Adhesion Molecules in Human Trophoblast - A Review. II. Extravillous Trophoblast

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Adhesion molecules in human trophoblast – A review

II Extravillous trophoblast

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1. Introduction

Migratory trophoblast undergoes a radical alteration in its repertoire of adhesion molecules as it escapes from the villous placenta and colonises maternal decidua (Figure 1a), spiral arteries and myometrium. Following the discovery that the expression of adhesion molecules by extravillous trophoblast (EVT) is dysregulated in pathological pregnancy, interest in this area of research has intensified. Nonetheless, data from functional studies are scarce, reflecting the limitations of current *in vitro* culture models. In the following review we summarise current knowledge of adhesion systems in extravillous trophoblast. Further information on the cell biology of placental development and trophoblast invasion can be found elsewhere [1, 2].

2. Integrins

A plethora of studies carried out over the past twenty years has documented the expression of integrin subunits in villous and extravillous trophoblast, and described the integrin switch that characterizes acquisition of an invasive phenotype, as trophoblasts differentiate, detach and invade the decidua (Figure 1a). Cells in the columns upregulate the fibronectin receptor integrin $\alpha 5 \beta 1$ [3-11], the fibronectin, VCAM-1 and EMILIN-1 receptor $\alpha 4 \beta 1$ [12], $\alpha V \beta 3$ [12], which binds fibronectin and other RGD-containing ligands in extracellular matrix (ECM), the laminin/collagen receptor $\alpha 1 \beta 1$ and the laminin receptor $\alpha 6 \beta 1$. They exhibit a reciprocal loss of expression of the laminin receptor $\alpha 6 \beta 4$ [4, 6, 13-16].

Functional studies examining the role of integrins in mediating invasion of primary cytotrophoblasts (CTB) through a basement membrane-like matrix (Matrigel) and interstitial collagen have demonstrated the importance of $\alpha V \beta 3$ and $\alpha 1 \beta 1$ in promoting migration and of $\alpha 5 \beta 1$ in anchorage [5, 6, 8, 12]. Function-blocking antibodies raised against $\beta 1$ disturb anchorage and reduce column outgrowth when applied to villous explants, and shift the balance of interactions away from cell-matrix and towards cell-cell adhesion [8]. Antibodies against laminin, collagen IV or integrin $\alpha 1$ inhibit invasion *in vitro*, implicating a role for the

$\alpha 1\beta 1$ - laminin or $\alpha 1\beta 1$ -collagen IV interactions. Conditions of reduced trophoblast invasion
45 have also been observed when $\alpha 1\beta 1$ expression is either maintained (but combined with an
altered protease profile, as *in vitro* after $TNF\alpha$ treatment [13]) or reduced (as *in vitro* under
hypoxia [14]).

Initially, within and nearby the cell columns, CTB interacts with a type of provisional
50 ECM known as matrix-type fibrinoid (Figure 2a, b) that contains fibronectin [17], the ligand
for integrin $\alpha 5\beta 1$, and vitronectin, which ligates $\alpha v\beta 3$, as well as the basement membrane
ligands laminin and collagen IV which bind $\alpha 1\beta 1$. Fibrinoid accumulates between the cells
and at the periphery of columns and probably anchors the columns to the maternal surface
[18]. Decidual ECM is also enriched in laminins, collagen IV [19, 20] and fibronectin [19,
55 21]. Interaction of CTB with the stromal and vascular wall fibrillar component EMILIN-1
occurs by binding to integrin $\alpha 4\beta 1$, leading to haptotactic migration and upregulation of
matrix metalloproteinase (MMP) activity [22].

The effect of hypoxia on integrin expression in EVT has received attention as a
potential mediator of altered depth of trophoblast invasion in preeclampsia (see below). EVT
60 outgrowths from first trimester placental explants cultured on ECM substrates at 2%, 3%,
8% or 20% oxygen [8, 23, 24] express integrin $\alpha 5\beta 1$, indicating that an EVT differentiation
programme is initiated as cells contact the ECM. In some studies, integrin $\alpha 1$ upregulation
has been reported not to occur at 2% oxygen, correlating with a lower migratory activity in
Matrigel [14, 25]. It has been suggested that this effect on integrin $\alpha 1$ is controlled by HIF1 α
65 [24]. These data suggest that hypoxia permits initiation, but not completion of the normal
integrin switching program.

