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Review

Notch Signaling Pathway and Human Placenta

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Abstract

Notch signaling was evolutionarily conserved and critical for cell-fate determination, differentiation and many other biological processes. Growing evidences suggested that Notch signaling pathway played an important role in the mammalian placental development. All of the mammalian Notch family proteins had been identified in human placenta except Delta-like 3, which appeared to affect the axial skeletal system. However the molecular mechanisms that regulated the Notch signaling pathway remained largely unknown in human placenta. Therefore, additional research was needed to investigate expression pattern of Notch family members and the mechanisms for activation of Notch signaling pathway in human placenta, which might help elucidate the roles of Notch signaling pathway in human placentation. This review would focus on the roles of Notch receptors and ligands in the human placental trophoblasts function and placental angiogenesis. It might hopefully provide perspectives for future research about human placentation of pregnancy complicated by preeclampsia and other placenta associated diseases.

Key words: Notch pathway, human, placenta, preeclampsia.

Introduction

In 1917, Thomas Hunt Morgan first described the Notch gene following the observation of notches on the wings of fruit flies (*Drosophila melanogaster*) caused by partial loss of function of the *Notch* gene [1]. Notch signaling was an evolutionarily conserved pathway from *Drosophila* to humans and played an important role in the regulation of cellular proliferation, differentiation and apoptosis. It arose with the evolution of multicellular organisms and the concomitant need for juxtacrine cell-to-cell communication to coordinate development [2]. The mammalian Notch signaling pathway consisted of four Notch receptors and five Notch ligands [3, 4]. In general, interaction of Notch receptors and ligands between neighbor cells activated the Notch signaling pathway [2, 3, 5, 6]. The published studies demonstrated that Notch signaling pathway was necessary for the development of placenta. Defects in the Notch receptor-ligand system had adverse impact on the placentation [7]. There were some discrepancies between results from different studies about Notch family members in human placenta. And the mechanism for the role of Notch signaling pathway in human placenta was largely unknown. Therefore, this paper reviewed the studies about Notch signaling pathway in human placenta and provided basis for the further research of the role of Notch signaling pathway in human placenta.

Molecular structure of Notch

Notch functioned as a receptor. Mammals had four Notch receptors (Notch1, Notch2, Notch3 and Notch4). The ligands included Jagged 1 (Jag1), Jag2 (homologues of serrate) and Delta-like proteins (Dll1, Dll3, Dll4 in mammals) [3, 4]. Notch and its ligands were single-pass transmembrane heterodimers. The extracellular domains of both Notch and its ligands

consisted of multiple epidermal growth factor (EGF) repeats, which could be modified by the addition of sugar moieties. Notch receptors also contained several domains that maintained the receptor in an inactivated state in the absence of a ligand [8]. Notch protein consisted of the extracellular domain (NECD), the transmembrane domain (TM), and the intracellular domain (NICD) with transcriptional activity [9, 10]. The extracellular region of the Notch receptor contained 10–36 EGF-like repeats essential for ligand binding and three copies of juxtamembrane repeats motif known as Lin-12-Notch Repeats (LNR) which modulated interactions between the extracellular and intracellular domains of Notch [11]. The NICD was composed of several domains, including a Rbp-associated molecule (RAM) domain that was involved in interactions with CBF-1, Suppressor of Hairless, Lag-2 (CSL, also called recombination signal sequence-binding protein J, RBPJ), an ankyrin (ANK) repeat domain, a transcription activation domain (TAD) and a C-terminal region rich in proline (P), glutamic acid (E), serine (S) and threonine (T) [8]. It was becoming increasingly clear that the NICD was subject to a variety of post-translational modifications, including phosphorylation, ubiquitylation, hydroxylation and acetylation [12].

