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Minireview

Human Placental MicroRNAs and Preeclampsia[1]

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ABSTRACT

MicroRNAs are a class of noncoding small RNAs that regulate the expression of nearly 30% of all the human genes and participate in all fundamental cell processes. Genome-wide analysis has revealed that human placenta expresses more than 600 miRNA species, including placenta-specific ones with high levels of expression. Comparative analysis also has revealed many differentially expressed miRNAs with either high or low levels of expression in human placentas from normal versus preeclamptic pregnancies, indicating an important role of miRNAs in normal and pathological placental physiology. Although limited information is currently available as to how miRNA regulates human placental development and function, there are studies suggesting that preeclampsia-associated differentially expressed miRNAs possess critical roles in regulating placental development and function via targeting specific genes with diverse known functions. Herein we summarize the current findings regarding the expression of placental miRNAs and their function, especially in the trophoblast cells. We have recently found that the angiogenesis-associated miR-17-family miRNAs are upregulated in preeclamptic compared with normotensive placentas and they target the ephrin-B2/Eph receptor B4 (EPHB4) system. Because ephrin-B2 and EPHB4 has been previously shown to play a crucial role in trophoblast invasion into maternal spiral artery and vascular patterning during early human placental development, the miR-17-ephrin-B2/EPHB4 pathway seems to be a novel miRNA pathway for regulating normal and aberrant placental development during preeclampsia.

microRNA, placenta, placentation, preeclampsia, pregnancy

INTRODUCTION

Preeclampsia (PE) is a human pregnancy-specific disorder characterized by new onset of hypertension and proteinuria; it affects 5%–8% of all pregnancies worldwide and remains as a leading cause of maternal and neonatal mortality and morbidity [1, 2]. Although the etiology and pathogenesis of PE remain elusive, the placenta is definitely involved in the pathogenesis of PE because removal of the placenta eradicates the clinical manifestations of PE [3]. Both conventional and microarray approaches have identified a substantial number of differentially expressed (DE) genes in the transcriptome of human placentas from PE compared with normotensive healthy term deliveries [4–6], clearly suggesting differential gene expression is associated with the pathogenesis of PE. Genetic and epigenetic pathways have been suspected to cause the alterations of placental transcriptome by PE [7, 8]. However, how placental gene expression is differentially regulated in PE pregnancy is largely unknown.

The miRNAs represent a class of 21–25-nucleotide noncoding small RNAs that negatively regulate gene expression primarily at the level of posttranscription. Since the discovery of the first miRNA, *lin-4*, in *Caenorhabditis elegans* in 1993 [9, 10], a large number of miRNAs have been identified in many species. In human, more than 1000 miRNAs have been detected to date, and this number is still increasing [11, 12]. In mammals, miRNAs are highly conserved across species [13]. It is estimated that miRNAs regulate the expression of ~30% of all human genes [14], thereby participating in nearly all fundamental cellular processes, including cell differentiation, proliferation, migration and apoptosis, etc. [15]. In recent years, miRNAs have emerged as one of the hottest research topics in biology and disease-related pathologies including PE. Genome-wide analysis of the miRNA species (miRNAome) expressed in human placenta has identified a dozen PE-associated DE miRNAs. A few functional studies have also suggested that miRNAs serve as an important pathway for regulating placental development and function. In this review, with a brief introduction of human placental development and the biosynthesis and function of miRNAs, we summarize the latest findings on the expression and function of miRNAs in the placenta. According to our findings we propose a novel miRNA pathway for the regulation of placental development as related to the pathogenesis of PE.

PLACENTAL DEVELOPMENT AND THE PATHOGENESIS OF PE

Mammalian development is characterized by the early specification of the trophoblast lineage whose dedicated function lies in the survival of embryo and fetal development in utero. In eutherians, the polarized outer layer of epithelial cells termed trophectoderm surrounding the inner cell mass of the implanting embryo develops as cytotrophoblasts (CTBs) that function as placental stem cells during early placental development [16]. The CTBs further differentiate into specialized villous cell types via the villous and extravillous pathways [17, 18]. The villous pathway directs the proliferative mononucleated CTBs' fusion to form an external layer of

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terminally differentiated nonproliferative multinucleated syncytiotrophoblasts (STBs). STBs are endocrine cells synthesizing hormones for pregnancy, including human chorionic gonadotropin, placental lactogen, and steroid hormones; they cover all placental chorionic villi and come into direct contact with maternal blood, and thus are crucial for maternal-fetal exchanges; they are human leukocyte antigen-G (HLA-G)-positive cells and protect the allograft fetus from immune rejection [19]. In human, around Day 14 after implantation, CTBs break through the STB layer, differentiating via the extravillous pathway to form columns of the extravillous trophoblasts (EVTs) to invade the uterine stroma. The motile invasive tip cells of the EVT columns move laterally to form the trophoblastic shell and longitudinally to invade deeply the decidua and the deeper portion of the myometrium, where these cells profoundly remodel the uterine spiral arteries [20–22]. The endothelial and smooth muscle cells of the spiral arteries are replaced by EVTs, transforming the spiral arteries from high-resistance and low-flow vessels into dilated vessels with more than 10-fold-increased blood flow due to a much-reduced pressure [23]. This is a process of vascular mimicry, which is called endovascular transformation or pseudovasculogenesis [24] because the invading trophoblasts convert from an epithelial to an endothelial phenotype [25]. Trophoblast-induced spiral artery remodeling is crucial for a healthy pregnancy because shallow EVT invasion and incomplete endovascular transformation impairs spiral artery remodeling, resulting in reduced blood flow in the maternal-fetal and placental interface in the first trimester of gestation. This has been recognized as the leading pathology of various pregnancy disorders, including PE, gestational diabetes, intrauterine growth restriction, and low birth weight [4, 5, 26].

In the case of PE, eradication of the clinical manifestations after removal of the placenta [3] clearly indicates that aberrant placental development is involved in the pathogenesis of PE. Although its etiology and pathogenesis remain elusive, the current literature for the pathogenesis of PE favors a two-stage model hypothesis that applies to a majority of all PE cases [1, 27]. The first stage is poor placentation as evidenced by color Doppler ultrasound demonstration of a tight link between reduced spiral arterial blood flow before the 20th week of gestation and a greatly increased risk of developing PE [28, 29]. Contrasting to a spectrum of physiological changes that normally occur in the maternal spiral arteries, e.g., loss of endothelial lining and musculoelastic tissue, interstitial CTB invasion is often shallow and endovascular invasion does not proceed beyond the terminal portions of the vessels in PE. The mean external diameter of the myometrial vessels is less than half that of similar vessels from normal pregnancies [30–33]. In addition, fewer vessels show evidence of trophoblast invasion [34]. As a result, impaired spiral artery remodeling precludes an adequate response to gestation-related fetal demands of increased blood flow. This causes placental ischemia/hypoxia, resulting in trophoblast debris [35], and induces placental secretion of harmful factors, such as soluble *fms*-like tyrosine kinase 1 (sFlt1) [36, 37], inflammatory cytokines [38, 39], and antibodies of angiotensin-I receptors [40], to be circulated into the maternal blood. Overall, these changes lead to the second stage of the maternal manifestations.

The second stage of the disease is the maternal response to abnormal placentation, which is featured by systemic endothelial dysfunction [41]. Of note, endothelial dysfunction occurs not only in the maternal but also in the placental vasculature [42–45]. Many circulating factors, such as sFlt1, soluble endoglin, tumor necrosis factor-α, interleukin 1, fibronectin, and factor VIII antigen [36–39, 46, 47], etc., have been found to be elevated in the blood of pregnant women with PE [38, 39]. Concomitantly, the levels of endothelium-derived vasodilators such as nitric oxide (NO) [48–50] and prostacyclin [51] are decreased, and the level of *S*-nitrosoproteins (NO reservoir) is increased in maternal serum [52] and placenta [53] in PE. An imbalance between circulating harmful and beneficial factors further leads to systemic inflammatory responses and endothelial dysfunction in many organ systems, including the vasculature, kidney, liver, brain, etc. Eventually, the clinical manifestations of PE occur after the 20th week of gestation with a triad symptom of rapid onset of hypertension, proteinuria, and edema secondary to increased vascular permeability in the glomeruli and peripheral tissues [1, 2].

BIOGENESIS AND FUNCTION OF miRNAs

The miRNA biosynthesis begins with RNA polymerase II-dependent transcription of long primary transcripts (pri-miRNAs) from the genes encoding miRNAs, many of which reside in the introns of their host genes; these pri-miRNAs fold into distinctive stem-loop precursors [54, 55]. In mammals, the long pri-miRNAs are first processed in the nucleus by a ''microprocessor complex'' that is composed of the RNase III endonuclease Drosha and a double-stranded RNA (dsRNA)-binding protein, DGCR8, forming 60–70 nucleotide precursor miRNAs (pre-miRNAs) with stem-loop structure and 3′ overhangs. The pre-miRNAs are then exported from the nucleus into the cytoplasm by Exportin 5 (Exp 5) and further processed by another RNase III enzyme, Dicer, into miRNA duplexes [56, 57]. Finally, the miRNA duplex is unwound; one strand functions as the mature miRNA, which is incorporated into the RNA-induced silencing complex (RISC) that contains Argonaute (Ago) proteins at its core [58, 59] and other proteins including DEAD-box helicase protein DP103 [60], gemin4, and Moloney leukemia virus 10 (MOV10) [61]. The miRNA-RISC complex binds specific sites in the 3′-untranslated region (3′UTR) of target mRNAs, disabling them through deadenylation and destabilization, as well as translational repression [62, 63] (Fig. 1).

