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Differential Expression of ADAM (a Disintegrin and Metalloproteinase) Genes between Human First Trimester Villous and Extravillous Trophoblast Cells

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Abstract

A disintegrin and metalloproteinases (ADAMs) are members of the metzincin family of zinc-dependent metalloproteinases that play pivotal roles in the proteolytic degradation of the extracellular matrix for cell invasion. Few studies have investigated the ADAM subtypes that are expressed in first trimester trophoblast cells. The purpose of this study was to elucidate the differential expression profiles of ADAMs between first trimester villous trophoblast cells (VTs) and extravillous trophoblast cells (EVTs). We isolated EVTs from explanted human first trimester chorionic villi and investigated the mRNA expression levels of five members of the ADAM family (*ADAMTS1*, *ADAMTS2*, *ADAM10*, *ADAM12*, and *ADAM17*) using real-time PCR. Chorionic villous tips were defined as first trimester VTs. Of the differentially expressed ADAM genes between first trimester VTs and EVTs, *ADAMTS1* was expressed at a significantly higher level in EVTs than in VTs. In contrast, both *ADAM10* and *ADAM12* were expressed at significantly higher levels in VTs than in EVTs. No differences were found in the mRNA levels of *ADAMTS2* and *ADAM17* between the two cell types. Moreover, we demonstrated that in VTs, the expression level of *ADAM12* was significantly downregulated in the late first trimester (10–13 gestational weeks) compared to the middle first trimester (7–8 weeks). These results suggest that first trimester trophoblast cells express ADAM genes in cell type- and gestational age-dependent manners. Our data provide additional insight into the functions of ADAMs in the human placenta.
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Key words: a disintegrin and metalloproteinase, extravillous trophoblast cell, cell invasion, villous trophoblast cell, human first trimester placenta

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Introduction

Trophoblast cells are the critical cells of the placenta. "Progenitor" cytotrophoblast cells differentiate into both villous trophoblast cells (VTs) and extravillous trophoblast cells (EVTs)[1,2]. VTs fuse into a multinucleated cell, called the syncytiotrophoblast, which is in direct contact with maternal blood[3,4]. EVTs lose their cell-to-cell connections, secrete proteases, lyse surrounding tissues, and invade the endometrium, especially the uterine spiral arteries[4–6]. Unlike cancer cell invasion, EVT invasion is tightly regulated both spatially and temporally[7,8]. EVT invasion into the decidua begins during the 6th gestational week[5,6]. EVTs invade deeper through the decidua and into the inner third of the myometrium during the 10th week when EVT invasion is maximal[5,6] and ultimately into the wall of the uterine spiral arteries, particularly during weeks 10 to 14[5,9,10]. EVTs replace both vascular smooth muscle cells and endothelial cells, resulting in spiral artery remodeling, "physiological dilatation of spiral arteries", which brings abundant flow into the intervillous space, a crucial event in fetal growth and development[5,11,12]. Impaired decidual invasion of EVTs can cause various disorders, including miscarriage, fetal growth restriction, preeclampsia, and abruption[13–15].

Proteases are involved in multiple physiological functions, among which the relationship between proteases and cell invasion has attracted much attention [16,17]. The metzincins, zinc-dependent metalloproteinases, play pivotal roles in the proteolytic degradation of the extracellular matrix during cell invasion[18,19]. They metzincins include the matrix metalloproteinase (MMP) and the "a disintegrin and metalloproteinase" (ADAM) enzyme families. Studies on placental proteinases have focused mainly on the expression, production, and roles of MMP family members in EVT invasion[20–23]. MMP expression is dependent on the trophoblast cell type and gestational week. For example, MMP2 and MMP9 were differentially expressed in first trimester EVTs; MMP2 was expressed in proliferative and proximal invasive EVTs of the cell column, while MMP9 was mainly observed in distal invasive EVTs[20]. In human cytotrophoblast cells cultured from the 6th to 11th week of gestation, the production of MMP2 gradually decreased with each week, while MMP9 production increased during this period[21]. ADAMs are structurally classified into two groups: the membrane-anchored ADAMs and secreted ADAMs (ADAMs with thrombospondin motifs, ADAMTSs)[24–27]. Of the ADAMs expressed in the human placenta, Beristain et al. detected *ADAMTS1*, *ADAMTS2* and *ADAMTS12* mRNA in first trimester placental tissues and in EVTs using Southern blot analysis[28]. Yang et al. observed ADAM10 and ADAM17 in the villous trophoblast layer in third trimester placentas using immunohistochemistry[29]. In addition, Kokozidou et al. showed that ADAM12 was present in the villous trophoblast layer in first and third trimester placentas using in situ hybridization and immunohistochemistry[30]. However, except for the study by Beristain et al.[28], few have reported on the ADAM subtypes expressed in first trimester EVTs.

