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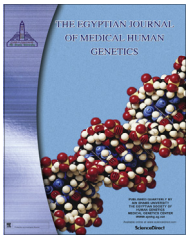

ORIGINAL ARTICLE

The role of microRNAs on angiogenesis and vascular pressure in preeclampsia: The evidence from systematic review

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KEYWORDS

Preeclampsia pathogenesis;
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Abstract *Background:* In pre-clinical stage of preeclampsia, placental angiogenesis is impaired leading to hypoxic placenta and dysregulation of pro- and anti-angiogenetic factors. As a consequence, these cause elevated systemic vascular resistance, vasoconstriction and hypertension in clinical stage of preeclampsia. Dysregulation of microRNAs (miRNAs) has been observed among preeclampsia patients and they are involved in several aspects of preeclampsia pathogenesis.

Aims: To evaluate the roles of miRNAs in angiogenesis and vascular pressure in preeclampsia.

Material and methods: Articles from MEDLINE database (between 2007 and February 2015) were searched by using the combination of Medical Subject Headings (MeSH terms) "preeclampsia", "pre-eclampsia", "miRNA" and "microRNA". All sources of miRNAs, all types of preeclampsia and all techniques used in measuring miRNAs were included. Furthermore, bibliographies of the articles were also retrieved for further relevant references.

Results: Data reveal that miRNAs interfere with angiogenesis during early pregnancy by dysregulating pro-angiogenic factors (such as placental growth factor, vascular endothelial growth factor, fibroblast growth factor, transforming growth factor and insulin-like growth factor) and their receptors including Fms-like tyrosine kinase-1 and fibroblast growth factor receptor 1. In addition, miRNAs are also involved on high vascular pressure during preeclampsia by targeting several vasodilators such as prostacyclin, 17β-estradiol, hydrogen sulfide and nitric oxide, and inducing the production of angiotensin type I receptor agonistic autoantibodies.

Conclusion: Data confirm that miRNAs are involved in pathobiology of preeclampsia including interference with angiogenesis during pre-clinical stage and induction of vascular resistance and vasoconstriction in clinical stage.

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1. Introduction

Preeclampsia is a disease of pregnancy characterized by the new onset of hypertension and proteinuria after 20 weeks of gestation and it is classified as mild and severe preeclampsia [1,2]. It has been estimated that preeclampsia affects 3–5% of pregnancies [3] and complicates 3–8% of pregnancies worldwide leading to a large disease burden [4].

Preeclampsia is a two-stage disorder: pre-clinical and clinical stage. In the first stage, the endothelialization of cytotrophoblasts is impaired and the invasion of spiral arteries into myometrium is inadequate leading to poor placentation, and ischemic and hypoxic placenta. In the second stage, the ischemic and hypoxic placenta releases anti-angiogenic factors such as soluble Fms-like tyrosine kinase-1 (sFlt-1), soluble endoglin (sEng), prostaglandins and cytokines into the maternal circulation and dysregulates the production of pro-angiogenic factors including vascular endothelial growth factor (VEGF), placental growth factor (PlGF), fibroblast growth factor (FGF), transforming growth factor-B (TGF-B) and insulin-like growth factor I (IGF-I) [5–8]. It is clear that the levels of sFlt1, sEng and other anti-angiogenic factors are increased and the concentrations of VEGF, PlGF and other pro-angiogenic factors are decreased in preeclampsia [9–22]. These changes induce systemic endothelial dysfunction and inflammatory response leading to elevated systemic vascular resistance, vasoconstriction, and activation of the coagulation cascade [23]. As final results, these cause clinical symptoms such as hypertension, proteinuria, hepatic dysfunction and hematological and neurological disturbances during clinical stage of preeclampsia.

One of the most important clinical features of preeclampsia is hypertension. Several mechanisms are involved in inducing hypertension among preeclampsia patients including down-regulation of pro-angiogenic factors [10,24], up-regulation of anti-angiogenic factors such as sFlt-1 and sEng [7,8,25], increase of vascular responsiveness to vasoconstrictors [8], the presence of angiotensin type I receptor agonistic autoantibody (AT1-AA) [26,27], high production of aldosterone and endothelin 1 [28,29] and low production of vasodilator such as nitric oxide (NO) [30] and hydrogen sulfide (H_2S) [31]. The production of these diverse molecules is regulated in a secure manner by different regulators, and the universal regulator such as miRNAs might have pivotal roles in dysregulating these molecules during preeclampsia.

Since 2007, several studies have been conducted to investigate the role of miRNAs in pathogenesis of preeclampsia in deep. Studies revealed that the expression of miRNAs was dysregulated in placentas and sera from preeclampsia pregnancies [32–74]. Therefore, the aim of this study was to evaluate the roles of miRNAs in angiogenesis during pre-clinical stage and pathobiology of hypertension during clinical stage of preeclampsia. This study is a part of our systematic review that has been conducted to evaluate the role of miRNAs on preeclampsia pathogenesis.

2. Methods

This study is a systematic review to assess the role of miRNAs on angiogenesis and vascular pressure among preeclampsia patients. Potential eligible studies from MEDLINE database from 2007 and February 2015 were searched by using keywords: "preeclampsia", "pre-eclampsia", "miRNA" and "microRNA". In 2007, the first investigation on the roles of miRNAs on preeclampsia pathogenesis was published; therefore, the year 2007 was used as cut point of the year. The bibliographies of the articles were retrieved for further relevant references. If an article evaluated the expression of miRNAs either from more than one set of patient-control and sources of miRNAs, each one of them was considered as one independent study. Only articles written in English were included.

Preeclampsia in this study is defined as the new onset of hypertension and proteinuria after 20 weeks of gestation. Hypertension is defined as systolic blood pressure of 140 mmHg or greater and diastolic blood pressure of 90 mmHg or greater measured on two occasions at least 4–6 h apart. Proteinuria is defined as at least 100 mg/dL of protein in random urine specimens collected at four-hour interval or as 300 mg or more of protein from 24 h of urine specimen and/or protein to creatinine ratio of >0.03. In this study preeclampsia is divided into preeclampsia and severe preeclampsia.

