissue transglutaminase (Li et al. 2002, 2006). Calcitonin promotes trophoblastic displacement of endometrial epithelial cells through calcium mobilization (Li et al. 2008). In rodents, it has been demonstrated that progesterone regulates calcitonin expression (Zhu et al. 1998b) and a reduction in implantation rate is observed if maternal calcitonin is blocked (Zhu et al. 1998a). Rising progesterone levels during the secretory phase in human probably induce endometrial calcitonin expression (Ding et al. 1994; Kumar et al. 1998; Zhu et al. 1998a). Calcitonin also acts to enhance trophectodermal surface expression of integrin $\alpha 5\beta 1$ in mouse blastocysts (Wang et al. 1998).

Members of the calbindin family of proteins are specifically up-regulated at the site of embryo attachment and dual ablation of two calbindins, CaBP-d9k and CaBP-d28k, in mouse prevents implantation (Nie et al. 2000; Luu et al. 2004). Thus regulators of calcium homeostasis clearly play an important role in the process of implantation.

As E-cadherin is found on luminal epithelium and also on trophectoderm, it has been suggested that it may be involved in the initial attachment of the embryo (Coutifaris et al. 1991). It is possible that E-cadherin (or other cadherins) possess a dual function. In the initial stages, expression at the cell surface may be required for epithelial continuity. However, cadherin-mediated adhesion may be subsequently down-regulated at the implantation site to enable blastocyst invasion.

β-Catenin interactions

In addition to its role in maintaining the integrity of cadherin-bearing cell–cell junctions, β-catenin is important in the transduction of cytosolic signals to the nucleus in a variety of cellular contexts. Signalling through the canonical Wnt pathway leads to the activation, accumulation and nuclear translocation of β-catenin (Widelitz, 2005). In mice, Wnt ligand secreted by the blastocyst activates the Wnt/B-catenin signalling pathway in the luminal epithelium of the uterus, inhibition of which severely reduces the rate of embryo implantation (Mohamed et al. 2005). β-Catenin expression has been observed in both proliferative and secretory phase human endometrium (Fujimoto et al. 1996; Shih et al. 2004) and mRNA data support the possibility that Wnt/β-catenin signalling may play a role during the receptive phase (Tulac et al. 2003).

The mucin MUC1 is highly expressed in secretory phase endometrium (Hey et al. 1994, 1995, 2003); its cytoplasmic domain interacts with β-catenin at a serine-rich motif (Yamamoto et al. 1997; Li et al. 1998) and the presence of associated adaptor proteins suggests a role in signal transduction (Wen et al. 2003). β-Catenin has also been found to be associated with EGFR at the cell surface and EGFR has been shown to phosphorylate MUC1 (Li et al. 2001). The MUC1 cytoplasmic domain has been localized to the nucleus in association with β-catenin. At sites of attachment of embryos to primary epithelial cells *in vitro*, MUC1 is observed to be cleared from cells beneath and adjacent to the embryo (Singh et al. unpublished observations; Meseguer et al. 2001), perhaps as a result of the proteolytic action of ADAM17 or MMP14 (Thathiah & Carson, 2004). These results raise the possibility that MUC1 cytoplasmic tail in association with β-catenin may be translocated to the nucleus, disrupting cadherin mediated cell–cell adhesion (Wen et al. 2003) and allowing the embryo to invade the epithelium.

Another mucin component of the luminal epithelial surface is MUC16, which inhibits cell–cell adhesion. Removal of this mucin during formation of uterodomes (bulbous projections from the apical surface of the epithelium that are often found during the implantation period) facilitates trophoblast adhesion *in vitro* (Gipson et al. 2008).

Desmosomes and tight junctions

Desmosomes are mechanical contacts associated with lateral epithelial borders (Dockery et al. 1988; Preston et al. 2004). The desmosomal plaque protein desmoplakin decreases in the luminal uterine epithelium during the preimplantation period of pregnancy in mice (Illingworth et al. 2000). The incidence of desmosomes (and also gap junctions) in lateral membranes is decreased at the time of implantation (Dockery & Burke, 2008), probably facilitating opening up of spaces and penetration of the luminal epithelium by trophoblast cells at implantation. There is sharing of apical junctional complexes and desmosomes between trophectoderm and luminal epithelial cells in *in vitro* attachment sites (Bentin-Ley et al. 2000; Lopata et al. 2002).

