CEOG Clinical and Experimental Obstetrics & Gynecology

Paternal contribution to the preeclampsia phenotype

T. Tsunemi, T. Sado, K. Naruse, H. Kobayashi

Department of Obstetrics and Gynecology, Nara Medical University, Nara (Japan)

Summary

Purpose of investigation: The aims of this study were to elucidate functional pathways of genes responsible for key events in trophoblast invasion and to compare differences in paternal and maternal fitness gene expression in preeclampsia (PE) placenta. *Materials and Methods:* The authors combined data across seven studies published between 1995 and 2014. All genes downloaded from public web sites were analyzed using the metaprofiling and highlighted differentially expressed genes, the chromosomal location of the candidate genes, enriched pathways, and genomic conflicting situations that may play an important role in the trophoblast invasion. *Results:* The majority of differentially expressed genes and their downstream targets associated with trophoblast invasion were mediated through the activation of MMP signaling pathways. The paternal fitness genes that favor trophoblast invasion and fetal growth were reduced in the PE placenta. Half of the differentially expressed genes were located in close proximity to known imprinted genes. Several genes identified in PE were located in a cluster of imprinted genes on chromosomes 1p31, 9q34, and 11p15.4. *Conclusion:* PE may be recognized as a paternal/fetal imprinting disease.

Key words: Preeclampsia; Trophoblast; Invasion; Imprinting; Matrix metalloproteinase.

Introduction

Preeclampsia (PE), one of the pregnancy-specific syndrome, complicates approximately 5% of pregnancies and affects maternal and fetal health worldwide [1]. One major underlying theory is that initial insults such as placental ischemia disrupt the deep trophoblast invasion into the uterine wall resulting in shallow implantation, abnormal spiral artery remodeling, and poor placentation [2]. The expression profiling of differentially expressed genes in PE placenta has been studied extensively with microarrays and genome-wide approaches as a tool for functional genomics studies [3, 4]. In order to understand the pathogenesis of PE, these analyses emphasized the dysfunctional decidualization as the main mechanism involved in the PE development [5], and can offer a potentially powerful way to infer the evolutionary dynamics.

The reproductive interests of fetus and mother lead to conflict over parental investment [6-8]. On the one hand, trophoblast invasion has been required to fulfill fetal demand maximally through intensive (epi)genetic selection, because paternal genetic interests are served by maximizing fetal growth. On the other hand, increased fetal body weight will be countered by a maternal genetic disagreement as an antagonistic coevolution. Understanding these (epi)genetic changes and predisposition could help to clarify the pathogenesis of the disease.

The objective of this study was to identify differentially expressed genes and essential signaling pathways of the trophoblast invasion in PE placenta. Next, the authors focused on the question if the imbalance of paternal and maternal fitness gene expression or genetic conflict could modulate the induction of trophoblast invasion in PE.

Materials and Methods

A Medline search of the literature was performed using the key words: ("preeclampsia" or "eclampsia" or "pregnancy-induced hypetension") and ("genome wide" or "gene expression" or "profiling" or "pathway analysis" or "susceptibility gene") and/or ("imprinting" or "maternal fetal conflict"). English-language publications in PubMed and references from relevant articles published between 1995 and 2014 were included.

The authors selected 12 studies that have used DNA microarrays and protein expression analysis to identify the expression signatures of human PE placenta [9-20]. Since each microarray study was limited by its relatively small sample sizes, seven available data were included in processing and analysis [9, 11, 13, 14, 17, 18, 20]. Individual genes were downloaded across publicly accessible PE expression databases. All data sets available were log transformed and data were provided in single composite excel file format. All genes downloaded from public web sites were analyzed using the comparative metaprofiling that identifies the multiple gene expression signatures from a diverse collection of data sets. These steps have been previously described in detail [21]. Genes are ranked within each dataset by the *p*-value. The authors identified differentially expressed genes between PE and normal placenta by applying the following thresholds: $p < 0.001$ and > ten -fold change. Gene set enrichment analyses across studies were determined by comparing rankings against a random distribution to improve gene-disease association prediction. Enriched terms were searched in functional-related gene categories in PE as described previously [4]. All genes listed in Table 1 were annotated by PubMed gene database (http://www.ncbi.nlm.nih.gov/gene/)

Revised manuscript accepted for publication June 16, 2016

that is an easily accessible and searchable web tool to assemble a relevant collection of gene function.

Imprinted gene information has been gathered from National Center for Biotechnology Information database geneimprint.com (http://www.geneimprint.com/). Patients of both severe PE and control groups were all in the second and third trimester of pregnancy.

Results

Functional categories of differentially expressed genes involved in the trophoblast invasion in PE

The aim of the present study was to detect the candidate genes involved in cell motility and invasion in PE. Among the 82 differentially expressed genes, 30 were upregulated and 37 were downregulated in the PE placenta as compared to normal placenta. In the remaining 15 genes, the authors could not identify the expression levels using a PubMed database search. The most meaningful sets of enriched categories were those involving the transcription factors/regulators (n=13), chemokines/cytokines (n=13), proteases/protein degradation (n=13), growth factors/regulators (n=10), and signal transduction (n=9) (Table 1). The remaining 24 genes could not be grouped into distinct groups.

First, the authors describe how these differentially expressed genes contribute to the dysregulation of the trophoblast invasion. The genes controlling 'transcriptional

Table 1. — *List of differentially expressed genes involved in trophoblast invasion in preeclampsia placenta.*
Left column, this table shows eighty-two differentially expressed genes and enriched pathways in preeclampsia placenta. Right column, known imprinted genes lie in close proximity of the known imprinted genes.