Decreased integrin $\alpha 1$ expression and reduced invasion is also noted when vascular
endothelial growth factor (VEGF), signaling through VEGF receptor-1 (VEGF-R1) or VEGFR-3,
is inhibited in primary first trimester CTB by a VEGFR-Fc fusion protein [26]. As VEGFR-1
70 and R-3 are highly expressed in the distal cell column and in interstitial EVT during the first

and second trimesters, with R-3 expression also prominent in endovascular trophoblast [26], it is conceivable that trophoblast-derived VEGF may promote invasion in an autocrine manner by enhancing $\alpha 1$ -mediated migration.

Insulin-like growth factors stimulate trophoblast migration [27], and the insulin-like growth factor binding protein-1 (IGFBP-1), a secretory product of decidual cells, may independently modulate trophoblast invasion in the placental bed. IGFBP-1 contains an RGD sequence motif that binds $\alpha 5\beta 1$ and disrupts trophoblast binding to fibronectin, resulting in enhanced migration in vitro [15]. CD9, a tetraspanin glycoprotein whose expression in the placenta is associated with $\alpha 3$ and $\alpha 5$ integrins, may also mediate trophoblast adhesion [28]. CD9 is absent from villous CTB, but is upregulated in cell columns and is highly expressed by EVT.

The presence of fibronectin molecules bearing a unique glycopeptide domain (termed oncofetal fibronectin or onfFN) within the type III connecting segment, has been noted within the placental bed. Immunohistochemical analysis has revealed the presence of onfFN in the extracellular matrix that connects extravillous anchoring trophoblasts and trophoblast cell columns to the uterine wall [21], and cell culture studies have confirmed that EVT produce and secrete onfFN *in vitro* [8, 21, 29]. However, not all cells in EVT subpopulations produce onfFN to the same extent. A subset of invasive EVT expressing $\alpha 6$ integrin and HLA-G produce high levels of gelatinases but secrete little onfFN, whereas EVT cells that express $\alpha 5$ integrin secrete low levels of gelatinases, produce large amounts of onfFN and show reduced invasion [30]. This suggests that alterations in invasive phenotype that lead to enhanced onfFN production may result in autocrine regulation of trophoblast adhesion. Transforming growth factor- $\beta 1$ (TGF- $\beta 1$) has been shown to stimulate onfFN production by trophoblast [29], suggesting that local gradients of exogenous cytokines within the placental bed could modulate trophoblast adhesion in defined locations. Further discussion can be found in a recent review [31].

$\alpha v\beta 3/\beta 5$ and $\beta 1$ integrins mediate adhesion of human CTB to endothelial cells *in vitro*, suggesting that they may facilitate endovascular trophoblast adhesion and migration within uterine arteries [32]. CTB expressing $\alpha 4$ integrin bind the vascular cell adhesion molecule VCAM-1, indicating that $\alpha 4\beta 1$ may mediate CTB-endothelium or CTB-CTB interactions during endovascular invasion [12]. Macaque CTB similarly express $\alpha v\beta 3$ and $\beta 1$ integrins, and upregulate $\beta 1$ in response to shear stress or co-culture with uterine microvascular endothelial cells [16, 33, 34]. Migration of macaque CTB toward vitronectin, and adhesion of CTB to myometrial endothelial cells in co-culture is mediated by integrin $\alpha v\beta 3/\beta 5$.

The serious pregnancy complication of pre-eclampsia is characterised by shallow interstitial EVT invasion, reduced endovascular invasion and an absence of remodelling in myometrial artery segments. Decreased invasion has been attributed to the failure of differentiating CTB to adopt an invasive phenotype: trophoblasts emigrating from cell columns show persistence of integrin $\alpha 6\beta 4$ expression and fail to upregulate $\alpha v\beta 3$ and $\alpha 1\beta 1$ [7, 35, 36]. These findings suggest that pre-eclampsia is associated with failure or delay of a CTB differentiation programme, with retention of cell adhesion molecules that under normal conditions are only expressed by villous progenitor cells and CTB in the proximal region of the cell column. However, another study has reported that expression of $\beta 1$ integrins in trophoblast is similar in healthy women and women with pre-eclampsia or pre-term labour [37]. Some heterogeneity in the expression of different integrin α subunits was noted, with variation being observed in each study group. Simultaneous expression of $\alpha 1$, $\alpha 3$, $\alpha 5$ and $\alpha 6$ integrins may be important for migration of EVT[38]. Atypical integrin expression has also been described in ectopic pregnancy: EVT have been reported to display prominent expression of $\alpha 6$ and $\beta 4$ subunits, despite exhibiting invasive behaviour [38].