Translational inhibition has been recognized as the main mechanism by which miRNA suppresses protein expression. The literature strongly suggests that this is largely achieved by destabilizing target mRNAs [64] based on the complementary pairing between the miRNA seed sequence centered on nucleotides 2–7 of the miRNA and the 3′UTR of the target mRNA [65]. As the pairing can be either a perfect or an imperfect complement, one miRNA is able to regulate the expression of multiple genes and one gene can be regulated by more than one miRNA. Although the majority of literature points to an inhibitory role of miRNA in regulating gene expression, there are studies also suggesting alternative mechanisms for miRNA to regulate gene expression. For example, miR-373 has been found to induce the expression of E-cadherin and cold-shock domain-containing protein C2 through targeting their complementary promoter sequences [66].

PLACENTA-SPECIFIC miRNAs

Since 2007, several studies have used miRNA microarray and real-time PCR approaches to profile the genome-wide expression of miRNAs in normal and pathological human placentas. These expression studies have revealed that many specific miRNAs are expressed in human placenta [67–70]. For example, our recent genome-wide screening study using miRNA array has identified that the miRNAome in normal healthy term human placenta contains more than 600 miRNAs

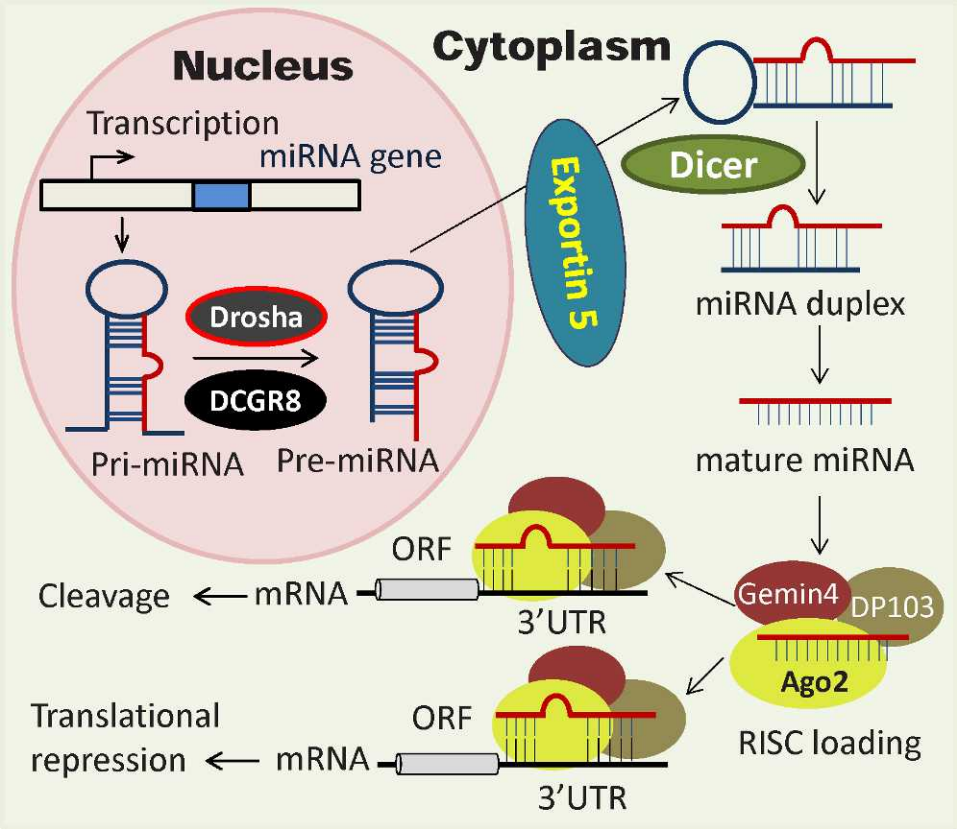

FIG. 1. Biogenesis and function of miRNAs. The miRNA biosynthesis begins with RNA polymerase II-dependent transcription of pri-miRNAs from miRNA genes that reside in the introns of their host genes. Pri-miRNAs fold into distinctive stem-loop precursors. In mammals, the long pri-miRNAs are first processed in the nucleus by a microprocessor complex that is composed of the RNase III endonuclease Drosha and a dsRNA-binding protein DGCR8, forming 60–70 nucleotide pre-miRNAs with stem-loop structure and 3′ overhangs. The pre-miRNAs are then exported from the nucleus into the cytoplasm by Exp 5 and further processed by another RNase III enzyme, Dicer, into miRNA duplexes. Finally, the miRNA duplex is unwound; one strand functions as the mature miRNA, which is incorporated into the RISC that contains Ago proteins at its core and other proteins including DEAD-box helicase protein DP103, gemin4, and MOV10. The miRNA-RISC complex binds specific sites in the 3′UTR of target mRNAs, disabling them through deadenylation, destabilization, and translational repression.

[71]. Other investigators have also identified more than 300 mature miRNAs in human placenta by using different arrays [72, 73].

The miRNAome in the human placenta contains numerous miRNAs with different expression levels. Through sequencing a small RNA library constructed from human placental villous tissue, it has been found that many placental miRNAs with high levels of expression are encoded by the C19MC-cluster miRNA genes located in chromosome 19 [70]. The C19MC cluster is the largest human miRNA gene cluster discovered to date. This cluster is primate specific and is expressed almost exclusively in the placenta [67–70]. The C19MC cluster spans about 100 kb at human chromosome 19q13.41, consisting of 54 predicted miRNAs; 43 have been cloned and sequenced [11, 74]. C19MC miRNAs are encoded and processed from introns of the non-protein-coding Pol-II transcripts [75]. Their expression pattern is associated with methylation status of a distal CpG-rich region located at 17.6 kb upstream of the miRNA cluster. DNA methylation profiling has identified that C19MC is regulated by genomic imprinting with only paternally inherited allele being expressed in the placenta, implicating its important role in human embryonic development similar to that of other imprinted genes such as insulin-like growth factor 2 [76–78]. Studies on the expression profile of the C19MC miRNAs in the primary human trophoblasts (PHTs) from term placenta demonstrate that they comprise the majority of human trophoblast miRNAs because they are expressed at a much higher level than the total level of all the other miRNAs expressed in the PHT. The highest-expressing C19MC miRNAs in the PHT include miR-517a, miR-517b, miR-516b, miR-525-5p, miR-512-3p, and miR-515-3p [79]. It is found that miR-517a-3p, miR-519a-3p, and miR-520c-3p are also abundantly expressed in placenta-derived mesenchymal stromal cells [80], indicating that their placenta-specific functions are not just restricted to the trophoblast. In addition, some of these placenta-specific miRNAs have been detected in the maternal circulation throughout gestation with significant decrease after pregnancy termination, including miR-515-3p, miR-516-5p, miR-517a, miR-517c, miR-518b, miR-520a*, miR-520h, miR-525, miR-526a, and miR-526b [81, 82]. These results suggest that maternal circulating miRNAs may have the potential to be novel diagnosis agents for pregnancy disorders. Although the biological functions of the C19MC cluster in the placenta are currently unknown, it is anticipated that they are important for the maintenance of normal placental physiology because of their high levels of expression.

Apart from the abundantly expressed C19MC cluster, numerous miRNAs with relatively low levels of expression are also expressed in the human placenta. Detailed information about human placental miRNAs can be found in the published work [67–73]. Although the biological functions of only a few miRNAs have been determined in placenta using established trophoblast cell models in vitro (see below), bioinformatics analysis of the diverse genes predicted to be the targets of

placental miRNAs suggests that miRNAs are essential for normal and abnormal placental physiology.

DIFFERENTIAL EXPRESSION AND REGULATION OF HUMAN PLACENTAL miRNAs

Genome-wide comparative analyses of the miRNAomes in human placentas from healthy term pregnancy and those complicated with PE [71–73, 83–85] and preterm labor [73] have identified more than a dozen DE miRNAs that are subjected to regulation in human placenta.

By using real-time quantitative RT-PCR with TaqMan primer sets, Pineles et al. [84] first determined the expression of 157 mature human miRNAs in placental samples from PE, small-for-gestational age (SGA), PE + SGA, and control group with spontaneous preterm labor and delivery (n = 9/group) [84]. They found 153 miRNAs expressed in placenta, and 7 miRNAs (miR-210, miR-155, miR-181b, miR-182*, miR-100b, miR-154*, and miR-183) were upregulated in the PE + SGA group compared with the control group. Zhu et al. [85] then compared the miRNAomes in human placentas from normal deliveries (n = 11) and those complicated by mild (n = 8) and severe PE (n = 15) by using the Exiqon miRNA microarray containing 455 human miRNAs, 344 mouse miRNAs, and 236 rat miRNAs. They found 34 PE-associated DE miRNAs, with 11 upregulated and 23 downregulated in severe-PE placentas compared with normal controls [71–73, 83–85]. Subsequently, several studies have analyzed the miRNAome in human placenta using microarrays with more miRNA probes. Enquobahrie et al. [72] used an Invitrogen microarray containing 1295 probes with 854 probes for human mature miRNAs and 379 novel small RNAs [72]. They found eight DE miRNAs, including upregulated miR-210, and seven downregulated miRNAs, including miR-328, miR-584, miR-139-5p, miR-500, miR-1247, miR-34c-5p, and miR-1, from PE compared with normotensive placentas (n = 20/group). In another study, Mayor-Lynn et al. [73] used a custom-developed microarray containing 1213 human probes targeting 820 mature miRNAs and 393 Invitrogen ''novel'' probes on 21 placental tissue samples from PE, spontaneous preterm delivery, and normal controls (n = 7/group) with elective cesarean delivery [73]. They found 20 DE miRNAs among the three groups and confirmed several DE miRNAs by real-time PCR. Lower expression of miR-15b, miR-181a, miR-210, and miR-483-5p was detected in the preterm group compared with the control group. However, when compared between PE and normal controls, no difference was observed for these miRNAs, except for miR-15b, with lower expression in PE.