All miRNA sources and all techniques that were used to measure the expression of miRNAs were included. If a study used microarray to measure miRNA expression profile at the first time then confirmed by quantitative reverse transcription polymerase chain reaction (qRT-PCR), the level of miRNAs expression used was validation level by qRT-PCR. For quality assessment, inhibition effect of a miRNA on a gene expression is based on Gene Ontology analysis and supported by direct miRNA inhibition on 3′untranslated region (3′UTR) of particular gene. Case series or reports, editorials, reviews without original data, letters to the editor were excluded from the systematic review.

The results of this systematic review are divided to two parts as miRNAs have diverse effects on preeclampsia pathogenesis. The first part is the roles of miRNAs on trophoblast function [75] and the second part is the roles of miRNAs on angiogenesis and vascular pressure dysregulation. In this article, the role of miRNAs in angiogenesis and dysregulation of pro-angiogenic and anti-angiogenic factors and other mechanisms that contribute to hypertension during preeclampsia are discussed.

3. Results

In this systematic review, the searches found 89 potential studies. Thirty-seven studies were excluded after further assessment and 52 studies were included in this study. Since the first report on the expression of miRNAs among preeclampsia patients was published in 2007 [32], intensive investigations have been conducted [33–74]. Among these studies, most of them were case–control study [32–40,42,43,45–74] and some of them were prospective cohort study [41,44,62]. Differential sources of miRNA expression have been investigated such as placenta [32–36,38,40,47–51,54–57,59–62,66,68,70–74], plasma [39,41–46,50,63–65,67], human umbilical vein endothelial cell (HUVEC) [69], peripheral blood mononuclear cell (PBMC) [53] and mesenchymal stem cell (MSC) of decidua or umbilical cord [37,53,58] from preeclampsia and normal pregnancies. The country of study also varies including China [33–35,37,40,42,43,45,50,52–58,63,64,67,69,72–74], USA [32,46–48,51,54,61,70,71], Canada [50,60], Switzerland [36], Norway [38], Italy [39], Spain

[41], Czech Republic [44], South Korea [49], Hungary [59], Japan [62], Turkey [65], Chile [66], and Germany [68].

4. Discussion

4.1. The role of miRNAs in angiogenic factor expression

Evidences suggest that failure of trophoblast invasion is linked to abnormal placental production of vasculogenic and pro-angiogenic factors, such as VEGF, PlGF, FGF, TGF-B, IGF-1 and angiopoietin 2 [5–8,76,77]. In maternal level, VEGF stabilizes endothelial cells, by stimulating the production of NO and prostacyclin and maintaining the health of fenestrated endothelium in the kidney, liver, and brain [8]. VEGF activities are mediated primarily by its receptors (Flt-1 and kinase-insert domain region (KDR)) that are selectively expressed on vascular endothelial cell surface. PlGF is a member of the VEGF and it amplifies VEGF signaling [8]. The function of these pro-angiogenic factors is interfered by anti-angiogenic factor such as sFlt-1 and sEng.

sFlt-1, an alternatively spliced and truncated version of Flt-1, is secreted prominently by syncytiotrophoblasts into the maternal circulation [8,10]. Because of its structure, sFlt-1 enables to bind VEGF and PlGF leading to reduced interaction with their receptors [78,79]. Karumanchi group, for the first time, demonstrated that administration of sFlt-1 and sEng in animal model produced almost all of clinical features of preeclampsia by interfering with the biological function of VEGF and PlGF [10,80]. Among preeclampsia patients, increased sFlt-1 was associated with decreased free VEGF and PlGF in the serum [10,24,81] and increase of sFlt-1 was correlated with preeclampsia severity [9,82,83]. Animal study found that exogenous sFlt-1 administration induced albuminuria, hypertension, and renal pathological changes of glomerular endotheliosis [10].

However, sFlt-1 excess is not sufficient to explain the other manifestations of preeclampsia including hyper-coagulation, liver dysfunction and seizures [84]. Evidences reveal that sEng, another anti-angiogenic factor, might be the causal factor for hyper-coagulation, liver dysfunction and seizures [80,85–87]. sEng, a truncated form of endoglin, prevents endoglin to interact with its receptor and sEng expression was up-regulated in preeclampsia patients [85]. Endoglin is co-receptor of TGF-β1 and TGF-β3 and it interacts with its receptor, activin-like kinases (ALK) and regulates the expression of DNA-binding protein inhibitor ID1, endothelial NO synthase (eNOS), and plasminogen activator inhibitor-1 (PAI-1) gene [88]. Therefore, endoglin is involved in regulation of angiogenesis, vascular tone, and coagulation and fibrinolytic balance [89,90]. Studies found that sEng inhibited endothelial capillary tube formation and promoted vascular permeability [80] and over-expression both of sEng and sFlt-1 induced severe proteinuria, hypertension, intrauterine growth restriction, HELLP syndrome (hemolysis, elevated liver enzyme levels, and low platelet levels) and increased vascular permeability that was associated with brain edema [87,80]. The roles of major pro- and anti-angiogenic factors in preeclampsia pathogenesis are summarized in Fig. 1.

It is clear that angiogenic factors are involved in preeclampsia pathogenesis but the regulation of their production is still not well understood. Interestingly, studies found that several miRNAs target directly angiogenic factors. It is indicating that miRNAs have critical roles in production of angiogenic factors during pre-clinical stage of preeclampsia. For example, Hu et al. [73] found that miR-16, miR-26b, miR-29b, miR-181a, miR-195, miR-222 and miR-335 were significantly higher in preeclamptic placentas than in normal placentas. The target genes of these miRNAs were related to angiogenic factors, such as VEGF-A and PlGF. This research revealed that miR-222, miR-335 and miR-195 targeted cysteine-rich 61 (CYR61), PlGF and VEGF-A, respectively. CYR61 is essential for vascular integrity and it is significantly decreased in preeclamptic placenta [91,92]. In addition, another study demonstrated that the expressions of VEGF-A and VEGF receptor-1 were also down-regulated in cytotrophoblasts of preeclamptic placenta [93].

