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Expression pattern and function of Notch2 in different subtypes of first trimester cytotrophoblast

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ABSTRACT

Introduction: Notch signalling has been shown to control cytotrophoblast (CTB) proliferation, differentiation and motility suggesting that the conserved signalling pathway could be critical for human placental development. Since individual Notch receptors have not been elucidated, we herein investigated expression pattern and function of Notch2 in different first trimester trophoblast subpopulations.
Methods: Localisation of Notch2 was analysed in first trimester placental and decidual tissues using immunofluorescence. Notch2 transcript and protein levels were studied by qRT-PCR and Western blotting in proliferative EGF receptor $(EGFR)^+$ and differentiated HLA-G^+ CTBs, respectively, isolated from early placentae by MACS. CTB migration through fibronectin-coated transwells as well as proliferation (EdU labelling) in floating villous explant cultures and primary CTBs were investigated in the presence of Notch2 siRNAs or specific antibodies blocking Notch2 cleavage.
Results: In tissue sections Notch2 expression was higher in HLA-G^+ distal cell column trophoblasts (dCCTs) compared to proximal CCTs. Accordingly, expression of Notch2 mRNA and protein were elevated in isolated HLA-G^+ CTBs compared to $EGFR^+$ CTBs. Notch2 was also detectable in interstitial CTBs as well as in intramural CTBs associated with maternal decidual vessels. Antibody-mediated inhibition of Notch2 signalling did not affect proliferation, but increased migration of SGHPL-5 cells and primary CTBs. Similarly, Notch2 siRNA treatment promoted trophoblast motility.
Discussion: Notch2 is present in differentiated cells of the extravillous trophoblast lineage, such as dCCTs, interstitial and intramural CTBs, suggesting diverse roles of the particular receptor. Notch2 signalling, activated by cell–cell contact of neighbouring dCCTs, could attenuate trophoblast migration.

1. Introduction

Human placentation involves the generation of diverse trophoblast subtypes which are essential for subsequent embryonic development. Whereas the villous syncytium produces hormones to maintain pregnancy, anchoring villi attaching to the maternal uterus give rise to extravillous trophoblasts (EVTs) invading decidual stroma and vessels. Interstitial cytotrophoblasts (iCTBs) approach the wall of maternal spiral arteries and promote vessel remodelling in conjunction with uterine NK (uNK) cells and endovascular cytotrophoblasts (eCTBs), the latter replacing maternal endothelial cells [1,2]. These processes are required for continuous, low pressure blood flow to the placenta, thereby ensuring adequate nutrition of the fetus and preventing oxidative stress [3]. Abnormal changes in myometrial vessel remodelling have been detected in pregnancy diseases such as preeclampsia and intrauterine growth restriction [4,5]; their causes however remain largely elusive. Failures in trophoblast proliferation and EVT differentiation could be involved since CTBs, isolated from preeclamptic patients, lack critical lineage marker genes and show changes in gene expression pattern when differentiating in vitro [6,7]. However, our knowledge about the differentiation program of the anchoring villus is only scarce. Whereas many different studies investigated soluble factors regulating trophoblast migration [8,9], numerous key questions concerning placental development, such as mechanisms controlling cell column proliferation and differentiation of progenitors into distinct EVT subtypes, have not been unravelled.

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The conserved Notch signalling pathway has been shown to control stem cell renewal, cell fate decisions and differentiation [10,11]. Upon cell–cell contact, membrane-anchored ligands, the Serrate-like ligands (Jagged1 and 2) and the Delta-like ligands (DLL1, 3 and 4), bind to the different Notch receptors (Notch1–4) and thereby provoke activation of the pathway. Subsequent downstream signalling involves two proteolytic cleavage steps executed by members of a disintegrin and metalloproteinase (ADAM) family and γ-secretase finally generating the Notch intracellular domain (NICD) which translocates to the nucleus. Upon binding NICD converts the transcription factor recombination signal binding protein for immunoglobulin kappa J region (RBPJκ) into a transcriptional activator. After recruitment of additional co-activators of the Mastermind-like (MAML) family the NICD-RBPJκ complex induces target genes involved in proliferation, cell lineage determination and differentiation [12].

