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Extravillous trophoblast and decidual natural killer cells: a remodelling partnership

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BACKGROUND: During pregnancy, maternal uterine spiral arteries (SAs) are remodelled from minimal-flow, high-resistance vessels into larger diameter vessels with low resistance and high flow. Fetal extravillous trophoblasts (EVT) have important roles in this process. Decidual natural killer cells (dNK cells) are the major maternal immune component of the decidua and accumulate around SAs before trophoblast invasion. A role for dNK cells in vessel remodelling is beginning to be elucidated. This review examines the overlapping and dissimilar mechanisms used by EVT and dNK cells in this process and how this may mirror another example of tissue remodelling, namely cancer development.

METHODS: The published literature was searched using Pubmed focusing on EVT, dNK cells and SA remodelling. Additional papers discussing cancer development are also included.

RESULTS: Similarities exist between actions carried out by dNK cells and EVT. Both interact with vascular cells lining the SA, as well as with each other, to promote transformation of the SA. EVT differentiation has previously been likened to the epithelial–mesenchymal transition in cancer cells, and we discuss how dNK–EVT interactions at the maternal–fetal interface can also be compared with the roles of immune cells in cancer.

CONCLUSIONS: The combined role that dNK cells and EVT play in SA remodelling suggests that these interactions could be described as a partnership. The investigation of pregnancy as a multicellular system involving both fetal and maternal components, as well as comparisons to similar examples of tissue remodelling, will further identify the key mechanisms in SA remodelling that are required for a successful pregnancy.

Key words: extravillous trophoblast / decidual natural killer cells / spiral artery remodelling / pregnancy / placenta

Introduction

A successful pregnancy is dependent on successful placentation, and placental development is not only reliant on the fetal trophoblast but also the maternal cells present in the decidua. To support the demands of the growing fetus, decidual spiral arteries (SAs) must be transformed into wide diameter, non-vasoactive vessels capable of transporting nutritional and oxygen requirements to the fetus. Disruption of this complex process is proposed to lead to poor SA transformation, which is associated with pregnancy disorders, such as intrauterine growth restriction (IUGR) and pre-eclampsia (Brosens *et al.*, 1967; Pijnenborg *et al.*, 1991; Cartwright *et al.*, 2010). There have been extensive studies on SA remodelling, particularly with regard to the role of fetal trophoblast invading the decidua; however, recent interest has turned to the contribution of maternal cells within the decidua.

During placental development, trophoblast cells at the tips of the branched placental villi differentiate into extravillous trophoblast (EVT). These grow from the villi in columns to form the trophoblast shell, from where they invade into the decidua as far as the inner third of the myometrium, where they are known as interstitial EVT. Trophoblast also form plugs in the decidual SAs during the first trimester of pregnancy, which disappear by the second trimester. These plugs, made up of what are termed endovascular EVT, are thought to arise either from EVT migration retrograde to flow from the trophoblast shell inside the arterial lumen or intravasation from the decidua (Knofler, 2010; Pijnenborg *et al.*, 2011). Aspects of reciprocal signalling between maternal and fetal components of the decidua have been characterized in recent years (Dimitriadis *et al.*, 2010; Chazara *et al.*, 2011). However, dissecting the cell types in the maternal decidua which provoke responses in trophoblast, and vice versa to lead to SA remodelling, is challenging. A number of studies have contributed to current knowledge stating that both endovascular and interstitial EVT are important in mediating the loss of arterial smooth muscle and endothelial cells (Pijnenborg *et al.*, 2006; Harris, 2011), likely through a combination of apoptosis (Ashton *et al.*, 2005; Harris *et al.*, 2005; Keogh *et al.*, 2007; Hamzic *et al.*, 2008; James *et al.*, 2011), destruction of the extracellular matrix (ECM) and induction of vascular smooth muscle cell (VMC) de-differentiation (Harris, 2011). However, evidence indicates that initiation of remodelling occurs in the presence of leukocytes but before the appearance of EVT (Smith *et al.*, 2009; Hazan *et al.*, 2010). It has been postulated that decidual NK (dNK) cells, with their secretion of numerous cytokines, enzymes and other factors, may play a role in the initiation of SA remodelling, as well as interacting with EVT to aid completion of this process (Harris, 2010). This review aims to first examine the evidence that dNK cells and EVT play a role individually in the remodelling of SAs and draw parallels between SA remodelling and tissue remodelling in cancer. We will then discuss how the functions ascribed to both EVT and dNK cells may lead to the concept that these cells may work in a 'partnership' to promote SA remodelling; both by the same mechanisms to produce the same outcome and by interacting to regulate each other's behaviour.

Search method

This review was prepared by systematically searching the published literature using PubMed with the search terms extravillous trophoblast and natural killer cells, and focusing on the literature describing SA remodelling. No restrictions were placed on year published; however, only English language literature was included. Additional papers discussing cancer development were also included in this review.

Decidual NK cells

Uterine NK cells are present in the normal human endometrium and the decidua. They increase in number in the late secretory phase of the menstrual cycle and accumulate in the decidua before the appearance of fetal trophoblast and during early pregnancy (Bulmer and Lash, 2005). Throughout pregnancy, dNK cells have been detected in both the decidua basalis and parietalis where trophoblast are present and absent, respectively (Bulmer and Lash, 2005). By the end of the first trimester, dNK cells comprise 70% of the leukocytes in the uterine environment (Kopcow *et al.*, 2010) and these cells are presumed to be proliferative, owing to the expression of the cell proliferation marker Ki67, as well as large numbers of metaphase cells detected in the endometrium and early stage decidua (Pace *et al.*, 1989; Peel, 1989). The importance of dNK cells has been demonstrated in placentation of both mouse and human (Manaster and Mandelboim, 2010) and here we will focus on the characteristics of these cells pertinent to SA remodelling and interaction with trophoblast.

dNK cells in murine pregnancy

Many of the studies implicating dNK cells in SA remodelling have been carried out in the mouse. In the murine decidua, vessel transformation requires a widening of the vessel lumen, an increase in the vessel length and a decrease in the thickness of the muscular wall (Ashkar *et al.*, 2000; Zhang *et al.*, 2011). The importance of dNK cells in mice was first demonstrated in the Tge26 strain of mouse, which lacks NK cells. Mice deficient in NK and T cells displayed abnormally high vessel wall to vessel lumen ratios, hypertensive vascular changes, irregular decidual organization, small yet morphologically intact placentae and 50% fetal death by Day 10 of gestation (Guimond *et al.*, 1997). Engraftment of bone marrow from severe combined immunodeficient mice, which lack T and B-lymphocytes but not NK cells, restored the NK population in the Tge26 mice. This reduced decidual abnormalities, restored fetal viability and demonstrated that the decidual changes were NK associated (Guimond *et al.*, 1998). The importance of cytokine signalling in remodelling was also demonstrated in these studies as interleukin (IL)-12 p40 null mice also showed abnormal decidual vasculature (Croy *et al.*, 1997). IL-12, along with IL-18, has more recently been shown to be a crucial signalling molecule in the murine decidua inducing production of interferon (IFN)-γ by NK cells and subsequent decidual artery modification through IFN-γ (Zhang *et al.*, 2003).