In addition, studies found that the expression of miR-182 and miR-182* were significantly higher in preeclampsia than the control group [32,33,47,68]. Interestingly, a previous study revealed that angiogenin and VEGF-B were the potential targets of miR-182 and miR-182*, respectively (Fig. 2) [32]. Additionally, other studies found that miR-29b increased significantly among preeclampsia patients [37,47,74] and it targeted VEGFA directly. Another study also found that VEGF-A is one of the putative targets of miR-16 and over-expression of miR-16 reduced the protein levels of VEGF-A [53].

Another study also found that preeclamptic placenta had up-regulation of miR-126 expression [62] and this miRNA directly targeted the 3′UTRs of sprouty-related, EVH1 domain-containing protein 1 (SPRED1) and phosphoinositide-3-kinase, regulatory subunit 2 (PIK3R2) [94,95]. SPRED1 and PIK3R2 are components that have a pivotal role in VEGF pathway [94]. See detailed explanation in angiogenesis section. A recent study found that miR-424 expression was up-regulated in placenta from severe preeclampsia patients [33] and it targeted FGF receptor 1 (FGFR1) [96].

Furthermore, other studies demonstrated that IGF-I was decreased in serum and placental tissue of women with preeclampsia [6,74,97]. Zhu et al. [74] documented a significant up-regulation of miR-30a-3p in preeclamptic placenta and it targeted IGF-1. IGF-I stimulates renal and placental 1,25-dihydroxyvitamin D [1,25-(OH)2D] and it is considered an important regulator of fetal growth. Other studies indicated that circulating IGF-I and 1,25-(OH)2D levels in both maternal and umbilical cord compartments were low in preeclampsia and it correlated with low weight and length at birth, high risk of preeclampsia and preeclampsia severity [97–99].

Surprisingly, a study found that Flt-1 and sFlt-1 were direct target of miR-10 [100]. This study found that inhibition of miR-10; both of sFlt-1 and Flt-1 were highly expressed and bound to VEGF and, in turn, interfered VEGF signaling for angiogenesis. The inverse condition was true for the presence of miR-10. It indicates that miR-10 is important to inhibit the production of anti-angiogenesis, sFlt-1. In preeclampsia, a study found that the expression of miR-10b was up-regulated in preeclamptic placentas [62]. There is no further study that confirms the role of miR-10 in preeclampsia.

4.2. The role of miRNAs in angiogenesis

The crucial roles of miRNAs in angiogenesis became obvious after several studies demonstrated that deletion of miRNAs

