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Original Article

The role of microRNAs in the proliferation, differentiation, invasion, and apoptosis of trophoblasts during the occurrence of preeclampsia—A systematic review

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ABSTRACT

Objectives: Dysregulation of trophoblast invasion into the decidual stroma and spiral arteries during early gestation is one of the major factors associated with the pathogenesis of preeclampsia. Therefore, the objective of this study was to evaluate, based on recent studies, the role of microRNAs (miRNAs) in trophoblast proliferation, differentiation, invasion, and apoptosis during the early gestation of pre-eclamptic pregnancies.

Materials and methods: This systematic review included articles between 2007 and 2015 that were obtained from the MEDLINE database. The articles were identified by searching using a combination of Medical Subject Headings (MeSH terms), namely "preeclampsia", "pre-eclampsia", "miRNA", and "microRNA". All sources of miRNAs, all types of preeclampsia, and all techniques used when measuring miRNAs were included in the reviewed papers.

Results: Confirmed upregulation of miR-125b-1-3p, miR-20a, miR-29b, miR-181a, miR-16, miR-210, and miR-155 and confirmed downregulation of miR-17, miR-19b1, miR-195, miR-378a-5p, miR-376c, and miR-675 were identified as involved in repressing the proliferation, differentiation, and invasion of trophoblast cells. In addition, upregulation of miR-29b and downregulation of miR-378a-5p and miR-376c were found to be associated with increased trophoblast cell apoptosis.

Conclusion: Overall, miRNAs have been confirmed to be involved in the shallow invasion by trophoblasts into the spiral arteries and decidual stroma during early gestation and these miRNAs are possible promising biomarkers that may help to predict preeclampsia in the future.

1. Introduction

Preeclampsia is a disease of pregnancy characterized by the new onset of hypertension and proteinuria after 20 weeks of gestation [1]. Preeclampsia affects 3–5% of pregnancies and complicates 3–8% of pregnancies, which leads to a high disease burden among pregnant women [2]. Preeclampsia causes a range of maternal complications, including renal failure, hemolysis, elevated liver enzymes and low platelet count (HELLP) syndrome, disseminated intravascular coagulation, placental abruption, liver failure, pulmonary edema, stroke, and cerebral edema with seizures [1,3]. As the placenta is involved in preeclampsia, this also increases the possibility of fetal complications, which include low birth weight, prematurity, intrauterine fetal growth restriction, oligohydramnios, bronchopulmonary dysplasia, and perinatal death [1,4,5].

Although the exact etiology of preeclampsia is not clear, it is believed that placental insufficiency is central to the pathogenesis of preeclampsia [6,7]. Several pieces of evidence support this theory including: (1) placenta from preeclampsia patients has been found to have infarcts and to show sclerotic narrowing of the arterioles; (2) placenta bed biopsies have identified that these samples show inadequate trophoblast invasion of the maternal decidual arterioles, which results in tight and constricted vessels; (3) various medical conditions involving underlying vascular insufficiency, such as chronic hypertension and diabetes, or conditions that decrease placental blood flow, such as multiple

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gestations or hydatiform moles, have been found to increase the risk of preeclampsia; (4) the creation of placenta insufficiency by disrupting uterine blood flow has been shown to cause preeclampsia-like events using animal models; and (5) when preeclampsia occurs in cases of extrauterine pregnancy, removal of the fetus alone is not sufficient and the symptoms persist until the placenta is removed [6–8].

During a normal healthy pregnancy, a highly coordinated program within the body ensures that the placenta and fetus are provided with an adequate blood supply; this process is essential for the provision of adequate oxygen and nutrients to the fetus. Cytotrophoblasts originating from the fetus migrate into the maternal vasculature and invade the maternal spiral arteries. These invasive trophoblast cells gradually replace the endothelial lining of these vessels and differentiate into endothelial-like cells. During this process (invasion and differentiation), trophoblasts change the expression of certain cytokines, various adhesion molecules, a range of extracellular matrix molecules and various metalloproteinases, as well as the expression of the major histocompatibility complex and the histocompatibility leukocyte antigen-G [7]. This process converts low-capacitance and high-resistance vessels into high-capacitance and low-resistance vessels [7,9,10]. In preeclampsia, this complex and coordinated process is significantly disrupted and normal remodeling of the spiral arteries does not occur. This results in retention in these mothers of small vessels that retain high resistance and low-capacity [10,11]. The major result of this lack of change is insufficient blood flow to the uteroplacental area.

