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Expression patterns of Notch receptors and their ligands Jagged and Delta in human placenta

F. Herr [a], I. Schreiner [b], N. Baal [b,c], C. Pfarrer [d], M. Zygmunt [a,*]

[a] Department of Obstetrics and Gynecology, University of Greifswald, Germany
[b] Department of Obstetrics and Gynecology, University of Giessen, Germany
[c] Department of Immunology and Transfusional Medicine, University of Giessen, Germany
[d] Department of Anatomy, University of Veterinary Medicine Hannover, Germany

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ABSTRACT

The establishment of an appropriate fetomaternal vessel system is a prerequisite for prevention of pregnancy associated pathologies. Notch receptors and ligands are manifoldly involved in vascular development and angiogenesis. To further characterize the process of human placental vasculo- and angiogenesis we investigated the expression pattern of Notch receptors and their ligands during pregnancy.

Real time RT-PCR, immunohistochemistry and flow cytometry analysis were performed in early (6–12) weeks of gestation (w.o.g.) and late placenta (37–41 w.o.g.). To specify the exact cellular localization immunofluorescent labelling of epithelial and endothelial cells (EC), respectively, with cytokeratin-7 and vonWillebrand factor (vWF) was done. One placenta from a patient with Alagille syndrome (AGS) was examined with real time RT-PCR and immunohistochemistry.

The receptors Notch2, -3, -4 and their ligands Jagged1, -2 and Delta1, -4 were detected at both the mRNA and protein level in early and late placenta. Notch1 was only detected at protein level. The expression was found mainly in the stromal compartment: placental EC expressed Notch1, Delta4, Jagged1 and Delta1. A strong Jagged1 expression was found in the endothelium of arteries and veins supporting a role in differentiation of capillaries. Hofbauer cells (HC) primarily displayed the receptors Notch2, -3 and -4. Placental stromal cells (SC) were positive for Jagged2. The syncytiotrophoblast (ST) and cytotrophoblast (CT) cells revealed a weak but detectable co-localization with cytokeratin-7 and Notch1, -3 and Delta1. These results were verified by flow cytometry of freshly isolated placental cells of placental tissue. Interestingly Jagged1 expression was absent in endothelial cells from an AGS placenta.

The Notch receptors and their ligands are expressed in human placental ST, CT, EC, SC and HC. The distribution pattern of Notch receptors and their ligands suggests their involvement in the process of placental vasculo- and angiogenesis via cell-cell communication between trophoblast, -stroma and endothelial cells.

Abbreviations: AGS, (Alagille syndrome); vWF, (vonWillebrand factor); CADASIL, (cerebral autosomal dominant arteriopathy with subcortical infarction and leukoencephalopathy); RBP- Jκ, (synonym for CSL, CBF-1, suppressor of hairless, LAG-1, DNA binding protein); w.o.g, (weeks of gestation); RT-PCR, (reverse transciptase polymerase chain reaction); ST, (syncytiotrophoblast); CT, (cytotrophoblast cells); HC, (Hofbauer cells); SC, (stromal cells); EC, (endothelial cells); Hope-solution, (Hepes Glutamic Acid Buffer mediated Organic Solvent Protection Effect); NCBI-BLASTn, (National Center of Biotechnical Information-Basic Local Alignment Search Tool-nucleotide); Cat.No, (catalogue number); TBS, (5 mM Tris, 15 mM NaCl); DAB, (3,3′-diaminobenzidine); CD, (cluster of differentiation); PBS, (phosphate buffered saline); FCS, (fetal calf serum); FITC, (fluorescence isothiocyanate); PE, (phycoerythrin); DAPI, (4′,6-diamidin-2-phenylindol); EDTA, (ethylendiamintetraacetat); FACS, (fluorescent activated cell sorter); PFA, (paraformaldehyde); VEGF, (vascular endothelial growth factor); hCG, (human chorionic gonadotropin); IGF-II, (insulin-like growth factor-II); AFP, (alpha-fetoprotein); n.s, (not significant).