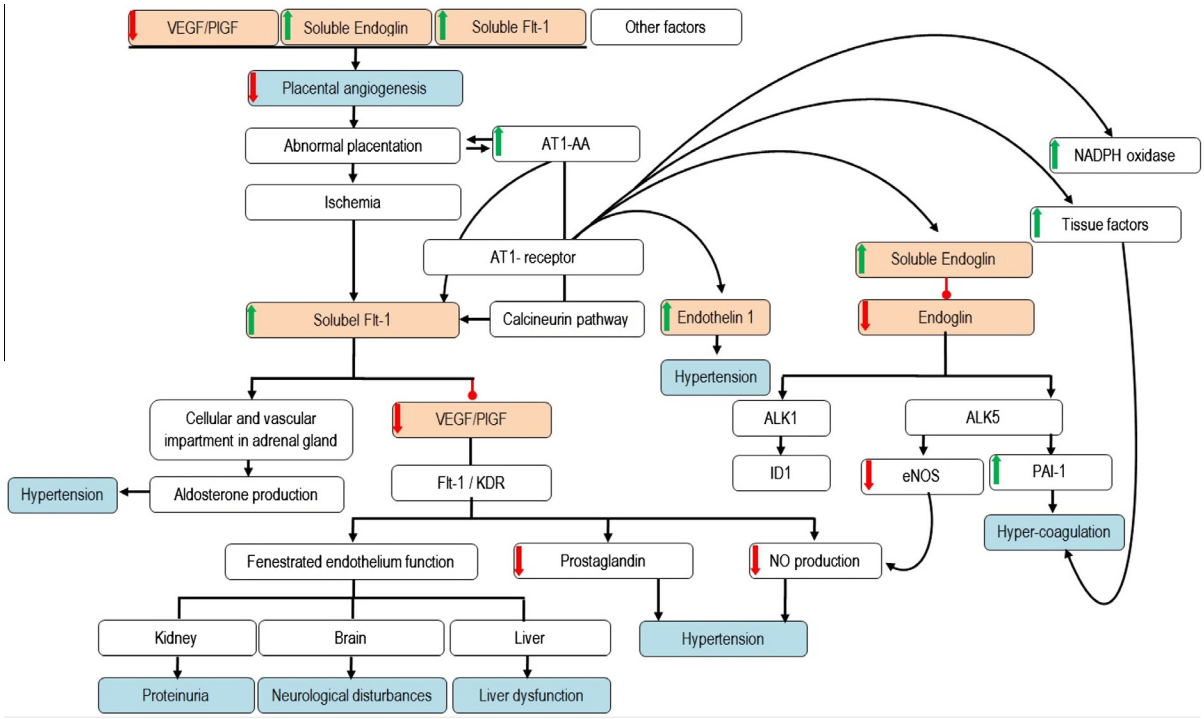

Figure 1 The role of angiogenic factors in preeclampsia pathogenesis. In the pre-clinical stage of preeclampsia, endothelialization of cytotrophoblasts is impaired leading to placenta ischemia and hypoxia. One of the important causal factors is imbalance pro-angiogenic factors (VEGF and PlGF) and anti-angiogenic (sFlt-1, sEng, AT1-AA). In the clinical stage, in response of ischemia condition, the placenta releases anti-angiogenic proteins and reduces the production of pro-angiogenic factors sFlt-1, reduces aldosterone levels in circulation and antagonizes the function of VEGF and PlGF. Reduction of interaction between pro-angiogenic factors (VEGF and PlGF) and their receptors leads to impaired fenestrated endothelial function in some organs and reduced the production vasodilators (prostaglandin and NO). These cause hypertension, proteinuria, neurological disturbances and high liver enzyme. sEng inhibits the production of NO by antagonizing TGF-β interaction to its receptor complex. TGF-β1 signaling is important to induce endothelium-dependent relaxation via an eNOS-dependent mechanism. sEng is also to be involved in hyper-coagulation and fibrinolytic imbalance by attenuating endoglin regulation on PAI-1 gene. One of the possible factors induces the production of sFlt-1 and sEng is AT1-AA. AT1-AA induces intracellular cascades and promoter activations in the nucleus lead to an up-regulation of endothelin-1, sFlt-1, sEng and other tissue factors. miRNAs could be an important factor either in up-regulation of sFlt-1 and sEng or down-regulation of VEGF and PlGF. Red arrow (down-regulation) and green arrow (up-regulation) indicate the confirmed expression level of molecule (as indicated) in preeclampsia condition. AT1-AA: the agonistic angiotensin II type 1 receptor autoantibody, AT1-receptor: angiotensin II type 1 receptor, eNOS: endothelial nitric oxide synthase, Flt-1: Fms-like tyrosine kinase-1 (also known as vascular endothelial growth factor receptor 1 (VEGFR-1)), KDR: kinase insert domain receptor (also known as VEGFR-2), PlGF: placenta growth factor, sEng: soluble endoglin, sFlt-1: soluble Fms-like tyrosine kinase-1, TGF-β: transforming growth factor beta, VEGF: vascular endothelial growth factor.

resulted in severe *in vivo* and *in vitro* angiogenesis defects [101–103]. Since then miRNAs were shown to play important roles in regulation of angiogenesis during development and normal physiological processes, as well as pathological angiogenesis [104].

In preeclampsia, several miRNAs are involved in angiogenesis by targeting various molecules including pro-angiogenic factors. A study found that miR-29b increased significantly among preeclampsia patients and it targeted VEGF-A directly (Fig. 2) [37,45,73]. Therefore, it has direct effect on angiogenesis. VEGF is a positive regulator of angiogenesis and plays a crucial role in vascular endothelial cell growth, blood vessel production as well as vascular permeability. Another study also found that VEGFA is one of the putative targets of miR-16 and over-expression of miR-16 reduced the protein levels of VEGFA (Fig. 2) [53]. A study confirmed that the expression of miR-16 was up-regulated among preeclampsia patients [33,37,53,73].

Wang et al. [53] found that angiogenesis-associated miRNAs (miR-17, miR-20a and miR-20b) were up-regulated among preeclampsia patients. These miRNAs target Ephrin-B2 and Ephrin type-B receptor 4 (EPHB4) (Fig. 2). Ephrin-B2 belongs to Ephrin ligands of Eph receptor, while EPHB4 belongs to families of Eph receptor. Interaction of Eph receptor and Ephrin ligands mediates vascular cell adhesion, repulsion, and migration [105]. A pro-angiogenic function of Ephrin-B2 was achieved by regulating internalization and signaling activities of VEGF receptor 2 (VEGFR2) and VEGFR3; therefore, both of EPHB4 and Ephrin-B2 have pivotal roles for angiogenesis during placentation [53].

In addition, miR-17, miR-20a and miR-20b also target other genes that are important for placental angiogenesis, including hypoxia-inducible factor 1-alpha (HIF1A), VEGFA, matrix metalloproteinase 2 (MMP2), metallopeptidase inhibitor 2 (TIMP2), IL-8 and TGF-β receptor (Fig. 2) [53,106]. HIF1A is a hypoxic-sensitive transcription factor

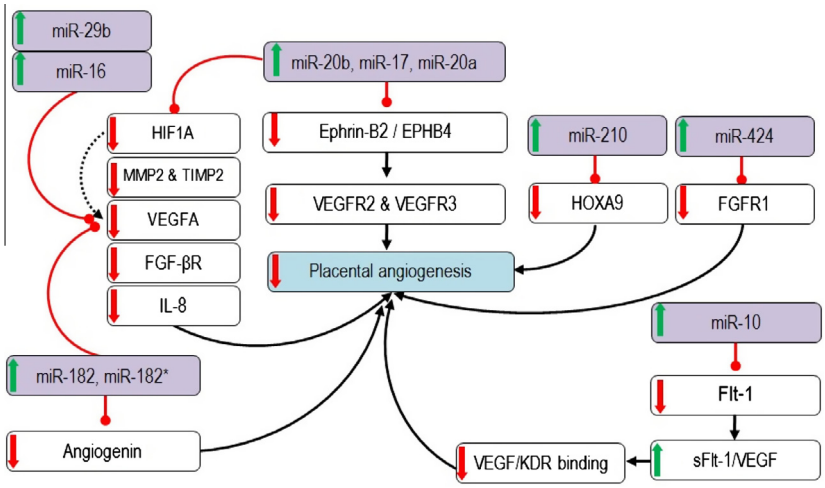

Figure 2 The molecular models of regulation of miR-17, miR-20a, miR-20b, miR-16, miR-29b, miR-210, miR-424 and miR-10 on angiogenesis and vascular integrity. Red arrow (down-regulation) and green arrow (up-regulation) indicate the confirmed expression level of miRNA or molecule (as indicated) in preeclampsia condition. EPHB4: ephrin type-B receptor 4, FGFR1: fibroblast growth factor receptor 1, FGF-βR: fibroblast growth factor beta receptor, Flt-1: Fms-like tyrosine kinase-1 (a receptor of VEGF), HIF1A: hypoxia-inducible factor 1-alpha, HOXA9: homeobox protein Hox-A9, KDR: kinase-insert domain region (a receptor of VEGF), MMP2: matrix metalloproteinase 2, sFlt-1: soluble Fms-like tyrosine kinase-1, TIMP2: TIMP metallopeptidase inhibitor 2, VEGFA: vascular endothelial growth factor A, VEGFR2: VEGF receptor 2, VEGFR3: VEGF receptor 2.

for placental development and function by regulating the expression of hypoxia-responsive genes including VEGFA, while MMP2 and TIMP2 are critical for regulating extracellular matrix degradation during initial angiogenic response. TGF-β1 induces angiogenesis through VEGF-mediated apoptosis [107]. Therefore, it is clear that miR-17, miR-20a and miR-20b regulate multiple steps during angiogenesis, including down-regulation of angiogenic factors expression, as well as inhibit matrix breakdown, endothelial cell proliferation, migration, and tube formation.