MicroRNAs (miRNAs), a member of the noncoding groups of RNAs, are involved in the specific regulation of both protein-coding and putatively noncoding genes via post-transcriptional silencing or, in rare cases, via gene activation [12]. miRNAs play important roles in physiological homeostasis and health, as well as in the pathophysiological derangements associated with various diseases [13]. The various physiological processes that miRNAs are involved in include the cell cycle [14], cell differentiation [15], development [15], metabolism [16], various immune responses [17], and a number of other processes [12]. miRNAs are also associated with various pathophysiological conditions such as various noninfectious diseases [18–20] and infectious diseases [21–24]. In terms of pregnancy, miRNAs have been found to play pivotal roles in a number of processes including homeostasis during the periimplantation period and placentation [25], embryonic stem cells regulation [26], fetal growth restriction [27], intrauterine growth retardation [28], small for gestational age [29], and preeclampsia [30,31].

Since 2007, a range of studies have been conducted that have investigated in depth the role of miRNAs in the pathogenesis of preeclampsia. This systematic review has been conducted to evaluate the role of miRNAs in preeclampsia pathogenesis. The aim is to evaluate the role(s) of miRNAs in trophoblast proliferation, differentiation, invasion, and apoptosis based on recent confirmed and published research findings.

2. Materials and methods

In this systematic review, articles present in the MEDLINE database between 2007 and 2015 were identified by searching using the keywords: “preeclampsia”, “pre-eclampsia”, “miRNA”, and “microRNA”. The bibliographies of the articles retrieved were scanned for any further relevant references. All studies that measured the presence of miRNAs in plasma, placenta material, human umbilical vein endothelial cells (HUVECs), peripheral blood mononuclear cells (PBMCs), and mesenchymal stem cells (MSCs) were included. In addition, any and all techniques that can be used to measure the expression of miRNAs, including microarray, quantitative reverse transcription polymerase chain reaction (qRT-PCR) and next-generation sequencing, were also included. In cases where the study applied a microarray approach that was then confirmed by qRT-PCR, the expression levels of the miRNAs presented in this systematic review are the miRNA levels that have been confirmed by qRT-PCR.

Because miRNAs regulate several pathological processes related to preeclampsia pathogenesis, the results of our systematic review have been divided into two parts. The present article describes the roles of miRNAs in trophoblast functioning, whereas the second outlines the roles of miRNAs in angiogenesis and the control of vascular pressure. Specifically, in this article, the role of miRNAs in the dysregulation of trophoblast proliferation, differentiation, and invasion, as well as their effect on apoptosis, all of which play a part in preeclampsia pathogenesis, are discussed.

3. Results

The role of miRNA in preeclampsia pathogenesis has been investigated intensively since 2007. The first report was published in 2007 and this found that the expression of two miRNAs was significantly higher in preeclamptic placentas than normal placentas [32]. Following this novel finding, a significant number of further studies have been published [33–74].

In general, these studies have investigated the differential expression of miRNAs using several sources of experimental material, including placenta [33–36,38,40,47–51,54–57,59–62,66,68,70–74], plasma [39,41–46,50,63–67], PBMCs [52], HUVECs [69], and MSCs from the decidua or the umbilical cord [37,53,58]; these samples were obtained from women who had preeclampsia and from women undergoing a normal pregnancy. These studies were carried out in The People's Republic of China [33–35,37,40,42,43,45,50,52–58,63,64,67,69,72–74], the USA [32,46–48,51,54,61,70,71], South Korea [49], Canada [50,60], Japan [62], Turkey [65], Chile [66], Germany [68], Hungary [59], Switzerland [36], Norway [38], Italy [39], Spain [41], and the Czech Republic [44] (see Table 1 for details).