The differentially expressed genes in PE					The imprinting genes which lie in close proximity on each chromosome				
Biological Processes	Official Symbol	Official Full Name	Location	The up- and down-regulated DEGs	Official Symbol	Official Full Name	Location	Status	Expressed Allele
The genes involved in the modulation of MMPs									
Transcription factors (n=13)	KLF8	Kr pel-like factor 8	Xp11.21	down					
	HMOX1	Heme oxygease 1	22q13.1	down					
	FOXM1	forkhead box M1	12p13	unknown	RBP5	retinol binding protein 5	12p13.31 AS	Imprinted	Maternal
	MYC	v-myc avian myelocytomatosis viral oncogene homolog	8q24.21	down					
	HIF1A	hypoxia inducible factor 1, alpha subunit (basic helix-loop-helix transcription factor)	14q23.2	up					
	RUNX2	runt related transcription factor 2	6p21	unknown	BTNL	2 butyrophilin like 2	6p21.3 AS	Predicted	Maternal
	CDX2	caudal type homeobox 2	13q12.3	up					
	FOSL1	FOS like antigen 1	11q13	unknown	ANO1	anoctamin 1, calcium activated chloride channel	11q13.3	Imprinted	Maternal
	FOS	FBJ murine osteosarcoma viral oncogene homolog	14q24.3	down					
	JUN	jun proto-oncogene	1p32-p31	up					
	CDX1	caudal type homeobox 1	5q32	down					
	TFAP2A	transcription factor AP-2 alpha (activating enhancer binding protein 2 alpha, AP-2α)	6p24	up					
	CALR	calreticulin	19p13.13	up	PPAP2C	phosphatidic acid phosphatase type 2C	19p13 AS	Predicted	Maternal
Chemokines (n=12)	SAA1	serum amyloid A1	11p15.1	down	PKP3	plakophilin 3	11p15	Predicted	Maternal
					KCNQ1OT1	KCNQ1 opposite strand/ antisense transcript 1	11p15	Imprinted	Paternal
	NODAL	nodal growth differentiation factor	10q22.1	up	CTNNA3	catenin alpha 3	10q22.2 AS	Provisional Data	Maternal
	IL17A	interleukin 17A	6p12	up	PRIM2	primase, DNA, polypeptide 2	6p12-p11.1 AS	Conflicting Data	
	TGFB1	transforming growth factor beta 1 (TGF-β1)	19q13.1	down	CHST8	carbohydrate (N-acetylgalactosamine 4-0) sulfotransferase 8	19q13.1	Predicted	Maternal
	LIF	leukemia inhibitory factor	22q12.2	up	FLJ20464		22q12.2	Predicted	Paternal
	CCL17	chemokine (C-C motif) ligand 17	16q13	unknown					
	OSM	oncostatin M	22q12.2	down	FLJ20464		22q12.2	Predicted	Paternal
	CXCL12	chemokine (C-X-C motif) ligand 12	10q11.1	down					
	TGFB3	tumor growth factor beta3	14q24	up					
	DCN	decorin	12q21.33	up	DCN	decorin	12q21.33 AS	Imprinted	Maternal
	CXCL6	chemokine (C-X-C motif) ligand 6	4q13.3	unknown					
	HLA-G	major histocompatibility complex, class I, G	6p21.3	down	BTNL	2 butyrophilin like 2	6p21.3 AS	Predicted	Maternal

	MMP2	Matrix metalloproteinase-2	16q12.2	down					
	MMP9	Matrix metalloproteinase-9	20q13.12	down	L3MBTL	l(3)mbt-like 1	20q13.12	Imprinted	Paternal
	ADAM12	DAM metallopeptidase domain 12	10q26	down	NKX6-2	NK6 homeobox 2	10q26	Imprinted	Maternal
					C10orf93	also known as CFAP46 (cilia and flagella associated protein 46)	10q26	Imprinted	Maternal
					VENTX	VENT homeobox	10q26	Imprinted	Maternal
					PAOX	polyamine oxidase (exo-N4-amino)	10q26	Imprinted	Maternal
					C10orf91	chromosome 10 open reading frame 91	10q26	Imprinted	Maternal
	PAPPA	pregnancy-associated plasma protein A, pappalysin 1	9q33.2	down					
	ADM2	adrenomedullin 2; also known as Intermedin (IMD)	22q13.33	down					
Proteases (n=11)	POFUT1	protein O-fucosyltransferase 1	20q11	unknown	PSIMCT-1	malignant T-cell amplified sequence 2, pseudogene	20q11	Imprinted	Paternal
					BLCAP	bladder cancer associated protein	20q11	Imprinted	Isoform Dependent
					NNAT	neuronatin	20q11	Imprinted	Paternal
					MCTS2	malignant T-cell amplified sequence 2, pseudogene	20q11	Imprinted	Paternal
	EDP	Elastin-derived peptides		unknown					
	CORIN	corin, serine peptidase; also known as atrial natriuretic peptide-converting enzyme		down					
	GALNT2	polypeptide N-acetylgalactosaminyltransferase 2	1q41-q42	unknown	OBSCN	obscurin, cytoskeletal calmodulin and titin-interacting RhoGEF	1q42	Predicted	Paternal
					HIST3H2BB	histone cluster 3, H2bb	1q42	Predicted	Maternal
	DPP4	Dipeptidyl peptidase IV	2q24.3	up					
	PRSS8	protease, serine 8	16p11.2	up					
	EGFL7	Epidermal growth factor-like domain multiple 7	9q34.3	down	LMX1B	LIM homeobox transcription factor 1, beta. I	9q34	Predicted	Maternal
					C9orf116	chromosome 9 open reading frame 116	9q34.3 AS	Predicted	Paternal
					EGFL7	EGF like domain multiple 7	9q34.3	Predicted	Paternal
					PHPT1	phosphohistidine phosphatase 1	9q34.3	Predicted	Maternal
	PRL	Prolactin	6p22.3	up					
	HGF	hepatocyte growth factor	7q21.1	down	MAGI2	membrane associated guanylate kinase, WW and PDZ domain containing 2	7q21 AS	Imprinted	Maternal
Growth factors /Hormones (n=10)					PEG10	paternally expressed 10	7q21	Imprinted	Paternal
					SGCE	sarcoglycan epsilon	7q21-q22 AS	Imprinted	Paternal
	HLX	H2.0-like homeobox	1q41	down					
	IGF2BP3	insulin like growth factor 2 mRNA binding protein 3	7p11	down					
	Activin A			up					
	hCG			up					
	GNRH1	gonadotropin releasing hormone 1	8p21-p11.2	unknown	PURG	purine-rich element binding protein G	8p11 AS	Predicted	Paternal
	IGFBP7	insulin like growth factor binding protein 7	4q12	up					
	ESR1	estrogen receptor 1	6q25.1	down					
	CDH2	cadherin 2; also known as N-cadherin	18q11.2	down					
	CLDN4	claudin 4	7q11.23	up	HOXA3	homeobox A3	7p15-p14 AS	Predicted	Maternal
	KISS1	KiSS-1 metastasis-suppressor	1q32	up	PTPN14	protein tyrosine phosphatase, non-receptor type 14	1q32.2 AS	Predicted	Maternal
	ADORA2B	adenosine A2b receptor	17p12	up					
Cell-cell interactions (n=8)	CD82	CD82 molecule	11p11.2	unknown					
	SPARC	secreted protein acidic and cysteine rich	5q31.3-q32	down	CSF2	colony stimulating factor 2	5q31.1	Predicted	Maternal
					VTRNA2-1	vault RNA 2-1	5q31.1 AS	Imprinted	Paternal
	LGALS1	lectin, galactoside-binding, soluble, 1	22q13.1	down					
	LAMA4	laminin subunit alpha 4	6q21	down	AIM1	absent in melanoma 1	6q21	Imprinted	Paternal
					LIN28B	lin-28 homolog B	6q21	Imprinted	Paternal