Surprisingly, each published work has reported a different set of DE miRNAs, with minimum overlapping among all the studies. The reason for the discrepancy is not known, but it likely originates from the different arrays and RNA samples used. The most commonly identified placental DE miRNA is miR-210, which is found to be upregulated in PE placentas compared with normotensive controls in most studies [72, 84–86], except that one study reported that miR-210 is downregulated in preterm compared with normal placentas, but with no significant difference between PE and control groups [73]. We recently used the LC Sciences miRNA microarray containing 894 unique probes to cover all miRNAs available in the miRBase database (version 14.0) for analyzing the miRNAomes in human placenta from severe PE compared with normotensive term deliveries (n = 3) and confirmed the DE miRNAs by real-time PCR. We also did not find significant changes in miR-210 in PE versus normotensive placentas. We found nine DE miRNAs between PE and normotensive placentas, including five upregulated miRNAs (miR-20b, miR-516a-5p, miR-512-3p, miR-2277, and miR-524-3p) and four downregulated miRNAs (miR-151-3p, miR-146a, miR-192, and miR-34c-5p). However, when real-time PCR with specific primers was used to confirm the DE miRNAs from 10 paired placental samples, we identified that the miR-17-family miRNAs (i.e., miR-17, miR-20a, and miR-20b) are significantly upregulated in PE compared with healthy term placentas [71].

Hypoxia plays a significant role in normal and abnormal placental development [26], and it is not surprising that hypoxia regulates trophoblast miRNA expression [87]. Mouillet et al. found seven DE miRNAs (miR-93, miR-205, miR-224, miR-335, miR-424, miR-451, and miR-491) in primary trophoblasts exposed to hypoxia using miRNA microarray and confirmed the DE ones by Northern blotting [87]. It has been found that miR-210 is hypoxia inducible in both endothelial cells and tumor cells [88, 89]. More recently, it has been reported that miRNA-210 expression was also elevated in cultured trophoblasts under hypoxic condition [86, 90].

There is one paper published recently comparing the miRNA expression profiles between the first- and third-trimester human placentas. Among the 191 DE miRNAs, miRNAs in the miR-17-92 cluster, C14MC, the miR-371 cluster, and C19MC were significantly higher in the first-trimester placentas, whereas miRNAs of the let-7 family, the miR-34 family, the miR-29 cluster, miR-195, and miR-181c were significantly upregulated in the third-trimester placentas [91]. The differential expression indicates the developmental changes of miRNA expression in placenta during pregnancy. Furthermore, it has been reported that the expression levels of both miR-517b and miR-519a change oppositely in association with the weight changes of placental villi during the first trimester. Both miR-517b and miR-519a are highly expressed in the trophoblast, and their expression is associated with the weight of villi in the same gestational week during the first trimester; miR-517b is upregulated in the light villi, whereas the expression of miR-519a is upregulated in the heavy villi. These data suggest their opposite role in regulation of trophoblast proliferation [92] and thus a role of miRNAs in placental development.

miRNA-DIRECTED GENE EXPRESSION IN PLACENTAL DEVELOPMENT AND FUNCTION

It is clear that miRNAs play an essential role in the regulation of placental development and function, because disabling miRNA machinery because of a mutation in mouse Ago2 causes malformation of placental labyrinthine layer with a greatly reduced thickness and death of the mutant embryos at midgestation [93]. Furthermore, global miRNA reduction in CTBs through small interfering RNA (siRNA)-mediated knockdown of Dicer significantly enhances cell proliferation in cultured first-trimester placental explants [94], implicating a negative regulatory effect of miRNAs in trophoblast cells. Gain and loss of function studies using established trophoblast cell lines and primary trophoblast cell culture models have also suggested critical roles of some DE miRNAs in placental development and angiogenesis as well as functionality of the placenta (see below). However, the current understanding of the roles that placenta-specific miRNAs play in the regulation of placental development and function is still in its very early stage. Most, if not all, of the published work has been focused on profiling miRNA expression in placenta from normal versus disease-affected pregnancies. Although bioinformatics analysis for target prediction suggests that placenta dysfunction-

TABLE 1. Target genes and their functions of miRNAs in the trophoblasts or placenta.

miRNA	Target genes	Trophoblast	Regulatory role in trophoblast	Reference
miR-17, miR-20a, miR-20b	VEGFA, EFNB2, EPHB4, MMP2, HIF1A	HUVEC, BeWo	Trophoblast differentiation (?), angiogenesis (?)	[71]
miR-34a	PAI-1 (possible)	JAR	Inhibit trophoblast invasion	[97]
miR-34a	Notch1, Jagged1	BeWo, JAR	Inhibit trophoblast invasion	[96]
miR-152	HLA-G	JEG-3	Increase NK cell-mediated cytolysis	[95]
miR-155	Cyclin D1	HTR8/SVneo	Inhibit cell proliferation and invasion, and increased cell number at the G1 stage	[103, 104]
miR-195	Acvr2a (ActRIIA)	HTR8/SVneo	Promote cell invasion	[105]
miR-210	Iron-sulfur cluster scaffold homologue (ISCU)	Swan 71	Inhibit cell invasion	[90]
miR-210	EFNA3, homeobox-A9	Primary CTB, JAR	Inhibit cell migration and invasion	[86]
miR-205	MED1	Primary CTB, HTR8/SVneo	?	[87]
miR-376c	ALK5 ALK7	HTR8/SVneo, placental explant	Induce cell proliferation, migration, and invasion and promote explant outgrowth	[107, 108]
miR-378a-5p	Nodal	HTR8/SVneo	Enhance cell survival, proliferation, migration, and invasion	[106]
miR-517b, miR-519a	?	Placental villi	Inhibit cell proliferation	[92]

associated DE miRNAs participate in the regulation of numerous fundamental cellular processes in the placenta, the biological functions of the majority of the placenta-specific miRNAs are awaiting determination. In Table 1, we summarize the current literature on the genes that have been identified to be regulated by placenta-specific miRNAs as related to placental development and function.

Functional studies with several widely used choriocarcinoma cell lines have suggested multiple regulatory roles of miRNAs in trophoblast cells. In human placental choriocarcinoma-derived JEG-3 cells, overexpression of miR-152 suppressed HLA-G expression and increased natural killer cell-mediated cytolysis, suggesting its function in the immune system [95]. In another human placental choriocarcinoma cell line, JAR cells, overexpression of miR-34a inhibits cell invasion by targeting Notch1 and Jagged1, and possibly plasminogen activator inhibitor-1 [96, 97]. The expression of miRNA-199b has been found to be downregulated in choriocarcinomas compared with noncancer trophoblast tissues; miR-199b seems to promote proliferation and tumorigenesis of trophoblast cells via inhibiting the protein phosphatase 2A inhibitor [98]. We have recently observed that forced miR-20b expression inhibits cell fusion (syncytialization) and induces the expression of a set of endothelial cell markers in BeWo cells (Wang and Chen, unpublished data), implicating a potential role of miR-17-family miRNAs in driving CTB differentiation via promoting the extravillous and inhibiting the villous pathways into EVTs and STBs, respectively [17, 18].

A few studies have also used PHT cells to characterize the biological function of the PE-associated DE miRNAs. Hypoxia induces the expression of many miRNAs, including miR-210 and miR-205, in cultured primary trophoblast cells [87, 99, 100]. Gain and loss of function studies by transfection of miR-210 precursors or antagomirs into primary trophoblast cells have shown that miR-210 inhibits trophoblast oxygen consumption, which might be responsible for placental mitochondria dysfunction in PE [100]. Forced miR-205 expression inhibits the expression of mediator 1 (MED1) that is known to regulate placental development [101]. In vitro, siRNA-silencing MED1 in primary CTBs perturbs the expression of several markers of trophoblast differentiation, implicating a role of miR-205 in placental adaptation to hypoxia [101]. Overexpression of PE-upregulated miR-155 [84, 102] inhibits proliferation and migration [103], but increases syncytialization of the human first-trimester EVT-derived HTR-8/SV-neo cells [104]. Overexpression of PE-downregulated miR-195 in HTR-8/SV-neo cells promotes cell invasion via targeting Acvr2a (ActRIIA), the type II receptor for activinA and Nodal [105]. Interestingly, stable overexpression of miR-378a-5p in HTR-8/SVneo cells enhances cell survival, migration, and invasion through downregulation of Nodal, a member of the transforming growth factor β (TGFβ) superfamily [106]. In addition, miR-376c indirectly regulates Nodal-TGFβ signaling. Overexpression of PE-downregulated miR-376c induces HTR-8/SVneo cell proliferation, migration, and invasion and promotes placental explant outgrowth via targeting activin receptor-like kinase 5, a type I receptor for TGFβ, and ALK7, a type I receptor for Nodal [107, 108]. The miR-378a-5p/Nodal-TGFβ pathway seems to be important for placental development because knockout of Nodal in the mouse uterus results in aberrant placental development and reduced proliferation combined with increased apoptosis in the decidual basalis at the maternal-fetal interface, leading to preterm birth [109].

The most common placental PE-associated DE miRNA, miR-210, has been demonstrated as one of the hypoxamirs, which are upregulated by hypoxia [88, 110–112]. This miRNA is evolutionarily conserved and ubiquitously expressed in different cell types and is specially induced by hypoxia-inducible factor-1 alpha (HIF1A) [88, 89] via directly binding to a hypoxia-responsive element on the promoter of miR-210 [113]. Hypoxia-driven miR-210 expression promotes angiogenesis by downregulating ephrin-A3, repressing vascular endothelial growth factor (VEGFA)-dependent endothelial cell migration and tubulogenesis [88]. In addition, miR-210 can target protein-tyrosine phosphatase 1B, inhibiting VEGFA

signaling in endothelial cells [114, 115]. A recent study using both rat ischemic brain cortexes and hypoxic models of human umbilical vein endothelial cells demonstrated that miR-210 activates Notch signaling and induces endothelial cell migration and tube formation [116]. Because Notch signaling plays a critical role in trophoblast endovascular invasion during placental development [117], these data indicate an important role of miR-210 in placentation.