The purpose of this study was to elucidate the differential expression profiles of ADAM genes between first trimester VTs and EVTs. We isolated EVTs from explanted human first trimester chorionic villi and investigated the mRNA expression levels of five ADAM family members (*ADAMTS1*, *ADAMTS2*, *ADAM10*, *ADAM12*, and *ADAM17*) using real-time PCR. We found that certain ADAM genes were expressed in a cell type- and gestational age-dependent manner in first trimester trophoblast cells.

Materials and Methods

Sample Collection

First trimester placental tissues (the 7–13th gestational week, n=17) were obtained from patients who underwent legal abortions and provided informed consent using protocols approved by the Jichi Medical University and the Nippon Medical School ethics committees. The samples were derived from the following gestational weeks: 7 (n=4), 8 (n=3), 9 (n=4), 10 (n=2), 11 (n=3), and 13 (n=1). The pregnancies were terminated with dilation and

Table 1 Primer information for real-time PCR analysis

Gene	Forward primer (5' → 3')	Reverse primer (5' → 3')
ADAMTS1	CGAGTGTGCAAAGGAAGTGA	CTACCCCATAATCCCACCT
ADAMTS2	CCTATGACTGGCTGCTGGAT	CTCCCAAAGTGCTGGGATAA
ADAM10	CCATCAACTTGTGCCAGTAC	CCCATTTGATAACTCTCTCG
ADAM12	CGAGGGGTGAGCTTATGGAAC	CACTCCGAACAGAGGCACTG
ADAM17	ATTGGTGGTAGCAGATCATCG	TGGGAGAGCCAACATAAGCTA
GAPDH	GCACCGTCAAGGCTGAGAAC	ATGGTGGTGAAGACGCCAGT

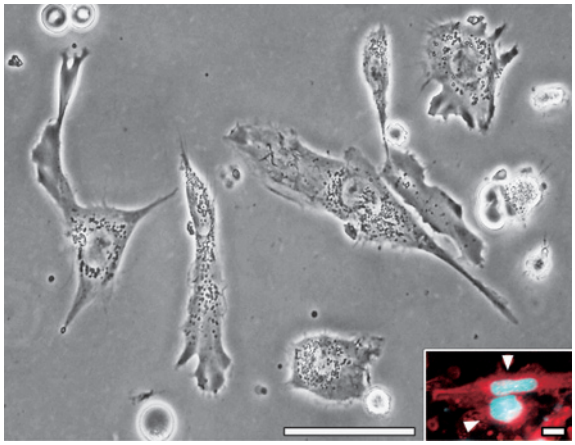

Fig. 1 A representative phase-contrast image of isolated extravillous trophoblast cells (EVTs). Bar=50 μm. (Inset) Immunohistochemistry of HLA-G in EVTs (arrowheads). A merged image of the red HLA-G signal and the blue 4',6-diamidino-2-phenylindole dihydrochloride nuclear staining. Bar=10 μm.

curettage in all but 2 cases, in which pregnancies were terminated with prostaglandin E1 (in the 11th and 13th weeks). Placental samples were used for quantitative real-time PCR and explant cultures. The gestational age was determined from the last menstrual period, as confirmed by the fetal crown-rump length.