IFN-γ signalling in the mouse is a key pathway inducing arterial modification during pregnancy. Peak IFN-γ expression is co-incident with the peak of NK cells in the mouse pregnant uterus (Ashkar and Croy, 1999). Its role was demonstrated using transgenic murine models, which lacked IFN-γ or its receptor, and these mice presented pregnancies with reduced modification of decidual arteries and necrotic decidua (Ashkar and Croy, 1999). Using models which are completely NK-cell deficient, Ashkar *et al.* (2000) engrafted bone marrow from mice possessing either NK cells unable to produce

IFN-γ, components of the IFN-γ signalling pathway or the IFN-γ receptor. These mice demonstrated different degrees of vessel modification and decidual disorganization, leading the authors to conclude that NK-derived IFN-γ was essential for normal pregnancy. This was further confirmed by the normal decidual morphology seen in NK-deficient mice treated with recombinant IFN-γ (Monk *et al.*, 2005). It has been suggested that IFN-γ may be able to promote cell adhesion, smooth muscle cell proliferation and caspase-dependent apoptosis (Boehm *et al.*, 1997; Boehm *et al.*, 1998; Murphy *et al.*, 2009). Whether NK cells have a similar role in humans regarding IFN-γ has not yet been elucidated.

dNK cells in human pregnancy

The evidence for a role of dNK cells in murine decidual artery modification has led to renewed interest in human dNK cells. In human pregnancy, the major subset of dNK cells expresses the surface markers $CD56^{bright}CD16^{-}CD160^{-}$, as opposed to peripheral blood (pb) NK cells, which are predominantly $CD56^{dim/-}CD16^{bright}CD160^{+}$ (King *et al.*, 1998, Searle *et al.*, 1999). CD56 is also known as neural cell adhesion molecule. Although similar in surface marker expression to the small population of $CD56^{bright}CD16^{-}$ pbNK cells, dNK cells display a large number of differentially expressed genes compared with both peripheral blood subsets and are a distinct subset of NK cells (Koopman *et al.*, 2003). Unique phenotypic differences of dNK include the surface expression of CD69, CD9, NKp44 and the absence of L-selectin (Koopman *et al.*, 2003; El Costa *et al.*, 2009). Other differences of dNK cells compared with pbNK cells include increased expression of the receptors NKG2C, NKG2E, NKG2A, KIR2DL4, CD31, CXCR3 (chemokine C-X-C motif) and CXCR4 (Hanna *et al.*, 2003; Koopman *et al.*, 2003; Tabiasco *et al.*, 2006; Manaster and Mandelboim, 2010).

Owing to their unique expression profile, dNK cells are generally considered to have a cytokine-secreting role rather than having the predominantly cytotoxic defence role of pbNK cells (Tabiasco *et al.*, 2006). Although dNK cells express cytotoxic proteins, including perforin, granzymes A and B, and granulysin and thus have cytolytic capacity, this cytotoxic machinery does not cause death of the invading trophoblast except potentially when responding to infection (Le Bouteiller *et al.*, 2011). The pattern of inhibitory and activating receptors expressed on the surface of dNK cells may contribute to the reduced cytotoxicity which they display. For example, engagement of the NKp46 receptor on pbNK but not dNK cells induces IFN-γ release (El Costa *et al.*, 2008) and the co-expression of the NKG2A inhibitory receptor on dNK blocks perforin polarization and target cell killing via NKp46 engagement (El Costa *et al.*, 2008; El Costa *et al.*, 2009). The ligand for NKG2A (HLA-E, expressed on trophoblast) may be responsible for this effect. An alternative reason for decreased cytotoxicity may involve signalling of the 2B4 inhibitory receptor (Vacca *et al.*, 2006; Manaster and Mandelboim, 2010).

The role of dNK cells in SA remodelling has been indicated by immunohistochemical studies of serial sections from staged samples of first trimester human placentation. It is accepted that trophoblasts are involved in SA modifications in human pregnancy; however, Craven *et al.* (1998) demonstrated that some initial changes occur prior to any cellular contact with the invading EVT. It was subsequently shown that the initial loss of VSMCs and breaks in the endothelial cell (EC) layer takes place in the presence of lymphocytes but in the absence of invading EVT (Smith *et al.*, 2009). These studies described a sequence of temporal stages of SA remodelling with classification into four distinct stages. In Stage I of this process, intact VSMCs and ECs were found with no indication of either leukocytes or EVTs in close proximity. During Stage II, there was evidence of disruption and partial loss of VSMCs, some breaks in the EC layer and extensive disorganization and separation of layers. Both dNK cells (identified by CD56 positivity) and macrophages (identified by CD68 positivity) were found to be infiltrating the vessel walls during this stage of actively remodelling vessels, but not trophoblast. This was also concurrent with a proportion of VSMC and EC undergoing apoptosis, detected by terminal deoxynucleotidyltransferase-mediated dUTP nick-end labelling. In Stage III, there was substantial loss of both VSMCs and ECs, and EVT were present by this stage, with some adhering to the vessel walls. Some apoptosis induction could be attributed to the invading EVT (Smith *et al.*, 2009). By Stage IV, the endothelium and VSMC were lost, fibrinoid deposition was present and endovascular EVT lined the vessel.

The histological study of Smith *et al.* (2009) therefore has demonstrated further evidence of trophoblast-independent SA remodelling. The direct influence of dNK cell-derived soluble factors on SA remodelling has also been demonstrated. For example, myometrial SAs (obtained from non-pregnant, premenopausal women undergoing hysterectomy) were cultured with dNK cell culture supernatant. These arteries showed evidence of disruption, with altered VSMC alignment and rounded nuclei compared with control arteries, where VSMCs were aligned in compact layers. A uterine priming phenomenon may therefore be occurring where dNK cells are able to generate factors that initiate destabilization of vascular structures and thus SA transformation, prior to EVT interaction. The factors secreted by dNK cells include chemokines and cytokines, such as IL-8, tumour necrosis factor (TNF)α, IFN-γ and leukaemia inhibitory factor (LIF; Saito *et al.*, 1993; Lash *et al.*, 2006). Decidual NK cells can also generate a number of vasoactive factors capable of stimulating VSMC destabilization and disorganization, including angiopoietin (Ang)-1, Ang-2, IFN-γ and vascular endothelial growth factor (VEGF)-C, whose corresponding receptors VEGF-R2 and Tie-2 are expressed by SAs (Lash *et al.*, 1999, 2006; Hu *et al.*, 2006; Kalkunte *et al.*, 2009; Schiessl *et al.*, 2009).

Circumstantial evidence from disease also suggests a role in the human for dNK cells in SA remodelling. Reduced numbers of dNK cells have been demonstrated in patients with pre-eclampsia and IUGR (Williams *et al.*, 2009), which are conditions associated with poor SA remodelling and reduced trophoblast invasion in the decidua (Stallmach *et al.*, 1999). However, the association of dNK cells with pre-eclampsia has not been consistently demonstrated (Bachmayer *et al.*, 2006). Uterine NK cells have also been associated with aberrant angiogenesis in recurrent spontaneous abortion (Quenby *et al.*, 2009).

