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Paracrine communication modulates production of Wnt antagonists and COX1-mediated prostaglandins in a decidual-trophoblast co-culture model

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ABSTRACT

Wnt signalling has important roles in decidualisation, implantation and placentation. We investigated the role of decidua-trophoblast communication and Wnt signalling in the placenta using a co-culture model. Expression of a wide range of Wnt-related genes was observed in both decidual and trophoblast cells using PCR array, with remarkably similar expression profiles. Co-culture induced altered expression of several Wnt-related proteins, with the Wnt inhibitors sFPR4 and DKK1 being among the most differentially expressed genes. Media concentrations of sFRP4 and DKK1 were increased with co-culture, coincident with a decrease in canonical Wnt signalling activity. Expression of PTGS1 mRNA and COX1 protein was also increased with co-culture as were media PGE_2 concentrations; these changes were replicated by addition of exogenous DKK1 and sFRP4. Collectively, these data suggest that paracrine interactions between decidua and trophoblast stimulate Wnt antagonist secretion leading to increased placental prostaglandin production. This may be important for implantation and placental function.

1. Introduction

The Wnt family of proteins are highly-conserved secreted pleiotropic cysteine-rich glycoproteins that act as signalling molecules and activators of transcription factors. The Wnt signalling pathway plays important roles during early embryonic development, adult tissue homeostasis, stem cell renewal, proliferation, differentiation, motility and cancer cell progression (Clevers and Nusse, 2012; Niehrs, 2012). Nineteen mammalian Wnt proteins have been identified, divided into 12 subfamilies (Cadigan and Peifer, 2009; Niehrs, 2012). Wnts interact with target cells by binding to a heterodimeric receptor complex consisting of a frizzled [1](FZD)-receptor and lipoprotein-receptor-related protein (LRP) 5/6 (Clevers and Nusse, 2012; Wang et al., 2006). Three pathways are known to be activated by the binding of Wnts to their receptors: the canonical Wnt/β-catenin cascade, the non-canonical planar cell polarity (PCP) pathway, and the non-canonical Wnt/Ca^{2+} pathway (Cadigan and Peifer, 2009). The central signalling molecule of the canonical pathway is β-catenin which in the absence of Wnts (off-state) is phosphorylated by casein kinase1 (CK1) and glycogen synthase kinase 3 (GSK3), resulting in its degradation through an ubiquitin/proteasome pathway (Aberle et al., 1997; Stamos and Weis, 2013). In presence of Wnt ligands (on-state) dishevelled (DVL) is activated resulting in accumulation of β-catenin in the cytoplasm followed by translocation to the nucleus where it activates transcription factors such as T-cell factor (TCF) and lymphocyte enhancer factor (LEF) (Clevers and Nusse, 2012; Niehrs, 2012).

An important aspect of regulation of Wnt signalling is its modulation by several secreted antagonists. These extracellular antagonists are broadly divided in two classes (Kawano and Kypta, 2003). The first class of inhibitors, includes secreted frizzled related proteins (sFRPs), Wnt inhibitory factor 1 (WIF1) and Cerberus (Rattner et al., 1997). The second class includes the members of the dickkopf (Dkk) family. Proteins from the first class bind directly to the Wnt ligands, whereas the second class of antagonists bind to the low density lipoprotein receptor-related protein (LRP) 5/LRP6 component of the Wnt receptor complex, causing a rapid removal

Abbreviations: CK, casein kinase; COX, cyclooxygenase; Dkk, dickkopf; DVL, dishevelled; FZD, frizzled; GSK3, glycogen synthase kinase 3; HOXA10, Homeobox A10; LRP, low density lipoprotein receptor-related protein; MSX, Msh homeobox; PCP, planar cell polarity; PGs, prostaglandins; PGE_2, prostaglandin E_2; PTGS, prostaglandin-endoperoxide synthase; PTGES, prostaglandin E synthase; sFRP, secreted frizzled related proteins; TCF, T-cell factor; WIF1, Wnt inhibitory factor 1; WISP, Wnt-inducible protein.