Villous Explant Culture and Isolation of EVTs

EVTs from explanted human chorionic villi were isolated using the method described by Sato et al[31,32]. Briefly, minced chorionic villi of first trimester placental tissues were placed in 10-cm-diameter dishes coated with type 1 collagen (Iwaki, Chiba, Japan). The cells that grew out from the adherent villous tips were dispersed using TrypLE Express (Gibco, Chagrin Falls, OH, USA), passed through a nylon strainer with a 40-μm pore diameter (Becton Dickinson, Franklin Lakes, NJ, USA), and then re-plated on type 1 collagen-coated dishes. After washing with PBS, the remaining trophoblast cells were considered isolated EVTs. To validate the identity of these EVTs, the cells were immunostained with mouse anti-HLA-G (catalog # ab7758; Abcam, Cambridge, Cambridgeshire, UK), an EVT marker. Minced villous tips were considered to be the first trimester VTs.

Real-time PCR

Total RNA was isolated from tissues using ISOGEN (Wako, Osaka, Japan) and from cultured cells using RNAiso Plus (Takara Bio, Otsu, Japan). Real-time PCR was performed on the ABI 7300 platform (Applied Biosystems, Foster City, CA, USA). SYBR Premix ExTaq II (Takara Bio, Mountain View, CA, USA) was used for quantitative analysis of the *ADAMTS1*, *ADAMTS2*, *ADAM10*, *ADAM12* and *ADAM17* mRNAs. Primers used for real-time PCR are listed in **Table 1**. Glyceraldehyde 3-phosphate dehydrogenase (*GAPDH*) expression was evaluated as the endogenous internal control.

Statistics

Statistical analyses were performed using the statistical software package JMP (version 9) for Macintosh (SAS Institute, Cary, NC, USA). The significance of between-group differences was assessed using the Student's paired *t*-test or ANOVA followed by Tukey's test. Data are presented as means±standard error (SE); p-value <0.05 was indicative of significance.

Results

The majority of the isolated cells were composed of spindle- and oval-shaped cells (**Fig. 1**). We