One of the factors causing preeclampsia is a deficient trophoblast invasion of the placental bed spiral arterioles; this alters the trophoblast-mediated remodeling of the spiral arteries and results in reduced uteroplacental perfusion. It is clear that shallow trophoblast invasion and impaired spiral arterial remodeling can be considered to be the initial pathological step in preeclampsia. One of the target areas of the miRNAs that forms a link with preeclampsia pathogenesis is the dysregulation of trophoblast proliferation, proliferation, and invasion. This occurs during early pregnancy and leads to the development of preeclampsia. A range of miRNAs have been confirmed to play pivotal roles in this process by targeting a number of different genes. In addition, miRNAs have also been found to be involved in deficiencies in trophoblast invasion that are associated with the induction of trophoblast cell apoptosis. Both of these mechanisms have the effect of producing a significant reduction in uteroplacental blood flow.

4. Discussion

4.1. The roles of miRNAs in trophoblast proliferation, survival and invasion

A number of studies have found that expression of miR-125b-1-3p in placenta samples from severe preeclampsia patients is significantly upregulated compared to control samples [33,35]. Further evidence has revealed that higher expression of miR-125b-1-3p decreases the invasiveness of trophoblasts and that this occurs

Table 1
Summary of studies related to differential expression of miRNAs in preeclampsia.

Country	Patients	Sample & method	Upregulation of miRNA(s)	Downregulation of miRNA(s)	Reference
The People's Republic of China	13 sPE vs. 25 control	Placenta RT-qPCR	miR-1, miR-16, miR-19b, miR-20a,miR-125b-1-3p, miR-181a, miR-182, miR-210, miR-355, miR-424, miR-1469	miR-29a-3p, miR-200c,miR-335,miR-363,miR-584,miR-744, miR-1826	[33]
The People's Republic of China	115 PE vs. 115 control	Placenta RT-qPCR	—	miR-126	[34]
The People's Republic of China	13 sPE vs. 25 control	Placenta RT-qPCR	miR-125b-1-3p	—	[35]
Switzerland	15 sPE vs. 14 control	Placenta RT-qPCR	—	miR455-3p, miR455-5p	[36]
The People's Republic of China	20 sPE and PE vs. 20 control	MSC-decidual RT-qPCR	miR-136, miR-495, miR-16, miR-29b, miR-140-5p, miR-30a, miR-100, miR-494, miR-221	miR-1207-5p	[37]
Norway	23 PE vs. 23 control	Placenta RT-qPCR	—	miR-1301, miR-223, miR-224	[38]
Italy	24 sPE vs. 24 control	Plasma TaqMan microarray	miR-1233, miR-650, miR-520a, miR-215, miR-210, miR-25, miR-518b, miR-193a-3p, miR-32, miR-204, miR-296-5p, miR-152	miR-126, miR-335, miR-144, miR-204, miR-668, miR-376a, miR-15b	[39]
The People's Republic of China	15 sPE vs. 26 control	Placenta RT-qPCR	miR-210	—	[40]
Spain	31 PE vs. 44 control	Plasma RT-qPCR	miR-192, miR-143, miR-125b	miR-127, miR-942, miR-126#, miR-221	[41]
The People's Republic of China	9 sPE vs. 22 control	Plasma RT-qPCR	miR-155	—	[42]
The People's Republic of China	20 sPE vs. 33 control	Plasma RT-qPCR	miR-210, miR-30a-3p, miR-518b, miR-524, miR-17-3p, miR-151, miR-193b	miR-195, miR-223, miR-218, miR-379, miR-411, miR-17, miR-18a, miR-19b1, miR-92a1	[43]
The People's Republic of China	20 sPE vs. 33 control	Plasma RT-qPCR	miR-210	miR-18a, miR-19b1, miR-92a1	[43]
Czech Republic	63 PE vs. 55 controls	Plasma RT-qPCR	miR-516-5p, miR-517*, miR-520a*, miR-525, miR-526a	—	[44]
The People's Republic of China	16 PE vs. 32 control	Plasma RT-qPCR	miR-141, miR-29a	miR-144	[45]
The People's Republic of China	22 sPE vs. 32 control	Plasma RT-qPCR	miR-141, miR-221, miR-29a	—	[45]
USA	33 PE vs. 34 control	Plasma RT-qPCR	miR-210	—	[46]
USA	20 PE vs. 56 control	Plasma RT-qPCR	miR-210	—	[46]
USA	16 PE vs. 12 control	Placenta RT-qPCR	miR-210, miR-182*	—	[47]
USA	19 PE vs. 40 control	Placenta RT-qPCR	—	miR-149	[48]
South Korea	21 sPE vs. 20 control	Placenta RT-qPCR	miR-92b, miR-197, miR-342-3p, miR-296-4p, miR-26b, miR-25, miR-296-3p	miR-21, miR-223	[49]
Canada	11 PE vs. 25 control	Placenta RT-qPCR	—	miR-376c	[50]
The People's Republic of China	15 PE vs. 22 control	Placenta RT-qPCR	—	miR-376c	[50]
The People's Republic of China	13 PE vs. 13 controls (preterm)	Plasma RT-qPCR	—	miR-376c	[50]
The People's Republic of China	16 PE vs. 31 control	Plasma RT-qPCR	—	miR-376c	[50]
USA	8 PE vs. 8 control	Placenta RT-qPCR	miR-106a, miR-19b	—	[51]
The People's Republic of China	12 PE vs. 12 control	PBMC qRT-PCR	—	miR-126	[52]
The People's Republic of China	20 sPE vs. 20 control	MSC-decidua qRT-PCR	miR-16	—	[53]
USA	6 sPE vs. 6 control	Placenta qRT-PCR	miR-210	—	[54]
The People's Republic of China	10 sPE vs. 24 control	Placenta qRT-PCR	—	miR-675	[55]
The People's Republic of China	15 sPE vs. 17 control	Placenta qRT-PCR	—	pri-miR-195, miR-195	[56]
The People's Republic of China	24 sPE vs. 26 control	Placenta qRT-PCR	miR-29b	—	[57]
The People's Republic of China	13 PE vs. 16 control	MSC-decidua qRT-PCR	miR-181	—	[58]
The People's Republic of China	11 PE vs. 16 control	MSCs-umbilical cord qRT-PCR	miR-181	—	[58]