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of the LRP receptors via Kremen-mediated endocytosis (Kawano and Kypta, 2003).

Human reproduction is a complex and highly regulated process that requires successful completion of several discrete events. Uterus and blastocyst must both undergo synchronous development and differentiation respectively for implantation (Cha et al., 2012). This delicate process involves a complex sequence of signalling events, mediated by a large number of molecular regulators (Minas et al., 2005; Zhang et al., 2013a). A series of complex interactions at the interface between the implanting blastocyst and the endometrial cells lead to the formation of decidua, a differentiated maternal tissue to supports embryo growth and early pregnancy (Ramathal et al., 2010). Accumulating evidence suggests that, among a range of identified signalling pathways, the Wnt signalling pathway plays a particularly important role in implantation, decidualisation and placentation (Chen et al., 2009; Nayeem et al., 2014; Sonderegger and Pollheimer, 2010).

A large number of Wnt-related genes have all been identified as being expressed in endometrial samples and in endometrial epithelial/stromal cells (Bui et al., 1997; Tulac et al., 2003). However, only a few studies have examined Wnt signalling in trophoblast and placenta (Nayeem et al., 2014). Expression of 14 Wnt ligands (out of 19) and 8 FZD receptors (out of 10) was detected in the human placenta by Sonderegger et al. (2007). Expression of TCF3/4 and β-catenin are markedly increased in invading trophoblast (Pollheimer et al., 2006). In a primate placental expression study, the antagonist sFRP4 was found to be expressed predominantly in the villous syncytiotrophoblast and the invasive cytotrophoblast, as well as in the amnion (the inner fetal membrane) (White et al., 2009). In the Matrigel invasion assay, it was demonstrated that Wnt3A stimulated both trophoblast invasion and migration, which could be blocked by the application of DKK1, suggesting that abnormal activation of the Wnt/β-catenin signalling pathway may contribute to trophoblastic hyperplasia and local invasion (Pollheimer et al.). Several other studies have investigated Wnt signalling in the mouse, both in fetal (Carroll et al., 2005; Kobayashi et al., 2004; Mericskay et al., 2004; Miller and Sassoon, 1998; Mohamed et al., 2004) and in maternal cells (Hayashi et al., 2009; Yip et al., 2013), but studies in human gestational tissues are lacking.

Prostaglandins (PGs) play a key role in the inflammatory response. They are generated from arachidonate by the action of cyclooxygenase (COX) isoenzymes (Ricciotti and Fitzgerald, 2011). COX1 and COX2 are encoded by genes prostaglandin-endoperoxide synthase 1 (*PTGS1)* and *PTGS2,* respectively. Female *Ptgs2*$^{-/-}$ mice have multiple reproductive deficits and are infertile. *Ptgs-1* null mice have normal fertility but show parturition defects (Lim et al., 1997). In cancer cells, COX2 is directly up-regulated by Wnt signalling (Araki et al., 2003; Nuñez et al., 2011).

Our understanding of the paracrine Wnt signalling interactions between trophoblast and decidua remain incomplete. In a co-culture experiment examining the effects on global gene expression of the interaction between human trophoblast and endometrial stromal cells, it was shown that presence of trophoblasts upregulates *DKK1* and *Wnt-inducible protein (WISP)* expression in endometrium (Popovici et al., 2006). In another study, the effect of trophoblast-conditioned media on decidualised endometrial stromal cell gene expression was investigated, demonstrating down-regulation of *WNT4* and *FZD* among other genes (Hess et al., 2007). However, to the best of our knowledge there have been no studies looking specifically at secreted Wnt antagonists and their role in cell-to-cell communication within placental tissues.

