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# Signalling Pathways Regulating the Invasive Differentiation of Human Trophoblasts: A Review

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The invasive differentiation pathway of trophoblasts is an indispensable physiological process of early human placental development. Formation of anchoring villi, proliferation of cell columns and invasion of extravillous cytotrophoblasts into maternal decidual stroma and vessels induce vascular changes ensuring an adequate blood supply to the growing fetus. Extravillous trophoblast differentiation is regulated by numerous growth factors as well as by extracellular matrix proteins and adhesion molecules expressed at the fetal—maternal interface. These regulatory molecules control cell invasion by modulating activities of matrix-degrading protease systems and ECM adhesion. The differentiation process involves numerous signalling cascades/proteins such as the GTPases RhoA, the protein kinases ROCK, ERK1, ERK2, FAK, PI3K, Akt/protein kinase B and mTOR as well as TGF-β-dependent SMAD factors. While an increasing number of signalling pathways regulating trophoblast differentiation are being unravelled, downstream effectors such as executing transcription factors remain largely elusive. Here, we summarise our current knowledge on signal transduction cascades regulating invasive trophoblast differentiation. We will focus on cell model systems which are used to study the particular differentiation process and discuss signalling pathways which regulate trophoblast proliferation and motility.
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**Keywords:** Human trophoblast; Signal transduction; Differentiation; Cell invasion

**Abbreviations:** ALKs, activin-receptor-like kinases; bHLH, basic helix-loop-helix; CaMKII, Ca/calmodulin-dependent kinase II; CTB, cytotrophoblast; eCTB, endovascular cytotrophoblast; ECM, extracellular matrix; EGF, epidermal growth factor; EMSA, elotrophorectic mobility shift assay; ERK, extracellular signal-regulated kinase; EVT, extravillous trophoblast; FGF, fibroblast growth factor; FAK, focal adhesion kinase; GAP, GTPase activating protein; GEF, guanine nucleotide exchange factor; GSK-3β, glycogen synthase kinase 3β; GPCR, G-protein-coupled receptor; hCG, human chorionic gonadotrophin; HGF, hepatocyte growth factor; ICTB, interstitial cytotrophoblast; IGF, insulin-like growth factor; IGFBP, insulin-like growth factor binding protein; IκB, inhibitor of κB; JAK, Janus kinase; JNK, c-Jun N-terminal kinase; LRP-5, low density lipoprotein receptor-related protein 5; MAPK, mitogen-activated protein kinase; MMP, matrix metalloproteinase; mTOR, mammalian target of rapamycin; PAK, p21-activated kinase; PI3K, phosphoinositide 3-kinase; PIP2, phosphatidylinositol-4,5-bis-phosphate; PKC, protein kinase C; PAI-1, plasminogen activator inhibitor 1; PTEN, phosphatase and tensin homolog deleted on chromosome 10; PTK, protein tyrosine kinase; ROCK, Rho-associated-kinase; RTK, receptor tyrosine kinase; STAT, signal transducer and activator of transcription; S, syncytium; TCP, T-cell factor; TGF-β, transforming growth factor-β; uPA, urokinase plasminogen activator; vEGF, vascular endothelial growth factor; Wnt, vertebrate homologue of wingless.

## INTRODUCTION

Cellular signalling is controlled by protein kinases via sequential steps of protein phosphorylation representing the most common controlling mechanism of protein function in the cell. Signalling cascades are initiated by various stimuli such as growth factors, cytokines, hormones, extracellular matrix adhesion and cell to cell contact, governing multiple cellular responses. Hence, at the cell membrane integrin receptors, receptor tyrosine kinases (RTKs) and G-protein-coupled receptors (GPCRs) transduce extracellular signals that activate various cascades such as focal adhesion kinase (FAK), mitogen-activated protein kinase (MAPK) or the PI3K (phosphoinositide 3-kinases)/Akt pathway. All of these

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cascades have been implicated in the control of a diverse range of biological processes, including cell cycle control, differentiation, cell migration and apoptosis [1–5]. Signalling became a main topic in molecular biology over the past years, since deregulation is linked with diseases such as cancer. Consequently, it is of great interest to study signalling pathways in the context of their specific role in pathological but also in physiological processes.

Extravillous trophoblast formation represents a remarkable example of a physiological differentiation process, which is controlled by fetal as well as maternal factors of the placental bed [6,7]. Extravillous trophoblasts (EVT) originate from anchoring villi attached to the uterine stroma during the first weeks of pregnancy (Figure 1). Cytotrophoblast (CTB) stem cells residing at the villous basement membrane lose their polarised structure and form proliferating cell columns. At distal sites invasive EVT are generated which detach from the columns and invade decidual stromal compartments (interstitial CTB) as well as spiral arteries (endovascular cytotrophoblasts) of the decidua and upper part of the myometrium [8]. Differently to tumour cells, EVT quit cell growth and become polyploid (4N–8N) during invasive differentiation [9]. In addition, endovascular trophoblasts invade maternal spiral arteries, replace the maternal endothelium and acquire a vascular adhesion phenotype [10]. These modifications contribute to the transformation of spiral arteries into vessels with low resistance thereby promoting uteroplacental circulation [11]. The lack of endovascular trophoblasts was noticed in the placental bed of women with preeclampsia or cases of severe intrauterine growth restriction (IUGR) [12–15] suggesting that failures in EVT formation/invasion could play a critical role. Therefore, elucidation of the regulatory mechanisms controlling differentiation of EVT in humans would be helpful to better understand the pathogenesis of the gestational diseases.