Signal (n=8)	STMN1	stathmin 1	1p36.11	unknown	DVL1	dishevelled segment polarity protein 1	1p36 AS	Predicted	Maternal
	LIMK1	LIM domain kinase 1	7q11.23	down	TMEM60	transmembrane protein 60	7q11.23 AS	Predicted	Paternal
	NOTCH1	notch 1	9q34.3	down	LMX1B	LIM homeobox transcription factor 1, beta	9q34	Predicted	Maternal
					C9orf116	chromosome 9 open reading frame 116	9q34.3 AS	Predicted	Paternal
					EGFL7	EGF like domain multiple 7	9q34.3	Predicted	Paternal
					PHPT1	phosphohistidine phosphatase 1	9q34.3	Predicted	Maternal
	S1PR1	sphingosine-1-phosphate receptor 1	1p21	down					
	TWIST1	twist family bHLH transcription factor 1	7p21.2	down					
	STAT5A	signal transducer and activator of transcription 5A	17q11.2	unknown	PYY2	peptide YY, 2 (pseudogene)	17q11	Predicted	Paternal
	EFNA1	ephrin-A1	1q21-q22	unknown					
	EFNA2	ephrin-A2	19p13.3	unknown	PPAP2C	Phosphatidic acid phosphatase type 2C	19p13 AS	Predicted	Maternal
Cell cycle regulation (n=3)	CD44	CD44 molecule	11p13	up	WT1-Alt trans		11p13 AS	Imprinted	Paternal
	GADD45A	growth arrest and DNA damage inducible alpha	1p31.2	up	DIRAS3	DIRAS family GTP binding RAS like 3	1p31 AS	Imprinted	Paternal
	RGC32	RGCC (Regulator of cell cycle)	13q14.11	up	HTR2A	5-hydroxytryptamine receptor 2A.	13q14-q21 AS	Conflicting Data	Maternal
Ubiquitination (n=2)	CUL1	cullin 1	7q36.1	down					
	CUL3	cullin 3	2q36.2	down					
Adipocytes (n=1)	LEP	leptin	7q31.3	up					
Channel (n=1)	SCNN1A (ENaC)	epithelial sodium channel	12p13	down	RBP5	retinol binding protein 5	12p13.31 AS	Imprinted	Maternal
Parentally expressed imprinted genes (n=1)	PEG10	paternally expressed 10	7q21	down	MAGI2	membrane associated guanylate kinase, WW and PDZ domain containing 2	7q21 AS	Imprinted	Maternal
					SGCE	sarcoglycan epsilon	7q21-q22 AS	Imprinted	Paternal
Others (n=2)	PLAC1	placenta specific 1	Xq26	up					
	NR4A1	nuclear receptor subfamily 4 group A member 1; also known as NUR77	12q13	down	SLC26A10		12q13	Predicted	Maternal
					SLC38A4		12q13 AS	Unknown	Unknown
					HOXC4		12q13.3	Predicted	Maternal
					HOXC9		12q13.3	Predicted	Maternal
The genes not involved in the modulation of MMPs									
Cell-cell interaction (n=4)	SLIT2	slit guidance ligand 2	4p15.2	up					
	CNN3	calponin 3	1p22-p21	unknown	GFI1	growth factor independent 1 transcription repressor	1p22 AS	Predicted	Paternal
	SIGLEC1	sialic acid binding Ig like lectin 1	20p13	up					
	MBL2	mannose binding lectin 2	10q11.2	up					
Protease (n=2)	HTRA4	High temperature requirement A4 (HtrA4) serine peptidase 4	8p11.22	up	PURG	purine-rich element binding protein G	8p11 AS	Predicted	Paternal
	SERPINB5	serpin peptidase inhibitor, clade B (ovalbumin), member 5; also known as Maspin	18q21.33	up					
Parentally expressed imprinted genes (n=1)	PHLDA2	pleckstrin homology like domain family A member 2	11p15.4	up	OSBPL5	oxysterol binding protein like 5	11p15.4 AS	Imprinted	Maternal
					KCNQ1DN	KCNQ1 downstream neighbor (non-protein coding)	11p15.4	Imprinted	Maternal
					ZNF215	zinc finger protein 215	11p15.4	Provisional Data	Maternal
					PKP3	plakophilin 3	11p15	Predicted	Maternal
					KCNQ1OT1	KCNQ1 opposite strand/ antisense transcript 1	11p15	Imprinted	Paternal
Signal (n=1)	DAB2IP	DAB2 interacting protein	9q33.1-q33.3	down					
Chemokines (n=1)	CXCL3	chemokine (C-X-C motif) ligand 3	4q21	down					
Others (n=1)	PIF	PreImplantation Factor		down					

factors/regulators' were functionally enriched for the regulation of increased cell migration, invasion, and motility: most of which are implicated in cancer invasion. These genes, such as KLF8 [22], HMOX1 [23], FOXM1 [24], MYC [25], HIF1A [26], RUNX2 [27], CDX2 [28], FOSL1 [29], FOS [29], JUN [30], CDX1 [31], TFAP2A [32], and CAL [33], are thought to be attractive targets for cancer therapy due to their role in tumor invasion, metastasis, and progression via regulating MMPs. These transcription regulators act as upstream target genes that were coupled with the regulation of expression and activation of MMPs, possibly resulting in the modulation of trophoblast invasion in decidual tissue. MMP deficiency during early pregnancy is associated with suppression of trophoblast invasion and spiral artery remodeling, and then leads to increased risk of several pregnancy complications, such as PE, fetal growth restriction (FGR), and recurrent pregnancy loss [34,35].

The next prominent biological pathway of the candidate target genes was identified as a subset of cellular process related to chemokines, cytokines or immune modulations, including SAA1 [36], NODAL [37], IL17A [38], TGFB1 [39], LIF [40], CCL17 [41], OSM [42], CXCL12 [43], TGFB3 [44], DCN [45], CXCL6 [46], HLA-G [47], and CXCL3 [48]. Chemokines (CXCL) and chemokine receptors (CXCR) have been implicated in a pivotal role in malignant transformation and tumor invasion [49]. Chemokines of the CXC family are thought to play key roles in the regulation of trophoblast differentiation and invasion [50]. Additionally, the CXCL-CXCR axis is also involved in regulation of decidual leukocyte trafficking to the maternal-fetal interface [50]. Thus, these genes have important roles in decidualization, embryo implantation, trophoblast invasion, and placentation, thus providing decidual-blastocyst communication in the peri-implantation period.