The actions of dNK cells in promotion of successful placentation in normal pregnancy are therefore beginning to be elucidated. Decidual NK cells are not the only myeloid cell type in the decidua which has been implicated in placentation and vessel remodelling. Macrophages, T-lymphocytes and dendritic cells are also found within the maternal decidua. Macrophages comprise ~20% of the decidual leukocyte population, and have been associated with phagocytosis of apoptotic

cells (Abrahams *et al.*, 2004; Bulmer *et al.*, 2010), cytokine production (Li *et al.*, 2009; Svensson *et al.*, 2011) and regulation of trophoblast invasion (Renaud *et al.*, 2007). T cells make up ~10% of the decidual leukocyte population. The main function of T cells in the decidua (particularly CD4+ and regulatory T-cells) is thought to be the promotion of tolerance to the fetus (Guerin *et al.*, 2009; Piccinni, 2010). A predominantly Th2 phenotype was originally presumed to be beneficial to pregnancy; however, because a variety of different phenotypes of T cells are present in the decidua the complex interactions of this cell type have not been completely defined (Bulmer *et al.*, 2010). CD8+ T cells have been associated with cytokine production and regulation of trophoblast invasion (Scaife *et al.*, 2006). Natural killer T cells are also present and may act in a similar manner to dNK cells, although little is known at present (Piccinni, 2010). Finally, dendritic cells make up a small percentage of the decidual leukocyte population, being present at ~1–2% (Bulmer *et al.*, 2010). Dendritic cells are thought not to interact with trophoblast directly (Huang *et al.*, 2008) but may modulate responses of T and NK cells (Dietl *et al.*, 2006). They may also be important for decidualization, as studies in mice (Krey *et al.*, 2008; Plaks *et al.*, 2008) and some evidence in humans (Barrientos *et al.*, 2009) has demonstrated. Therefore, as for dNK cells, roles for these three cell types (macrophoges, dendritic cells and T-lymphocytes) in SA and decidual remodelling are being elucidated, although it is beyond the scope of this review to discuss these in great detail (Dietl *et al.*, 2006; Bulmer *et al.*, 2010; Hazan *et al.*, 2010; Blois *et al.*, 2011).

The extravillous trophoblast

The role of EVT in SA remodelling has been widely investigated (Harris, 2010; Whitley and Cartwright, 2010). Interstitial EVT, which are found surrounding the SAs within the decidua, are proposed to remodel the arteries by destroying the arterial media and mediating loss of the vascular smooth muscle outer layers, partly by inducing vascular cell apoptosis (Harris *et al.*, 2006; Keogh *et al.*, 2007). Endovascular trophoblasts, present in the lumen, temporarily replace the ECs constituting the inner layer of the SAs (Brosens *et al.*, 1967; Kam *et al.*, 1999).

EVT differentiation and invasion

The differentiation status of the trophoblast, from villous cytotrophoblast into EVT, involves a switch from a proliferative to an invasive, cytokine-secreting phenotype. Further differentiation into endovascular trophoblast expressing endothelial-like markers then occurs, and these differentiation stages are important in regulating how remodelling takes place (Zhou *et al.*, 1997). The control of the differentiation into EVT has been attributed to both an intrinsic programme (McMaster *et al.*, 1994) as well as the hypoxic environment provided by endovascular trophoblast plugs in the first trimester, which is detected in EVT by hypoxia-sensitive molecules, such as hypoxia inducible-factor (HIF) 1α and Id1 (James *et al.*, 2006). The differentiation into the invasive EVT phenotype is linked to changes in the expression of a number of genes, which can be broadly categorized as a loss of epithelial phenotype and the gain of an invasive, more mesenchymal phenotype. This phenotypic change includes increased production of cytokines, proteases and adhesion molecules, allowing the EVT to migrate and invade into the uterine environment, and interact with different decidual cell types (Knofler, 2010).

The phenotypic adhesion molecule change demonstrated by EVT has been termed 'integrin switching', and enables the expression of molecules which are typically expressed by the endothelium that they replace. This process also allows EVT to invade and interact with the ECM. These changes in gene expression include an up-regulation of $\alpha_1\beta_1$ and $\alpha_5\beta_1$ integrin (Damsky *et al.*, 1992), VE-(endothelial) cadherin, platelet endothelial adhesion molecule, vascular endothelial adhesion molecule, α_4-integrins and α_V-β_3 and a decrease in E-cadherin and connexin-40 (Zhou *et al.*, 1997; Wright *et al.*, 2006; Arimoto-Ishida *et al.*, 2009; James *et al.*, 2010). The importance of decidual secreted factors in controlling this differentiation has been demonstrated (Godbole *et al.*, 2011). For example, blocking epidermal growth factor receptor (EGFR/HER1) activation in an EVT cell line incubated with decidual cell-conditioned media prevented both differentiation mediated by EGF and heparin-binding EGF (HB-EGF; Wright *et al.*, 2010) and the differentiation-associated decrease in connexin-40 (Wright *et al.*, 2006) and transforming growth factor (TGF)-α signalling can also decrease the expression of α_1 and α_6--integrins (Leach *et al.*, 2004). Other decidual molecules which play an important role in the switch to a pro-invasive phenotype include insulin-like growth factor-binding protein-1, TGF-β (Irving and Lala, 1995) and IL-8 (Jovanovic *et al.*, 2010).

In addition to changes in the expression of adhesion molecules, EVT up-regulate a range of proteases to aid invasion through the ECM. Included in these are matrix metalloproteinase (MMP)-2 (Staun-Ram *et al.*, 2009), MMP-3 (Husslein *et al.*, 2009) and MMP-9 (LaMarca *et al.*, 2005; Naruse *et al.*, 2009a, b). Up-regulation of cathepsins (Varanou *et al.*, 2006) and urokinase plasminogen activator (uPA), which can activate several MMPs, has also been described (Liu *et al.*, 2003). In addition, cellular motility—a key aspect of the invasive process—is up-regulated by external factors. Hepatocyte growth factor (HGF) has been implicated in trophoblast migration, by interacting with its receptor, the c-met proto-oncogene, a tyrosine kinase receptor expressed by EVT (Kauma *et al.*, 1999). HGF promotes EVT motility by induction of inducible nitric oxide synthase, via the phosphatyklinositol-3-kinase signalling pathway (Cartwright *et al.*, 1999; Cartwright *et al.*, 2002; Harris *et al.*, 2008). Furthermore, EGF can activate RhoA (a member of the Ras homologue gene family), causing actin rearrangement in trophoblasts, thus aiding trophoblast migration (Han *et al.*, 2010). The factors affecting invasiveness of EVT are therefore of key importance in the remodelling process (Knofler, 2010).

EVT and vascular cell interactions

The acquisition of this altered phenotype of adhesion molecules and invasive capability described above enables the actions of EVT in SA remodelling. One additional role EVT play in SA remodelling is through induction of vascular cell apoptosis. SA remodelling is characterized by loss of the outer layer of VSMCs, the ECM and the inner layer of ECs, which is replaced by endovascular EVT by the end of the first trimester (Robertson *et al.*, 1967; Pijnenborg *et al.*, 2006). Various *in vitro* studies investigating EVT-dependent remodelling of SAs have suggested that apoptotic mechanisms are involved (Whitley and Cartwright, 2009). Apoptosis has been described in

vascular cells in a mouse model of trophoblast invasion (Red-Horse *et al.*, 2006) and has been detected in VSMC of arteries undergoing remodelling in human first trimester decidua (Smith *et al.*, 2009). Most mechanisms investigated *in vitro* have involved the TNF family of cytokines. EVT have been shown to produce the pro-apoptotic cytokine Fas-ligand, which can bind to its receptor on vascular cells to induce caspase-dependent death. There is evidence that Fas-ligand is involved in the induction of apoptosis of both ECs (Ashton *et al.*, 2005; James *et al.*, 2011) and VSMC (Harris *et al.*, 2006; Harris *et al.*, 2007) caused by endovascular and interstitial EVT. A related cytokine, TNF-alpha-related apoptosis-inducing ligand (TRAIL) was also found to be expressed by EVT and involved in the induction of VSMC death (Keogh *et al.*, 2007).