Another miRNA that might be important on angiogenesis pathobiology during the first stage of preeclampsia is miR-210. Although, a couple of studies found that miR-201 expression was down-regulated in mild preeclampsia [71,74], up-regulation of miR-210 is robust in preeclampsia patients [33,39,40,43,46,47,54,62,65–67,77]. Previous studies found that miR-210 targeted homeobox protein Hox-A9 (HOXA9) (Fig. 2) [67]. HOXA9 is a member of homeobox gene family and has a crucial role in angiogenesis by regulating the EPHB4 receptor to modulate endothelial cell tube formation [108]. In addition, ablation of HOXA9 gene in endothelial cells inhibited *in vitro* sprout formation and cell migration [108]. Furthermore, a study also revealed that the levels of mRNA and protein of HOXA9 were significantly lower in preeclamptic placentas compared to healthy control [67].

In addition, a study found that there was an up-regulation of miR-424 in placentas from severe preeclampsia patients [33] and FGF receptor 1 (FGFR1) was a target of miR-424 (Fig. 2) [96]. FGFR1 also has pivotal roles in many signaling pathways that control cellular proliferation, differentiation, survival, and angiogenesis [96].

One of the most robust miRNA functions on angiogenesis is shown by miR-126. Studies found that miR-126 directly targeted the 3′UTRs of vascular cell-adhesion molecule-1 (VCAM-1), sprouty-related, EVH1 domain-containing protein 1 (SPRED1), and phosphoinositide-3-kinase, regulatory subunit 2 (PIK3R2) (Fig. 3) [94,95]. VCAM-1 is a stimulator of angiogenesis [109], while SPRED1 and PIK3R2 are pivotal components of VEGF pathway [94]. SPRED1 inhibits RAF1 kinase activity, decreases ERK phosphorylation and, as the final result, reduces VEGF signaling that related to angiogenesis and vascular integrity. In addition, miR-126 regulates VEGF (and other growth factor signaling) also by targeting PIK3R2. PIK3R2 is an anti-angiogenic factor and a negative regulator of phosphatidylinositide 3-kinases (PI3) kinase activity signaling cascades [52]. By targeting PIK3R2, PIK3R2 reduces the PI3 kinase activity and AKT, and as the final consequence it reduces VEGF signaling (Fig. 3).miR-126 also regulates EphrinB2 and regulator of G-protein signaling 5 (RGS5) [94]. EphrinB2 is an inhibitor of MAP kinase, a component of signal cascade downstream of VEGF. In reduced miR-126 expression, EphrinB2 is up-regulated and it causes reduced MAP kinase signaling pathway for angiogenesis (Fig. 3). RGS5 represses phosphorylation of ERK. A study found that RGS5 protein was up-regulated in endothelial cells when miR-126 expression was down-regulated and RGS5 protein inhibited tubulogenesis by reducing ERK phosphorylation [110]. Therefore, it is clear that miR-126 has pivotal roles in angiogenesis signaling pathways. However, there is a contradictive result of miR-126 expression from preeclampsia patients. Although profiling analysis found that expression of miR-126 was up-regulated in preeclamptic placentas [63], another study confirmed that miR-126 expression was down-regulated [34].

4.3. The role of miRNAs in vascular tone

4.3.1. Regulation of renin angiotensin system (RAS) and AT1-AA

Angiotensin II has a critical role in preeclampsia pathogenesis. In normal pregnancy, renin, aldosterone, and angiotensin II

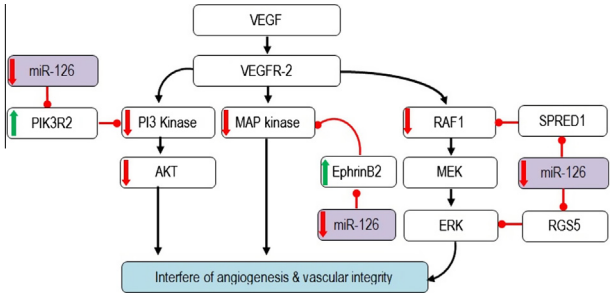

Figure 3 The molecular models of miR-126 regulation on angiogenesis and vascular integrity. miR-126 targets several molecules that are involved in VEGF signaling cascade (such as PIK3R2 and EphrinB2) and MEK/ERK signaling (such as SPRED1 and RGS5). Both of these signaling cascades are important for angiogenesis and vascular integrity. Red arrow (down-regulation) and green arrow (up-regulation) indicate the confirmed expression level of miRNA or molecule (as indicated) in preeclampsia condition. AKT: known as protein kinase B (PKB), is a protein kinase that has a key role in multiple cellular processes including apoptosis, cell proliferation, transcription and cell migration, ERK: extracellular signal-regulated kinases, FGFR1: fibroblast growth factor receptor 1, MEK: mitogen-activated protein kinase kinase, PI3: phosphatidylinositide 3-kinases, PIK3R2: phosphoinositide-3-kinase, regulatory subunit 2, RGS5: regulator of G-protein signaling 5, SPRED1: sprouty-related, EVH1 domain-containing protein 1, VEGFR-2: vascular endothelial growth factor receptor type 2, also known as kinase-insert domain region (KDR).

are increased; however, pregnant women remain normotensive because they are resistance to vasoconstriction effect of angiotensin II [111]. In contrast, preeclamptic women have increased vascular responsiveness to angiotensin II and other vasoconstrictors [8]. AbdAlla et al. [112] found that an up-regulation of heterodimerization of angiotensin type I (AT1) and the bradykinin B2 receptor in circulation increased vascular responsiveness to angiotensin II among patients with preeclampsia.