Desmosomal plasma membrane proteins (desmocollins and desmogleins) are members of the cadherin superfamily but have cytoplasmic regions that differ from the classical cadherins, allowing the recruitment of plakoglobin (γ-catenin), desmoplakin, and plakophilin (but not β-catenin), which form links to the intermediate filament cytoskeleton. There are three subtypes of desmocollins and desmogleins, which are expressed in a tissue- and differentiation-specific manner. Desmosomal cadherins form heterotypic interactions, in contrast to the homotypic interactions of classical cadherins. Desmosomal cadherins can initiate and maintain cell–cell adhesion in the absence

of classical cadherins. Desmoglein-2 null mice and a proportion of heterozygotes fail at implantation because of inner cell mass defects (Eshkind et al. 2002). Desmoglein 1 and 2 are observed in rat uterine epithelium (Preston et al. 2004). Further analysis is required of the tissue phenotype in human endometrium.

Tight junctions are evident in endometrial luminal epithelial cells in the apical junctional complex, and their depth and complexity are higher at days 14–16 than at days 24–25 (Murphy et al. 1992; Dockery & Burke, 2008). In rats there is a transformation of the apical plasma membrane at the time of implantation with increased depth of tight junctions, flattening of microvilli and associated changes in the apical cytoskeleton (Lindsay & Murphy, 2008). The fencekeeping function of tight junctions in endometrium is deserving of further examination – many adhesion molecules that could be involved in interactions with trophectoderm at implantation are located principally, though not exclusively, in the lateral membrane domain (Aplin, 2006) such that regulated diffusion to the apical domain may be of potential functional importance. Previous authors have suggested some loss or alteration of epithelial polarity may occur in the receptive phase (Denker, 1993, 1994; Lindsay & Murphy, 2008).

Immunoglobulin superfamily members

Members of the immunoglobulin superfamily (IgSF) are involved in the control of cell behaviour by acting as cell–cell adhesion receptors or signal transducing receptors or both (Aricescu & Jones, 2007). IgSF members can be involved in homophilic binding or act as ligands for integrins to mediate heterophilic cell–cell adhesion.

ICAM-1

Intercellular adhesion molecule-1 (ICAM-1 or CD54) is a ligand for β_2 integrins. ICAM-1 adhesive interactions mediate transendothelial migration of leukocytes and various other immunological functions (van de Stolpe & van der Saag, 1996). ICAM-1 is immunolocalized to the apical surface of endometrial epithelial cells throughout the menstrual cycle (Thomson et al. 1999).

A soluble circulating form of ICAM-1 (sICAM-1) is released from the cell surface by proteolysis of transmembrane ICAM-1. In addition, sICAM-1 interferes with immunological functions by which refluxed endometrial cells escape immune surveillance and its shedding may be related to the pathogenesis of endometriosis (Defrere et al. 2005).

C-CAM and N-CAM

Other IgSF family members present on the surface of trophectoderm at the time of implantation are homophilic cell–cell adhesion molecule 105 (C-CAM) (Svalander et al. 1987) and neural cell adhesion molecule (N-CAM) (Kimber et al. 1994). C-CAM protein expression is also detected in the luminal epithelium under the influence of estrogen, whereas expression is down-regulated in the progesterone-primed uterus (Svalander et al. 1990).

CD146

CD146 (Mel-CAM; MUC18) is expressed in invasive cytotrophoblasts in the first trimester of pregnancy but not in non-invasive trophoblast (Shih et al. 1998b; Liu et al. 2004). A functional role in trophoblast invasion *in vitro* has been demonstrated (Shih et al. 1998a). In mice, CD146 is specifically expressed in receptive maternal uteri and invasive embryonic trophoblasts, but is completely absent in non-pregnant uteri. Blocking CD146 function by antibody *in vivo* and *in vitro* potently inhibited blastocyst attachment and subsequent trophoblastic invasion, leading to pregnancy failure (Liu et al. 2008a).

Basigin

Basigin (BSG) is also known as EMMPRIN or CD147. It is a highly glycosylated transmembrane protein with multiple binding partners at the cell surface. It may be activated by homotypic interaction between adjacent cells, but is also known as an activator of matrix metalloproteinase (MMP)-mediated proteolysis. It is involved in multiple molecular associations at the cell surface including complexing with caveolin, monocarboxylate transporters and β1 integrins (Iacono et al. 2007).