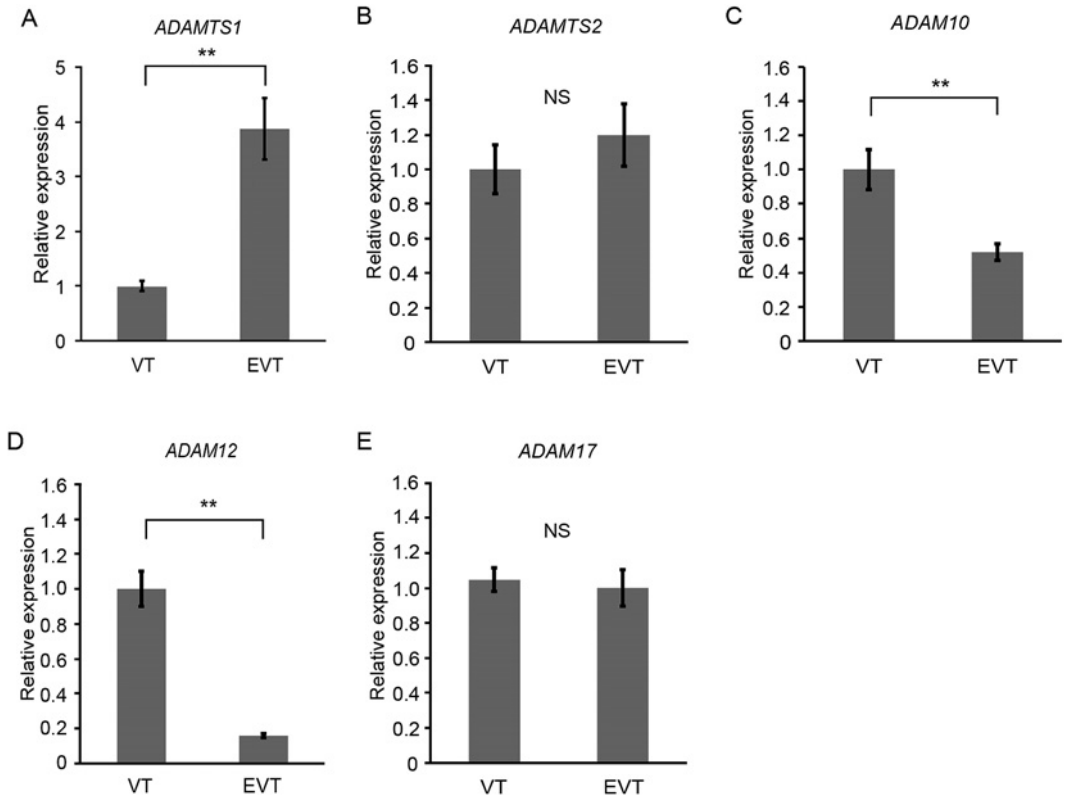

Fig. 2 Real-time PCR analysis of five a disintegrin and metalloproteinase (ADAM) family members, (A) *ADAMTS1*, (B) *ADAMTS2*, (C) *ADAM10*, (D) *ADAM12*, and (E) *ADAM17*, in villous trophoblast cells (VTs; n=17) and extravillous trophoblast cells (EVTs; n=17). Expression levels were normalized to *GAPDH*. The VT expression level was set to 1.0. The significance of the differences between the cell types was assessed by Student's paired *t*-test. Data represent means ± SE; $**p<0.01$, NS: not significance.

performed immunohistochemistry of HLA-G, which is a known EVT marker. Approximately 95% of the isolated cells were positive for HLA-G (**Fig. 1**). The results are consistent with previous findings that cells sprouting from the explanted villous tips comprised mainly EVTs[32,33].

We focused on five ADAM genes (*ADAMTS1*, *ADAMTS2*, *ADAM10*, *ADAM12*, and *ADAM17*) that are expressed in the human placenta[28–30]. Real-time PCR was performed to investigate the differential expression of the genes between first trimester VTs and EVTs (**Fig. 2**). *ADAMTS1* was expressed at a significantly higher level in EVTs than in VTs (**Fig. 2A**). In contrast, both *ADAM10* and *ADAM12* were expressed at significantly higher levels in VTs than in EVTs (**Figs. 2C and 2D**). No differences were found in *ADAMTS2* and *ADAM17* mRNA levels between the two cell types (**Figs. 2B and 2E**).

In addition, we compared the expression of the genes in each cell type of middle (7–8th gestational week) versus late (10–13th week) first trimesters (**Fig. 3**). EVTs expressed significantly higher *ADAMTS1* and significantly lower *ADAM12* levels than did VTs during both middle and late first trimesters (**Fig. 3A and D**). VTs expressed significantly more *ADAM10* than did EVTs during the middle first trimester (**Fig. 3C**). No significant differences in the expression levels of *ADAMTS2* and *ADAM17* were observed between VTs and EVTs during either middle or late first trimesters (**Figs. 3B and 3E**). Interestingly, we found that the differential expression of *ADAM12* in first trimester VTs was dependent on the gestation week. In VTs, the expression level of *ADAM12* was significantly downregulated during late relative to middle first trimester (**Fig. 3D**).

