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Beyond pregnancy: modulation of trophoblast invasion and its consequences for fetal growth and long-term children's health

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ABSTRACT

Invasion of extravillous trophoblast cells (EVTs) into the maternal tissues is a key step in the development of a successful pregnancy, excessive and insufficient EVT invasion being associated with pregnancy complications. These pregnancy complications include preeclampsia and fetal growth restriction, both of which are associated with maternal and fetal morbidity and mortality at the time of birth and with increased risk of cardiovascular disease, diabetes and obesity in adult life for infants born from these conditions. In addition, women who develop preeclampsia are also at a greater risk of cardiovascular disease in later life. Many factors, protein and environmental, have been shown to both up- and down-regulate this process *in vitro via* different mechanisms. The redundancy observed in the regulation of this system suggests that dysregulation of one factor may not contribute to the pathological conditions of EVT invasion and that the relative local concentrations of many different factors may be more important. This review article explores the possibility that the modulation of EVT invasion as a therapeutic target for pregnancies affected by preeclampsia and fetal growth restriction may not be possible or needs to concentrate on the modulation of cell activity as a whole and not of one particular factor.

1. Introduction

Invasion of fetally derived extravillous trophoblast cells (EVTs) into the maternal decidua and inner third of the myometrium is one of the key processes in the establishment of early human pregnancy. This is a tightly regulated process with many different factors playing roles. Dysregulated EVT invasion is associated with several complications of pregnancy such as placenta accreta (excessive EVT invasion), preeclampsia (shallow EVT invasion) and fetal growth restriction (shallow EVT invasion). *In utero* programming for adult health is currently a hot topic and it is now clear that incomplete EVT invasion and remodeling of the spiral arteries contribute to this programming. Therefore, a lot of research has focused on determining the factors that regulate EVT invasion so that novel therapeutic targets for these conditions can be identified.

1.1. Trophoblast

The human placenta is an intricate organ that is made up of a variety of different specialist cell types and vascular networks, which allow it to achieve its main functional role of promoting fetal growth and viability. The major

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cell type of the placenta is the trophoblast, which has three main sub-types: villous cytotrophoblast (CTB), syncytiotrophoblast, and EVT (Gude et al., 2004; Fitzgerald et al., 2008). EVT and CTB can be distinguished by the differential expression of various phenotypical markers such as cell adhesion molecules, integrins, growth factors, and HLA molecules (Norwitz et al., 2001). The villous cytotrophoblast cells fuse to form the multinucleated syncytiotrophoblast cell layer, which covers floating chorionic villi in the intervillous space. In contrast, the cytotrophoblast cells of the anchoring villi differentiate from a proliferative phenotype into an invasive phenotype (EVT), anchoring the placenta to the underlying decidua (Irving and Lala, 1995). The EVT invade through the decidua as far as the inner third of the myometrium *via* two distinct pathways, the interstitial and the endovascular, forming four populations of EVT: interstitial mononuclear, interstitial multinuclear (giant cells), intramural, and endovascular. In interstitial invasion EVT cells invade through the decidua and inner third of the myometrium, while in endovascular invasion EVT cells move up the lumen of the spiral arteries in a retrograde fashion, again ceasing in the inner third of the myometrium. Interstitial mononuclear and multinuclear EVT are found throughout the decidua and inner third of the myometrium, it is assumed that multinuclear interstitial EVT are formed from the fusion of mononuclear interstitial EVT, although the mechanism underlying this is not known. Endovascular EVT are found in the lumen of spiral arteries while intramural EVT are located embedded in fibrinoid material within the wall of spiral arteries during and after spiral artery remodeling.