Canonical and non-canonical Notch signal pathways

Notch could canonically and non-canonically exerted its biological functions. The canonical Notch pathway initiated when Notch ligands bound to the EGF repeats 11-12 and 24-29 of the NECD from adjacent cells, followed by sequential cytoplasmic cleavage of NICD [13-15]. At the molecular level, triggering of Notch receptor by ligand binding promoted two proteolytic cleavage events at the Notch receptor [16]. The first proteolytic step after binding of Notch receptors to their ligands was mediated by metalloprotease tumor necrosis factor-alpha-converting enzyme, also known as a disintegrin and metalloprotease 17 (ADAM17), on extracellular part of the receptor. The cleaved extracellular subunit of the receptor was"trans-endocytosed" by the neighbouring ligand-expressing cells [17]. This process seemed to be controlled by Neutralized or Mindbomb E3 ubiquitin ligases [18]. Binding of extracellular ligand to Notch also induced the second proteolytic cleavage event at the transmembrane region by a γ-secretase that depended on presenilin-1 [19, 20]. This cleavage could release a membrane tethered form of the Notch NICD [14]. The liberated NICD translocated to the nucleus, where NICD interacted with the DNA-binding transcription factor CSL resulting in the transcriptional activation of Notch targeting genes. The Notch targeting genes included the *Hairy-Enhancer of Split (HES)* and *HES-related proteins* (*HERP*, also called *Hey/Hesr/HRT/CHF/gridlock*) genes [21-24]. In the absence of NICD, the DNA-binding protein CSL recruited corepressor complexes to repress transcription of Notch targeting genes [25]. However, in the presence of NICD, the NICD interacted with CSL, followed by recruiting a coactivator complex composed of mastermind-like proteins (MAML-1) and other chromatin modifying transcription factors, which resulted in the transcriptional activation of Notch targeting genes [26]. The canonical Notch pathway was very simple: there were no second messengers. Non-canonical Notch signaling was CSL-independent and could be either ligand-dependent or independent. The most well-studied and conserved effect of non-canonical Notch function was regulation of Wnt/β-catenin signaling: Notch bound and titrated levels of the obligate Wnt-signaling component active β-catenin [27].

It was reported that interaction between ligands and receptors could both activate and inhibit Notch signaling pathway. Cell-cell interaction (trans-interactions) could regulate the Notch signaling pathway. And Notch ligands could also regulate the Notch signaling pathway by binding to the Notch receptors within the same cell (cis-interactions) [28]. In general, the trans-interactions between Notch receptors and ligands activated the Notch signaling pathway, whereas the cis-interactions were believed to inhibit the Notch signaling pathway [3].

Notch signaling pathway and human placenta

Notch signaling pathway might exert a role throughout the pregnancy. Afshar et al. found that Notch1 signaling modulated uterine decidualization which was essential for implantation [29]. Another paper from Afshar et al. demonstrated that Notch1 underwent up-regulation by chorionic gonadotropin in combination with estrogen and progesterone, followed by down-regulation during the peri-implantation period of pregnancy. It was crucial for a successful pregnancy [30]. Members of the Notch signaling pathway had been detected in the developing placenta and had been shown to play an important role in the normal development and function of the placenta [31, 32]. It was also found that Notch members were activated in subsets of trophoblasts [33].

For successful placentation to occur, a highly orchestrated control of trophoblast functions, vasculogenesis, and angiogenesis was required [34].

Trophoblast functions included differentiation, proliferation, migration and invasiveness (interstitial invasion and endovascularinvasion). The cytotrophoblast differentiated into the syncytiotrophoblast and the extravillous cytotrophoblast. Trophoblast differentiation to the invasive extravillous phenotype was integral to implantation and invasion of the uterus. As a result, trophoblasts underwent a phenotype change from an epithelial to an endothelial one, which was described as pseudovasculogenesis [35-38]. Pseudovasculogenesis was one of the key processes that became impaired in the placentas of women with preeclampsia (PE) [37].

Adequate invasion of the human placenta during the first weeks of pregnancy was a critical step to ensure both fetal and maternal health. It was now clear that inadequate placental invasion had been associated with such reproductive complications as PE, fetal growth retardation, and recurrent pregnancy loss [39, 40].