In both mice and rats, basigin is expressed in luminal epithelium on day 1 of pregnancy under the influence of estrogen, is down-regulated but then reappears locally in response to an embryonic stimulus on day 4 (Xiao et al. 2002a,b). It has been reported that basigin expression is regionally elevated at the site of embryo apposition in the uterine endometrium, and mutant mice lacking the basigin gene show severely compromised implantation rates (Igakura et al. 1998).

In human endometrium it is weakly expressed on luminal (lateral) epithelial surfaces with strong expression on both glandular and stromal cells (www.proteinatlas. org/). Moderate apical distribution is also observed in Ishikawa cells by immunofluorescence and cell surface proteomic analysis. Menstrual cycle-dependent molecular variants have been observed in human endometrium (Noguchi et al. 2003).

ALCAM (CD166)

ALCAM (CD166), an activated leukocyte-cell adhesion molecule, is a transmembrane glycoprotein belonging to the immunoglobulin superfamily and a ligand for CD6 that is expressed on T-lymphocytes. ALCAM–ALCAM homotypic

adhesion has been shown to play an important role in regulation of stem cell differentiation (van Kempen et al. 2001). ALCAM is expressed on endometrial luminal and glandular epithelial cell surfaces and also the blastocyst cell surface (Fujiwara et al. 2003). It activates MMP-mediated proteolysis (Lunter et al. 2005). Expression is significantly decreased in endometriosis compared to normal endometrium (Zhang et al. 2006). ALCAM–ALCAM interaction in endothelial cells is involved in tube formation in angiogenesis (Ohneda et al. 2001). Null mice are overtly normal and fertile.

Trophinin

Trophinin is an apical transmembrane glycoprotein identified based on its involvement in the adhesion of human trophoblastic and endometrial cell lines (Fukuda et al. 1995; Fukuda & Sugihara, 2008). The trophinin cytoplasmic domain forms complexes with the proteins bystin and tastin. Trophinin appears to mediate calcium-dependent homophilic cell adhesion and acts as a molecular switch for trophoblast activation; its ligation leads to ErbB4 phosphorylation in the presence of HB-EGF, a signalling event that is suggested to promote proliferation and motility in trophoblast. Although trophinin is absent from cycling endometrium, the combination of chorionic gonadotrophin (produced by the implanting embryo), and interleukin (IL)-1β delivers a juxtacrine signal leading to local up-regulation of trophinin in luminal epithelium (Sugihara et al. 2008). In mice, trophinin is expressed by both the blastocyst and uterus between 3.5 and 5.5 days *post coitum*; however, it is not absolutely required during early implantation (Fukuda & Sugihara, 2008). At macaque and rhesus monkey implantation sites, trophinin expression is observed at the apposed apical surfaces of both luminal epithelium and trophoblast.

CD164

CD164 (endolyn, MUC24) is a highly O-glycosylated type I transmembrane protein containing two extracellular mucin domains (I and II) interrupted by a cysteine-rich non-mucin domain (Chan et al. 2001) and a short cytoplasmic tail of 13 amino acids (Ihrke et al. 2000). Alternative splicing leads to production of molecular variants including a soluble form. CD164 regulates the proliferation, adhesion, and migration of human haematopoietic stem cells (Zhou et al. 2006). Blocking CD164 on prostate cancer cell lines reduced their adherence to bone marrow endothelial cells and invasion of extracellular matrices (ECMs) (Havens et al. 2006).

Immunolocalization studies on endometrial epithelial cells and endometrial biopsies indicate that CD164 is expressed on the apical surface of luminal epithelial cells along with glandular and stromal cells, suggesting that it could play a role during embryo attachment. However, indirect immunofluorescence of embryo attachment site *in vitro* with anti-CD164 showed no perceptible disturbance in distribution (Singh et al. unpublished observations).

Integrins and their binding partners

Integrins are a family of transmembrane glycoproteins, formed by non-covalent association of α and β subunits. Each subunit comprises an extracellular domain, a transmembrane region and an intracellular domain. They act as receptors for ECM components, secreted glycoproteins, complement and other cells. The assembly of integrins into focal adhesion sites in response to ligand binding leads to the recruitment of a network of cytoskeletal proteins and intracellular signalling complexes (Arnaout et al. 2007). The role of integrins in implantation has been widely reviewed (Aplin, 1997, 2006; Lessey, 1998, 2000, 2002; Lessey et al. 2000; Aplin & Kimber, 2004; Kimber, 2008) so key points are here summarized briefly.