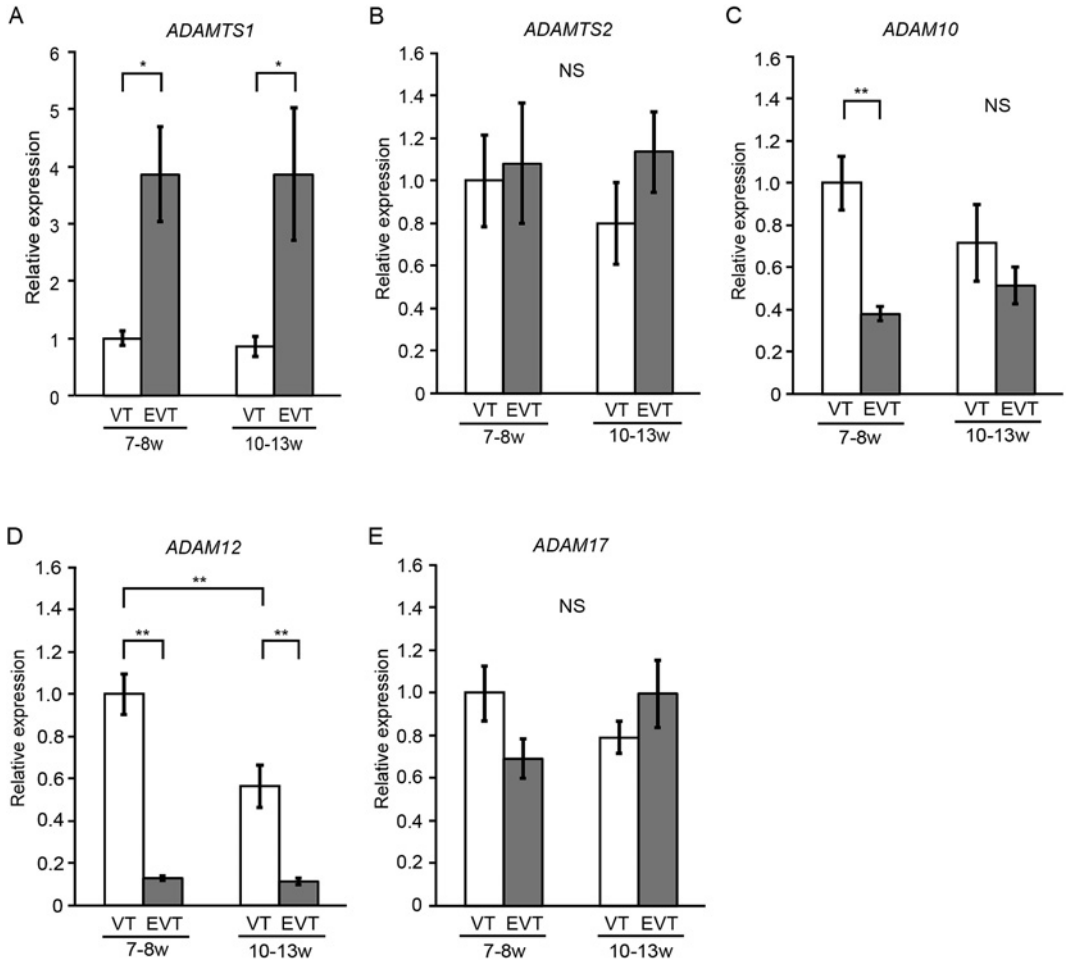

Fig. 3 Expression levels of five ADAM genes, (A) *ADAMTS1*, (B) *ADAMTS2*, (C) *ADAM10*, (D) *ADAM12* and (E) *ADAM17*, between the middle (7–8th gestational week; n=7) and late (10-13th week; n=6) first trimesters in each trophoblast cell type. Expression levels were normalized to *GAPDH*. The VT expression level during middle first trimester (7–8th gestational week) was set to 1.0. The significance of the differences was assessed by ANOVA and Tukey's test. Data represent means ± SE; $*p<0.05$, $**p<0.01$, NS: not significance.

Discussion

In the present study, we revealed differential expression of *ADAM* family genes between first trimester VTs and EVTs. EVTs highly expressed *ADAMTS1* compared to VTs; conversely, VTs highly expressed both *ADAM10* and *ADAM12* compared to EVTs. The results indicate that the expression of the *ADAM* family genes depends on the trophoblast cell type. Moreover, we demonstrated the downregulation of *ADAM12* expression in VTs during the late compared to the middle first trimester, suggesting that *ADAM12* expression in VTs depends on the first trimester gestation age. To our knowledge, the present study is the first to demonstrate a gestational age-dependent change of *ADAM12* expression in first trimester trophoblast cells.

