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Original contribution

Alteration of Delta-like ligand 1 and Notch 1 receptor in various placental disorders with special reference to early onset preeclampsia ☆

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Summary Notch signaling pathway has been shown to be dysregulated in placentas with preeclampsia, but there has been a lack of studies on methylation of Notch family genes in this disorder. We therefore executed methylation-specific polymerase chain reaction and immunostaining for Notch 1 receptor and the activating ligand, Delta-like (DLL) 1, with placental tissues from cases of preeclampsia (early onset, n = 18; late-onset, n = 19) and other placental disorders, including maternal complications such as diabetes mellitus and collagen disease (n = 10), fetal growth restriction (n = 17), fetal anomaly (n = 23), preterm birth (n = 15), miscarriage (n = 25), and hydatidiform moles (n = 9) as well as term births (n = 12). The frequency of *DLL1* methylation was higher in early onset preeclamptic placentas (61%) than the other subjects (0%-36%; $P \leq .016$). Appreciable samples (36%) of miscarriage also represented *DLL1* methylation. None of the samples studied showed Notch 1 methylation. On gestational period–matched analysis, the rate of *DLL1* methylation was higher in early onset preeclampsia (83.3%) than preterm birth (13.3%; $P < .001$), with no significant differences in clinical backgrounds between the two. In this analysis, increase of syncytial knots and accelerated villous maturation were most prominent in *DLL1*-methylated placentas with early onset preeclampsia. Notch 1 and DLL1 expressions in villous trophoblasts and endothelial cells were significantly lower in early onset preeclamptic placentas as compared with preterm birth controls. In conclusion, altered Notch signaling via methylation of *DLL1* is likely involved in possible disease-related mechanisms of early onset preeclampsia. Alternatively, *DLL1* methylation in early onset preeclampsia could be a manifestation of a lack of placental maturation, similar to miscarriage.

1. Introduction

Preeclampsia (PE) is one of the important complications of human pregnancy and a leading cause of both maternal and neonatal morbidity and mortality; women with PE are at increased risk of cardiovascular complications [1] and intrauterine growth restriction as an important trigger of

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perinatal mortality [2] and development of coronary heart disease, hypertension, and diabetes in adult life with low–birth-weight babies [3]. The 2 stage model of PE proposes that a poorly perfused placenta (stage 1) produces factors leading to clinical manifestations (stage 2); stage 1 is not sufficient to cause the maternal syndrome but interacts with maternal constitutional factors (genetic, behavioral, or environmental) to result in stage 2 [4,5]. Although the pathophysiology of PE remains largely unknown, shallow trophoblast invasion and deficient remodeling of uterine spiral arteries are thought to contribute to the disease pathogenesis [6,7]. In addition, morphological studies have confirmed an increase of syncytial knots or terminal villi vessels, thickening of vasculosyncytial membrane, and the presence of larger fibrin deposits in PE-complicated placental tissue [8,9]. It is also known that PE can be further subclassified into early onset and late onset, with differences regarding clinical presentation and outcome [2,10] as well as morphological characteristics of the placental tissues [11].

The Notch signaling family of receptor-ligands regulates cellular processes as diverse as proliferation, apoptosis, differentiation, invasion, and adhesion, with particularly important roles in vascular patterning [12,13]. In humans, there are 4 Notch receptors (1-4) and 5 membrane-bound Delta-like ligands (DLL1, 3, 4) and Jagged (1, 2) ligands for canonical Notch signaling [14]. Notch ligand-receptor binding leads to receptor cleavage by proteases and release of the intracellular domain that translocates to the nucleus and induces gene transcription of Notch target genes [15]. The Notch pathway has been demonstrated to be involved in multiple aspects of vascular development and angiogenesis [16,17]. In vitro study, during ischemia-induced arteriogenesis, shows endothelial DLL1 expression was strongly induced, and angiogenic growth factors synergistically activated Notch signaling by induction of DLL1 in human arterial endothelial cells [16]. Although DLL4-mediated activation has been shown to suppress vascular endothelial growth factor (VEGF) pathway components in growing capillary beds, DLL1-Notch signaling was required for VEGF receptor expression, and in the absence of DLL1 function, VEGF receptor were down-regulated in mutant mouse arteries [17].

Angiogenesis as an essential component of placenta development occurs in the placental bed in which extravillous trophoblast cells within the maternal decidual tissue transform the spiral arteries into resistance vessels; in this line, great emphasis has been focused on placenta tissue in which vascular remodeling is important for normal fetal development and growth and in which alterations of these elements cause PE [4,5]. Several studies have identified dysregulated expression of Notch family members in placentas with PE [18-26]. For Notch 3, case results have been varied, that is, up-regulated [20], down-regulated [23,25], or normoregulated [26], whereas Notch 1, Notch 2, and Notch 4 expression appears to be consistently down-regulated in placentas with this disorder [18,23]. At the same time, DLL4 expression has been shown to be increased in PE-complicated placentas [26]. Epigenetic alterations of nonimprinted gene such as *TIMP3* or *maspin* are implicated in the pathogenesis of PE [27,28]. In this line, epigenetic variation of the Notch signal pathway may be associated with the disease-related mechanisms of PE, but there has been a relative lack of epigenetic studies on Notch family genes in this disorder.

We therefore investigated the methylation status of *DLL1* and *NOTCH 1* and expression of those proteins in placental tissues with early onset or late-onset PE as compared with other diseases. In addition, we assessed clinicopathological differences in early onset PE with and without methylation of these Notch family genes.

2. Materials and methods

2.1. Patients and materials

The materials for our study were 148 samples of placental tissue (from 136 patients and 12 healthy individuals) obtained by vaginal delivery, cesarean section, or intrauterine curettage at Juntendo University Hospital between 2011 and 2014. These comprised PE (early onset, 21-33 weeks of gestation at onset/25-35 weeks of gestation at delivery, n = 18; late onset, 34-39 weeks at onset/34-40 weeks at delivery, n = 19); maternal complications (34-39 weeks of gestation, n = 10) including gestational diabetes mellitus (n = 2), diabetes mellitus (n = 3), and collagen disease (n = 5); fetal growth restriction (25-40 weeks of gestation, n = 17); fetal anomaly (31-39 weeks of gestation, n = 23); preterm birth (24-32 weeks of gestation, n = 15); miscarriage before 21 weeks of gestation (6-20 weeks of gestation, n = 25); and hydatidiform mole (8-11 weeks of gestation, n = 9) as well as term births (37-40 weeks of gestation, n = 12). A review of all the medical records of patients or individuals who had their placental tissues included in this study was conducted, aimed at collecting information on the gynecologic background.

Preeclampsia was defined as hypertension (systolic blood pressure ≥140 mm Hg and diastolic blood pressure ≥90 mm Hg after 20 weeks of gestation) and proteinuria (≥300 mg in a 24-hour urine collection or 1 dipstick measurement of ≥1+) according to the criteria of the National High Blood Pressure Education Program Working Group on High Blood Pressure in Pregnancy [29]. Preeclampsia was further subclassified into early onset (<34 weeks) and late onset (≥34 weeks) according to previous reports [2,10].

This study was approved by the ethical committee of our hospital (registration no. 2012174).

2.2. Methylation analysis of DLL1 and Notch1

Genomic DNA was extracted from 5 10-μm-thick formalin-fixed, paraffin-embedded sections of one representative block for each case using a QIAamp DNA FFPE