Apoptosis of SAs has not, however, been consistently described (Bulla *et al.*, 2005). One difficulty in the detection of apoptotic vascular cells in decidual SAs may be related to rapid phagocytosis, as the ability of EVT to phagocytose apoptotic ECs has been demonstrated (Chen *et al.*, 2005). Successful physiological change of vessels and EVT invasion is also reliant on other mechanisms, such as elastolytic processes carried out by EVT, which ensure the breakdown of the elastic fibres found at the basal sections of decidual SAs and amongst all the myometrial segments of the SAs. MMPs produced by EVT are proposed to aid migration through breaks in the internal elastic lamina *in vivo* and disruption of the ECM around SAs may aid EVT remodelling (Naruse *et al.*, 2009a, b; Smith *et al.*, 2009). MMP-12 is an elastolytic protease expressed by trophoblast which is capable of degrading several ECM components, including collagen type IV, laminin, fibronectin, vitronectin and heparin sulphate proteoglycans. Furthermore, in other examples of vessel remodelling MMP-12 is capable of activating pro-MMP-2 and pro-MMP-3, as well as processing α1-anti-trypsin and latent TGF-β (Lagente *et al.*, 2009), and this may be a mechanism by which MMP-12 production by EVT may influence VSMC disruption and de-differentiation. Therefore, the role of the EVT in the remodelling of SAs is crucial.

Cell parallels: the tumour microenvironment

The invasion and migration of EVT into the maternal decidua shares similarities with the characteristic properties of malignant cells and metastasizing cells at the invasive front of a tumour (Fig. 1). There are, however, two major differences displayed by EVT: the strict restrictions temporally and spatially to the pregnant decidua and myometrium and the loss of proliferative ability. The differentiation from cytotrophoblast into EVT has been likened to the common, but uncontrolled, epithelial–mesenchymal transition (EMT) seen in cancer (Denker, 1993; Kalluri and Weinberg, 2009). This transition involves the change from a column of cells in contact by a range of adhesion molecules to individual cells capable of migrating long distances, and is made possible by changes in cytoskeletal proteins and the production of proteases which break down the ECM. The acquisition of these properties allows cells to migrate to different regions of the body, and also occurs in embryo development and wound healing. However, the transition undergone by EVT and cancer cells is thought to be an intermediate phenotype of the EMT (Kalluri and Weinberg, 2009).

The initiation of EMT in cancer cells is proposed to be dependent on signals from the tumour stroma such as EGF, HGF and TGF-β (Kalluri and Weinberg, 2009). Similarly, as cytotrophoblast differentiates into EVT, strikingly similar molecules from the decidua induce

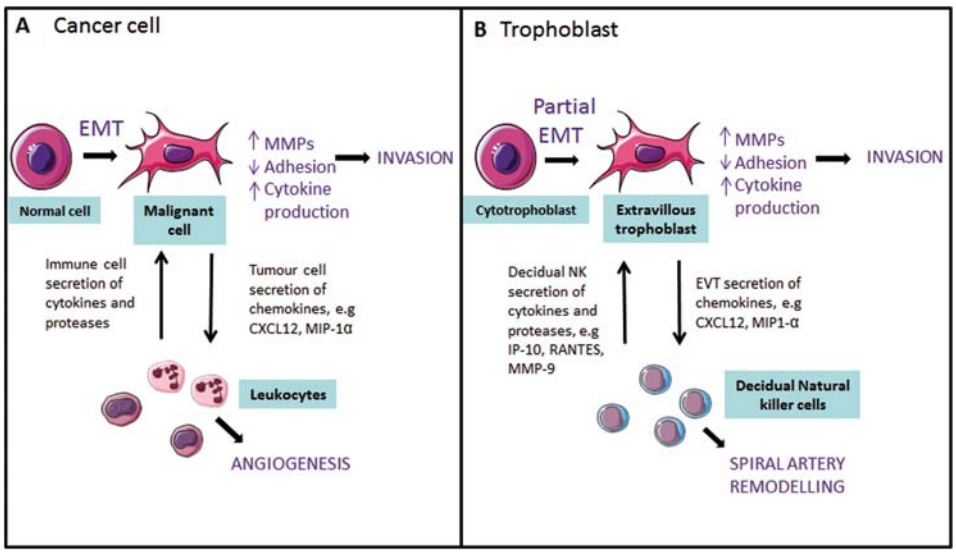

Figure 1 Similarities between the EMT in cancerous cells and cytotrophoblast differentiation into extravillous trophoblast. (**A**) During malignant transformation, tumour cells undergo an EMT into invasive cells, which secrete increased levels of proteases and cytokines, leading to increased invasion. Secretion of chemokines, such as chemokine (C-X-C motif) ligand-12 (CXCL12) and MIP-1α, leads to chemoattraction of leukocytes which play a role in tumour angiogenesis, and may secrete further cytokines which act upon the tumour cells. (**B**) During cytotrophoblast differentiation, trophoblast undergoes a transformation into invasive EVT and up-regulates proteases and cytokines. Interactions between decidual natural killer (NK) cells and EVT, for example the secretion of chemokines and proteases, are proposed to contribute to SA remodelling.

this change, and many of the same transcription factors are activated in both tumour cells and EVT, which orchestrate the phenotypic changes in EMT. For example, tumour cell EGFR phosphorylation has wide-ranging effects including the down-regulation of adhesion molecules (Kalluri and Weinberg, 2009) and up-regulation of MMP-2 (Xu *et al.*, 2010), giving the combined effect of increased invasion. In placental development, the phosphorylation of EGFR1 in cytotrophoblast by HB-EGF up-regulates the pro-migratory EGFR2 receptor (Wright *et al.*, 2010), down-regulates the inter-cellular adhesion molecule connexin 40 (Wright *et al.*, 2006) and increases MMP-2 secretion (Staun-Ram *et al.*, 2009). EGFR phosphorylation can also activate the transcription factor Snail, a molecule which in many cancer types is also induced by TGF-β1 and TGF-β2 signalling and leads to the most common change in EMT, a loss of E-cadherin expression and increased invasive capability (Medici *et al.*, 2008). In placental development, Snail is regulated by hypoxia and in turn regulates the repression of E-cadherin expression, which up-regulates integrins critical for ECM remodelling and therefore decidual invasion (Arimoto-Ishida *et al.*, 2009). Finally, HGF has been identified in cancer as a critical molecule promoting metastasis (Birchmeier *et al.*, 2003), while EVT motility is significantly stimulated by HGF (Cartwright *et al.*, 2002). The signalling pathways described here are by no means the only ones showing similarity between these two processes and are described extensively elsewhere (Ferretti *et al.*, 2007).

However, the close similarity with the EMT is not the only parallel seen between EVT and tumour cells. For example, it is interesting that, similar to EVT, many tumours express HLA-G (Amiot *et al.*, 2011). The expression of soluble HLA-G has been demonstrated to induce cytokine and chemokine production (e.g. IL-8, IL-6, TNF-α, IFN-γ and IL-6) in both dNK and pbNK cells via internalization of the KIR2DL4 receptor (Rajagopalan *et al.*, 2006; Li *et al.*, 2009). This may contribute to tissue remodelling in both the maternal decidua and in tumours. Both soluble and membrane-bound HLA-G are also proposed to act in both situations as a method of avoiding immune surveillance (Poehlmann *et al.*, 2006; Amiot *et al.*, 2011). However, the role of HLA-G in both cancer and trophoblast is still largely unknown (Apps *et al.*, 2008), and other parallels with the immune system may be important in tissue remodelling in both examples.