During development, the Notch signaling pathway played a critical role in patterning tissues by regulating proliferation, cell death and specifying cell fate determination [3]. Trophoblast differentiation, proliferation and invasiveness were essential for the development of placenta. Therefore the Notch signaling pathway might regulate the trophoblast function. In fact, there were growing evidences that trophoblast expressed some Notch receptors and ligands, which supported the idea that the Notch signaling pathway regulated the trophoblast function.

Notch signaling pathway and trophoblast

The reports about expression of Notch receptors and ligands in human placenta were inconsistent. Table 1 showed the expression of Notch receptors and ligands in human trophoblasts from published literatures.

De Falco et al. and Herr et al. showed that Notch1 was expressed in cytotrophoblasts (CTBs) [31, 42]. However, Hunkapiller et al. found that CTBs did not express Notch1 [41]. There were also some contradictions to results about other Notch receptors and ligands from different studies. These differences may be attributed to the use of different antibodies, differences in the experimental systems and the heterogeneity of the placental tissues. In addition, Hunkapiller et al. revealed the complex spatial and temporal expression patterns of Notch receptors and ligands in human placenta [41]. For example, they found that immunostaining for Notch2 was either absent or weak in CTB progenitors while the expression was dramatically upregulated in the CTB cell columns as invasion began. But the mechanisms by which Notch molecules were expressed spatially and temporally remained to be elucidated. Taken together, the presence of Notch receptors and ligand in different placental trophoblasts might suggest an involvement of Notch pathway in trophoblast differentiation program and invasion of EVT cells. There was no direct proof that Notch signaling pathway involved in human trophoblast proliferation so far. Sahin et al. found that the decrease of Notch proteins immunostaining in fetal growth retardation (FGR) placentas coincided with a reduction in placental weight [43]. From this result, they speculated that Notch proteins might also play a role in cell proliferation within the placenta. So further studies were needed to elucidate the role of Notch signaling pathway in trophoblast proliferation. As shown in Table 1, several Notch proteins were coexpressed in the same cell. Rizzo et al. found that Notch1 overexpression up-regulated Notch4 expression, whereas Notch 1 knockdown down-regulated Notch 4 in breast cancer cell lines [44]. Hence, it seemed likely that there was some relationship between these Notch proteins coexpressed in the same placental cell, which further studies were necessary to decipher.

Notch signaling pathway and human placental angiogenesis

Notch receptors and ligands were involved in vascular development and angiogenesis. The immunostaining of Notch family members in the vessels of normal term placenta was confirmed by some studies. Herr et al. revealed the immunohistochemical localization of Notch receptors and ligands in human placental vessels [42]. They found that Notch1 receptor and the Notch ligands, Jag1, Dll4 and Dll1 were mainly localized in EC in tertiary villi, while Jag1 was additionally detected in perivascular cells. And it was also found that Jag1 was mainly expressed in large vessels and perivascular cells, whereas Dll4 was found in capillaries of placental villi. Moreover, they examined one Alagille syndrome (AGS) placenta with a mutation of *Jagged1* and detected no Jag1 expression in EC of the placenta. It was intriguing that the number and types of vessels seemed not to be reduced in AGS placenta. Maybe a further systematic analysis of those criteria in a population of AGS could verify the functional role of Jag1 in human placenta. Sahin et al. reported that the endothelial cells of normal human placentas were intensely immunostained for Notch1 in both basal (maternal) side and chorionic plate (fetal) side [43]. The data from De Falco et al. showed that a moderate Notch1, intense Nontch4 and intense Jag1 immunopositivity was evident in the cytoplasm of endothelial cells of placental villi [31]. Kume re-

viewed that Notch1, Notch4, Dll1, Dll4 and Jag1 were predominantly expressed in vascular endothelia cells [45], which was in agreement with the description in Table 1. The discrepancy about expression of Notch receptors and ligands in placental vessels from different papers might mirror the spatial and temporal expression pattern of Notch family members in placental angiogenesis. Further studies about the exact expression of Notch family members by placenta endothelial cells were needed to perform. The results from the other study supported the association of Notch receptors and their ligands with the vascular system [46]. Taken together, the immunostaining of Notch family members in the endothelial cells of human placenta suggested the role of Notch signaling pathway in placental angiogenesis process. However, the molecular processes by which members of the Notch pathway exerted their roles were still not well understood.