This study also suggests that the genes controlling 'proteases' are most likely to be essential for extracellular matrix degradation and trophoblast invasion, including MMP2 [51], MMP9 [51], ADAM12 [52], PAPPA [53], ADM2 [54], POFUT1 [55], EDP, CORIN [56], GALNT2 [57], DPP4 [58], PRSS8 [59], HTRA4 [60], and SERPINB5 [61]. These genes were recognized as important factors mediating tumor invasion. The majority of these genes listed in this study can regulate the expression and function of MMPs that are direct or indirect targets of these genes. Overall, MMPs are regulated by DNA methylation and downregulated in PE [62].

One of the common differentially expressed genes belongs to the pathway regulating 'growth factors and hormones', which includes EGFL7 [63], PRL [64], HGF [65], HLX [66], IGF2BP3 [67], Activin A [68], hCG [69], GNRH1 [70], IGFBP7 [71], and ESR1 [72]. These genes modulate tumor growth, invasion, and distant metastasis. The encoded proteins also play a role in regulating trophoblast proliferation, invasion, angiogenesis, and microenvironment modification in normal pregnancy. Members of the IGF family were found to be downregulated in placental tissue from women with PE [73].

The 'signal transduction'-related genes are candidate regulators of trophoblast invasion. Aberrant expression of these genes, such as STMN1 [74], LIMK1 [75], NOTCH1 [76], S1PR1 [77], TWIST1 [78], STAT5A [79], EFNA1 [80], and EFNA2 [80], was identified as potential tumor-associated genes and may be involved in the growth, invasion, metastasis, and epithelial mesenchymal transition of tumor cells. These candidate genes promote trophoblast proliferation, migration, and invasion, whereas knockdown of these genes inhibited these processes. The majority of these genes were downregulated in placental villi tissue and trophoblasts from patients with PE.

Transcriptional regulation of 'other genes' also shows an enrichment of terms involved in cell functions that also coordinates various programs essential for trophoblast invasion. Collectively, these differentially expressed genes and their downstream targets play important roles in trophoblast invasion, which may be mediated mostly through the activation of MMP signaling pathways. These genes were found to suppress trophoblast invasion experimentally through downregulation and inactivation of members of the MMP family.

Genomic conflict arising from an imbalance between maternal and paternal fitness genes

Second, the authors assessed parental investment and adaptations function to benefit maternal and fetal reproductive interests. Profiling of differentially expressed genes and potential downstream targets predict the molecular pathways underlying the pathological control of PE development. The authors found that the majority of the differentially expressed genes that might promote MMP production were downregulated, while most upregulated genes inhibit MMP expression in the placentae of women with PE (Table 1, see 'the genes involved in the modulation of MMPs'). Among 82 genes, 68 (81.7%) genes were negatively correlated with trophoblast invasion and fetal growth, namely paternal fitness genes. These results support the previous idea that PE may be associated with an imbalance in the maternal-paternal (fetal) genetic response [3]. The parentally fitness genes perform different roles during fetal development. For example, IGF2BP3 (insulin-like growth factor-2 mRNA-binding protein 3) is expressed specifically in placenta as a paternal fitness gene and activates IGF2-dependent signaling pathways required for fetal development through phosphatidylinositide 3-kinase (PI3K) and mitogen-activated protein kinase (MAPK) cascades. Most components of the paternal fitness genes including IGF induces cell invasion by a mechanism that involves the MMP activation. IGF2BP3 can promote both cancer cell and trophoblast invasion [81]. Thus, members of the IGF family are evolved for the benefit of the trophoblast

invasion and fetal development as the paternal/fetal fitness gene [82]. In contrast, IGFBP7 has been identified as a tumor suppressor gene and plays a key role in the suppression of trophoblast proliferation and invasion [83]. Maternal allele IGFBP can inhibit trophoblast invasion and fetal growth as the maternal fitness gene. Alterations in the IGF axis, low IGF, and high IGFBP placental concentrations, may influence trophoblast invasion and fetal growth, representing a risk factor for PE and FGR susceptibility. Indeed, the IGF/IGFBP ratio was lower in PE group than in healthy pregnancy group [84].

Genes involved in trophoblast invasion are located in close proximity to the imprinting region

Finally, the authors found that, among the 82 differentially expressed genes, 36 (43.9%) genes involved in trophoblast invasion lay in close proximity to the chromosome imprinting region that displays a parental-specific imprinting (Table 1, right column, see 'the imprinting genes which lie in close proximity on each chromosome'). More than 200 loci are imprinted in humans through the online PubMed database. Although it is unclear whether these 36 genes are regulated by a specific imprinting center, the number of genes that mapped in close proximity to the imprinting region was higher than those expected by chance alone. Although the role of imprinted genes in the genesis of PE is still unknown, some differentially expressed genes were located in or in close proximity to an imprinted cluster on chromosomes 1p31, 9q34, and 11p15.4. Loss of heterozygosity in a region on chromosome 1p31 was frequently observed in breast and ovarian cancer and male germ-cell tumors [85]. Chromosome 9q34 is thought to be a common deletion site in various cancers and their premalignant lesions [86]. Genes located on chromosome 11p15 is frequently methylated and silenced at an imprinted cluster. These imprinting center regions specifically regulate parentally-expressed genes. Since the IGF2 gene located on chromosome 11p15 is maternally imprinted in humans, disruption of the paternal Igf2 allele causes severe FGR in mice. Alterations in expression and methylation of imprinted genes may be involved in in utero growth [87]. It has been hypothesized that the maternal fitness genes inhibit fetal growth to counteract the deleterious effects of growth enhancement by the paternal fitness genes [88]. Trophoblast invasiveness and then fetal growth may be controlled by the tug-of-war between paternal and maternal fitness genes [89].

Discussion

The present research focused on the identification of differentially expressed genes and biological pathways underlying trophoblast invasion in PE placenta, and the existence of genetic conflict and genomic imprinting situations on the parental control of trophoblast invasion.

First, the authors identified significantly enriched molecular functions concerning trophoblast invasion. The differentially expressed genes were involved in five key pathways. Extracellular matrix degradation and remodeling by proteolytic enzymes, such as MMPs, are required for each step of trophoblast invasion and spiral artery remodeling [90]. This study showed a close similarity with cancer cells in respect to their invasive behavior and capacity of MMPs production. Indeed, human PE placenta is accompanied by the decreased expression of MMPs compared with normal placenta [34]. Downregulation of members of the MMP family likely results in failure to achieve the establishment and maintenance of normal pregnancy and may participate in the pathological processes underlying PE and FGR.