Furthermore, a study revealed that immune system participated in activation of RAS and increased vascular responsiveness in preeclampsia through the development of AT1-AA of the IgG isotype [111]. AT1-AA was originally detected by Wallukat et al. [113] based on the ability of this autoantibody to activate AT1 receptor (AT1-R). A study found that administration of AT1-AA (from pregnant women) in animal models caused hypertension, proteinuria, placental abnormalities, and glomerular endotheliosis [114].

The binding of AT1-AA to AT1-R induces sFlt-1 and sEng (Fig. 1) [115,116]. A population based study found that preeclampsia patients with AT1-AA had higher sFlt-1 level, lower VEGF level, and greater insulin resistance than preeclampsia patients without AT1-AA [117]. AT1-AA also promotes IL-6 production, in turn, induces endothelin production and stimulates placental oxidative stress [118].

Another effect of AT1-AA is inducing the synthesis and secretion of PAI-1 by trophoblast cells of the placenta (Fig. 1) [119]. Elevated PAI-1 contributes to the hyper-coagulation and fibrinolytic imbalance in preeclampsia [90]. Another study also found that administration of AT1-AA and AT1-R in human mesangial cell culture stimulated PAI-1 synthesis and secretion, a feature that may contribute to kidney damage leading to proteinuria in preeclampsia [90]. AT1-AA also stimulates production of tissue factors and NADPH oxidase, features that may play a role in vascular injury and oxidative stress, respectively (Fig. 1) [120]. Overexpression of tissue factors also contributed to hyper-coagulation in preeclampsia [90].

Some miRNAs contribute to RAS function and AT1-AA production. miR-155 regulates human AT1-R expression in fibroblast cells by targeting 3′-UTR AT1-R directly [121]. They also demonstrated that inhibition of miR-155 increased AT1-R expression and enhanced activation of angiotensin II-induced phospho-ERK1/2.

In preeclampsia, although, studies found that miR-155 expression was up-regulated in preeclampsia [32,35,69,72], another study revealed that miR-155 from HUVECs of severe preeclampsia was less mature compared to miR-155 from controls [69]. Therefore, Cheng et al. [69] speculated that the function of miR-155 in preeclampsia was decreased. In addition, they also demonstrated that RAS expressions, especially angiotensin II and AT1-R, were significantly increased in HUVECs from patients with severe preeclampsia [69].

In addition, AT1-R levels could also be regulated by another miRNA. In human intestinal epithelial cell line, a bioinformatics study found that miR-802 could directly interact with 3′UTR AT1-R [122]. This study also demonstrated that loss of miR-802 function resulted in augmented AT1-R levels and enhanced angiotensin II-induced signaling. However, there is no report related to miR-802 expression in preeclampsia patients.

Besides targeting AT1-R production, miRNAs also contribute to RAS by involving in regulation of AT1-AA production. A previous study found that miR-181a expression was significantly increased in placentas from women with preeclampsia [33,58,63,73]. miR-181a enhanced mRNA expression of IL-6 and indoleamine 2,3-dioxygenase (IDO) by activating p38 and c-Jun N-terminal kinases (JNK) signaling pathways, respectively [58]. Interestingly, increased IL-6 levels could stimulate production of AT1-AA (Fig. 4) [58]. A previous study also supports that the level of IL-6 in plasma was elevated in preeclampsia patients [123].

IDO is an enzyme that mediates the conversion of tryptophan to kynurenine and it regulates T-cell activity and endothelial-derived relaxing factor. A previous study found that mice with IDO knockdown suffered from renal glomerular endotheliosis, proteinuria, endothelial dysfunction, intrauterine growth restriction, and elevated blood pressure [124]. However, Liu et al. [58] found that IDO mRNA level was increased about 10 times in mesenchymal stem cells from preeclampsia patients compared to normal pregnancy. Therefore, the role of IDO in preeclampsia is still debatable.

In addition, another study found that miR-1301 was significantly down-regulated in preeclampsia and miR-1201 targeted leptin gene (LEP) [38]. Furthermore, over-expression of LEP in preeclampsia (because of miR-1301 down-regulation) increased IL-6 production, and as a result, induced the production of AT1-AA (Fig. 5D) [38]. It also confirmed that down-regulation of miR-1301 was correlated with increasing maternal blood pressure [38]. Therefore, there are enough evidences to support that that miRNAs are involved in RAS dysregulation during clinical stage of preeclampsia. The existing data reveal that miRNAs interfere with RAS by targeting AT1-R

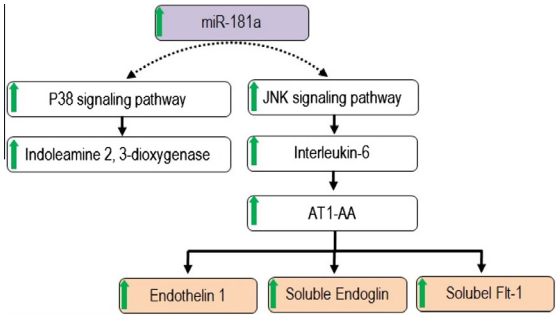

Figure 4 The role of miR-181a in AT1-AA production. Up-regulation of miR-181a in preeclampsia increases the activation of JNK signaling, in turn, it increases the production of AT1-AA. As a consequence AT1-AA activates AT1 receptor and induces the production of ET-1, sEng, sFlt-1 and aldosterone. Red arrow (down-regulation) and green arrow (up-regulation) indicate the confirmed expression level of miRNA or molecule (as indicated) in preeclampsia condition. AT1-AA: angiotensin type I receptor agonistic autoantibody, Flt-1: Fms-like tyrosine kinase-1, JNK: c-Jun N-terminal kinases.

and induce the production of AT-AA. However, further studies are needed to investigate other possible mechanism on how miRNAs regulate RAS.