β_1 Integrins

Integrins $\alpha_2\beta_1$ and $\alpha_3\beta_1$ are constitutively expressed during the menstrual cycle, and, as receptors for collagen and other components of interstitial ECM, may be used in re-epithelialization after menstruation. Integrins $\alpha_1\beta_1$ and $\alpha_4\beta_1$ are expressed on days 20–24 of the cycle. Subunit localization data suggests that integrins are present, albeit in varying amounts, at apical, basal and lateral epithelial surfaces. However, β_1 often shows a pronounced lateral distribution (Quenby et al. 2007).

Mouse blastocysts lacking the integrin β_1 subunit fail to implant, apparently because of an inability to adhere to or invade the subepithelial stroma (Brakebusch et al. 1997). Antibody inhibition experiments suggest a role for integrin $\alpha_4\beta_1$ in mouse implantation (Basak et al. 2002).

α_v Integrins

α_v Integrins bind ECM or secreted proteins containing an arginine-glycine-aspartic acid (RGD) peptide motif. Mid-secretory phase increase in endometrial epithelial $\alpha_v\beta_3$ results from an increase in β_3 abundance after day 19 (Lessey, 2002). This is an indirect effect of steroids mediated by the transcription factor HoxA10 (Daftary et al. 2002). Aberrant $\alpha_v\beta_3$ integrin has been associated with unexplained infertility and other endometrial pathologies (Lessey et al. 1992, 1994, 1995, 1996; Apparao et al. 2002; Tei et al. 2003). Up-regulation of β_3 subunit by the blastocyst has been demonstrated in cocultured human endometrial epithelial cells, an effect possibly mediated by the embryonic IL-1 system (Simon et al. 1997). Integrins $\alpha_v\beta_5$ and $\alpha_v\beta_6$ are also present in endometrium (Aplin et al. 1996). Integrins of this family are expressed by trophectoderm at

the time of implantation (Campbell et al. 1995b; Bloor et al. 2002). Blocking $\alpha_v\beta_3$ interactions in mouse or rabbit models impairs implantation (Illera et al. 2000; Illera et al. 2003). However, mice null for α_v are fertile.

Osteopontin and other integrin ligands

It may be that integrins present on both the trophoblast and uterine epithelial surface bind to a bifunctional extracellular bridging ligand to mediate embryonic adhesion. Osteopontin (OPN; also known as SPP1) is a secreted glycoprotein that contains an RGD motif and is capable of multimerization (Johnson et al. 2003). It is regulated by progesterone and reaches a maximum in the secretory phase of the cycle (Apparao et al. 2001; Quenby et al. 2007) when it immunolocalizes predominantly to apical LE and GE cells. In pregnant ewes osteopontin mRNA in glands is progesterone-dependent and the resultant protein is secreted between day 11 and 17, a period that corresponds to the adherence and attachment phase of early implantation in this species (Johnson et al. 2000; Spencer et al. 2004). Indeed, OPN localizes precisely to the interface between trophectoderm and luminal epithelium (Johnson et al. 1999). mRNA encoding osteopontin is also up-regulated by progesterone in human trophoblasts (Omigbodun et al. 1997). OPN has multiple binding partners including integrins $\alpha_4\beta_1$, $\alpha_v\beta_3$, $\alpha_v\beta_5$, $\alpha_v\beta_6$ and specific variants of CD44 (v3, v6). The increase in its expression matches increased expression of integrin $\alpha_v\beta_3$ in luminal epithelium during the mid-secretory phase in humans. Thus, complexes of OPN with receptors including $\alpha_v\beta_3$ and CD44 (see below) may occur at the apical surface of luminal epithelium to promote embryo attachment. OPN is also expressed in mouse endometrium (White et al. 2006). However, OPN-null mice are fertile.

Other integrin ligands are also present. Fibronectin has been described in association with the zona pellucida of human embryos (Turpeenniemi-Hujanen et al. 1995). The heparan sulphate proteoglycan (HSPG) perlecan is present on the outer surface of the mouse blastocyst (Carson et al. 1993) and its core protein can act as a ligand for $\alpha_v\beta_3$ integrin. Thrombospondin is a ligand for $\alpha_v\beta_3$ and is expressed by trophectoderm as well as by glandular epithelium and decidua (O'Shea et al. 1990).

Integrin $\alpha_6\beta_4$

Integrin $\alpha_6\beta_4$ is basally expressed in endometrial epithelium (Murray et al. 1999). It acts as a receptor for laminins, mediating cell adhesion to the basement membrane and regulating cell polarity. It can also be involved in migration over basement membranes. Integrin $\alpha_6\beta_4$ occupancy can amplify intracellular signalling from erbB2 and confer resistance to apoptotic stimuli (Guo et al. 2006; Friedland et al. 2007).