3. Immunoglobulin family cell adhesion molecules (CAMs)

Neural cell adhesion molecule (NCAM; CD56) is a cell surface glycoprotein that mediates homophilic binding. Human endovascular trophoblasts express a unique polysialylated form of NCAM which may moderate the strength of trophoblast interactions [39]. NCAM expression has been observed in trophoblast plugs within the spiral arteries but is absent from villous CTB, syncytiotrophoblast (STB) and cell columns. A similar distribution of NCAM has been noted in the macaque: endovascular and perivascular trophoblast stain for NCAM, but intramural trophoblast exhibit little immunoreactivity [40]. These findings suggest that NCAM is necessary for endovascular invasion, aiding binding of trophoblast to the arterial endothelium or to vascular extracellular matrix components.

Platelet-endothelial cell adhesion molecule-1 (PECAM-1; CD31)[41] is an immunoglobulin superfamily member that acts as both a heterophilic and homophilic adhesion molecule and may mediate the attachment of trophoblast to the vascular endothelium. Its proposed ligands include integrin $\alpha v \beta 3$ [41]. Like NCAM, PECAM-1 expression has been described in human perivascular and endovascular trophoblasts [12, 42, 43], but also in interstitial EVT and in CTB of the distal column [36]. However, these findings have been disputed by other groups, who observed PECAM-1 expression exclusively in spiral artery endothelium throughout gestation and not in villous or extravillous trophoblast [44, 45]. It has been reported that PECAM-1 upregulation by endovascular trophoblast fails in pre-eclampsia [12], although other studies have shown no difference in its expression between normotensive and pre-eclamptic pregnancies [44, 45]. Interestingly, PECAM-1 is expressed by endovascular trophoblasts in healthy rats as well as in a rat model of pre-eclampsia [46], and PECAM-1 expression is upregulated at points of contact between CTB and endothelial cells *in vitro* [42, 43]. PECAM-1 is also expressed in endovascular trophoblasts and vascular endothelial cells of the macaque, although expression is often reduced in EVT distal to the arterial lumen. The spiral artery endothelium is observed to retain PECAM-1 reactivity even after vessel remodelling [47]. Since PECAM-1 plays a role in

transendothelial migration of leukocytes [41], it is possible that it may function in trophoblast invasion, as cells cross either from the vessel lumen to the wall, or in the reverse direction.

Melanoma cell adhesion molecule (Mel-CAM; MUC18; CD164) is an adhesion molecule that mediates heterophilic binding to an unidentified ligand and has been shown to be important in tumor progression. Mel-CAM is expressed by the majority of EVT, is present on endothelial and myometrial cells but is absent from villous CTB and STB [48, 49]. It is present on invasive trophoblast in the mouse, where antibody-mediated blockade leads to pregnancy failure [50]. Cell culture studies have demonstrated the presence of a putative Mel-CAM ligand on uterine smooth muscle cells and have shown that disruption of Mel-CAM binding promotes migration of an EVT cell line [51].

Intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1) are other members of the immunoglobulin superfamily that mediate adhesion of leukocytes to the vascular endothelium. ICAM-1 is expressed by vascular endothelial cells throughout the decidua; however, expression of VCAM-1 is variable at the implantation site and it is only seen in vascular endothelial cells in the decidua basalis [39]. VCAM-1 is expressed by CTB in the distal portion of the cell column, and by both interstitial and endovascular EVT [36]. In common with the failure to alter their integrin repertoire, invasive CTB from pregnancies complicated with pre-eclampsia do not upregulate VCAM-1 expression.

There has been some speculation that ICAM-1, VCAM-1 and PECAM-1 may mediate adhesion of endovascular trophoblasts to the walls of spiral arteries. Studies of early gestation macaque trophoblast have shown that ICAM-1-blocking antibodies reduce transendothelial migration *in vitro* [52]. The same study demonstrated that antibodies against the mucin MUC-1, which is expressed by macaque endovascular EVT, reduced adhesion of isolated trophoblast to uterine endothelial cells and inhibited trophoblast transendothelial migration. These data predict that both ICAM-1 and MUC-1 may be

mediators of mural invasion in human pregnancy. It should be noted that MUC-1 antibodies mark a subset of human interstitial EVTs, suggesting that the EVT population contains cells that vary in their potential to colonise vessels [53].