4.3.2. Regulation of the production of prostacyclin, 17β-estradiol, hydrogen sulfide and leptin

Several factors are also involved in vascular pressure regulation including prostacyclin (PGI2), 17β-estradiol, hydrogen sulfide (H_2S) and leptin. PGI2 is an anti-platelet aggregator and vasodilator that is participating in pathogenesis of preeclampsia. PGI2 is produced from arachidonic acid metabolism by the cyclooxygenase (COX)-1 and COX-2. A previous study found that the level of PGI2 product, 6-keto-prostaglandin F1α, in plasma and urine were lower in severe preeclampsia compared to normal pregnancies [125]. Recent data reveal that miR-144 contributes in low level of PGI2 in preeclampsia. A study found a down-regulation of miR-144 among preeclampsia patients compared to control and miR-144 targeted CUG triplet repeat-binding protein 2 (CUGBP2) [35,39]. CUGBP2 is a ubiquitously expressed RNA-binding protein that interacts and inhibits COX-2 translation (Fig. 5A).

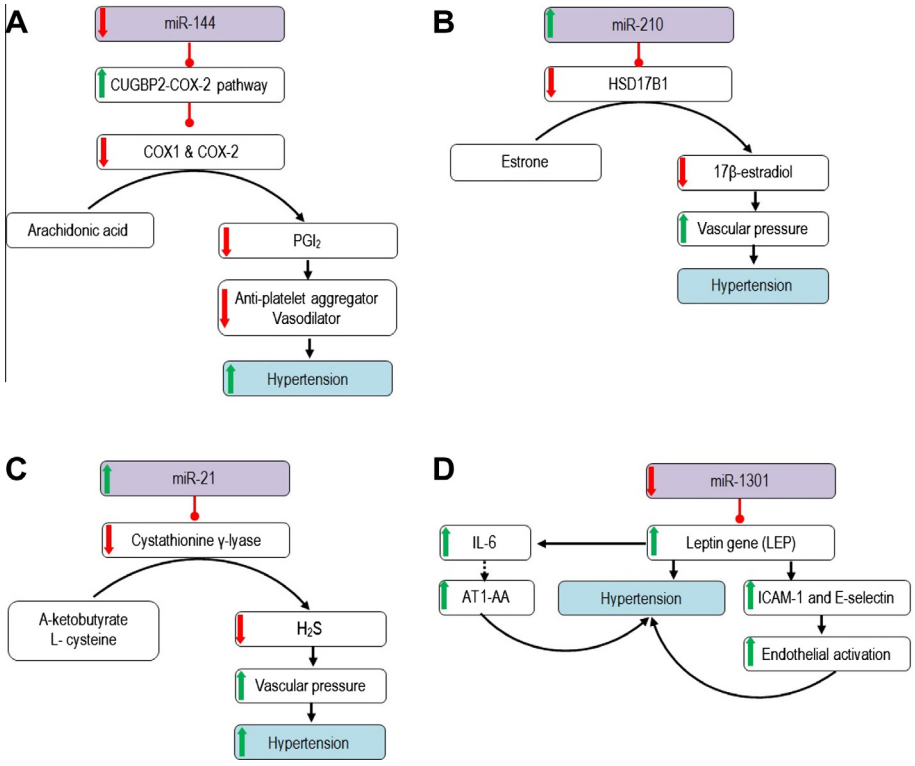

Figure 5 The role of miRNAs in regulation of the production of PGI2, 17β-estradiol, H_2S and leptin. Several miRNAs are involved in high vascular pressure in preeclampsia by reducing the production of potential vasodilators and inducing of endothelial activation. (A) Down-regulation of miR-144 in preeclampsia reduces the production of enzymes (COX1 and COX2) that produce prostacyclin. As a consequence, the production of prostacyclin is reduced. (B) miR-210 reduces the production of 17β-estradiol by targeting HSD17B1, enzyme that induces the production of 17β-estradiol. (C) miR-21 reduces the production of H_2S by targeting cystathionine γ-lyase. (D) Down-regulation of miR-1301 increases the production of leptin and it induces hypertension, either increases the production of AT1-AA or activates endothelial. As a result, these increase responsiveness of endothelial to vasoconstrictors. Red arrow (down-regulation) and green arrow (up-regulation) indicate the confirmed expression level of miRNA or molecule (as indicated) in preeclampsia condition. AT1-AA: angiotensin II type I receptor agonistic autoantibody; COX1: cyclooxygenase 1, COX2: cyclooxygenase 2, CUGBP2: CUG triplet repeat-binding protein 2, H_2S: hydrogen sulfide, HSD17B1: 17-beta-hydroxysteroid dehydrogenase, ICAM1: intercellular adhesion molecule 1, PGI2: prostacyclin.

Inhibition of COX-2 translation, in turn, reduces the production of PGI_2 and as the consequence increases vascular tone and increases platelet count in preeclampsia.

17β-Estradiol is a primary female sex hormone. Interestingly, 17β-estradiol induced a relaxant response in Sprague–Dawley rats [126] and it retained the capability for relaxing omental artery rings from preeclamptic women [127]. Several studies revealed an increase in expression of mi-R210 in preeclampsia [32,33,39,40,43,46,54,62,65–67,70]. A study by Ishibashi et al. [62] found that miR-210 targeted 17-beta-hydroxysteroid dehydrogenase (HSD17B1) in preeclampsia (Fig. 5B). HSD17B1 is an enzyme that catalyzes 17β-estradiol production from estrone [128]. A previous study identified that HSD17B1 gene was down-regulated in preeclamptic placentas compared to normal placentas [62]. A prospective cohort study also found a low HSD17B1 plasma level before the onset of preeclampsia [63]. Therefore, over-expression of miR-210 in preeclampsia decreases 17β-estradiol and increases vascular pressure as a final consequence.

In addition, H_2S, a new vasodilator, also has a pivotal role in regulating vascular pressure in preeclampsia. H_2S decreases vascular tone by targeting ATP-sensitive K^+ (KATP) channels in vascular smooth muscle cells [129] and probably interacts with NO [130]. A study found that the expression of cystathionine gamma-lyase (CSE), an enzyme that catalyzes H_2S production from α-ketobutyrate and L-cysteine was repressed by miR-21 [131]. A study revealed a significant up-regulation of miR-21 expression and down-regulation of CSE protein and mRNA expression in preeclamptic placentas compared to normal placentas [130]. Therefore, reduction of CSE expression (consequently decreasing of H_2S expression) contributes to high vascular tone in preeclampsia (Fig. 5C).