CD9

A member of the transmembrane-4 superfamily, CD9 is a receptor for pregnancy-specific glycoproteins (PSG) produced by trophoblast (Park et al. 2000; Wynne et al. 2006) and associates with integrins α_6, α_3 and β_1 in endometrial epithelial cells (Hirano et al. 1999; Park et al. 2000). It is predominantly distributed in lateral membranes of luminal epithelial cells as well as being expressed in blastocysts. Functional analysis using blocking antibodies to CD9 in an *in vitro* mouse embryo attachment model demonstrated no effect on attachment rate, but stimulated trophoblast outgrowth. In mice, an increase in implantation sites was observed after antibodies to CD9 were injected on day 4 of pregnancy (Liu et al. 2006). Antibody to CD9 increases migration of BeWo choriocarcinoma cells, perhaps suggesting that the integrin association is function-inhibiting (Hirano et al. 1999). Embryos without CD9 implant normally in CD9-null mothers (Wynne et al. 2006) in agreement with the hypothesis that CD9 is an inhibitor or regulator of implantation.

Carbohydrate-binding receptors and implantation

A rich diversity of sugar structures in the secretory phase glycocalyx and in secretory material has been demonstrated using lectin- and immuno-histochemistry (Aplin, 1991; Hey et al. 1994; Jones et al. 1998). Some structures are hormonally regulated in a species-specific fashion (Jones et al. 1998). A cell surface glycan code has been postulated to play a role in regulating trophectoderm-epithelial attachment (Jones & Aplin, 2008), which in species with epitheliochorial placentation is much longer-lived than is the case in human (Jones & Aplin, 2004). Carbohydrate-binding proteins at the epithelial-trophoblast interface could provide one way of mediating such an interaction.

CD44

CD44 is a single-pass transmembrane glycoprotein and shows a complex pattern of alternative splicing with several isoforms observed in endometrium (Behzad et al. 1994; Horne et al. 2002). It is associated with cell migration and has numerous binding partners including hyaluronate and osteopontin (Behzad et al. 1994; Poncelet et al. 2002; Cichy & Pure, 2003).

In endometrium, CD44 shows a predominantly lateral distribution in both glandular and luminal epithelium (Behzad et al. 1994). Its expression on lateral membrane is increased in the secretory phase (Albers et al. 1995) with maximum levels during mid-secretory phase (Afify et al. 2006). In addition to the variability caused by differential splicing of CD44, glycosylation introduces a degree of structural polymorphism (Brown et al. 1991). The epithelial-

specific sequences have the characteristics of mucin-like domains, being rich in serine, threonine and other hydrophilic amino acids, offering a high potential for glycosylation. Specific extracellular domain splice variants are associated with metastatic spread of carcinoma cells, suggesting a role in epithelial cell adhesion or migration. Thus it is possible that CD44 plays a role in the integrity of the epithelial sheet (Cichy & Pure, 2003). CD44 is also expressed in pre-implantation human embryos (Campbell et al. 1995a). CD44-null mice are fertile.

Galectins

Galectins are a family of calcium-independent β-galactoside-binding proteins that belong to the lectin superfamily. Several functions have been described for galectins: immunomodulation, cell adhesion and chemotaxis (regulation of endometrial leukocytes), cell surface receptor residency and scaling of growth factor responses, and defence against invading microorganisms. Galectins do not possess a signal peptide or transmembrane spanning domain, and are secreted from cells by a nonclassical pathway. They probably act by cross-linking carbohydrate chains on the cell surface and/or the ECM (Lau et al. 2007; Rabinovich et al. 2007).

Cycle-dependent expression of galectin-1 (LGALS1) is observed in human endometrial stromal cells and of galectin-3 (LGALS3) in epithelial cells (von Wolff et al. 2005; Aplin & Singh, 2008). Galectin-1 is thought to have both adhesive and anti-adhesive roles and is an immunomodulator with functions in maternofetal tolerance (Blois et al. 2007). Expression of transcripts in the trophectoderm of late blastocysts indicates that it may play a role in embryo implantation. However, galectin-1-null mouse embryos develop normally and do not produce any overt phenotypic abnormalities (Poirier & Robertson, 1993).