4. Selectins

Selectins are a family of single-chain transmembrane glycoproteins that bind oligosaccharides and permit transient tethering and shear stress-dependent rolling. Both E- and P-selectin are expressed by human vascular endothelial cells in the decidua basalis, but are absent from the vessels of the decidua parietalis [39]. Thus they may be candidates for mediating endovascular trophoblast invasion/migration. Indeed, adhesion of isolated CTB to endothelial cells *in vitro* is partially blocked by antibodies to P-selectin [32]. In contrast, L-selectin is expressed by CTB in columns, with high expression noted at the distal ends [54-56]. L-selectin is also strongly expressed by EVT and may be important for establishing and maintaining column architecture, based on the observation that function-perturbing antibodies inhibit column formation *in vitro* [55].

5. Cadherins

VE-cadherin (VE-cad), a marker of the endothelial cell lineage, is absent from villous CTB but is expressed by trophoblast cell columns, interstitial EVT and spiral artery endothelial cells [12, 36, 38]. Following loss of the vascular endothelium during arterial remodelling, VE-cad expression is further upregulated in endovascular and mural CTB, reflecting their acquisition of certain features of the endothelial phenotype [12, 42]. Antibodies to VE-cad reduce CTB adhesion, migration and invasion across Matrigel barriers *in vitro*, suggesting a role in interaction with extracellular matrix [12, 42]. VE-cad expression is also required for first trimester trophoblast cells to bind to decidual endothelial cells *in vitro* [36], and failure of invasive CTB to upregulate VE-cad expression is noted in pre-eclampsia [12, 36, 57].

In contrast to VE-cad, E-cadherin (E-cad) expression is observed throughout
205 anchoring villi, with cells detaching from the distal ends of columns and individual interstitial
EVT exhibiting reduced, discontinuous expression [57]. When present in aggregates,
interstitial, intraluminal, perivascular and endovascular EVT express E-cad, although
discontinuous membrane expression is observed in lone cells [58]. Isolated CTB cultured on
basement membrane-like ECM express E-cad, and anti-E-cad antibodies enhance CTB
210 invasion [12], suggesting a role in CTB-CTB attachment. Invasive CTB in pre-eclamptic
placentas have been shown to retain expression of E-cad [35].

Cadherin-11 (Cad-11) expression has been documented in EVT where it is located at
the tips of cell columns [59]. Although little work has been done to elucidate its role, Cad-
11 may be important in promoting adhesion of trophoblast to maternal stromal cells, where
215 it is upregulated during decidualization [60]. Similarly, expression of dysadherin, a cell
surface glycoprotein thought to reduce cell-cell adhesion and increase motility, is increased
in CTB developing into columns [61, 62]. Dysadherin is believed to promote cell migration
via effects on the actin cytoskeleton and downregulation of E-cadherin expression [57, 58].

220 **6. Kisspeptins**

Kisspeptin, the 145 residue polypeptide product of the KiSS-1 gene, is proteolytically
processed to generate smaller peptides containing 54 (Kp-54; metastin), 14 (Kp-14), 13
(Kp-13) or 10 (Kp-10) amino acids. Kisspeptins are endogenous ligands for the G protein-
coupled KiSS-1 receptor (KiSS-1R) and both kisspeptin and KiSS-1R are highly expressed
225 during the first trimester of pregnancy. KiSS-1/Kp-54 protein expression is restricted to the
STB, whereas KiSS-1R is expressed by the STB and by villous and extravillous CTB [63-66].
In addition, Kp-54, Kp-14, Kp-13 and Kp-10 are produced by first trimester trophoblast *in*
vitro, although only Kp-10 has been shown to mediate significant physiological effects. Kp-
10 reduced CTB outgrowth from villous explant culture and inhibited migration and MMP-2
230 expression in isolated human first trimester trophoblasts. As KiSS-1R is expressed on both

villous and extravillous CTB, secretion of kisspeptins by the syncytium may allow paracrine regulation of invasion and column formation.