Table 1 (*continued*)

Country	Patients	Sample & method	Upregulation of miRNA(s)	Downregulation of miRNA(s)	Reference
Hungary	31 PE 28 vs. control	Placenta qRT-PCR	—	miR-325	[59]
Canada	15 PE vs. 22 control	Placenta qRT-PCR	—	miR-378a-5p	[60]
USA	10 sPE vs. 10 control	Placenta qRT-PCR	miR-17, miR-20a, miR-20b	—	[61]
Japan	8 PE vs. 10 control	Placenta qRT-PCR	miR-210, miR-193b, miR-144, miR-193b, miR-18a, miR-185, miR-19a, miR-590–5p, miR-142–3p, miR-451, miR-22, miR-526b, miR-520a-3p, miR-10b, miR-20a, miR-518f, miR-146b-5p, miR-517c, miR-518c, miR-525–5p, miR-519e, miR-126	miR-145, miR-143, miR-188-5p, miR-107, miR-133a, miR-105, miR-224, miR-551b, miR-542-5p, miR-186	[62]
The People's Republic of China	10 sPE vs. 9 control	Plasma qRT-PCR	miR-24, miR-26a, miR-103, miR-130b, miR-181a, miR-342-3p, miR-574-5p	—	[63]
The People's Republic of China	2 PE and 2 sPE vs. 1 control	Plasma NGS	miR-521, miR-520h, miR-517c, miR-519d, miR-517b, miR-542-3p, miR-136, let-7f-1, miR-125b, miR-125a-5p, miR-519a, miR-29a	let-7f, miR-223, miR-1260, let-7d, miR-320c, miR-185, miR-1272	[64]
Turkey	20 PE vs. 20 control	Plasma qRT-PCR	miR-210	miR-152	[65]
Chile	52 PE and 31 PE + SGA vs. 72 control	Placenta qRT-PCR	miR-210	—	[66]
The People's Republic of China	15 PE and 15 sPE vs. 15 controls	Plasma qRT-PCR	miR-210	—	[67]
Germany	5 sPE vs. 5 control	Placenta qRT-PCR	let-7b, miR-302*, miR-104, miR-128a, miR-182*, miR-133b	—	[68]
China	5 sPE vs. 5 control	HUVEC qRT-PCR	miR-155		[69]
USA	20 PE vs. 20 control	Placenta qRT-PCR	miR-210	miR-328, miR-584, miR-139-5p, miR-500, miR-1247, miR-34c-5p, miR-1	[70]
USA	6 PE vs. 5 control	Placenta qRT-PCR	miR-496	miR-15b, miR-181, miR-210, miR-483—5p	[71]
The People's Republic of China	20 sPE vs. 20 control	Placenta qRT-PCR	miR-155		[72]
The People's Republic of China	24 sPE vs. 26 control	Placenta qRT-PCR	miR-16, miR-29b, miR-195, miR-26b, miR-181a, miR-335, miR-222	—	[73]
The People's Republic of China	8 PE and 15 sPE vs. 11 control	Placenta qRT-PCR	miR-152	miR-210, miR-411, miR-377	[74]
The People's Republic of China	15 sPE vs. 11 control	Placenta qRT-PCR	miR-210, miR-152, miR-518b	miR-411, miR-377, miR-18a, miR-363, miR-542-3p	[74]
USA	9 PE vs. 9 spontaneous preterm labor	Placenta qRT-PCR	miR-182, miR-210	—	[32]
USA	9 PE + SGA vs. 9 spontaneous preterm labor	Placenta qRT-PCR	miR-210, miR-155, miR-181b, miR-182*, miR-200b, miR-154*, miR-183	—	[32]