Recent evidence suggests that the Notch signalling pathway could also be critically involved in placental development and function. Mice harbouring homozygous deletions of different Notch signalling components displayed defects in chorioallantoic branching, labyrinth formation/function or chorion-allantoic fusion [13]. Moreover, *Notch2* mutant mice die around E11.5 due to reduced blood delivery to the placenta and lower numbers of blood sinuses within the labyrinth [14]. Conditional deletion of *Notch2* in progenitors of the invasive trophoblast lineage reduced endovascular invasion, diameter of trophoblast-lined vascular canals and

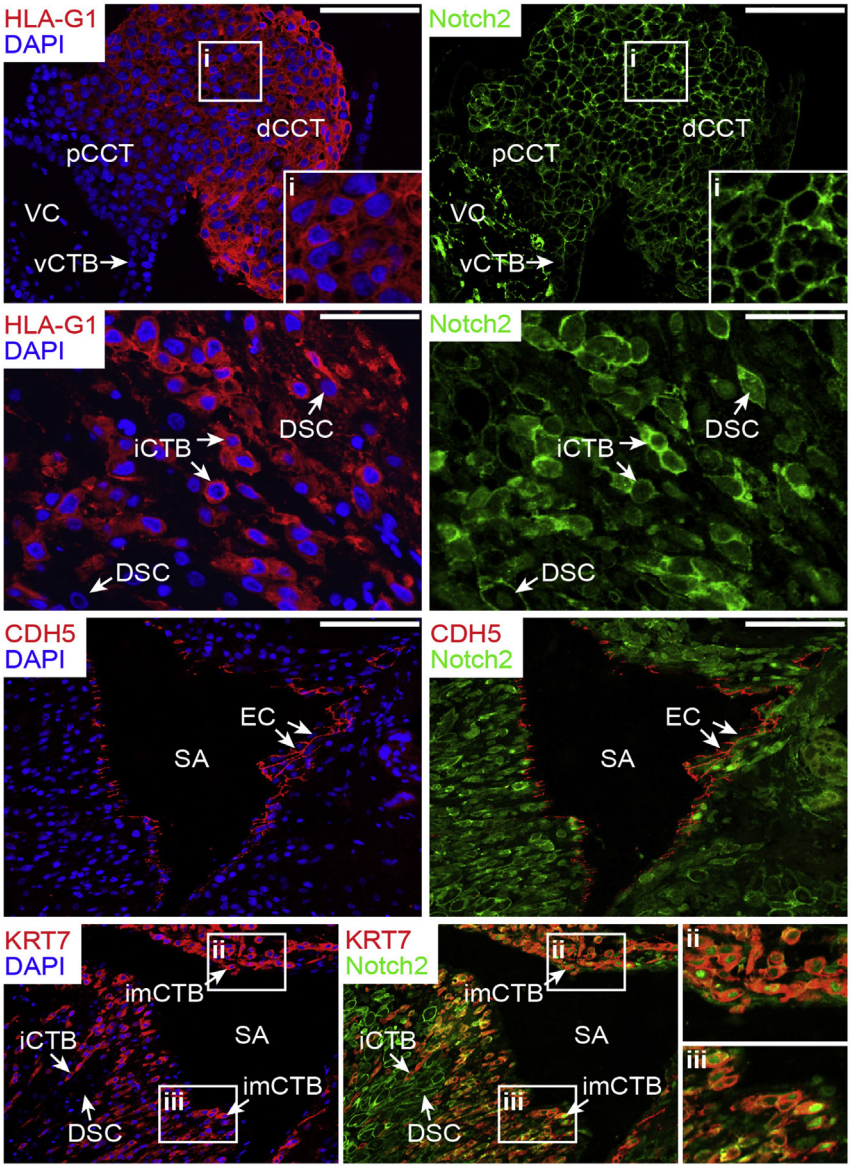

Fig. 1. Expression of Notch2 in first trimester placental and decidual tissues. Cytokeratin 7 (KRT7) and HLA-G were used to mark all CTB subtypes and EVTs. Nuclei are stained with DAPI. Representative examples showing localisation of Notch2 in cell column trophoblasts (first panel; dCCT, distal cell column trophoblast; pCCT, proximal cell column trophoblast; 12th week placenta, scale bars represent 100 μm), in interstitial cytotrophoblasts (iCTBs) of the decidua basalis (second panel; 12th week, scale bars represent 50 μm), and in intramural cytotrophoblasts (imCTB), associated with maternal blood vessels (fourth panel; 12th week decidua basalis, digitally zoomed). In all placentae analysed (n = 4, between 6th and 12th week of gestation) expression was weaker in pCCTs and villous cytotrophoblasts (vCTB) compared to non-proliferative, HLA-G$^+$ dCCTs. Inserts (i) depict digital magnifications, showing HLA-G and Notch2 in dCCTs. Notch2 was also present in the villous core (VC) as well as in decidual stromal cells (DSC), some of which showed nuclear staining as published [26]. VE-cadherin (CDH5) stained endothelial cells (EC) in spiral arteries (SA) present in maternal decidua (third panel; 12th week decidua basalis, scale bars represent 100 μm). Partial disruption of the maternal EC layer/VE-cadherin staining depicted in (ii) suggested on-going vessel remodelling. imCTBs in (ii) and (iii) showed nuclear Notch2 staining. Selected areas (ii) and (iii) on the right-hand side represent magnified overlays of KRT7$^+$/Notch2$^+$ imCTBs with nuclear N2ICD.