One such example may be the role of immune cells in cancer, which has recently been identified as an important aspect of tumour biology (Mantovani *et al.*, 2008). The tumour microenvironment is commonly infiltrated by several types of leukocyte including macrophages, neutrophils and NK cells, and these cells of the innate immune system are proposed to infiltrate tumours by chemoattraction to pro-inflammatory cytokines secreted by the cancer cells. These immune cells have been shown to contribute to tissue remodelling within cancer, including promotion of metastasis of tumour cells and angiogenesis (de Visser *et al.*, 2006). NK cells were so named in recognition of their cytotoxicity towards tumour cells, and the role of NK cells in a variety of tumours has been long studied and clinical trials exploiting their effects are underway (Ljunggren and Malmberg, 2007; Vivier *et al.*, 2011). In mouse models, early studies showed that the depletion of NK cells by antibodies increased tumour growth (Seaman *et al.*, 1987). In humans, low pbNK cytotoxic activity is associated with increased risk of cancer (Imai *et al.*, 2000) and NK cell presence in tumour is positively associated with prognosis (Villegas *et al.*, 2002; Qiu *et al.*, 2009; Levy *et al.*, 2011). Engagement of receptors on the NK cell, particularly NKp46, NKp30, NKp44 and NKG2D, is important for tumour cell cytotoxicity (Vivier *et al.*, 2011).

As described earlier in this review, pbNK and dNK cells display different phenotypes. Tumour-associated NK cells have also been found to express a unique phenotype, possibly related to the microenvironment in which they are found. NK cells appear to be important in immediate rejection of tumour cells by cytotoxicity, rather than the control of established tumours, which may be more attributed to T lymphocytes (Levy *et al.*, 2011). NK cells are found in low numbers in tumours, and so most studies have been performed on pbNK rather than on isolated tumour NK cells. However, NK cells isolated from ovarian cancer display reduced cytotoxicity in a classical tumour cytotoxicity assay and decreased CD16 expression, an effect which could be mirrored in pbNK incubated with cancer cells (Carlsten *et al.*, 2009). Additionally, NK cells isolated from lung cancer showed a lower cytotoxicity compared with those from normal lung or peripheral blood, and increased expression of the activation marker CD69 and the receptor NKp44 (Carrega *et al.*, 2008). The receptor for HLA-E, NKG2A, is also more frequently expressed by NK cells which are associated with tongue cancer (Katou *et al.*, 2007). These are all characteristics of dNK cells (although dNK expression of NKp44 is disputed; Kusumi *et al.*, 2006; Male *et al.*, 2011). Therefore, whether the phenotype of NK cells in an established tumour microenvironment changes, to be more similar to the cytokine secreting, low cytotoxic phenotype of dNK cells, is unknown. Whether NK cells contribute with tumour cells to remodelling in cancer is also unknown. However, the role of macrophages and neutrophils in tissue remodelling in cancer has been more intensively studied in cancer biology.

The most common infiltrating immune cell into cancer is often the macrophage (Dirkx *et al.*, 2006). Tumour-associated macrophages are increased in many cancer types including colon (Forssell *et al.*, 2007), breast (Bingle *et al.*, 2006) and melanoma (Hussein, 2006) and are often associated with poor prognosis (Salvesen and Akslen, 1999; Mantovani *et al.*, 2011). Subpopulations of macrophages which have different phenotypes and promote tumour progression in different ways have been identified. In general, it has been suggested that the macrophages found in tumours are predominantly of a 'M2' phenotype, also known as 'alternatively activated' and associated with processes such as wound healing, as opposed to pathogen killing and inflammatory cytokine production (Mantovani *et al.*, 2008; Mantovani *et al.*, 2011). However, the phenotype of macrophages in cancer may not be neatly divided into these two categories and, instead, they share overlapping features and at least six phenotypes have been identified, which promote features including tumour cell survival, metastasis and immunosuppression (Qian and Pollard, 2010). One main contribution by macrophages to the growth of tumours is thought to be angiogenesis. In an important study, macrophages were shown to be essential for the initiation of angiogenesis in breast cancer (Lin *et al.*, 2007). One subset of macrophages, which are identified by the presence of the Tie2 marker, expresses a number of angiogenic factors and enhances tumour growth in mouse models (Venneri *et al.* 2007; Qian and Pollard, 2010). Factors secreted by macrophages include VEGF, fibroblast growth factor-2 and IL-8, amongst other pro-angiogenic chemokines (Nesbit *et al.* 2001; Bingle *et al.* 2006; Lamagna *et al.*, 2006; Venneri *et al.*, 2007). Macrophages can also promote tumour invasion and metastasis, through the secretion of

remodelling proteases including MMP12 and MMP-9 (Luo *et al.*, 2006; Yang *et al.*, 2007). The phenotype and actions shown by tumour-associated macrophages therefore display similarities with the difference in phenotype and activities between pbNK and dNK cells.

It is then possible to draw parallels between cancer cells and immune cells interacting with vasculature within the tumour, and EVT and dNK cell interactions with vascular cells to remodel SAs. EVT secrete cytokines for which dNK cells express receptors, and the resulting cross-talk can induce dNK cell migration: these include the ligand CXCL12 (Hanna *et al.*, 2003) and macrophage inflammatory protein (MIP)-1α (Drake *et al.*, 2001). By a similar mechanism, secretion of chemokines by tumour cells has been demonstrated to chemoattract immune cells: examples include IL-8 and CXCL12-mediated chemoattraction of neutrophils and macrophages in breast cancer (Yao *et al.*, 2007; Welford *et al.*, 2011), IL1-β-mediated infiltration of macrophages in lung cancer (Kimura *et al.*, 2007), CXCL1-induced chemoattraction of neutrophils to endometrial adenocarcinoma (Wallace *et al.*, 2009) and MIP1-α-mediated infiltration of macrophages (Wu *et al.*, 2008). During placentation, therefore, expression of chemokines by EVT may augment the decidual stroma's role of homing dNK cells further to the SAs.

Macrophages and neutrophils can also promote the invasion and metastasis of cancer cells. This is achieved through the secretion of proteases, including MMP-9 and MMP-12, which break down the ECM and facilitate cell migration (Luo *et al.*, 2006; Ardi *et al.*, 2007; Yang *et al.*, 2007). In the decidua, dNK cells are thought to fulfil a similar role through their production of MMP-2, 9, tissue inhibitor of metalloproteinase-2 (Naruse *et al.*, 2009b) and uPA (Naruse *et al.*, 2009a), which may facilitate migration of EVT. Additionally, similar signalling pathways may be activated in EVT as in tumour cells in response to immune cells. For example, IL-8, which is produced both by the tumour microenvironment and immune cells, induces production of MMP-2 in melanoma (Luca *et al.*, 1997) and MMP-9 in prostate cancer (Inoue *et al.*, 2000) which increases tumour invasion. In the decidua, IL-8 is produced by dNK cells and this increases EVT MMP-2 expression and invasion (De Oliveira *et al.*, 2010), as does CXCL10 expression via the CXCR1 and CXCR3 receptors, respectively (Hanna *et al.*, 2006).