Furthermore, another factor that might contribute to vascular pressure in preeclampsia is indirect effect of leptin production. Leptin, a pro-inflammatory factor, promotes Th-1 responses and contributes to vascular pressure regulation [130]. Dysregulation of placental leptin production in preeclampsia contributes to excessive systemic pro-inflammatory response. In non-pregnant rabbits, for example, leptin administration increased blood pressures [132]. In pregnant rats, leptin administration increased the circulating concentration of endothelial activation markers (ICAM-1 and E-selectin), and caused hypertension and proteinuria [133]. In addition, leptin also increased sympathetic nervous response [134].

The expressions of miR-1301, miR-223 and miR-224 were down-regulated in preeclampsia and these miRNAs target leptin gene (LEP) [38]. Further evidence reveals that circulating leptin or placental LEP and placental miR-1301 were inversely correlated [38]. In addition, miR-1301 was inversely correlated with both maternal systolic and diastolic blood pressure [38]. Therefore, these data indicated that down-regulation of miR-1301 in preeclampsia increases maternal blood pressure (Fig. 5D). Down-regulation of miR-1301 also induces AT1-AA production (see previous explanation).

4.3.3. Regulation of NO production

NO, a biological mediator synthesized from L-arginine by NO synthases (NOS), plays a pivotal role in regulation of vascular resistance and hemodynamic changes during normal pregnancy and preeclampsia. During normal pregnancy, the production and activity of NO are increasing because of high activity of NOS; however, the same does not occur with preeclampsia [135].

Studies found that the levels of NO, placenta NOS activity, cyclic guanosine monophosphate (the effector of NO) as well as nitrate and nitrite were significantly lower in preeclampsia than in normal pregnancy [135]. In addition, in preeclampsia, endothelial-derived vasoactive factors are predominated by vasoconstrictors (endothelin, thromboxane A2) over vasodilators (NO, prostacyclin) [136].

Although, large data exist regarding the regulation of endothelial NOS (eNOS) enzyme activity, little information is available about the role of miRNAs in regulation of eNOS expression. Davis et al. [137] found that shear stress was an important activator of eNOS expression and it increased the expression of miR-21 (Fig. 6). In addition, phosphatase and tensin homolog (PTEN), a known target of miR-21, was down-regulated. In addition, over-expression of miR-21 increased eNOS phosphorylation and NO production [138]. Interestingly, a recent study found that the expression of miR-21 was down-regulated in placentas from women with preeclampsia [135]. It indicates that down-regulation of miR-21 in preeclampsia leads to reduced eNOS phosphorylation and NO production.

A previous study found that Dicer-knockdown mice had elevated eNOS protein and transfection with miR-221/miR-222 restored the elevated eNOS [101]. This indicates that miR-221/miR-222 induces the production of eNOS (Fig. 6). However, prediction sites of these miRNAs are not located within eNOS 3′UTR [104]. Interestingly, in severe preeclampsia, the level of miR-222 was increased significantly [73]. In addition, a couple of studies also found that the expression of miR-211 either in MSC-decidual and plasma from patients with severe preeclampsia was up-regulated [37,45]. However, miR-211 expression was down-regulated in plasma from patients with mild preeclampsia [41].

Previously, a study found that lipopolysaccharide (LPS) could induce iNOS expression through toll-like receptor (TLR) signaling; and increasing the activity of miR-146a, a

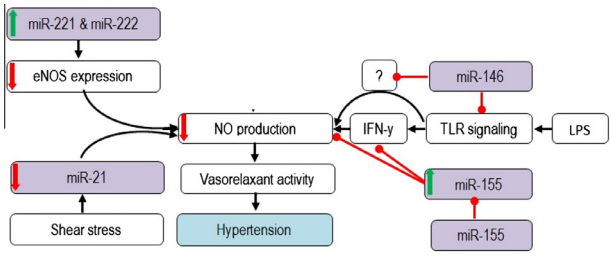

Figure 6 The role miRNAs in regulation of eNO production. miR-21 increases the production of NO by inducing eNOS phosphorylation. Enhancing the activity of miR-146a inhibits the expression of LPS-induced iNOS and NO. Up-regulation of miR-155 in preeclampsia patients inhibits the production of NO. Red arrow (down-regulation) and green arrow (up-regulation) indicate the confirmed expression level of miRNA or molecule (as indicated) in preeclampsia condition. IFN-γ: interferon gamma, LPS: lipopolysaccharide, NO: nitric oxide, NOS: endothelial NO synthases, TLR: toll-like receptor.

negative regulator of TLR signaling, significantly inhibited LPS-induced iNOS and NO expression [139]. However, there is no study that confirms the expression of miR-146a in preeclampsia patients.

Additionally, a previous study found that miR-155 targeted eNOS directly [140]. They validated that over-expression of miR-155 decreased eNOS expression and NO production, whereas inhibition of miR-155 increased eNOS expression and NO production in HUVEC. In addition, another study also found that miR-155 also reduced iNOS expression by targeting iNOS-upstream regulators [141]. Studies found that miR-155 expression was up-regulated among preeclampsia patients [32,35,69,72]. Therefore, it indicates that over-expression of miR-155 in preeclampsia patients inhibits the production of NO and as a result it causes vasoconstriction.

5. Conclusion

One of the major causal factors of preeclampsia is impaired angiogenesis during placentation and impaired endothelialization of cytotrophoblasts and the invasion of spiral arteries into myometrium leading to poor placentation. It causes over-expression of sFlt-1, sEng and other anti-angiogenic factors and down-regulation of major pro-angiogenic factors such as VEGF and PlGF. Our previous study concludes that miRNAs could be a potential causal factor on pathobiology of preeclampsia [142]. Data reveal that miRNAs interfere with angiogenesis process during early pregnancy by dysregulating these angiogenic factors and their receptors. Dysregulation of these angiogenetic factors also induce hypertension during the clinical stage of preeclampsia. In addition, miRNAs also induce hypertension by inducing the production of AT1-AA and targeting several vasodilators such as prostacyclin, 17β-estradiol, H_2S and NO.

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Conflict of interest

None.

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