* Corresponding author. Department of Obstetrics and Gynecology, University of Greifswald, Ferdinand-Sauerbruch-Strasse, D-17475 Greifswald, Germany. Tel.: +49 3834 86 65 00; fax: +49 3834 86 65 01.

E-mail addresses: Friederike.Herr@uni-greifswald.de (F. Herr), isabel.schreiner@web.de (I. Schreiner), Nelli.Baal@immunologie.med.uni-giessen.de (N. Baal), Christiane.Pfarrer@tiho-hannover.de (C. Pfarrer), Zygmunt@uni-greifswald.de (M. Zygmunt).

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1. Introduction

The process of differentiation of cells in human placenta is essential for the establishment of placental vascular system and is a prerequisite for an adequate nutrient and substrate supply of the developing fetus. An insufficient placental vascular development results in intrauterine growth retardation and is associated with increased cardiovascular morbidity in adult life [1]. To date, the role of vascular cell differentiation in the process of placental vasculo- and angiogenesis is not completely understood. The differentiation of cells in human placenta seems to result from intensive interaction between mesoderm, trophoblast and endothelial cells [2].

The Notch pathway is involved in many developmental and cell type specification processes. Notch signaling is strictly context-dependent and differs in strength, timing and cell type. The Notch receptors and ligands are highly conserved in vertebrates [3]. Mammals have four Notch receptor proteins (Notch1-4) that are membrane-bound single-pass transmembrane receptors. The large extracellular domain contains a variable number of epidermal growth factor (EGF)-like repeats that are critical for binding interactions [4]. The Notch ligands Delta1, Delta3, Delta4, Jagged1 and Jagged2 are also membrane bound and contain a variable number of EGF-like repeats [3].

The specificity of a Notch receptor to its ligand results from glycosylation [5]. Via ligand binding Notch receptor activation results in proteolytic cleavage of the intracellular domain and its translocation into the nucleus. Within the nucleus this domain interacts with the DNA-binding protein RBP-Jκ. Therefore Notch signaling is often viewed as a transcription cascade [6].

Binding of Notch receptors to their ligands plays a central role in the process of differentiation of unspecific cells in many different species. Interestingly, the differentiation process can either be activated or inhibited via receptor-ligand binding [7]. With Notch being expressed in both the inner cell mass and the trophectoderm of human blastocysts [8] evidence is given that Notch and their ligands are involved in very early cell fate decisions in the placenta.

Defects in the Notch receptor-ligand system have major impact on various organ functions. For example, a mutation of JAGGED1 in human results in the autosomal dominant AGS with a variability in clinical findings, including cholestasis caused by bile duct paucity and pulmonary tree stenosis, as well as posterior embryotoxon in the eye, typical facial features and butterfly vertebrae [9]. The incidence of AGS is 1:70,000 while the mortality is approximately 10% with vascular accidents, cardiac disease, and liver disease accounting for most of the deaths [10].

Further evidence for a critical function of Notch in vascular development and homeostasis can be concluded from the symptoms observed in a mutation of the human NOTCH3 gene, which causes stroke and vascular dementia and is called CADASIL (cerebral autosomal dominant arteriopathy with subcortical infarction and leukoencephalopathy) [11].

A role for Notch receptor and their ligands in angio- and vasculogenesis has been described for several species and compartments. Initially, the Notch signaling pathway was implicated as a prime player in zebrafish, specifically in establishing arterial versus venous vessel type [12]. Mice embryos with a homozygous defect of DELTA4 major arteries and veins develop until they reveal severe defects in artery development from day 8,5 on. A reduced aortic diameter, an abnormal accumulation of EC in apical portion of intersomitic vessels resulting in misdirection between aorta to lateral vessels in this region are characteristic. Furthermore, the yolk sac vascular plexus reveal a highly abnormal circulation with angiogenic remodelling defects and persistence of primary capillary bed [13]. DELTA4 seems to act on the arterial endothelium through the receptors Notch1 and -4 primarily in an autocrine manner. Double mutants of NOTCH1 and -4 have a similar phenotype as DELTA4 knockout mice, including the loss of arterial specification [14]. Besides that, knockout mice for Notch1 die at embryonic stage due to vascular defects including a fail of vessels invading the placental labyrinth [15]. The involvement of Notch into cancer development led to numeral experimental designs of anti-cancer therapy in addition to established anti-VEGF medication [16].