There are numerous studies that have shown a key role of miRNAs in angiogenesis [118, 119]. The first evidence came from a study of mouse embryos with homozygous deletion of exon 1 and 2 of Dicer gene resulting in embryo death between Days 12.5 and 14.5 of gestation with severely compromised blood vessel formation, which indicates an essential role of Dicer in mouse embryonic angiogenesis [120]. In vitro studies with cultured endothelial cells provide more evidence on the roles of Dicer in endothelial cell biology and angiogenesis processes, including proliferation, migration, capillary sprouting, and tube formation [121–124]. All of these important cellular processes are required during placenta development. Recently, it has been shown that miR-155 may be important for placental angiogenesis because it targets the PE-downregulated angiogenic factor cysteine-rich angiogenic inducer 61 [102, 125, 126] that promotes cell proliferation, differentiation, and adhesion [127]. Although how miRNA regulates placental angiogenesis is unknown, this is an important area that warrants further investigation because angiogenesis is critical for the formation and expansion of the maternal, fetal, and placental interface vascular network that carries out bidirectional maternal-fetal exchanges [1, 128, 129].

miR-17-FAMILY miRNAs (miR-17, miR-20a, AND miR-20b) AND ephrinB2/Eph RECEPTOR B4 IN THE PLACENTA

By using microarray and real-time quantitative PCR analysis, we have identified significantly greater levels of miR-17, miR-20a, and miR-20b in PE versus normotensive term human placentas ($n = 10$/group) [71]. They comprise the miR-17 family that belongs to the miR-17-92 cluster important for angiogenesis [118, 119]. The cluster contains six miRNAs, including miR-17, miR-18, miR-19a, miR-19b-1, miR-20a, and miR-92a-1, that are processed from a common precursor transcript. Based on their seed sequences, they are grouped into four families: the miR-17 family (miR-17 and miR-20a), the miR-18 family, the miR-19 family, and the miR-92 family. The cluster has two paralogs in mammals, miR-106a-363 and miR-106b-25, that are derived from ancient gene duplications. They contain homologous miRNAs to those encoded by miR-17-92 [130]. The miRNA miR-20b is located in the miR-106a-363 cluster and belongs to the miR-17 family. Sequence analysis revealed that miR-17, miR-20a, and miR-20b share identical seed sequence [71], leading to an assumption that they possibly possess overlapping functions by targeting similar sets of genes. Bioinformatics analysis using target prediction databases suggests many putative target genes of miR-17, miR-20a, and miR-20b, including HIF1A, interleukin 8, tissue inhibitor of metalloproteinase 2, matrix metallopeptidase 2, VEGFA, ephrin-B2 (EFNB2) and Eph receptor B4 (EPHB4).

Among the potential targets of miR-17-family miRNAs, EFNB2 and EPHB4 are of importance because they have been shown to play a critical role in directing CTB invasion toward the EFNB2-expressing uterine spiral arterioles, but not the EPHB4-expressing uterine veins at the maternal-fetal interface during early human placentation [131]. EPHB4 and EFNB2 are the molecular markers of veins and arterials [132], respectively; they belong to the large family of Eph receptor tyrosine kinases and their ephrin ligands, respectively. EFNB2 is a membrane-bound molecule that acts via close cell contact (up to 20 nm) to control not only arterial endothelial cell growth but also the motility and adhesion of vascular smooth muscle cells and pericytes for growing arterial endothelial cell tubes [133]. EFNB2 binds its receptor EPHB4 specifically with very high affinity. EPHB4 expression occurs in veins of all diameters [134]; it has been implicated in tumor angiogenesis and is considered a target for antiangiogenic tumor therapy [135]. Because both EFNs and EPHs are receptor tyrosine kinases, ligation induces bidirectional signaling that is essential and specific for delineating arterial-venous boundaries [136]. EFNB2 forward signaling through EPHB4 receptor inhibits cell adhesion, whereas EPHB4 reverse signaling to EFNB2 induces cell attachment [137, 138] and differentiation [139]. Interaction of Eph receptors and cell surface-bound ephrin ligands induces signal transduction into both receptor- and ligand-expressing cells, mediating various cellular responses such as cell adhesion, repulsion, and migration [140, 141]. It is also possible that they possess a role in angiogenesis because of a proangiogenic function of EFNB2 in regulating the internalization and signaling activities of VEGFA receptor VEGFR2 and VEGFR3 in endothelial cells [142, 143].

During human pregnancy, the trophoblast expressions of both EFNB2 and EPHB4 are significantly decreased with gestation stage and are involved in building the functional placental structures [144, 145]. During placentation, human CTBs switch from venous $EPHB4^+$ to arterial $EFNB2^+$ phenotype as they differentiate to become EVTs [131]. This arterial endovascular transformation of CTBs seems to account for the unique replacement of endothelial cells by human EVTs in the spiral arteries but not veins. In this regard, work from Susan Fisher's group has elegantly shown that the Eph-ephrin interactions pattern CTB invasion via generating repulsive signals to direct CTB invasion towards the uterine wall and allowing invasive EVTs to migrate only to the spiral arteries ($EFNB2^+$) but not veins ($EPHB4^+$) during human placentation [117, 131, 146]. During mouse pregnancy, trophoblasts express high levels of EFNB2 and EPHB4 at early Gestational Day 6.5 (gd6.5) and both decrease continuously during the course of gestation, implicating their critical roles in placental development. Meanwhile, the phenotype of spiral arteries switches from $EFNB2^+/EPHB4^-$ at gd6.5 to $EFNB2^+/EPHB4^+$ at gd10.5 and onwards [147], possibly because of increased blood flow, because shear has been found to increase arterial EPHB4 expression in tumors [148]. This switch may reduce trophoblast-mediated spiral artery remodeling by repulsing trophoblast cells to signal the completion of placentation.

It is noteworthy that until we have verified both EFNB2 and EPHB4 as the direct targets of miR-17-family miRNAs by luciferase reporter gene expression assays and gain and loss of function studies [71], how placental EFNB2/EPHB4 expression is regulated has never been reported. In addition, we have also observed significantly lower EFNB2 mRNA expression in severe PE versus normotensive term placentas [71]. In keeping with the inverse relationship between placental expressions of miR-17-family miRNAs and EFNB2/EPHB4 in severe PE versus normotensive term placentas and the known functions of EFNB2/EPHB4 in mouse and human placentation, it is highly suspicious that miR-17-family miRNAs play key roles in trophoblast differentiation, invasion, endovascular transformation, and spiral artery remodeling during placental development via regulating EFNB2 and EPHB4 expression.

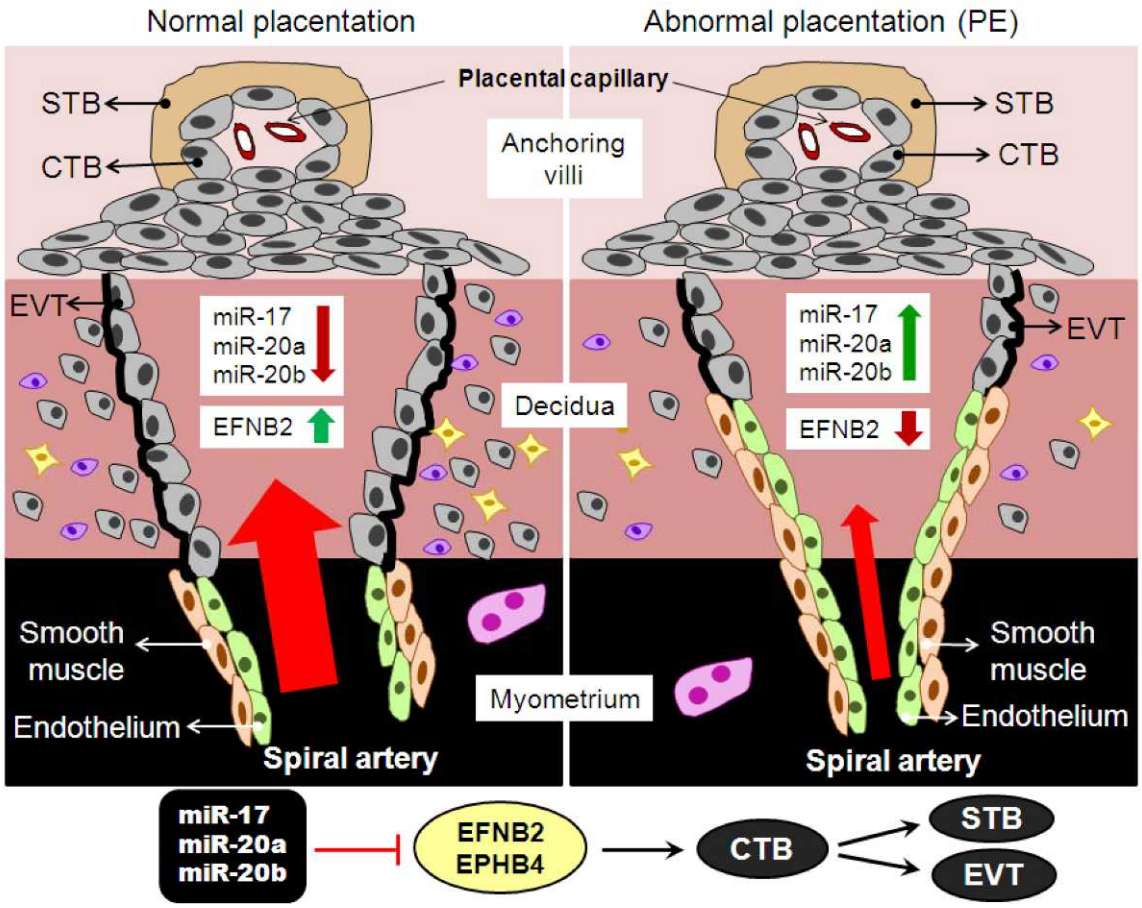

FIG. 2. A putative miRNA pathway for placental development and the pathogenesis of PE. During normal placentation, low levels of miR-17-family miRNAs (mir-17, miR-20a, and miR-20b) allow higher expression of EPHB4 and EFNB2 in the placenta. This facilitates the differentiation of CTB progenitors into invasive EVTs for invading the maternal decidua and for remodeling the uterine spiral arteries. The spiral arteries are transformed into low-resistance vessels with a much-reduced pressure, thereby causing blood flow to rise for facilitating the bidirectional mother-fetus exchanges of nutrients and respiratory gases (oxygen and carbon dioxide) and exclusion of fetal metabolic wastes. However, during abnormal placentation in PE, increased miR-17-family miRNAs suppress EFNB2/EPHB4 expression, further inhibiting endovascular transformation of CTBs and invasion of EVTs for spiral artery remodeling. Failed spiral artery remodeling constrains maternal-fetal interface blood flow, which contributes to the pathogenesis of PE.