Second, the authors identified the shared preeclamptic molecular features over which conflict is expected, and then reviewed the literature for evidence of their occurrence. Two opposite signaling pathways, e.g., fetal growth and decidualization processes, were related with the pathogenesis of PE as a genetic consequence of maternal-fetal conflict situations [3]. Paternal genes may favor fetal growth, whereas maternal gene has a protective role against fetal growth. Members of the IGF family are genes evolved for the benefit of the trophoblast invasion, spiral artery remodeling, and placental and fetal development as the paternal fitness genes to achieve maximal benefit for fetal growth [82]. In contrast, growth factor suppressors such as members of the IGFBP family, have been identified as the maternal fitness genes that function as the first line of defence against invading trophoblast cells. Recent study showed that the majority of genes evolved as the defense and protective mechanism for the mother, namely maternal fitness genes, were also downregulated in PE placenta [4]. Maternal fitness genes are considered to be involved in the 'decidualization process' [3]. These results suggest that insufficient decidualization plays a significant role in the pathogenesis of PE.

The present authors hypothesize that PE may be associated with genomic conflict arising from an imbalance between maternal and paternal fitness genes [3, 4]. The paternal genes attempt to outshine the maternal genes for optimal benefit. The paternal fitness genes are unable to cope with a physiologic genetic conflict, possibly through inactivation of the genes involved in trophoblast invasion and fetal growth. Therefore, the balance between fetal energy demand and nutrient supply is impaired in the PE patients.

Finally, the authors found that the high frequency of the differentially expressed genes involved in trophoblast invasion is located in close proximity to the imprinting region. Obstetricians typically recognize PE as maternal disease [91]. However, the present molecular genomic, genetic, and imprinting study suggests the paternal contribution. PE may be originated by a destiny marked since birth. Previous study indicated that the paternal fitness genes were predominant in PE placenta [3]. Here the present au-

thors demonstrated that, among the paternal fitness genes, the majority of MMP-related genes were inactivated. MMP dysfunction is considered to be a critical component of PE pathogenesis. One possibility is that epigenetic dysregulation of paternal fitness genes in the blastocyst stage may be the leading mechanism for developing PE. Furthermore, altered epigenetic regulation in a specific couple may occur at the embryonic stage which is sensitive to alter the DNA methylation profile. Epigenetic modifications may be detected not only in the exposed individual, but also in subsequent progeny.

In conclusion, PE may be associated with either: 1) inactivation of the MMP-related genes that are located in close proximity to known imprinted genes, 2) aberrant expression of paternal fitness genes that are related to nutrient demand, 3) an imbalance in the maternal-fetal genetic conflict, or 3) an interaction of the three. PE may be recognized as a paternal/fetal imprinting disease. Future experiments will focus further on several of these differentially expressed genes.

Conclusion

PE may be recognized as a paternal/fetal imprinting disease.

Acknowledgments

This study was supported by Grant-in-aid for Scientific Research from the Ministry of Education, Science, and Culture of Japan to the Department of Obstetrics and Gynecology, Nara Medical University (H. Kobayashi).

References

[1] Steegers E.A., von Dadelszen P., Duvekot J.J., Pijnenborg R.: "Pre-eclampsia". *Lancet*, 2010, *376*, 631.

[2] Hussein W., Lafayette R.A.: "Renal function in normal and disordered pregnancy". Curr. Opin. Nephrol. Hypertens., 2014, *23*, 46.

[3] Kobayashi H.: "The impact of maternal-fetal genetic conflict situations on the pathogenesis of preeclampsia". *Biochem. Genet.*, 2015, *53*, 223.

[4] Kobayashi H.: "Characterization of the down-regulated genes identified in preeclampsia placenta". *Hypertens. Pregnancy*, 2016, *24*, 1.

[5] Rabaglino M.B., Post Uiterweer E.D., Jeyabalan A., Hogge W.A., Conrad K.P.: "Bioinformatics approach reveals evidence for impaired endometrial maturation before and during early pregnancy in women who developed preeclampsia". *Hypertension*, 2015, *65*, 421.

[6] Haig D.: "Genetic conflicts in human pregnancy". *Q. Rev. Biol.*, 1993, *68*, 495.

[7] Haig D.: "Maternal-fetal conflict, genomic imprinting and mammalian vulnerabilities to cancer". *Philos. Trans. R. Soc. Lond. B. Biol. Sci.*, 2015, *370*, pii: 20140178.

[8] Haig D.: "Coadaptation and conflict, misconception and muddle, in the evolution of genomic imprinting". *Heredity (Edinb.)*, 2014, *113*, 96.

[9] Louwen F., Muschol-Steinmetz C., Reinhard J., Reitter A., Yuan J.: "A lesson for cancer research: placental microarray gene analysis in preeclampsia". *Oncotarget*, 2012, *3*, 759.

[10] Staun-Ram E., Shalev E.: "Human trophoblast function during the implantation process". *Reprod. Biol. Endocrinol.,* 2005, *3*, 56.

[11] Tsai S., Hardison N.E., James A.H., Motsinger-Reif A.A., Bischoff S.R., Thames B.H., Piedrahita J.A.: "Transcriptional profiling of human placentas from pregnancies complicated by preeclampsia reveals disregulation of sialic acid acetylesterase and immune signalling pathways". *Placenta*, 2011, *32*, 175.

[12] Jia R.Z., Zhang X., Hu P., Liu X.M., Hua X.D., Wang X., Ding H.J.: "Screening for differential methylation status in human placenta in preeclampsia using a CpG island plus promoter microarray". *Int. J. Mol. Med.*, 2012, *30*, 133.

[13] Lapaire O., Grill S., Lalevee S., Kolla V., Hösli I., Hahn S.: "Microarray screening for novel preeclampsia biomarker candidates". *Fetal Diagn. Ther.*, 2012, *31*, 147.

[14] Sado T., Naruse K., Noguchi T., Haruta S., Yoshida S., Tanase Y., *et al.*: "Inflammatory pattern recognition receptors and their ligands: factors contributing to the pathogenesis of preeclampsia". *Inflamm. Res.,* 2011, *60*, 509.

[15] Johansson A., Curran J.E., Johnson M.P., Freed K.A., Fenstad M.H., Bjørge L., *et al.*: "Identification of ACOX2 as a shared genetic risk factor for preeclampsia and cardiovascular disease". *Eur. J. Hum. Genet.,* 2011, *19*, 796.

[16] Rajakumar A., Chu T., Handley D.E., Bunce K.D., Burke B., Hubel C.A., *et al.*: "Maternal gene expression profiling during pregnancy and preeclampsia in human peripheral blood mononuclear cells". *Placenta*, 2011, *32*, 70.

[17] Løset M., Mundal S.B., Johnson M.P., Fenstad M.H., Freed K.A., Lian I.A., *et al.*: "A transcriptional profile of the decidua in preeclampsia". *Am. J. Obstet. Gynecol.*, 2011, *204*, 84.e1.