We hypothesise that reciprocal Wnt signalling between maternal and placental cells during gestation is important for implantation, placentation and decidualisation with key roles likely to be played by the secreted Wnt antagonists in decidual and trophoblastic tissues. We analysed expression of 84 Wnt related components, including several secreted Wnt antagonists, in trophoblast and decidual cells and assessed their interactions using a co-culture model. We further investigated the paracrine effects of the Wnt antagonists and found a link between Wnt signalling and COX1 activity, demonstrating that Wnt inhibitors derived from decidua enhance the production of prostaglandins in trophoblast cells.

2. Materials and methods

2.1. Trophoblast cell isolation

Placentas were obtained with informed consent from women after delivery by Caesarean section at term as approved by the Human Research Ethics Committee of the Women and Newborn Health Service, Perth, WA. Cytotrophoblast cells were extracted from term placenta as previously described with modifications (Aye et al., 2010). To isolate trophoblast cells, around 80–100 g of villous tissue was dissected free of membranes and blood vessels, washed in Dulbecco's Phosphate-Buffered Saline (D-PBS) and digested in 0.25% *w/v* (2.4 U/ml) dispase II (Life Technologies, Grand Island, NY, USA) for 1 h at 37 °C, with 2.5 μg/ml DNAse I (Roche Diagnostics Australia Pty., Castle Hill, NSW, Australia) added 15 min prior to the end of digestion. Tissue digests were then filtered through 70 μm cell filters and erythrocytes removed by incubating cell pellets in red cell lysis buffer (50 mM NH_4Cl, 10 mM $NaHCO_3$, and 0.1 mM EDTA). Trophoblasts were purified by centrifugation at 1200 *g* for 20 min on a discontinuous Percoll gradient (20–60%) and cells which migrated between the 20% and 40% Percoll bands were collected.

2.2. Decidua cell isolation

Primary decidual cell cultures were prepared of modifications of a previously published method (Keelan et al., 2010). Membranes were cut from the placenta, blood clots and vessels were removed, and then decidua was then scraped from the underlying chorioamnion using a glass slide. The collected decidual tissue was washed in D-PBS, digested for 1.5–2 h at 37 °C in 100 ml 0.012% collagenase A/0.25% dispase II. DNase I (400 μg; Roche Diagnostics) was added for the final 15–30 min of incubation. Tissue digests were then filtered through 70 μm cell filters and erythrocytes were removed by incubation in lysis buffer for 5 min. Decidual stromal cells were purified by centrifugation at 1200 *g* for 20 min on a discontinuous Percoll gradient (40%/60%). Cells lying above the 40% layer were recovered by aspiration, washed in media and recovered by centrifugation.

2.3. Cell culture and treatments

Collected trophoblast cells were seeded at 1×10^6 cells/ml (2.1×10^5 cells/cm^2) and cultured in M199 (Life Technologies) media. Collected decidual cells were seeded at 5×10^5 cells/ml (1.05×10^5 cells/cm^2) and cultured in F12/DMEM (Sigma-Aldrich, St. Louis, MO, USA) media. Both types of media were supplemented with 10% fetal calf serum (Bovogen, Keilor East, VIC, Australia), 10 ng/ml epidermal growth factor (Life Technologies), 5 ng/ml insulin, 10 ng/ml transferrin, 0.2 nM sodium selenite (Life Technologies), and penicillin/streptomycin (100 U/ml; Life technologies) in a 95% air/5% CO_2 humidified atmosphere at 37 °C. Cells were washed twice with D-PBS after an overnight incubation and media were replaced. The isolated primary trophoblast and decidua cells were grown in a co-culture setting using 6 and 24-well plates (BD Biosciences, Franklin Lakes, NJ, USA) containing 24 or 6.5 mm diameter Transwell inserts (Sigma-Aldrich) comprising of a 0.4 μm polyester (PET) microporous membrane which allows mixing of media and secreted factors between compartments. Trophoblast cells were seeded in the upper (inner) compartment to attach to the apical