PERSPECTIVES: A miR-17/EFNB2-EPHB4 PATHWAY FOR THE PATHOGENESIS OF PE

We have recently shown that miR-17-family miRNAs negatively regulate EFNB2-EPHB4 expression in human placentas because of the following observations [71]. First, by using microarray and real-time RT-PCR we have found that miRNA-17-family miRNAs are upregulated in human placentas complicated by PE compared with normotensive pregnancies, which is inversely correlated to EFNB2 expression in placenta. Second, bioinformatics analysis predicts the direct binding of miR-17-family miRNAs to the 3′UTR of EFNB2 and EPHB4, which is confirmed by luciferase reporter assays. Third, overexpression of miR-17-family miRNAs downregulates EFNB2 whereas transfection of miR-17 antagomirs upregulates EPHB4 expression in BeWo cells [71]. On the other hand, work from Fisher's group has elegantly shown that EPHB4 and EFNB2 play a critical role in regulating trophoblast differentiation and migration during early human placental development because of the following findings. EPHB4 and EFNB2 are DE in different populations of trophoblasts. CTB progenitors and STB in placenta express high levels of EPHB4. When CTB progenitors differentiate into invasive CTBs, EPHB4 expression is downregulated, with a concomitant upregulation of EFNB2 and other members of the EFN/EPH family such as EFNB1 and EPHB2. The invasive CTBs that line maternal spiral arteries, i.e., the endovascular subpopulation of CTBs, also express high levels of EFNB2, B1, and EPHB2. When CTB progenitors are cultured on laminin substrates that are spotted with either recombinant EFNB2 or EPHB4 proteins, they specially avoid substrates containing EPHB4, but not EFNB2. CTBs rapidly migrate to the EFNB2-expressing NIH 3T3 cells to form aggregates when cocultured, whereas CTB migration is largely inhibited by coculture with EPHB4-expressing NIH 3T3 cells [131]. Moreover, endometrial EFNB2/EPHB4 expression changes during mouse pregnancy. The spiral arteries transit from an $EFNB2^{+}$-high state to an $EFNB2^{+}$-low state accompanied by a strong gain of EPHB4 expression between gd10.5 and 12.5. The gain of EPHB4 expression by midgestation spiral arteries may serve as a repulsion signal for completion of spiral artery remodeling [147].

The aforementioned findings lead us to propose a novel miR-17/EFNB2-EPHB4 pathway for regulating early placental development, which may contribute to the pathogenesis of PE as follows. During normal placentation, low levels of miR-17-family miRNAs allow higher expression of EPHB4 and EFNB2 in the placenta. This facilitates the differentiation of CTB progenitors into EVTs for invading the maternal decidua and for remodeling the uterine spiral arteries. Therefore, the

uterine arteries can be transformed into low-resistance vessels to facilitate increased blood flow with a much-reduced pressure, thereby facilitating the bidirectional mother-fetus exchanges of nutrients and respiratory gases (oxygen and carbon dioxide) and exclusion of fetal metabolic wastes. However, during abnormal placentation in PE, increased miR-17-family miRNAs suppress the expression of EFNB2 and EPHB4; deregulated EFNB2/EPHB4 expression further inhibits endovascular transformation of CTBs and invasion of EVTs for spiral artery remodeling. Failed remodeling of spiral artery will constrain maternal-fetal interface blood flow, which contributes to the pathogenesis of PE (Fig. 2).

REFERENCES

1. Redman CW, Sargent IL. Latest advances in understanding preeclampsia. Science 2005; 308:1592–1594.
2. Kanasaki K, Kalluri R. The biology of preeclampsia. Kidney Int 2009; 76:831–837.
3. Roberts JM, Cooper DW. Pathogenesis and genetics of pre-eclampsia. Lancet 2001; 357:53–56.
4. Enquobahrie DA, Meller M, Rice K, Psaty BM, Siscovick DS, Williams MA. Differential placental gene expression in preeclampsia. Am J Obstet Gynecol 2008; 199:566.e1–e11.
5. Sitras V, Paulssen RH, Gronaas H, Leirvik J, Hanssen TA, Vartun A, Acharya G. Differential placental gene expression in severe preeclampsia. Placenta 2009; 30:424–433.
6. Mouillet JF, Chu T, Sadovsky Y. Expression patterns of placental microRNAs. Birth Defects Res A Clin Mol Teratol 2011; 91:737–743.
7. Chelbi ST, Vaiman D. Genetic and epigenetic factors contribute to the onset of preeclampsia. Mol Cell Endocrinol 2008; 282:120–129.
8. Kulkarni A, Chavan-Gautam P, Mehendale S, Yadav H, Joshi S. Global DNA methylation patterns in placenta and its association with maternal hypertension in pre-eclampsia. DNA Cell Biol 2010; 30:79–84.
9. Lee RC, Feinbaum RL, Ambros V. The *C. elegans* heterochronic gene lin-4 encodes small RNAs with antisense complementarity to lin-14. Cell 1993; 75:843–854.
10. Wightman B, Ha I, Ruvkun G. Posttranscriptional regulation of the heterochronic gene lin-14 by lin-4 mediates temporal pattern formation in *C. elegans*. Cell 1993; 75:855–862.
11. Bentwich I, Avniel A, Karov Y, Aharonov R, Gilad S, Barad O, Barzilai A, Einat P, Einav U, Meiri E, Sharon E, Spector Y, et al. Identification of hundreds of conserved and nonconserved human microRNAs. Nat Genet 2005; 37:766–770.
12. miRBase [Internet]. www.miRbase.org. Release 19. Accessed November 10, 2012.
13. Chen K, Rajewsky N. The evolution of gene regulation by transcription factors and microRNAs. Nat Rev Genet 2007; 8:93–103.
14. Baek D, Villen J, Shin C, Camargo FD, Gygi SP, Bartel DP. The impact of microRNAs on protein output. Nature 2008; 455:64–71.
15. Zhang C. MicroRNomics: a newly emerging approach for disease biology. Physiol Genomics 2008; 33:139–147.
16. Lunghi L, Ferretti ME, Medici S, Biondi C, Vesce F. Control of human trophoblast function. Reprod Biol Endocrinol 2007; 5:6.
17. Douglas GC, VandeVoort CA, Kumar P, Chang TC, Golos TG. Trophoblast stem cells: models for investigating trophectoderm differentiation and placental development. Endocr Rev 2009; 30:228–240.
18. Pfeffer PL, Pearton DJ. Trophoblast development. Reproduction 2012; 143:231–246.
19. Benirschke K, Kaufmann P, Baergen R. Pathology of Human Placenta, 5th ed. New York, NY: Springer; 2006.
20. Loregger T, Pollheimer J, Knofler M. Regulatory transcription factors controlling function and differentiation of human trophoblast—a review. Placenta 2003; 24(suppl A):S104–S110.
21. Huppertz B, Peeters LL. Vascular biology in implantation and placentation. Angiogenesis 2005; 8:157–167.
22. Myatt L, Webster RP. Vascular biology of preeclampsia. J Thromb Haemost 2009; 7:375–384.
23. Burton GJ, Woods AW, Jauniaux E, Kingdom JC. Rheological and physiological consequences of conversion of the maternal spiral arteries for uteroplacental blood flow during human pregnancy. Placenta 2009; 30:473–482.
24. Damsky CH, Fisher SJ. Trophoblast pseudo-vasculogenesis: faking it with endothelial adhesion receptors. Curr Opin Cell Biol 1998; 10: 660–666.
25. Zhou Y, Fisher SJ, Janatpour M, Genbacev O, Dejana E, Wheelock M, Damsky CH. Human cytotrophoblasts adopt a vascular phenotype as they differentiate. A strategy for successful endovascular invasion? J Clin Invest 1997; 99:2139–2151.
26. Steegers EA, von Dadelszen P, Duvekot JJ, Pijnenborg R. Pre-eclampsia. Lancet 2010; 376:631–644.
27. Roberts JM, Hubel CA, Taylor RN. Endothelial dysfunction yes, cytotoxicity no! Am J Obstet Gynecol 1995; 173:978–979.
28. Gomez O, Martinez JM, Figueras F, Del Rio M, Borobio V, Puerto B, Coll O, Cararach V, Vanrell JA. Uterine artery Doppler at 11–14 weeks of gestation to screen for hypertensive disorders and associated complications in an unselected population. Ultrasound Obstet Gynecol 2005; 26:490–494.
29. Hershkovitz R, de Swiet M, Kingdom J. Mid-trimester placentation assessment in high-risk pregnancies using maternal serum screening and uterine artery Doppler. Hypertens Pregnancy 2005; 24:273–280.
30. Brosens IA, Robertson WB, Dixon HG. The role of the spiral arteries in the pathogenesis of preeclampsia. Obstet Gynecol Annu 1972; 1: 177–191.
31. Gerretsen G, Huisjes HJ, Elema JD. Morphological changes of the spiral arteries in the placental bed in relation to pre-eclampsia and fetal growth retardation. Br J Obstet Gynaecol 1981; 88:876–881.
32. Pijnenborg R, Bland JM, Robertson WB, Brosens I. Uteroplacental arterial changes related to interstitial trophoblast migration in early human pregnancy. Placenta 1983; 4:397–413.
33. Pijnenborg R, Bland JM, Robertson WB, Dixon G, Brosens I. The pattern of interstitial trophoblastic invasion of the myometrium in early human pregnancy. Placenta 1981; 2:303–316.
34. Khong TY, De Wolf F, Robertson WB, Brosens I. Inadequate maternal vascular response to placentation in pregnancies complicated by pre-eclampsia and by small-for-gestational age infants. Br J Obstet Gynaecol 1986; 93:1049–1059.
35. Chen LM, Liu B, Zhao HB, Stone P, Chen Q, Chamley L. IL-6, TNFalpha and TGFbeta promote nonapoptotic trophoblast deportation and subsequently causes endothelial cell activation. Placenta 2009; 31: 75–80.
36. Maynard SE, Min JY, Merchan J, Lim KH, Li J, Mondal S, Libermann TA, Morgan JP, Sellke FW, Stillman IE, Epstein FH, Sukhatme VP, et al. Excess placental soluble fms-like tyrosine kinase 1 (sFlt1) may contribute to endothelial dysfunction, hypertension, and proteinuria in preeclampsia. J Clin Invest 2003; 111:649–658.
37. Levine RJ, Maynard SE, Qian C, Lim KH, England LJ, Yu KF, Schisterman EF, Thadhani R, Sachs BP, Epstein FH, Sibai BM, Sukhatme VP, et al. Circulating angiogenic factors and the risk of preeclampsia. N Engl J Med 2004; 350:672–683.
38. Chen Q, Chen L, Liu B, Vialli C, Stone P, Ching LM, Chamley L. The role of autocrine TGFbeta1 in endothelial cell activation induced by phagocytosis of necrotic trophoblasts: a possible role in the pathogenesis of pre-eclampsia. J Pathol 2010; 221:87–95.
39. Chen Q, Ding JX, Liu B, Stone P, Feng YJ, Chamley L. Spreading endothelial cell dysfunction in response to necrotic trophoblasts. Soluble factors released from endothelial cells that have phagocytosed necrotic shed trophoblasts reduce the proliferation of additional endothelial cells. Placenta 2010; 31:976–981.
40. Xia Y, Kellems RE. Receptor-activating autoantibodies and disease: preeclampsia and beyond. Expert Rev Clin Immunol 2011; 7:659–674.
41. Roberts JM, Taylor RN, Musci TJ, Rodgers GM, Hubel CA, McLaughlin MK. Preeclampsia: an endothelial cell disorder. Am J Obstet Gynecol 1989; 161:1200–1204.
42. Wang Y, Lewis DF, Gu Y, Zhang Y, Alexander JS, Granger DN. Placental trophoblast-derived factors diminish endothelial barrier function. J Clin Endocrinol Metab 2004; 89:2421–2428.
43. Zhang Y, Zhao S, Gu Y, Lewis DF, Alexander JS, Wang Y. Effects of peroxynitrite and superoxide radicals on endothelial monolayer permeability: potential role of peroxynitrite in preeclampsia. J Soc Gynecol Investig 2005; 12:586–592.
44. Gu Y, Lewis DF, Zhang Y, Groome LJ, Wang Y. Increased superoxide generation and decreased stress protein Hsp90 expression in human umbilical cord vein endothelial cells (HUVECs) from pregnancies complicated by preeclampsia. Hypertens Pregnancy 2006; 25:169–182.
45. Wang Y, Lewis DF, Alexander JS, Granger DN. Endothelial barrier function in preeclampsia. Front Biosci 2007; 12:2412–2424.
46. Friedman SA, Schiff E, Emeis JJ, Dekker GA, Sibai BM. Biochemical corroboration of endothelial involvement in severe preeclampsia. Am J Obstet Gynecol 1995; 172:202–203.
47. Venkatesha S, Toporsian M, Lam C, Hanai J, Mammoto T, Kim YM, Bdolah Y, Lim KH, Yuan HT, Libermann TA, Stillman IE, Roberts D, et