[18] Founds S.A., Conley Y.P., Lyons-Weiler J.F., Jeyabalan A., Hogge W.A., Conrad K.P.: "Altered global gene expression in first trimester placentas of women destined to develop preeclampsia". *Placenta*, 2009, *30*, 15-24.

[19] Laivuori H.: "Genetic aspects of preeclampsia". *Front. Biosci.,* 2007, *12*, 2372.

[20] Zhou R., Zhu Q., Wang Y., Ren Y., Zhang L., Zhou Y.: "Genomewide oligonucleotide microarray analysis on placentae of pre-eclamptic pregnancies". *Gynecol. Obstet. Invest.*, 2006, *62*, 108.

[21] Rhodes D.R., Yu J., Shanker K., Deshpande N., Varambally R., Ghosh D., *et al.*: "Large-scale meta-analysis of cancer microarray data identifies common transcriptional profiles of neoplastic transformation and progression". *Proc. Natl. Acad. Sci. USA*, 2004, *101*, 9309.

[22] Yang Z., Bai B., Luo X., Xiao X., Liu X., Ding Y., *et al.*: "Downregulated Krüppel-like factor 8 is involved in decreased trophoblast invasion under hypoxia-reoxygenation conditions". *Reprod. Sci.*, 2014, *21*, 72.

[23] Zhao H., Ozen M., Wong R.J., Stevenson D.K.: "Heme oxygenase-1 in pregnancy and cancer: similarities in cellular invasion, cytoprotection, angiogenesis, and immunomodulation". *Front. Pharmacol.*, 2015, *5*, 295.

[24] Xie Y., Cui D., Sui L., Xu Y., Zhang N., Ma Y., *et al.*: "Induction of forkhead box M1 (FoxM1) by EGF through ERK signaling pathway promotes trophoblast cell invasion". *Cell Tissue Res.*, 2015, *362*, 421.

[25] Sun M., Chen H., Liu J., Tong C., Meng T.: "MicroRNA-34a inhibits human trophoblast cell invasion by targeting MYC". *BMC Cell. Biol.*, 2015, *16*, 21.

[26] Highet A.R., Khoda S.M., Buckberry S., Leemaqz S., Bianco-Miotto T., Harrington E., *et al.*: "Hypoxia induced HIF-1/HIF-2 activity alters trophoblast transcriptional regulation and promotes invasion". *Eur. J. Cell. Biol.*, 2015, *94*, 589.

[27] Peng B., Zhu H., Klausen C., Ma L., Wang Y.L., Leung P.C.: "GnRH regulates trophoblast invasion via RUNX2-mediated MMP2/9 expression". *Mol. Hum. Reprod.*, 2015, *22*, 119.

[28] Jia R.Z., Ding G.C., Gu C.M., Huang T., Rui C., Wang Y.X., Lu Q.: "CDX2 enhances HTR-8/SVneo trophoblast cell invasion by altering the expression of matrix metalloproteinases". *Cell. Physiol. Biochem.*, 2014, *34*, 628.

[29] Renaud S.J., Kubota K., Rumi M.A., Soares M.J.: "The FOS transcription factor family differentially controls trophoblast migration and invasion". *J. Biol. Chem.*, 2014, *289*, 5025.

[30] Peng B., Zhu H., Ma L., Wang Y.L., Klausen C., Leung P.C.: "AP-1

"transcription factors c-FOS and c-JUN mediate GnRH-induced cadherin-11 expression and trophoblast cell invasion". *Endocrinol.*, 2015, *156*, 2269.

[31] Jia R.Z., Rui C., Li J.Y., Cui X.W., Wang X.: "CDX1 restricts the invasion of HTR-8/SVneo trophoblast cells by inhibiting MMP-9 expression". *Placenta*, 2014, *35*, 450.

[32] Zhang Z., Zhang L., Jia L., Cui S., Shi Y., Chang A., *et al.*: "AP-2α suppresses invasion in BeWo cells by repression of matrix metalloproteinase-2 and -9 and up-regulation of E-cadherin". *Mol. Cell. Biochem.*, 2013, *381*, 31.

[33] Shi Z., Hou W., Hua X., Zhang X., Liu X., Wang X., Wang X.: "Overexpression of calreticulin in pre-eclamptic placentas: effect on apoptosis, cell invasion and severity of pre-eclampsia". *Cell. Biochem. Biophys.*, 2012, *63*, 183.

[34] Majali-Martinez A., Hiden U., Ghaffari-Tabrizi-Wizsy N., Lang U., Desoye G., Dieber-Rotheneder M.: "Placental membrane-type metalloproteinases (MT-MMPs): Key players in pregnancy". *Cell. Adh. Migr.*, 2016, *10*, 136.

[35] Wu L.Z., Liu X.L., Xie Q.Z.: "Osteopontin facilitates invasion in human trophoblastic cells via promoting matrix metalloproteinase-9 in vitro". *Int. J. Clin. Exp. Pathol.*, 2015, *8*, 14121.

[36] Sandri S., Urban Borbely A., Fernandes I., de Oliveira E.M., Knebel F.H., Ruano R.,*et al.*: "Serum amyloid A in the placenta and its role in trophoblast invasion". *PLoS One*, 2014, *9*, e90881.

[37] Law J., Zhang G., Dragan M., Postovit L.M., Bhattacharya M.: "Nodal signals via β-arrestins and RalGTPases to regulate trophoblast invasion". *Cell. Signal.*, 2014, *26*, 1935.

[38] Wu H.X., Jin L.P., Xu B., Liang S.S., Li D.J.: "Decidual stromal cells recruit Th17 cells into decidua to promote proliferation and invasion of human trophoblast cells by secreting IL-17". *Cell. Mol. Immunol.*, 2014, *11*, 253.

[39] Cheng J.C., Chang H.M., Leung P.C.: "Transforming growth factor-β1 inhibits trophoblast cell invasion by inducing Snail-mediated down-regulation of vascular endothelial-cadherin protein". *J. Biol. Chem.*, 2013, *288*, 33181.

[40] Winship A., Correia J., Zhang J.G., Nicola N.A., Dimitriadis E.: "Leukemia inhibitory factor (LIF) inhibition during mid-gestation impairs trophoblast invasion and spiral artery remodelling during pregnancy in mice". *PLoS One*, 2015, *10*, e0129110.

[41] Li C.M., Hou L., Zhang H., Zhang W.Y.: "CCL17 Induces Trophoblast Migration and Invasion by Regulating Matrix Metalloproteinase and Integrin Expression in Human First-Trimester Placenta". *Reprod Sci.*, 2014; Jan 8. pii: 1933719113519170.