However, dNK cells are also proposed to play a role in the inhibition of EVT invasion to regulate excessive decidual remodelling. This is potentially achieved by production of the cytokines TNFα, TGF-β1 and IFN-γ. Additionally, differences in the repertoire of cytokines produced by dNK cells have been demonstrated at different gestational ages (Lash *et al.*, 2010a, b, c). It is therefore possible that the role of dNK cells alters as gestation progresses. Decidual NK cells have been shown to be more stimulatory as gestation advances, possibly as EVT at lower gestational ages are more intrinsically invasive and therefore less responsive to external factors (Lash *et al.*, 2010a, b, c).

The remodelling partnership: dNK cells and EVT

Similar to the mechanisms employed by immune cells and cancer cells to promote tumour growth, dNK cells and EVT may also be interacting to carry out SA remodelling. As described, the panel of surface receptors expressed by dNK is distinct from those in pbNK cells (Koopman *et al.*, 2003; Sharkey *et al.*, 2008; El Costa *et al.*, 2009). Receptors displaying differing levels of expression between pbNK and dNK include the natural cytotoxicity receptors NKp46, NKp30 and Nkp44 (El Costa *et al.*, 2009) and ILT2 as well as the inhibitory receptors NKG2A and NKG2C and the killer cell immunoglobulin-like receptors (KIR; El Costa *et al.*, 2009)]. Interactions exist between these dNK cell receptors and EVT major histocompatibility complex (MHC) molecules. The receptor NKG2A/CD94 on dNK cells binds to the MHC class I molecule, HLA-E on the invading trophoblast (King *et al.*, 1998; Hanna *et al.*, 2006) and engagement of this receptor and ligand pairing is thought to override cytotoxic signals from the NKp46 natural cytotoxicity receptor expressed by dNK (El Costa *et al.*, 2008). Engagement of the NKp44 receptor by trophoblast can also induce secretion of various cytokines (Hanna *et al.*, 2006). The interaction between dNK KIR expression and fetal trophoblast HLA-C expression is interesting, as particular combinations are associated with an increased risk of pre-eclampsia, fetal growth restriction and recurrent spontaneous abortion (Hiby *et al.*, 2004; Hiby *et al.*, 2010). The HLA-C-specific KIR receptor genotype can be divided into two groups dependent on the presence (A) or absence (B) of activatory KIR receptors. HLA-C has two allotypes, C1 and C2. Increased risk of pregnancy complication is found when mothers which have the AA KIR genotype are paired with fetal trophoblast cells with a C2 allotype (Hiby *et al.*, 2004), potentially related to a premature halting of trophoblast-mediated vessel transformation directed by dNK cells (Parham, 2004). Decidual NK–EVT interactions are crucial in the maintenance of a normal pregnancy and SA remodelling. We will now discuss the additional mechanisms by which interactions between dNK cells and EVT may achieve SA remodelling. The combined role that these cell types play could be described as a 'partnership'.

Contribution to artery remodelling: dNK cell actions on EVT

Studies investigating the effect of dNK cells on EVT have established that cytokines produced by dNK cells can promote trophoblast invasion. For example, Hanna *et al.* (2006) demonstrated that dNK cells are able to produce IL-8 and INF-γ inducible protein (IP)-10, even in the presence of low concentrations of IL-15. Corresponding to this, it was also found that invasive EVT expressed CXCR1 and CXCR3, the receptors for IL-8 and IFN-γ-induced protein-10, respectively. This suggested that cross-talk between dNK cells and EVT is taking place via stimulation of these receptor pathways. Both *in vitro* and *in vivo* trophoblast migration experiments revealed that dNK cells are able to stimulate recruitment and migration of EVT by their production of IL-8 and IP-10, ensuring their recruitment to the SAs (Hanna *et al.*, 2006). The same authors demonstrated that dNK cells produce the RANTES chemokine (regulated on activation, normal T-cell expressed and secreted), which regulates macaque trophoblast migration (Thirkill *et al.*, 2005). We have also demonstrated that HGF production by dNK cells induces motility in an EVT cell line (Fraser *et al.*, 2011). EVT invasion is dependent on proteases and their inhibitors produced by the EVT themselves. IL-8 promotes the up-regulation of MMP-2 and MMP-9 and the invasion of an

EVT cell line (De Oliveira *et al.*, 2010; Jovanovic *et al.*, 2010), and also reduces EVT apoptosis (Knofler, 2010).

As previously described, EVT differentiation into an invasive phenotype is required for successful trophoblast invasion. Adhesion molecules altered in this differentiation, such as integrins expressed by EVT, can also be altered by chemokines which are secreted by dNK cells; for example, IL-8 increases expression of integrins α1 and β5 (Jovanovic *et al.*, 2010). LIF is produced by dNK cells (Reister *et al.*, 2006) and has been associated with Stat3 activation (Poehlmann *et al.*, 2005) which can regulate trophoblast invasion (Corvinus *et al.*, 2003) and is involved in the EMT. In addition, the ability of dNK-cell secreted factors to indirectly induce a key step in trophoblast differentiation in the rat has been described, as factors including VEGF increase oxygen delivery to the site of placentation and thus the differentiation of trophoblast via the hypoxic response element HIF 1α (Chakraborty *et al.*, 2011).

Further effects on EVT by dNK cells include the ability to promote the 'endothelial-like' properties of endovascular trophoblast. Decidual NK cell conditioned media not only induced increased migration of primary and cell line EVT but also promoted their formation into capillary tubes and increased expression of the endothelial-like adhesion molecule, ICAM (Hu *et al.*, 2010). Additionally, VEGF-C expression by dNK cells has been demonstrated to be important in increasing EVT resistance to cytotoxicity, as well as promoting the assembly of EVT into networks of tube-like structures (Kalkunte *et al.*, 2009). Therefore the signalling from dNK cells to EVT may also promote the differentiation of EVT, a hypothesis which is also reinforced by the promotion of EVT migration from placental explants by dNK cell-conditioned medium, implying a switch from villous cytotrophoblast to EVT (Lash *et al.*, 2010a, b, c).

Contribution to artery remodelling: EVT actions on dNK cells

EVT may also influence dNK cells in order to promote SA remodelling. Previously described in this review is the secretion of CXCL12 by EVT within the decidua and SAs, inducing migration of dNK cells (Hanna *et al.*, 2003). MIP-1α production by cytotrophoblast has also been demonstrated, and this is a chemotactic factor to dNK cells and may therefore function to position dNK cells in the vicinity of SAs (Drake *et al.*, 2001). The expression of GnRH-induced CXCL chemokines by EVT has also been demonstrated to be chemotactic to dNK cells (Cavanagh *et al.*, 2009).