al. Soluble endoglin contributes to the pathogenesis of preeclampsia. Nat Med 2006; 12:642–649.
48. Sladek SM, Magness RR, Conrad KP. Nitric oxide and pregnancy. Am J Physiol 1997; 272:R441–R463.
49. VanWijk MJ, Kublickiene K, Boer K, VanBavel E. Vascular function in preeclampsia. Cardiovasc Res 2000; 47:38–48.
50. Roberts JM, Hubel CA. Oxidative stress in preeclampsia. Am J Obstet Gynecol 2004; 190:1177–1178.
51. Mills JL, DerSimonian R, Raymond E, Morrow JD, Roberts LJ II, Clemens JD, Hauth JC, Catalano P, Sibai B, Curet LB, Levine RJ. Prostacyclin and thromboxane changes predating clinical onset of preeclampsia: a multicenter prospective study. JAMA 1999; 282: 356–362.
52. Tyurin VA, Liu SX, Tyurina YY, Sussman NB, Hubel CA, Roberts JM, Taylor RN, Kagan VE. Elevated levels of S-nitrosoalbumin in preeclampsia plasma. Circ Res 2001; 88:1210–1215.
53. Zhang HH, Wang YP, Chen DB. Analysis of nitroso-proteomes in normotensive and severe preeclamptic human placentas. Biol Reprod 2011; 84:966–975.
54. Cai X, Hagedorn CH, Cullen BR. Human microRNAs are processed from capped, polyadenylated transcripts that can also function as mRNAs. RNA 2004; 10:1957–1966.
55. Denli AM, Tops BB, Plasterk RH, Ketting RF, Hannon GJ. Processing of primary microRNAs by the Microprocessor complex. Nature 2004; 432: 231–235.
56. Hutvagner G, McLachlan J, Pasquinelli AE, Balint E, Tuschl T, Zamore PD. A cellular function for the RNA-interference enzyme Dicer in the maturation of the let-7 small temporal RNA. Science 2001; 293:834–838.
57. Lund E, Guttinger S, Calado A, Dahlberg JE, Kutay U. Nuclear export of microRNA precursors. Science 2004; 303:95–98.
58. Meister G, Landthaler M, Patkaniowska A, Dorsett Y, Teng G, Tuschl T. Human Argonaute2 mediates RNA cleavage targeted by miRNAs and siRNAs. Mol Cell 2004; 15:185–197.
59. Tomari Y, Du T, Haley B, Schwarz DS, Bennett R, Cook HA, Koppetsch BS, Theurkauf WE, Zamore PD. RISC assembly defects in the Drosophila RNAi mutant armitage. Cell 2004; 116:831–841.
60. Yan X, Mouillet JF, Ou Q, Sadovsky Y. A novel domain within the DEAD-box protein DP103 is essential for transcriptional repression and helicase activity. Mol Cell Biol 2003; 23:414–423.
61. Meister G, Landthaler M, Peters L, Chen PY, Urlaub H, Luhrmann R, Tuschl T. Identification of novel argonaute-associated proteins. Curr Biol 2005; 15:2149–2155.
62. Jaskiewicz L, Filipowicz W. Role of Dicer in posttranscriptional RNA silencing. Curr Top Microbiol Immunol 2008; 320:77–97.
63. Sinkkonen L, Hugenschmidt T, Berninger P, Gaidatzis D, Mohn F, Artus-Revel CG, Zavolan M, Svoboda P, Filipowicz W. MicroRNAs control de novo DNA methylation through regulation of transcriptional repressors in mouse embryonic stem cells. Nat Struct Mol Biol 2008; 15: 259–267.
64. Grosshans H, Filipowicz W. Molecular biology: the expanding world of small RNAs. Nature 2008; 451:414–416.
65. Bartel DP. MicroRNAs: target recognition and regulatory functions. Cell 2009; 136:215–233.
66. Place RF, Li LC, Pookot D, Noonan EJ, Dahiya R. MicroRNA-373 induces expression of genes with complementary promoter sequences. Proc Natl Acad Sci U S A 2008; 105:1608–1613.
67. Barad O, Meiri E, Avniel A, Aharonov R, Barzilai A, Bentwich I, Einav U, Gilad S, Hurban P, Karov Y, Lobenhofer EK, Sharon E, et al. MicroRNA expression detected by oligonucleotide microarrays: system establishment and expression profiling in human tissues. Genome Res 2004; 14:2486–2494.
68. Landgraf P, Rusu M, Sheridan R, Sewer A, Iovino N, Aravin A, Pfeffer S, Rice A, Kamphorst AO, Landthaler M, Lin C, Socci ND, et al. A mammalian microRNA expression atlas based on small RNA library sequencing. Cell 2007; 129:1401–1414.
69. Liang Y, Ridzon D, Wong L, Chen C. Characterization of microRNA expression profiles in normal human tissues. BMC Genomics 2007; 8: 166.
70. Luo SS, Ishibashi O, Ishikawa G, Ishikawa T, Katayama A, Mishima T, Takizawa T, Shigihara T, Goto T, Izumi A, Ohkuchi A, Matsubara S, et al. Human villous trophoblasts express and secrete placenta-specific microRNAs into maternal circulation via exosomes. Biol Reprod 2009; 81:717–729.
71. Wang W, Feng L, Zhang H, Hachy S, Satohisa S, Laurent LC, Parast M, Zheng J, Chen DB. Preeclampsia up-regulates angiogenesis-associated microRNA (i.e., miR-17, −20a, and −20b) that target ephrin-B2 and EPHB4 in human placenta. J Clin Endocrinol Metab 2012; 97: E1051–E1059.
72. Enquobahrie DA, Abetew DF, Sorensen TK, Willoughby D, Chidambaram K, Williams MA. Placental microRNA expression in pregnancies complicated by preeclampsia. Am J Obstet Gynecol 2011; 204:178.e12–e21.
73. Mayor-Lynn K, Toloubeydokhti T, Cruz AC, Chegini N. Expression profile of microRNAs and mRNAs in human placentas from pregnancies complicated by preeclampsia and preterm labor. Reprod Sci 2011; 18: 46–56.
74. Zhang R, Wang YQ, Su B. Molecular evolution of a primate-specific microRNA family. Mol Biol Evol 2008; 25:1493–1502.
75. Bortolin-Cavaille ML, Dance M, Weber M, Cavaille J. C19MC microRNAs are processed from introns of large Pol-II, non-protein-coding transcripts. Nucleic Acids Res 2009; 37:3464–3473.
76. Tsai KW, Kao HW, Chen HC, Chen SJ, Lin WC. Epigenetic control of the expression of a primate-specific microRNA cluster in human cancer cells. Epigenetics 2009; 4:587–592.
77. Noguer-Dance M, Abu-Amero S, Al-Khtib M, Lefevre A, Coullin P, Moore GE, Cavaille J. The primate-specific microRNA gene cluster (C19MC) is imprinted in the placenta. Hum Mol Genet 2010; 19: 3566–3582.
78. St-Pierre J, Hivert MF, Perron P, Poirier P, Guay SP, Brisson D, Bouchard L. IGF2 DNA methylation is a modulator of newborn's fetal growth and development. Epigenetics 2012; 7:1125–1132.
79. Donker RB, Mouillet JF, Chu T, Hubel CA, Stolz DB, Morelli AE, Sadovsky Y. The expression profile of C19MC microRNAs in primary human trophoblast cells and exosomes. Mol Hum Reprod 2012; 18: 417–424.
80. Flor I, Neumann A, Freter C, Helmke BM, Langenbuch M, Rippe V, Bullerdiek J. Abundant expression and hemimethylation of C19MC in cell cultures from placenta-derived stromal cells. Biochem Biophys Res Commun 2012; 422:411–416.
81. Kotlabova K, Doucha J, Hromadnikova I. Placental-specific microRNA in maternal circulation–identification of appropriate pregnancy-associated microRNAs with diagnostic potential. J Reprod Immunol 2011; 89: 185–191.
82. Miura K, Miura S, Yamasaki K, Higashijima A, Kinoshita A, Yoshiura K, Masuzaki H. Identification of pregnancy-associated microRNAs in maternal plasma. Clin Chem 2010; 56:1767–1771.
83. Hu Y, Li P, Hao S, Liu L, Zhao J, Hou Y. Differential expression of microRNAs in the placentae of Chinese patients with severe pre-eclampsia. Clin Chem Lab Med 2009; 47:923–929.
84. Pineles BL, Romero R, Montenegro D, Tarca AL, Han YM, Kim YM, Draghici S, Espinoza J, Kusanovic JP, Mittal P, Hassan SS, Kim CJ. Distinct subsets of microRNAs are expressed differentially in the human placentas of patients with preeclampsia. Am J Obstet Gynecol 2007; 196:261.e1–e6.
85. Zhu XM, Han T, Sargent IL, Yin GW, Yao YQ. Differential expression profile of microRNAs in human placentas from preeclamptic pregnancies vs normal pregnancies. Am J Obstet Gynecol 2009; 200:661.e1–e7.
86. Zhang Y, Fei M, Xue G, Zhou Q, Jia Y, Li L, Xin H, Sun S. Elevated levels of hypoxia-inducible microRNA-210 in pre-eclampsia: new insights into molecular mechanisms for the disease. J Cell Mol Med 2012; 16:249–259.
87. Mouillet JF, Chu T, Nelson DM, Mishima T, Sadovsky Y. MiR-205 silences MED1 in hypoxic primary human trophoblasts. FASEB J 2010; 24:2030–2039.
88. Fasanaro P, D'Alessandra Y, Di Stefano V, Melchionna R, Romani S, Pompilio G, Capogrossi MC, Martelli F. MicroRNA-210 modulates endothelial cell response to hypoxia and inhibits the receptor tyrosine kinase ligand Ephrin-A3. J Biol Chem 2008; 283:15878–15883.
89. Huang X, Ding L, Bennewith KL, Tong RT, Welford SM, Ang KK, Story M, Le QT, Giaccia AJ. Hypoxia-inducible mir-210 regulates normoxic gene expression involved in tumor initiation. Mol Cell 2009; 35:856–867.
90. Lee DC, Romero R, Kim JS, Tarca AL, Montenegro D, Pineles BL, Kim E, Lee J, Kim SY, Draghici S, Mittal P, Kusanovic JP, et al. miR-210 targets iron-sulfur cluster scaffold homologue in human trophoblast cell lines: siderosis of interstitial trophoblasts as a novel pathology of preterm preeclampsia and small-for-gestational-age pregnancies. Am J Pathol 2011; 179:590–602.
91. Gu Y, Sun J, Groome LJ, Wang Y. Differential miRNA expression profiles between the first and third trimester human placentas. Am J Physiol Endocrinol Metab 2013; 304:E836–E843.
92. Wang D, Song W, Na Q. The emerging roles of placenta-specific