Notch signaling might also be important in determining the basic pattern and number of branch points within the process of angiogenesis. Several in vitro studies show a role for active Notch signaling in restricting branching morphogenesis and in vivo mutation studies support this. Loss of DELTA4 [13] or NOTCH1 [14] lead to excessive and misdirected intersomitic branching, while activation of Notch signaling in the endothelium suppresses branching of vessels. This suggests that during normal development, activation of the Notch signaling pathway in developing vessels is required for a proper differentiation and maturation of blood vessels.

Concerning the fetomaternal unit only a few studies have been published on the expression pattern of Notch receptors and their ligands. With Notch1, -4 and Jagged1 just a limited number of Notch family members have been localized in human placenta [17]. An analysis of Notch1 expression in total human tissues revealed an intermediate to high immunohistochemical expression in the decidua, which was in contrast with low levels observed in placental villous syncytiotrophoblast. In the arterial endothelium a moderate immunostaining was shown, whereas the level of expression was almost undetectable in veins [18].

Determining the localization of all Notch receptors and ligands within the human placenta is essential for a better understanding of their role in differentiation process of cells in the fetomaternal unit.

2. Materials and methods

2.1. Placental tissue

For mRNA and immunohistochemical analysis human placental tissue of first and third trimester was used with a written consent as approved by the ethics committee of the Justus-Liebig-University. Placental tissue from first trimester was collected from legal abortions (6. -12. w.o.g. $n = 14$). Third trimester placental tissue was received from term pregnancies following cesarean sections (37. -41. w.o.g., $n = 12$). Exclusion criteria were preterm delivery, amnion infection syndrome, gestational diabetes or intrauterine growth retardation. The placenta from the patient with AGS was 36. w.o.g. Termination of pregnancy of AGS patient was done by cesarean section due to cardiotocography changes, growth retardation and breech presentation. The patient had typical facial features like prominent forehead, deep-set eyes with moderate hypertelorism, pointed chin, and saddle nose with a bulbous tip, spinal cord scoliosis with butterfly vertebrae, skin dryness from hypercholesterolemia and intermittend cholestasis, and pulmonary stenosis. The newborn child was growth retarded (1750 g, 40 cm) and developed AGS in later life. Tissue was taken from three different localizations of all placentae: marginal zone, central fetal- and central maternal– site. Extravillous trophoblast was excluded via macroscopic visual tissue sampling: Villous trees were collected from early first trimester placentae, whilst third trimester cotelydons were dissected and the amnion, chorionic plate and decidua discarded. The purity of isolated villous tissue was verified by flow cytometry (cytokeratin-7 for trophoblast and vimentin for stromal cells and vWF for endothelial cells). Placental tissue was immediately transferred into cryotubes at −198 °C to reduce RNAase activity for mRNA analysis. In parallel, pieces of $1 \times 1 \times 1$ cm were directly transferred into Hope-solution (Hepes Glutamic Acid Buffer mediated Organic Solvent Protection Effect DCS Innovative Diagnostik Systeme, Hamburg, Germany) followed by paraffin embedding for immunohistochemical analysis or transferred to mechanical and enzymatic digestion for flow cytometry analysis.

2.2. Real time RT-PCR for Notch receptors and ligands in human placental tissue of first and third trimester

Real time RT-PCR with SYBR green chemistry was performed as described before [19]. Briefly, total RNA was isolated from either freshly acquired or frozen placental tissue (−80 °C) using a Qiashredder (Qiagen, Hilden, Germany) and Trizol (Invitrogen, Darmstadt, Germany) following the manufacturer's protocol. Purity and yield of the