1.2. Decidua

Successful pregnancy requires a highly receptive endometrium during the implantation window, which involves decidualization and a symbiotic signaling process between the blastocyst and the mother (Aplin, 2000). The decidua is composed of luminal and glandular epithelium, stromal cells, spiral arteries, lymphatics, and leucocytes. In the first trimester of pregnancy, approximately 30–40% of decidual stromal cells are leucocytes, primarily uterine natural killer (uNK) cells, macrophages, and T lymphocytes (Bulmer et al., 1991), although other, less abundant, but functionally important endometrial leukocyte populations are also present, including dendritic cells (Gardner and Moffett, 2003), natural killer T (NKT) (Tsuda et al., 2001) cells and regulatory T cells (Heikkinen et al., 2004). Leucocytes are prominent at the implantation site, where they come into close contact with the implanting blastocyst and with invading EVT. The decidua is a rich source of cytokines, growth factors, and proteases that may contribute to regulation of EVT invasion (Lala and Chakraborty, 2003).

1.3. Trophoblast invasion

Cellular invasion is a complex process that is tightly regulated in EVT, unlike in metastatic cancers (Lala et al., 2002). In simple terms, there are three features of cellular invasion; attachment to the extracellular matrix (ECM), proteolytic breakdown of the ECM, and then movement into that cleared space prior to reattachment. EVT are a naturally highly invasive cell type, although their ability to invade in *in vitro* models decreases with increasing gestational age, with EVT from 8 to 10 weeks gestation being twice as invasive as those from 12 to 14 weeks, 16 to 20 weeks or term (Genbacev et al., 1996; Lash et al., 2006a). EVT invasiveness is associated with their phenotype, for example, EVT express a unique repertoire of cell surface integrins, distinct from those expressed by CTB. In particular, EVT are characterized by the expression of $\alpha1\beta1$ and $\alpha5\beta1$ integrins, while CTB express $\alpha6\beta4$ integrin (Damsky et al., 1992). This switch in integrin expression appears to be essential for the invasive phenotype of EVT (Damsky et al., 1994). In addition, EVT express a wide range of proteases, both secreted and cell surface-associated, dipeptidyl peptidase IV (Sato et al., 2002), carboxypeptidase-M (Nishioka et al., 2003), matrix metalloproteinases (MMPs) (Bischof et al., 2000; Anacker et al., 2011), and the urokinase plasminogen activator (uPA) system (Chakraborty et al., 2002). The gelatinases, MMP-2 and MMP-9, appear to be the most important proteases secreted by EVT for their invasive behavior with the ratio of MMP-2 and MMP-9 altering throughout gestation (Shimonovitz et al., 1994; Bischof et al., 2000) and inhibition of MMP-9 completely inhibiting EVT invasion *in vitro* (Librach et al., 1991). Disruptions in the tightly controlled process of EVT invasion can lead to placental deficiencies, which affect the maternal vascular homeostasis, resulting in pregnancy complications, such as early miscarriage (Khong et al., 1987; Hustin et al., 1990), late miscarriage (Ball et al., 2006), preeclampsia (Pijnenborg et al., 1991), fetal growth restriction (Khong et al., 1986), pre-term birth (Kim et al., 2003), and placenta accreta (Khong and Robertson, 1987; Hannon et al., 2012). Despite the importance of trophoblast invasion in pregnancy very little is understood about the factors that control this process *in vivo*, although decidual factors are likely to play an important role (Fitzgerald et al., 2008).

1.4. Spiral artery remodeling

Remodeling of the uterine spiral arteries is another key maternal adaptation to pregnancy that requires EVT invasion for completion of the process (Pijnenborg et al., 2006). During spiral artery remodeling the blood vessels supplying the uterus undergo significant alterations that result in the decidual and superficial myometrial portions of the vessels losing their musculoelastic wall, which is replaced by fibrinoid and intramural EVT (Pijnenborg et al., 2006). This remodeling process allows for maternal blood that is not under vasoactive control to be delivered to the fetal–placental unit. The underlying pathological features of preeclampsia and fetal growth restriction are associated with reduced spiral artery remodeling, likely due to reduced EVT invasion. However, the molecular triggers of spiral artery remodeling are not known and exactly how EVT contribute to this process is not known either.

1.5. In utero programming

The concept of developmental/fetal origins of adult disease, known as the 'Barker hypothesis,' is based upon the