The unique properties of the HLA-G molecule expressed by EVT may also influence the actions of dNK cells in SA remodelling. HLA-G has only been found to bind to two receptors on dNK (KIR2DL4 and LILR-1) owing to differences in contact residues which bind to KIRs on HLA-G compared with the classical MHC molecules such as HLA-C (Gonen-Gross *et al.*, 2010) Interactions between HLA-G-expressing cells and dNK cells can reduce the expression of some cytokines by dNK cells, including TNF-α (Rieger *et al.*, 2002; Ntrivalas *et al.*, 2006). A further mechanism that has been demonstrated is via the actions of soluble HLA-G (sHLA-G), which is proposed to have a different role to its membrane-bound form, and is secreted by EVT in the decidual environment. It has been suggested that by binding to KIR2DL4 on dNK cells, sHLA-G alters mRNA levels of numerous cytokines in NK cells, including IL-6, IL-8 and IFN-γ (Rajagopalan *et al.*, 2006). This study was carried out using pbNK cells; however, endometrial NK cells have also been demonstrated to secrete IFN-γ and increase proliferation in response to sHLA-G (van der Meer *et al.*, 2007). Additionally, the homodimer form of sHLA-G has been demonstrated to increase secretion of IL-6, IL-8 and TNF-α in dNK cells (Li *et al.*, 2009), and is therefore proposed to promote a phenotype of dNK cells capable of participating in SA remodelling. The importance of the HLA-G–KIR2DL4 interaction in the decidua is however still unknown, with the suggestion that it is not necessary for reproduction in humans (Gomez-Lozano *et al.*, 2003; Nowak *et al.*, 2011).

Finally, EVT recruitment of dNK cells may be achieved by an indirect mechanism. It has also been proposed that EVT may alter chemokine secretion by cells making up SAs, which may alter dNK cell recruitment (Hazan *et al.*, 2010). However, expression of MMP-12 by VSMC and EVT may act to indirectly inhibit dNK cell recruitment by the cleavage and inactivation of leukocyte chemotactic proteins (Harris *et al.*, 2010; Harris, 2011), and so the picture is far from clear.

Contribution to artery remodelling: similar mechanisms

Some of the repertoire of angiogenic factors, cytokines and enzymes which contribute to SA remodelling are secreted by both EVT and dNK cells, and may perform the same and possibly redundant functions in SA remodelling. For example, both cell types express the proteases MMP-2 and MMP-9, as described above (Naruse *et al.*, 2009b). The expression of a number of similar angiogenic factors and cytokines has been highlighted by recent studies using multiplex arrays and enzyme-linked immunosorbent assay to examine factors secreted from isolated primary EVT and dNK cells (Lash *et al.*, 2006; Engert *et al.*, 2007; Lash *et al.*, 2010a, b, c; Naruse *et al.*, 2010). These include the expression of VEGF-C by trophoblast which is proposed to disrupt the VSMC layer of SAs (Lash *et al.*, 2011) and expression of VEGF-C by dNK cells may perform a similar function (Kalkunte *et al.*, 2009). The angiopoietins (Ang1 and Ang 2) have well described roles in blood vessel stabilization or breakdown, and are also expressed by both cell types (Lash *et al.*, 2006; Schiessl *et al.*, 2009). EVT and dNK cells also secrete the angiogenic factors IL-8, platelet-derived growth factor BB homodimer, placenta growth factor and FGFb (Saito *et al.*, 1993; Lash *et al.*, 2006; De Oliveira *et al.*, 2010; Lash *et al.*, 2010a). While the functions of these proteins secreted by dNK cells and EVT have not been described in all cases, it is possible that the actions of these on SAs induce similar responses, and may even be redundant. The unique and overlapping secreted factors of dNK cells and EVT are summarized in Table I. Discrepancies exist in the literature between the presence and absence of factors, and may be related to sampling method or gestation age of samples examined.

Apoptotic signalling has been implicated in SA remodelling by trophoblast cells and various studies have demonstrated the induction of apoptotic death of vascular cells by invasive trophoblast contact, as described above (Ashton *et al.*, 2005; Chen *et al.*, 2005; Harris *et al.*, 2005). There may also be a similar pathway used by dNK cells. Apoptotic markers can be detected in SA VSMC when dNK cells but not EVT are present (Smith *et al.*, 2009). Additionally, recent evidence from our group has revealed that dNK cells induce vascular cell

Table I Factors secreted by human primary dNK cells and EVT at a stage when SA remodelling is taking place during pregnancy

Factor secreted during SA remodelling	dNK cells	EVT	References
Ang-1	✓	✓	Lash *et al.* (2006); Schiessl *et al.* (2009); Lash *et al.* (2010a, b, c)
Ang-2	✓	✓	Lash *et al.* (2006); Schiessl *et al.* (2009); Lash *et al.* (2010a, b, c)
Angiogenin	✓/NE	✓	Engert *et al.* (2007); Lash *et al.* (2010a, b, c)
CXCL1	✓	NE	Engert *et al.* (2007)
CXCL10	✓	NE	Hanna *et al.* (2003); Hanna *et al.* (2006)
CXCL12	✓	✓	Hanna *et al.* (2003); Engert *et al.* (2007)
EGF	✓	✓	Engert *et al.* (2007)
Ena-78	✓	NE	Engert *et al.* (2007); El Costa *et al.* (2008)
FasL	✓	✓	Ashton *et al.* (2005); Harris *et al.* (2006); Fraser *et al.* (2011)
FGFb	✓	✓/NE	Lash *et al.* (2006); Lash *et al.* (2010a, b, c); Lash *et al.* (2011)
GM-CSF	✓	✓/NE	Engert *et al.* (2007); Naruse *et al.*
HGF	✓	NE	Clark *et al.* (1996); Fraser *et al.* (2011)
IFN-γ	✓	✓/NE	Saito *et al.* (1993); Engert *et al.* (2007); Lash *et al.* (2010a, b, c)
IL-1β	✓	✓	Engert *et al.* (2007); Naruse *et al.*
IL-2	NE	NE	Engert *et al.* (2007)
IL-3	✓	✓	Engert *et al.* (2007)
IL-4	✓	✓/NE	Engert *et al.* (2007); Lash *et al.* (2010a, b, c)
IL-5	NE	NE	Engert *et al.* (2007); Lash *et al.* (2010a, b, c)
IL-6	✓/NE	✓	Engert *et al.* (2007); Lash *et al.* (2010a, b, c)
IL-7	NE	NE	Engert *et al.* (2007)
IL-8 (CXCL8)	✓	✓	De Oliveira *et al.* (2010); Saito *et al.* (1994); Hanna *et al.* (2006), Naruse *et al.*
IL-10	✓	✓/NE	Engert *et al.* (2007); Lash *et al.* (2010a, b, c)
IL-12p70	✓	✓	Engert *et al.* (2007); Naruse *et al.*, 2010
IL-13	NE	✓/NE	Engert *et al.* (2007); Lash *et al.* (2010a, b, c); Naruse *et al.* (2010)
IL-15	✓	NE	Engert *et al.* (2007)
KGF	✓	NE	Lash *et al.* (2006); Naruse *et al.* (2010)
Leptin	✓	✓	Engert *et al.* (2007)
LIF	✓	NE	Saito *et al.* (1993); Sharkey *et al.* (1999); Reister *et al.* (2006)
MCP-1	✓	✓	Engert *et al.* (2007); Naruse *et al.* (2010)
MCP-2	✓	✓	Engert *et al.* (2007); El Costa *et al.* (2008)
MIP1α (CCL3)	✓	✓	Drake *et al.* (2001)
MIP1β (CCL4)	✓	✓	Lee *et al.* (2001); Konishi *et al.* (2004); El Costa *et al.* (2008)
MIG	NE	NE	Hanna *et al.* (2006); Engert *et al.* (2007)
MMP-2	✓	✓	Naruse *et al.* (2009); Staun-Ram *et al.* (2009); Anacker *et al.* (2011)
MMP-3	✓	✓	Husslein *et al.* (2009; Anacker *et al.* (2011)
MMP-9	✓	✓	LaMarca *et al.* (2005); Naruse *et al.* (2009); Anacker *et al.* (2011)
MMP-12	✓	✓	Anacker *et al.* (2011); Harris *et al.* (2010)
MSCF	✓	✓	Engert *et al.* (2007)
Oncostatin M	✓	✓	Engert *et al.* (2007); El Costa *et al.* (2008)
PDGF-BB	✓	✓	Lash *et al.* (2006, 2010a, b, c)
PlGF	✓	✓	Hanna *et al.* (2006); Lash *et al.* (2006); Naruse *et al* (2010)
RANTES	✓	✓	Engert *et al.* (2007); Naruse *et al.* (2010)
TGF-β1	✓	✓	Lash *et al.* (2006); Lash *et al.* (2010a, b, c)
TNF-α	✓	✓	Saito *et al.* (1993); Knofler *et al.* (2000); Hu *et al.* (2010)
TRAIL	NI	✓	Keogh *et al.* (2007)
VEGF-A	✓	✓	Lash *et al.* (2006, 2010a, b, c); Schiessl *et al.* (2009)
VEGF-C	✓	✓	Hu *et al.*; Lash *et al.* (2006, 2010a, b, c); Kalkunte *et al.* (2009); Schiessl *et al.* (2009)