Regarding signal transduction in trophoblast invasion various differential stages including proliferation, differentiation and migration/invasion need to be precisely coordinated and integrated at all times in order to guarantee successful placental development. Here, we review experimental data demonstrating a role of different signalling proteins such ERKs, PI3K, Rho/ROCK, FAK or PI3K/Akt/mTOR in extravillous trophoblast differentiation and discuss growth factors of the fetal–maternal interface acting through these pathways.

## MODEL SYSTEMS FOR STUDIES OF INVASIVE TROPHOBLAST DIFFERENTIATION

Various model systems have been developed to study human EVT differentiation/invasion in vitro. The most common way to study these processes is the use of tumorigenic (choriocarcinoma) or non-tumorigenic cell lines because they can be indefinitely propagated in the laboratory. Similarities/differences between established trophoblast cell lines with respect to trophoblast-specific gene expression have been investigated recently [16]. Here, the cell lines mainly used by investigators

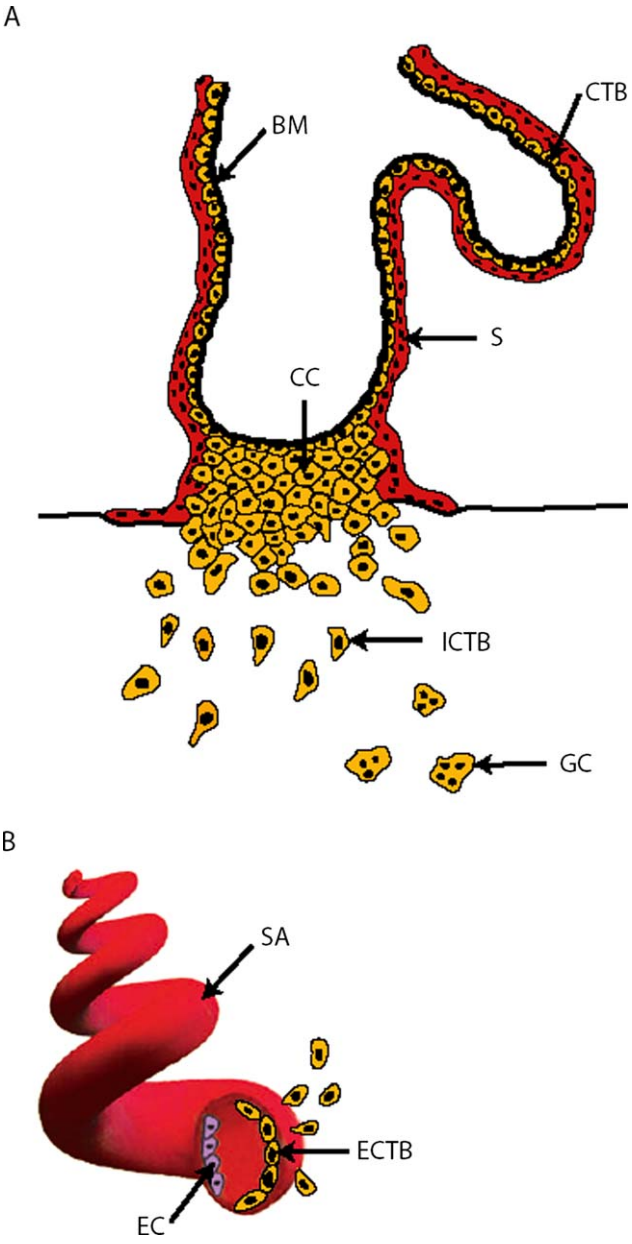


**Figure 1.** Formation and differentiation of extravillous trophoblasts. After anchorage of a mesenchymal villus at the uterine basement membrane cytotrophoblast (CTB) stem cells give rise to proliferative cell columns (CC). At distal sites non-proliferating, extravillous trophoblasts are formed which detach from the cell columns and migrate into stromal areas of the maternal decidua (formation of interstitial cytotrophoblasts, ICTB). ICTB differentiate to giant cells (GC) in deeper areas of the placental bed. Endovascular trophoblasts (ECTB), which play a role in remodelling of the vasculature, migrate into spiral arteries, replace the maternal endothelium (EC) and acquire molecular characteristics of endothelial cells. In floating villi surrounded by maternal blood, CTB stem cells fuse to form the multinucleated syncytium (S).

are described. HTR-8 cells and its SV40 large T antigen-transformed derivative, HTR-8/SVneo cells, have been generated from adherent cells after plating of minced chorionic villi of first trimester placental tissue [17,18]. Cells generated this way express markers of the EVT such as α1, α3, α5 and β1 integrin subunits [19]. The two cell lines share characteristics of EVT such as expression of cytokeratin 8 and 18 and