Galectin-3 (LGALS3) is a galactose-specific lectin, expression of which increases significantly during the secretory phase of the menstrual cycle (von Wolff et al. 2005). It can modulate cell adhesion by binding to ligands including laminin, fibronectin and integrins after its secretion from epithelial cells. Mice lacking both galectins-1 and -3 implant normally but a further family member, galectin-5, is also present and may compensate (Colnot et al. 1998).

Galectins bind to both H-type I and type II sugar chains (Leffler & Barondes, 1986) and lacto-N-fuco-pentaose I (LNF-1), which is a potential ligand for both galectins-1 and -3 for which there is evidence for a role in mouse implantation (Lindenberg et al. 1988). MUC1 bears the Thomsen–Friedenreich disaccharide, binding of which to galectin-3 causes redistribution of MUC1 on the cell surface and promotion of cancer cell adhesion to endothelium by revealing epithelial adhesion molecules such as E-selectin and CD44H that are otherwise concealed by MUC1 (Yu et al. 2007).

Galectin-9, which has two non-identical carbohydrate-recognition domains (Popovici et al. 2005), has been identified in mid- and late-secretory and decidual phases in human endometrium, with expression in glandular and luminal epithelial but not stromal or immune cells (Smalley & Ley, 2005). Expression of galectin-9 on uterodomes suggests that galectin-9 may play a role during the initial events of human embryo implantation (Shimizu et al. 2008). Galectin 15 (OVGAL 11) is induced by progesterone and secreted from the endometrial luminal epithelium in sheep, where it has a prospective role in trophectoderm attachment (Farmer et al. 2008).

Selectins

Selectins are calcium-dependent cell adhesion molecules that contain a large, highly glycosylated extracellular domain, a single transmembrane domain and a small intracellular cytoplasmic tail (Barthel et al. 2007). Selectins bind heterotypically to fucosylated and sialylated glycoproteins such as sialyl lewis^x (sLe^x) and sialyl lewis^a (sLe^a). There is evidence for L-selectin expression on the blastocyst (Genbacev et al. 2003) and the physiological importance of an interaction with oligosaccharide ligand on the maternal surface has been suggested. L-selectin ligands (including sialyl lewis^x associated with MUC1) are immunolocalized to the luminal and glandular epithelium, with increased expression during the window of implantation (Hey & Aplin, 1996; Red-Horse et al. 2004; Lai et al. 2005). Elevated L-selectin ligand has been associated with improved implantation (Wang et al. 2008). Suppression of the fucosyltransferase FUT7, which catalyzes the synthesis of sLe^X, both reduces sLe^X and decreases implantation in an *in vitro* model (Liu et al. 2008b). Conversely, implantation can be increased by FUT7 overexpression (Zhang et al. 2008). However, in homozygotic mutant mice null for each of the three selectins, and in mice lacking two or all three selectins, embryonic development, implantation and pregnancy appear normal (Bullard et al. 1996; Robinson et al. 1999; Collins et al. 2001).

Identification of molecular mediators of implantation

Advances in knowledge of the key attributes that confer receptive status to endometrium suggest a cascade of adhesive interactions beginning with carbohydrate-mediated binding to the glycocalyx and progressing to tighter binding involving OPN, members of the IgSF, integrin and cadherin families, trophinin and CD44, each of which involves a set of accessory molecules both within the plasma membrane (basigin, CD9; growth factor receptors including Wnt, EGFR and Erb4) and in association with molecules at its cytoplasmic face (tastin, bystin, catenins). Activation of proteases including MMPs and ADAMs may well be important in

these molecular assemblies. Components of the lateral epithelial membrane including desmosomes detach and reassemble as trophectoderm extends between maternal epithelial cells.

Uterine epithelial cells in the receptive state possess cytoplasmic/membrane architecture of a modified type with reduced apico-basal polarity and an apical cell pole that is equipped with appropriate sets of adhesion molecules. Perturbations in epithelial polarization that occur at implantation may allow molecules which are otherwise involved in epithelial cohesion to play a transient role in embryonic attachment.

Thus an increasing body of data suggests that trophoblast binding initiates a cascade of signalling events in epithelial cells to mediate progression to interstitial implantation. Definitive functional information regarding the adhesion molecules that determine uterine receptivity to implantation has immediate translational application to the improvement of pregnancy rates in IVF and the possibility of a contraceptive method that targets the endometrium. Novel *in vivo* approaches, including modifying embryo culture, improved embryo selection, and intrauterine release of adhesion-promoting factors, are possible approaches to increase implantation rates and diminish embryo wastage.

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