microRNAs in regulating trophoblast proliferation during the first trimester. Aust N Z J Obstet Gynaecol 2012; 52:565–570.
93. Cheloufi S, Dos Santos CO, Chong MM, Hannon GJ. A dicer-independent miRNA biogenesis pathway that requires Ago catalysis. Nature 2010; 465:584–589.
94. Forbes K, Farrokhnia F, Aplin JD, Westwood M. Dicer-dependent miRNAs provide an endogenous restraint on cytotrophoblast proliferation. Placenta 2012; 33:581–585.
95. Zhu XM, Han T, Wang XH, Li YH, Yang HG, Luo YN, Yin GW, Yao YQ. Overexpression of miR-152 leads to reduced expression of human leukocyte antigen-G and increased natural killer cell mediated cytolysis in JEG-3 cells. Am J Obstet Gynecol 2010; 202:592.e1–e7.
96. Pang RT, Leung CO, Ye TM, Liu W, Chiu PC, Lam KK, Lee KF, Yeung WS. MicroRNA-34a suppresses invasion through downregulation of Notch1 and Jagged1 in cervical carcinoma and choriocarcinoma cells. Carcinogenesis 2010; 31:1037–1044.
97. Umemura K, Ishioka SI, Endo T, Ezaka Y, Takahashi M, Saito T. Roles of microRNA-34a in the pathogenesis of placenta accreta. J Obstet Gynaecol Res 2013; 39:67–74.
98. Chao A, Tsai CL, Wei PC, Hsueh S, Chao AS, Wang CJ, Tsai CN, Lee YS, Wang TH, Lai CH. Decreased expression of microRNA-199b increases protein levels of SET (protein phosphatase 2A inhibitor) in human choriocarcinoma. Cancer Lett 2010; 291:99–107.
99. Donker RB, Mouillet JF, Nelson DM, Sadovsky Y. The expression of Argonaute2 and related microRNA biogenesis proteins in normal and hypoxic trophoblasts. Mol Hum Reprod 2007; 13:273–279.
100. Muralimanoharan S, Maloyan A, Mele J, Guo C, Myatt LG, Myatt L. MIR-210 modulates mitochondrial respiration in placenta with preeclampsia. Placenta 2012; 33:816–823.
101. Landles C, Chalk S, Steel JH, Rosewell I, Spencer-Dene B, Lalani el N, Parker MG. The thyroid hormone receptor-associated protein TRAP220 is required at distinct embryonic stages in placental, cardiac, and hepatic development. Mol Endocrinol 2003; 17:2418–2435.
102. Zhang Y, Diao Z, Su L, Sun H, Li R, Cui H, Hu Y. MicroRNA-155 contributes to preeclampsia by down-regulating CYR61. Am J Obstet Gynecol 2010; 202:466.e1–e7.
103. Dai Y, Qiu Z, Diao Z, Shen L, Xue P, Sun H, Hu Y. MicroRNA-155 inhibits proliferation and migration of human extravillous trophoblast derived HTR-8/SVneo cells via down-regulating cyclin D1. Placenta 2012; 33:824–829.
104. Dai Y, Diao Z, Sun H, Li R, Qiu Z, Hu Y. MicroRNA-155 is involved in the remodelling of human-trophoblast-derived HTR-8/SVneo cells induced by lipopolysaccharides. Hum Reprod 2011; 26:1882–1891.
105. Bai Y, Yang W, Yang HX, Liao Q, Ye G, Fu G, Ji L, Xu P, Wang H, Li YX, Peng C, Wang YL. Downregulated miR-195 detected in preeclamptic placenta affects trophoblast cell invasion via modulating ActRIIA expression. PLoS One 2012; 7:e38875.
106. Luo L, Ye G, Nadeem L, Fu G, Yang BB, Honarparvar E, Dunk C, Lye S, Peng C. MicroRNA-378a-5p promotes trophoblast cell survival, migration and invasion by targeting Nodal. J Cell Sci 2012; 125: 3124–3132.
107. Fu G, Ye G, Nadeem L, Ji L, Manchanda T, Wang Y, Zhao Y, Qiao J, Wang YL, Lye S, Yang BB, Peng C. MicroRNA-376c impairs transforming growth factor-beta and nodal signaling to promote trophoblast cell proliferation and invasion. Hypertension 2013; 61: 864–872.
108. Ye G, Fu G, Cui S, Zhao S, Bernaudo S, Bai Y, Ding Y, Zhang Y, Yang BB, Peng C. MicroRNA 376c enhances ovarian cancer cell survival by targeting activin receptor-like kinase 7: implications for chemoresistance. J Cell Sci 2011; 124:359–368.
109. Park CB, DeMayo FJ, Lydon JP, Dufort D. NODAL in the uterus is necessary for proper placental development and maintenance of pregnancy. Biol Reprod 2012; 86:194.
110. Camps C, Buffa FM, Colella S, Moore J, Sotiriou C, Sheldon H, Harris AL, Gleadle JM, Ragoussis J. hsa-miR-210 is induced by hypoxia and is an independent prognostic factor in breast cancer. Clin Cancer Res 2008; 14:1340–1348.
111. Giannakakis A, Sandaltzopoulos R, Greshock J, Liang S, Huang J, Hasegawa K, Li C, O'Brien-Jenkins A, Katsaros D, Weber BL, Simon C, Coukos G, et al. miR-210 links hypoxia with cell cycle regulation and is deleted in human epithelial ovarian cancer. Cancer Biol Ther 2008; 7: 255–264.
112. Pulkkinen K, Malm T, Turunen M, Koistinaho J, Yla-Herttuala S. Hypoxia induces microRNA miR-210 in vitro and in vivo ephrin-A3 and neuronal pentraxin 1 are potentially regulated by miR-210. FEBS Lett 2008; 582:2397–2401.
113. Huang X, Le QT, Giaccia AJ. MiR-210–micromanager of the hypoxia pathway. Trends Mol Med 2010; 16:230–237.
114. Fasanaro P, Greco S, Lorenzi M, Pescatori M, Brioschi M, Kulshreshtha R, Banfi C, Stubbs A, Calin GA, Ivan M, Capogrossi MC, Martelli F. An integrated approach for experimental target identification of hypoxia-induced miR-210. J Biol Chem 2009; 284:35134–35143.
115. Hu S, Huang M, Li Z, Jia F, Ghosh Z, Lijkwan MA, Fasanaro P, Sun N, Wang X, Martelli F, Robbins RC, Wu JC. MicroRNA-210 as a novel therapy for treatment of ischemic heart disease. Circulation 2010; 122: S124–S131.
116. Lou YL, Guo F, Liu F, Gao FL, Zhang PQ, Niu X, Guo SC, Yin JH, Wang Y, Deng ZF. miR-210 activates notch signaling pathway in angiogenesis induced by cerebral ischemia.Mol Cell Biochem 2012; 370: 45–51.
117. Hunkapiller NM, Gasperowicz M, Kapidzic M, Plaks V, Maltepe E, Kitajewski J, Cross JC, Fisher SJ. A role for Notch signaling in trophoblast endovascular invasion and in the pathogenesis of pre-eclampsia. Development 2011; 138:2987–2998.
118. Wang S, Olson EN. AngiomiRs—key regulators of angiogenesis. Curr Opin Genet Dev 2009; 19:205–211.
119. Wu F, Yang Z, Li G. Role of specific microRNAs for endothelial function and angiogenesis. Biochem Biophys Res Commun 2009; 386: 549–553.
120. Yang WJ, Yang DD, Na S, Sandusky GE, Zhang Q, Zhao G. Dicer is required for embryonic angiogenesis during mouse development. J Biol Chem 2005; 280:9330–9335.
121. Kuehbacher A, Urbich C, Zeiher AM, Dimmeler S. Role of Dicer and Drosha for endothelial microRNA expression and angiogenesis. Circ Res 2007; 101:59–68.
122. Poliseno L, Tuccoli A, Mariani L, Evangelista M, Citti L, Woods K, Mercatanti A, Hammond S, Rainaldi G. MicroRNAs modulate the angiogenic properties of HUVECs. Blood 2006; 108:3068–3071.
123. Suarez Y, Fernandez-Hernando C, Pober JS, Sessa WC. Dicer dependent microRNAs regulate gene expression and functions in human endothelial cells. Circ Res 2007; 100:1164–1173.
124. Suarez Y, Fernandez-Hernando C, Yu J, Gerber SA, Harrison KD, Pober JS, Iruela-Arispe ML, Merkenschlager M, Sessa WC. Dicer-dependent endothelial microRNAs are necessary for postnatal angiogenesis. Proc Natl Acad Sci U S A 2008; 105:14082–14087.
125. Gellhaus A, Schmidt M, Dunk C, Lye SJ, Kimmig R, Winterhager E. Decreased expression of the angiogenic regulators CYR61 (CCN1) and NOV (CCN3) in human placenta is associated with pre-eclampsia. Mol Hum Reprod 2006; 12:389–399.
126. Gellhaus A, Schmidt M, Dunk C, Lye SJ, Winterhager E. The circulating proangiogenic factors CYR61 (CCN1) and NOV (CCN3) are significantly decreased in placentae and sera of preeclamptic patients. Reprod Sci 2007; 14:46–52.
127. Chen Y, Du XY. Functional properties and intracellular signaling of CCN1/Cyr61. J Cell Biochem 2007; 100:1337–1345.
128. Reynolds LP, Borowicz PP, Vonnahme KA, Johnson ML, Grazul-Bilska AT, Redmer DA, Caton JS. Placental angiogenesis in sheep models of compromised pregnancy. J Physiol 2005; 565:43–58.
129. Reynolds LP, Redmer DA. Angiogenesis in the placenta. Biol Reprod 2001; 64:1033–1040.
130. Ventura A, Young AG, Winslow MM, Lintault L, Meissner A, Erkeland SJ, Newman J, Bronson RT, Crowley D, Stone JR, Jaenisch R, Sharp PA, et al. Targeted deletion reveals essential and overlapping functions of the miR-17 through 92 family of miRNA clusters. Cell 2008; 132: 875–886.
131. Red-Horse K, Kapidzic M, Zhou Y, Feng KT, Singh H, Fisher SJ. EPHB4 regulates chemokine-evoked trophoblast responses: a mechanism for incorporating the human placenta into the maternal circulation. Development 2005; 132:4097–4106.
132. Wang HU, Chen ZF, Anderson DJ. Molecular distinction and angiogenic interaction between embryonic arteries and veins revealed by ephrin-B2 and its receptor Eph-B4. Cell 1998; 93:741–753.
133. Foo SS, Turner CJ, Adams S, Compagni A, Aubyn D, Kogata N, Lindblom P, Shani M, Zicha D, Adams RH. Ephrin-B2 controls cell motility and adhesion during blood-vessel-wall assembly. Cell 2006; 124:161–173.
134. Chi JT, Chang HY, Haraldsen G, Jahnsen FL, Troyanskaya OG, Chang DS, Wang Z, Rockson SG, van de Rijn M, Botstein D, Brown PO. Endothelial cell diversity revealed by global expression profiling. Proc Natl Acad Sci U S A 2003; 100:10623–10628.
135. Yancopoulos GD, Davis S, Gale NW, Rudge JS, Wiegand SJ, Holash J. Vascular-specific growth factors and blood vessel formation. Nature 2000; 407:242–248.