ADAMs are characterized by their metalloprotease and integrin receptor-binding activities and by a cytoplasmic domain with binding sites downstream of various signal transduction pathways[27]. ADAMs are involved in multiple pathogeneses, especially cancer invasion[34]. Tyan et al. reported that cancer-associated fibroblasts secreted ADAMTS1, which promoted cancer cell invasion[35]. EVTs have the capacity to "invade", similar to cancer cells, and we showed high expression of *ADAMTS1* in EVTs **(Fig. 2A)**.

Moreover, Ng et al. found that interleukin 1β and transforming growth factor β1, whose coordinated expression mediates decidual extracellular matrix remodeling, differentially regulated *ADAMTS1* mRNA and protein expression in human decidual stromal cells[36]. Our present data together with previous findings suggest that ADAMTS1 is associated with EVT invasion.

The expression of *ADAM10* was greater in VTs than in EVTs **(Fig. 2C)** for reasons not yet understood. A few studies have investigated ADAM10 in the human placenta. Zhao et al. demonstrated using immunohistochemistry that ADAM10 was significantly increased in the syncytiotrophoblast of preeclampsia placentas compared to that of normal placentas[37]. Recently, Yang et al. showed that the protein levels of ADAM10 and ADAM17 were significantly higher in preeclamptic placentas than in normal placentas and that H_2O_2, a source of oxidative stress, upregulated ADAM10 and ADAM17 in the trophoblastic cell model B6Tert-1[29]. Reactive oxygen species may increase ADAM10 and ADAM17 activation in trophoblast cells, resulting in adverse effects on the human placenta. In addition, we did not detect any differences in *ADAM17* mRNA levels between VTs and EVTs of normal first trimester placentas **(Fig. 2 E)**. Further investigation is needed to elucidate the functional roles of ADAM10 and ADAM17 in trophoblast cells under pathological conditions.

We also found that *ADAM12* was expressed at higher levels in VTs than in EVTs **(Fig. 2D)**. Kokozidou et al. demonstrated that ADAM12 primarily localized in the syncytiotrophoblast of the first trimester placenta[30]. Laigaard et al. reported that ADAM 12 in maternal serum was downregulated in Down syndrome pregnancies during the first trimester[38]. Moreover, Pidoux et al. noted that cultured cytotrophoblast cells isolated from trisomy 21 placentas exhibited delays in trophoblast fusion and differentiation[39]. Thus, it may be that ADAM 12 is involved in villous cytotrophoblast syncytialization, which is the fusion of cytotrophoblast cells into the overlaying syncytiotrophoblast of the human placenta. Recently, Cocquebert et al. showed that syncytialization was significantly higher in primary cultures of early trophoblasts than in those of late trophoblasts[40]. In the present study, we found that the expression of *ADAM12* in VTs was significantly greater in the middle first trimester than in late first trimester **(Fig. 3D)**. These gestational age-dependent changes may support an association between ADAM12 and syncytialization.

This study has certain limitations. For example, we investigated five ADAMs only at the mRNA level because the number of EVTs isolated from explanted human first trimester chorionic villi was limited. Further studies of protein expression, proteolytic activity, and cytoplasmic domain-mediated signal transduction are necessary to elucidate the functional roles of ADAMs in placental pathophysiological and developmental mechanisms, especially EVT invasion and VT syncytialization.

In conclusion, we showed the expression profiles of five ADAM family genes (*ADAMTS1*, *ADAMTS2*, *ADAM10*, *ADAM12*, and *ADAM17*) in VTs and EVTs from human first trimester placentas. Three ADAM genes examined in this study (*ADAMTS1*, *ADAM10* and *ADAM12*) were expressed in a trophoblast cell type-dependent manner. Moreover, *ADAM12* expression in first trimester VTs decreased in a gestational age-dependent manner. Our data provide additional insight into the functions of ADAMs in the human placenta.

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Conflict of Interest: We declare no conflicts of interest.

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