[42] Ko H.S., Kang H.K., Kim H.S., Choi S.K., Park I.Y., Shin J.C.: "The effects of oncostatin M on trophoblast cells: influence on matrix metalloproteinases-2 and -9, and invasion activity". *Placenta*, 2012, *33*, 908.

[43] Ren L., Liu Y.Q., Zhou W.H., Zhang Y.Z.: "Trophoblast-derived chemokine CXCL12 promotes CXCR4 expression and invasion of human first-trimester decidual stromal cells". *Hum. Reprod.*, 2012, *27*, 366.

[44] Zhao H., Jiang Y., Cao Q., Hou Y., Wang C.: "Role of integrin switch and transforming growth factor Beta 3 in hypoxia-induced invasion inhibition of human extravillous trophoblast cells". *Biol. Reprod.*, 2012, *87*, 47.

[45] Nandi P., Siddiqui M.F., Lala P.K.: "Restraint of trophoblast invasion of the uterus by decorin: role in pre-eclampsia". *Am. J. Reprod. Immunol.*, 2015, *75*, 351.

[46] Zhang H., Hou L., Li C.M., Zhang W.Y.: "The chemokine CXCL6 restricts human trophoblast cell migration and invasion by suppressing MMP-2 activity in the first trimester". *Hum. Reprod.*, 2013, *28*, 2350.

[47] Tang Y., Liu H., Li H., Peng T., Gu W., Li X.: "Hypermethylation of the HLA-G promoter is associated with preeclampsia". *Mol. Hum. Reprod.*, 2015, *21*, 736.

[48] Gui S., Ni S., Jia J., Gong Y., Gao L., Zhang L., Zhou R.: "Inconformity of CXCL3 plasma level and placenta expression in preeclampsia and its effect on trophoblast viability and invasion". *PLoS One*, 2014, *9*, e114408.

[49] Liu Y., Wu B.Q., Geng H., Xu M.L., Zhong H.H.: "Association of chemokine and chemokine receptor expression with the invasion and metastasis of lung carcinoma". *Oncol. Lett.*, 2015, *10*, 1315.

[50] Wang L., Li X., Zhao Y., Fang C., Lian Y., Gou W., *et al.*: "Insights into the mechanism of CXCL12-mediated signaling in trophoblast functions and placental angiogenesis". *Acta Biochim. Biophys. Sin. (Shanghai)*, 2015, *47*, 663.

[51] Suman P., Gupta S.K.: "Comparative analysis of the invasion-associated genes expression pattern in first trimester trophoblastic (HTR-8/SVneo) and JEG-3 choriocarcinoma cells". *Placenta*, 2012, *33*, 874.

[52] Aghababaei M., Perdu S., Irvine K., Beristain A.G.: "A disintegrin and metalloproteinase 12 (ADAM12) localizes to invasive trophoblast, promotes cell invasion and directs column outgrowth in early placental development". *Mol. Hum. Reprod.*, 2014, *20*, 235.

[53] Christians J.K., Beristain A.G.: "ADAM12 and PAPP-A: candidate regulators of trophoblast invasion and first trimester markers of healthy trophoblasts". *Cell. Adh. Migr.*, 2016, *10*, 147.

[54] Havemann D., Balakrishnan M., Borahay M., Theiler R., Jennings K, Endsley J,. Phelps J., *et al.*: "Intermedin/adrenomedullin 2 is associated with implantation and placentation via trophoblast invasion in human pregnancy". *J. Clin. Endocrinol. Metab.*, 2013, *98*, 695.

[55] Liu S., Wang J., Qin H.M., Yan X.M., Yang X.S., Liu C., Yan Q.: "LIF upregulates poFUT1 expression and promotes trophoblast cell migration and invasion at the fetal-maternal interface". *Cell Death Dis.*, 2014, *5*, e1396.

[56] Cui Y., Wang W., Dong N., Lou J., Srinivasan D.K., Cheng W., *et al.*: "Role of corin in trophoblast invasion and uterine spiral artery remodelling in pregnancy". *Nature*, 2012, *484*, 246.

[57] Liao W.C., Chen C.H., Liu C.H., Huang M.J., Chen C.W., Hung J.S., *et al.*: "Expression of GALNT2 in human extravillous trophoblasts and its suppressive role in trophoblast invasion". *Placenta*, 2012, *33*, 1005.

[58] Li Y., Li Z., Zhang J.: "Dipeptidyl peptidase IV reduces trophoblast invasion by inhibiting the activity of MMPs". *Int. J. Dev. Biol.*, 2014, *58*, 349.

[59] Yang Y., Zhang J., Gong Y., Liu X., Bai Y., Xu W., Zhou R.: "Increased expression of prostasin contributes to early-onset severe preeclampsia through inhibiting trophoblast invasion". *J. Perinatol.*, 2015, *35*, 16.

[60] Chen Y.Y., Chuang P.Y., Chen C.P., Chiu Y.H., Lo H.F., Cheong M.L., *et al.*: "Functional antagonism between high temperature requirement protein A (HtrA) family members regulates trophoblast invasion". *J. Biol. Chem.*, 2014, *289*, 22958.

[61] Shi X., Liu H., Cao J., Liu Q., Tang G., Liu W., et al.: "Promoter hypomethylation of maspin inhibits migration and invasion of extravillous trophoblast cells during placentation". *PLoS One*, 2015, *10*, e0135359.

[62] Vincent Z.L., Mitchell M.D., Ponnampalam A.P.: "Regulation of MT1-MMP/MMP-2/TIMP-2 axis in human placenta". *J. Inflamm. Res.*, 2015, *8*, 193.

[63] Massimiani M., Vecchione L., Piccirilli D., Spitalieri P., Amati F., Salvi S., *et al.*: "Epidermal growth factor-like domain 7 promotes migration and invasion of human trophoblast cells through activation of MAPK, PI3K and NOTCH signaling pathways". *Mol. Hum. Reprod.*, 2015, *21*, 435.

[64] Stefanoska I., Jovanović Krivokuća M., Vasilijić S., Ćujić D., Vićovac L.: "Prolactin stimulates cell migration and invasion by human trophoblast in vitro". *Placenta*, 2013, *34*, 775.

[65] Chen C.P.: "Placental villous mesenchymal cells trigger trophoblast invasion". *Cell. Adh. Migr.*, 2014, *8*, 94.

[66] Rajaraman G., Murthi P., Brennecke S.P., Kalionis B.: "Homeobox gene HLX is a regulator of HGF/c-met-mediated migration of human trophoblast-derived cell lines". *Biol. Reprod.*, 2010, *83*, 676.

[67] Li W., Liu D., Chang W., Lu X., Wang Y.L., Wang H., *et al.*: "Role of IGF2BP3 in trophoblast cell invasion and migration". *Cell Death Dis.*, 2014, *5*, e1025.