136. Zhang XQ, Takakura N, Oike Y, Inada T, Gale NW, Yancopoulos GD, Suda T. Stromal cells expressing ephrin-B2 promote the growth and sprouting of ephrin-B2(+) endothelial cells. Blood 2001; 98:1028–1037.
137. Hamada K, Oike Y, Ito Y, Maekawa H, Miyata K, Shimomura T, Suda T. Distinct roles of ephrin-B2 forward and EphB4 reverse signaling in endothelial cells. Arterioscler Thromb Vasc Biol 2003; 23:190–197.
138. Oike Y, Ito Y, Hamada K, Zhang XQ, Miyata K, Arai F, Inada T, Araki K, Nakagata N, Takeya M, Kisanuki YY, Yanagisawa M, et al. Regulation of vasculogenesis and angiogenesis by EphB/ephrin-B2 signaling between endothelial cells and surrounding mesenchymal cells. Blood 2002; 100:1326–1333.
139. Xing W, Kim J, Wergedal J, Chen ST, Mohan S. Ephrin B1 regulates bone marrow stromal cell differentiation and bone formation by influencing TAZ transactivation via complex formation with NHERF1. Mol Cell Biol 2010; 30:711–721.
140. Kuijper S, Turner CJ, Adams RH. Regulation of angiogenesis by Eph-ephrin interactions. Trends Cardiovasc Med 2007; 17:145–151.
141. Mosch B, Reissenweber B, Neuber C, Pietzsch J. Eph receptors and ephrin ligands: important players in angiogenesis and tumor angiogenesis. J Oncol 2010; 2010:135285.
142. Sawamiphak S, Seidel S, Essmann CL, Wilkinson GA, Pitulescu ME, Acker T, Acker-Palmer A. Ephrin-B2 regulates VEGFR2 function in developmental and tumour angiogenesis. Nature 2010; 465:487–491.
143. Wang Y, Nakayama M, Pitulescu ME, Schmidt TS, Bochenek ML, Sakakibara A, Adams S, Davy A, Deutsch U, Luthi U, Barberis A, Benjamin LE, et al. Ephrin-B2 controls VEGF-induced angiogenesis and lymphangiogenesis. Nature 2010; 465:483–486.
144. Chennakesava CS, Di Santo S, Ziemiecki A, Schneider H, Andres AC. Differential expression of the receptor tyrosine kinase EphB4 and its ligand Ephrin-B2 during human placental development. Placenta 2006; 27:959–967.
145. Goldman-Wohl D, Greenfield C, Haimov-Kochman R, Ariel I, Anteby EY, Hochner-Celnikier D, Farhat M, Yagel S. Eph and ephrin expression in normal placental development and preeclampsia. Placenta 2004; 25: 623–630.
146. Red-Horse K, Rivera J, Schanz A, Zhou Y, Winn V, Kapidzic M, Maltepe E, Okazaki K, Kochman R, Vo KC, Giudice L, Erlebacher A, et al. Cytotrophoblast induction of arterial apoptosis and lymphangiogenesis in an in vivo model of human placentation. J Clin Invest 2006; 116:2643–2652.
147. Zhang J, Dong H, Wang B, Zhu S, Croy BA. Dynamic changes occur in patterns of endometrial EFNB2/EPHB4 expression during the period of spiral arterial modification in mice. Biol Reprod 2008; 79:450–458.
148. Huang X, Yamada Y, Kidoya H, Naito H, Nagahama Y, Kong L, Katoh SY, Li WL, Ueno M, Takakura N. EphB4 overexpression in B16 melanoma cells affects arterial-venous patterning in tumor angiogenesis. Cancer Res 2007; 67:9800–9808.