Data were obtained by measuring factors secreted from isolated cells and immunohistochemistry studies of the placental bed. ✓, detected; NE, not expressed; NI , not investigated. See text for descriptions of all factors.

apoptosis via a FasL pathway (Fraser *et al.*, 2009) similar to EVT (Ashton *et al.*, 2005; Keogh *et al.*, 2005). This implies that the induction of apoptosis in vascular cells is a pathway used by more than one cell type in the decidua, and that cytokine and angiogenic factor signalling is not the only similarity between dNK cells and EVT.

These observations demonstrate many similarities between dNK cells and EVTs at the maternal–fetal interface. These interactions still need to be fully elucidated but they indicate that dNK cells and EVT may work in partnership, either by acting via similar mechanisms and/or by actions on each other, during the first trimester of pregnancy. However, recent data suggest that in co-culture, EVT and dNK cells decrease secretion of some angiogenic factors and chemokines (Lash *et al.*, 2011), and therefore there is still much to be elucidated regarding the redundancy of remodelling mechanisms.

When the partnership goes wrong

In some placental disorders, it has been postulated that the EMT is disrupted in trophoblast, in a similar yet opposite manner to the EMT disruption leading to uncontrolled cancer growth (Kokkinos *et al.*, 2010). This could lead to the inadequate trophoblast invasion seen in pre-eclampsia and IUGR. For example, Genbacev et al. described an inability of cytotrophoblasts to differentiate into an invasive phenotype, as well as increased trophoblast apoptosis in pre-eclamptic pregnancies (Genbacev *et al.*, 1999, 2000). Disruptions between cancer cell and immune cell signalling are also proposed to lead to disruptions in tumour growth and therapies which block signalling between these cell types are now being investigated (Squadrito and Palma, 2011; Tseng *et al.*, 2011). It is therefore possible that, mirroring the interactions between innate immune cells and tumour cells, the interaction between dNK cells and trophoblast can also be disrupted, playing a role in placental disorders.

The proposed pathological consequences of abnormal placental growth and SA remodelling include pre-eclampsia, recurrent spontaneous abortion and IUGR (Cartwright *et al.*, 2010). Both EVT and dNK cells individually have been implicated in the cause of these disorders. An association between pre-eclampsia and impaired EVT trophoblast invasion and reduced SA remodelling has long been evident (Brosens *et al.*, 1972; Pijnenborg *et al.*, 1991), with numerous studies investigating the mechanisms of this impairment (Kaufmann *et al.*, 2003; Whitley and Cartwright, 2009). Furthermore, increased dNK cell numbers has been linked to recurrent spontaneous abortion (Quenby *et al.*, 2009), although this is controversial (Tang *et al.*, 2011), and clinical trials aimed at decreasing dNK cell number in high-risk patients are underway (Tang *et al.*, 2009). There is also evidence for decreased dNK cells in third trimester pre-eclamptic and fetal-growth restricted pregnancies (Williams *et al.*, 2009). These associations between dNK cell numbers and disease indicate the possible therapeutic potential of investigating the functions of dNK signalling further.

To fully resolve all the cellular and molecular interactions associated with placentation and SA remodelling, studies which link the two cell types in disorders of SA remodelling may be required. The HLA-C/KIR interaction described in this review is one such mechanism (Hiby *et al.*, 2004; Hiby *et al.*, 2010; Chazara *et al.*, 2011), and this finding has recently been mirrored in the mouse. Expression of the murine classical MHC molecule H2-K on mouse trophoblast giant cells has been detected, and antigenic dissimilarity between paternal MHC and maternal NK is associated with increased decidual vessel dilation, fetal growth and placental weight, which may have implications for the study of reproductive pathologies in human pregnancy (Madeja *et al.*, 2011). Additional mechanisms other than MHC have also been investigated. It has been demonstrated that trophoblast co-cultured with decidual immune cells (60% dNK cells in this study) produce increased soluble-Flt, a protein associated with pre-eclampsia (Matsubara *et al.*, 2005). Moreover, dNK cells expressing granulysin, a cytotoxic protein, are increased in patients who have undergone a first trimester spontaneous abortion as compared with a first trimester termination of pregnancy. Granulysin-expressing-dNK cells are capable of inducing apoptosis in EVT, and therefore this may be an aspect of disrupted signalling leading to pregnancy disorders (Nakashima *et al.*, 2008). The expression of MMP-12 by trophoblast and other cell types, which inhibits dNK cell recruitment, is decreased in pre-eclampsia, further implying a connection between dNK cells and pathological conditions. Additionally, our studies of pregnancies with a high risk of developing pre-eclampsia show that dNK cells isolated from these patients are less able to induce EVT motility and fail to induce vascular cell apoptosis as compared with dNK cells isolated from low-risk pregnancies (Fraser *et al.*, 2009). As such, further studies of the interactions between dNKs and EVT in patients with pathologies associated with altered SA remodelling will be of great interest to the field.

Conclusions and future perspectives

Although many disorders of pregnancy, such as pre-eclampsia, present symptoms in the second or third trimester, the remodelling of SAs in the first trimester is a key process in setting up a successful pregnancy. The role of EVT in this process has been extensively studied, however not in conjunction with dNK cells, and the role of dNK cells in SA remodelling has only recently been explored (Hanna *et al.*, 2006; Harris, 2011). What is becoming apparent is that pregnancy disorders are likely to involve the actions of both fetal and maternal cells. The interactions between fetal and maternal cells are also complex, involving both the cytotoxic- and chemokine-producing capacity of dNK cells, and their roles alters as gestation progresses. The investigation of pregnancy as a multicellular system involving both fetal and maternal components, as well as comparison to other examples of tissue remodelling including cancer progression, is identifying key mechanisms in this process and will allow development of future therapeutic targets for pregnancy disorders.

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Authors' roles

A.E.W., R.F. and J.E.C. conceived the manuscript. A.E.W. and R.F. drafted the manuscript and A.E.W. drafted figures and tables. A.E.W., R.F. and J.E.C. revised the manuscript and approved the final version.

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Conflict of interest

None declared.

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