[68] Li Y., Klausen C., Cheng J.C., Zhu H., Leung P.C.: "Activin A, B,

and AB increase human trophoblast cell invasion by up-regulating N-cadherin". *J. Clin. Endocrinol. Metab.*, 2014, *99*, E2216.
[69] Tapia-Pizarro A., Argandoña F., Palomino W.A., Devoto L.: "Human chorionic gonadotropin (hCG) modulation of TIMP1 secretion by human endometrial stromal cells facilitates extravillous trophoblast invasion in vitro". *Hum. Reprod.*, 2013, *28*, 2215.
[70] Peng B., Zhu H., Leung P.C.: "Gonadotropin-releasing hormone regulates human trophoblastic cell invasion via TWIST-induced N-cadherin expression". *J. Clin. Endocrinol. Metab.*, 2015, *100*, E19.
[71] Liu Z.K., Liu H.Y., Fang W.N., Yang Y., Wang H.M., Peng J.P.: "Insulin-like growth factor binding protein 7 modulates estrogen-induced trophoblast proliferation and invasion in HTR-8 and JEG-3 cells". *Cell Biochem. Biophys.*, 2012, *63*, 73.
[72] Zhu X., Yang Y., Han T., Yin G., Gao P., Ni Y., *et al.*: "Suppression of microRNA-18a expression inhibits invasion and promotes apoptosis of human trophoblast cells by targeting the estrogen receptor α gene". *Mol. Med. Rep.*, 2015, *12*, 2701.
[73] Winn V.D., Gormley M., Paquet A.C., Kjaer-Sorensen K., Kramer A., Rumer K.K., *et al.*: "Severe preeclampsia-related changes in gene expression at the maternal-fetal interface include sialic acid-binding immunoglobulin-like lectin-6 and pappalysin-2". *Endocrinol.*, 2009, *150*, 452.
[74] Tian F.J., Qin C.M., Li X.C., Wu F., Liu X.R., Xu W.M., Lin Y.: "Decreased stathmin-1 expression inhibits trophoblast proliferation and invasion and is associated with recurrent miscarriage". *Am. J. Pathol.*, 2015, *185*, 2709.
[75] Zhou Y., Yuge A., Rajah A.M., Unek G., Rinaudo P.F., Maltepe E.: "LIMK1 regulates human trophoblast invasion/differentiation and is down-regulated in preeclampsia". *Am. J. Pathol.*, 2014, *184*, 3321.
[76] Yu Y., Wang L., Tang W., Zhang D., Shang T.: "RNA interference-mediated knockdown of Notch-1 inhibits migration and invasion, down-regulates matrix metalloproteinases and suppresses NF-κB signaling pathway in trophoblast cells". *Acta Histochem.*, 2014, *116*, 911.
[77] Yang W., Li Q., Pan Z.: "Sphingosine-1-phosphate promotes extravillous trophoblast cell invasion by activating MEK/ERK/MMP-2 signaling pathways via S1P/S1PR1 axis activation". *PLoS One*, 2014, *9*, e106725.
[78] Ng Y.H., Zhu H., Leung P.C.: "Twist modulates human trophoblastic cell invasion via regulation of N-cadherin". *Endocrinol.*, 2012, *153*, 925.
[79] Ospina-Prieto S., Chaiwangyen W., Pastuschek J., Schleussner E., Markert U.R., Morales-Prieto D.M.: "STAT5 is activated by epidermal growth factor and induces proliferation and invasion in trophoblastic cells". *Reprod. Sci.*, 2015, *22*, 1358.
[80] Yang Y., Min J.: "Effect of ephrin-A1/EphA2 on invasion of trophoblastic cells". *J. Huazhong Univ. Sci. Technolog. Med. Sci.*, 2011, *31*, 824.
[81] Li W., Liu D., Chang W., Lu X., Wang Y.L., Wang H., *et al.*: "Role of IGF2BP3 in trophoblast cell invasion and migration". *Cell Death. Dis.*, 2014, *5*, e1025.
[82] Tuteja G., Chung T., Bejerano G.: "Changes in the enhancer landscape during early placental development uncover a trophoblast invasion gene-enhancer network". *Placenta*, 2016, *37*, 45.
[83] Liu Z.K., Liu H.Y., Fang W.N., Yang Y., Wang H.M., Peng J.P.: "Insulin-like growth factor binding protein 7 modulates estrogen-induced trophoblast proliferation and invasion in HTR-8 and JEG-3 cells". *Cel. Biochem. Biophys.*, 2012, *63*, 73.
[84] van Dijk M., Visser A., Posthuma J., Poutsma A., Oudejans C.B.: "Naturally occurring variation in trophoblast invasion as a source of novel (epigenetic) biomarkers". *Front. Genet.*, 2012, *3*, 22.
[85] Peng H., Xu F., Pershad R., Hunt K.K., Frazier M.L., Berchuck A., *et al.*: "ARHI is the center of allelic deletion on chromosome 1p31 in ovarian and breast cancers". *Int. J. Cancer*, 2000, *86*, 690.
[86] Wong V.C., Chan P.L., Bernabeu C., Law S., Wang L.D., Li J.L., *et al.*: "Identification of an invasion and tumor-suppressing gene, Endoglin (ENG), silenced by both epigenetic inactivation and allelic loss in esophageal squamous cell carcinoma". *Int. J. Cancer*, 2008, *123*, 2816.
[87] Paganini L., Carlessi N., Fontana L., Silipigni R., Motta S., Fiori S., *et al.*: "Beckwith-Wiedemann syndrome prenatal diagnosis by methylation analysis in chorionic villi". *Epigenetics*, 2015, *10*, 643.
[88] FitzGerald J., Luo M., Chaudhury A., Berger F.: "DNA methylation causes predominant maternal controls of plant embryo growth". *PLoS One*, 2008, *3*, e2298.
[89] Fowden A.L., Moore T.: "Maternal-fetal resource allocation: co-operation and conflict". *Placenta*, 2012, *33*, e11.
[90] Myers J.E., Merchant S.J., Macleod M., Mires G.J., Baker P.N., Davidge S.T.: "MMP-2 levels are elevated in the plasma of women who subsequently develop preeclampsia". *Hypertens. Pregnancy*, 2005, *24*, 103.
[91] Dekker G.A.: "Pre-eclampsia - a disease of an individual couple". *Pregnancy Hypertens.*, 2014, *4*, 242.

Corresponding Author:
H. KOBAYASHI, M.D., Ph.D.
Department of Obstetrics and Gynecology
Nara Medical University, 840 Shijo-cho
Kashihara, 634-8522 (Japan)
e-mail: hirokoba@naramed-u.ac.jp