7. Ephrins

235 The receptor tyrosine kinase EPHB4 and its ligands ephrin-B1 and ephrin-B2 may play a role in trophoblast adhesion. In early gestation, ephrin-B1 is expressed by interstitial and endovascular EVT, and ephrin-B2 expression is observed in both villous CTB and EVT [61, 62]. Studies of EVT differentiation have revealed that acquisition of an invasive phenotype is associated with a rapid decrease in EPHB4 expression and enhanced expression of ephrin-
240 B1 and -B2. Indeed, during differentiation of villous CTB at the proximal region of the cell column, EPHB4 expression is downregulated in favour of enhanced expression of ephrin B1. As substrates containing an EPHB4-Fc fusion protein decrease adhesion and reduce migration of CTB *in vitro*, and EPHB4 is expressed by the uterine venous endothelium, this may explain why EVT do not colonize the uterine veins. Conversely, ephrin B2-Fc fusion
245 proteins support CTB migration *in vitro*, thus expression of ephrin-B2 by the spiral artery endothelium may promote preferential arterial remodelling.

8. Junctional proteins

Within the anchoring column, CTB are linked by desmosomes which are associated with
250 extensive arrays of cytoplasmic intermediate filaments (Figure 1b) [67, 68]. The tight junctional proteins occludin and zona occludens-1 (ZO-1) are also present, but it is not yet clear what functional contribution they may make [69]. Sometimes arrays of fine submembranous filaments are visible at cell interfaces (Figure 1c). Emigration of CTB from the column (Figure 1a) is concomitant with fracture and loss of desmosomes (Figure 2a) and
255 loss of ZO-1 and occludin [69, 70]. Similarly, connexin 40-containing gap junctions (Figure 2b) are decreased during differentiation of CTB into a more invasive phenotype [66]. Downregulation of connexin 40, or experimental inhibition of gap junction function, are both

associated with differentiation and an increase in expression of $\alpha 1$ integrin [70, 71]. Furthermore, factors secreted by decidua decrease connexin 40 in placental explants and trophoblast cell lines, resulting in enhanced migration and invasion [71].

9. Conclusion

Key transition points in trophoblast differentiation and invasion depend very obviously on alterations in intercellular adhesion: detachment of precursor EVT from the basement membrane, detachment of migratory EVT from distal cell columns, *de novo* adhesion to the uterine extracellular matrix, and migration into maternal vascular and interstitial tissue compartments require acquisition of motility and significant alterations in the adhesion molecule repertoire. Adhesion molecules upregulated during differentiation of CTB in the cell column include VCAM-1, PECAM-1, L-selectin, VE-cad, CAD-11, CD9, ephrin-B1 and -B2, and the integrins $\alpha 5\beta 1$, $\alpha 4\beta 1$, $\alpha 1\beta 1$ and $\alpha 6\beta 1$. A reciprocal decrease in the expression of Ecad, EphB4, desmosomes, Cx40 gap junctions and integrin $\alpha 6\beta 4$ is also observed. Endovascular and interstitial trophoblast maintain expression of VE-cad, PECAM-1, ephrin-B1 and -B2, and upregulate Mel-CAM; endovascular EVT also express NCAM.

Cell contact, and the signalling events that follow downstream, play a profound role in the control of proliferation, death, migration and invasion in the uterine environment. A better understanding of trophoblast migration and the regulation of the molecular switches that make it possible will be critical in addressing pathologies of pregnancy including preeclampsia [72-74], preterm labour [75], late miscarriage [76], hypertension, [77] and IUGR [74, 78], in which trophoblast migration and maternal vascular remodelling are impaired.

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Figure legends

515 **Figure 1:** a) Anchoring villus showing CTB forming a column from the distal edge of which
cytokeratin-positive cells detach to infiltrate the decidua (wax embedded first
trimester decidua, 17 weeks gestation, stained with an anti-pan cytokeratin
antibody); b) CTB cells in columns within the anchoring villus have arrays of
intermediate filaments (IF) associated with desmosomes (D) and elsewhere; c) Fine
520 submembranous filaments (F) can be seen in some areas; here they are prominent at
a cyto-syncytial interface in an anchoring villus.

Figure 2: Second trimester placenta, 18 weeks gestation. a) A cytotrophoblast (arrow) can
be seen apparently separated from the anchoring villus (though may be attached
525 outside the plane of section), with possible remnants of desmosomes (short white
arrows) visible on cell membranes. b) Dark fibrinoid extracellular matrix is present
between migrating cells and some gap junctions (arrows) can be seen between
adjoining cytotrophoblasts.

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