Placental Notch pathway and preeclampsia

As described above, the Notch signaling pathway exerted important role in normal development of human placenta. Therefore the defect of Notch signaling pathway would contribute to the pregnancy complications, such as PE. In fact, in PE complicated placentas, Sahin et al. detected a significant decrease in the immunoreactivity of Notch proteins compared to normal term placenta [43]. This was consistent with the data published by Cobellis et al. Cobellis et al. demonstrated that Notch proteins were decreased significantly in preeclamptic placentas compared with controls in the term of gestation, which had been proposed to explain the onset of PE [47]. Sahin et al. proposed that the decreased immunoreaction of Notch1 in vascular endothelial cells in PE placentas might mirror the disturbance of the feto-placental vascular system as a result of the distracted role of Notch proteins in vasculogenesis and angiogenesis [43]. Cobellis et al. also found the decrease of Notch1 expression level in placentas from PE, suggesting that normal Notch1 expression was required for normal human placental function [47]. Hunkapiller et al. failed to detect any differences in CTB Notch2-4 and Dll4 expression between two groups. However, they found that a CTB Jag1 signal was absent in many vessels from PE cases while it was intense in preterm labor placentas [41]. Taken together, they speculated that the correlation between reduced Jag1 expression and failed vascular remodeling in PE suggested an important functional role for Notch signaling in remodeling human spiral arterioles [41]. Overrepresented Notch signaling was also detected in the decidua basalis of PE by the transcriptional profile [48]. All of these studies indicated the roles of Notch signaling pathway in the pathogenesis of PE.

Table 1. Notch molecules expression in human placenta.

	cytotrophoblast			**syncytiotrophoblast**			**EVT**			**Stromal cell**			**EC**		
	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C
Notch1	++	–	++	+++		++	+++			++		–	++		++
Notch2		++	–			–									–
Notch3		++	++			++						++			–
Notch4	++	++	–	+		–	++			++			++		–
Jag1	+++	++	–	+++		–	+++	+++†		+++			+++		++
Jag2		–	–			–						++			–
Dll1		–	++			+									+++‡
Dll4		–	–			–		++							++

A De Falco et al. (2007) [31]
B Hunkapiller et al. (2011) [41]
C Herr et al. (2011) [42]
EC endothelial cell
EVT extravillous trophoblast
† perivascular and endovascular cytotrophoblasts
‡ placental blood vessels
+ faint immunopositivity
++ moderate immunopositivity
+++ intense immunopositivity

Conclusions

In summary, the presence of Notch receptors and ligands in different human placental trophoblasts might suggest an involvement of Notch pathway in trophoblast differentiation program. Reported studies supported the role of Notch pathway in invasion of EVT. And the expression of Notch receptors and ligands might be spatial and temporal. The expression of various Notch family proteins in placental blood vessels implied that the Notch signaling pathway was also involved in the placental angiogenesis. However, many questions remained to be answered. First, which factors induced the activation of Notch signaling pathway in human placentation? The upstream signaling pathways or factors that controlled the expression of Notch family members in human placental trophoblasts and blood vessels also remained largely unclear. Second, most of the studies about the roles of Notch signaling pathway in human placentation were descriptive analysis so far. Hence, to resolve these questions, further studies including *in vitro* and *in vivo* ones, were needed to undertake, especially the studies in primates whose placental structure was similar to human placenta.

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Competing Interests

The authors have declared that no competing interest exists.

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