HUVEC = human umbilical vein endothelial cell; MSC = mesenchymal stem cells; NGS = next-generation sequencing; PBMC = peripheral blood mononuclear cell; PE = preeclampsia; qRT-PCR = quantitative reverse transcription polymerase chain reaction; SGA = small for gestational age; sPE = severe preeclampsia.

directly via the expression of sphingosine-1-phosphate receptor 1 (S1PR1) [35]. S1PR1 is a G-protein-coupled receptor of sphingosine-1-phosphate and this protein plays a critical role in angiogenesis inhibition and the maintenance of vascular stability. An earlier study has revealed that activation of S1PR1 is able to promote the invasion of trophoblast cells [75]. Therefore, it is clear that the trophoblast invasion-inhibiting effect of miR-125b-1-3p occurs via a suppression of S1PR1 expression (Fig. 1).

Other studies have found that a downregulation of miR-18a occurs in severe preeclamptic placenta samples [43,74]. However, one study did find that the expression of miR-18a is upregulated in mild preeclamptic placenta samples [62]. Using a human trophoblast cell line, miR-18a was found to promote trophoblast invasion by targeting and suppressing Smad2 expression (Fig. 1) [43]. Smad proteins are able to modulate signaling using transforming growth factor β (TGF-β) and they play a major role as the central mediators of TGF-β signaling. The modulation by miR-18a of the TGF-β signaling pathway thus plays an essential role in the regulation of trophoblast cell activity. Other studies have found that the members of miR-17-92 cluster (miR-17, miR-19b1) are downregulated in severe preeclamptic placenta samples [43]. It has been found that miR-17 and miR-19b1 target Smad6/Smad7 and Smad4/Smad5, respectively (Fig. 1). However, one study by Wang et al [61] disagrees with the above findings. This investigation found that there was upregulation of miR-17 expression in severe preeclamptic placenta samples.

A number of studies have found that miR-20a is upregulated in preeclamptic placenta samples [33,61,62] and that this miRNA directly targets the forkhead box protein (FOX)A1 [76]. FOXA1, a member of the FOX family of transcription factors, plays key roles in cell proliferation and migration during organ development [77]. One study using an *in vitro* model found that overexpression of miR-20a significantly inhibited the proliferation, migration, and invasion of a trophoblast-like cell line and that this occurred by direct regulation of FOXA1 (Fig. 1) [76].

One of the other miRNAs found to be involved in trophoblast proliferation, proliferation, and invasion is miR-29b. Studies have confirmed that expression of miR-29b is upregulated in placenta samples from severe preeclampsia patients [45,73] and in MSCs from the decidual tissue [37] from severe preeclampsia patients.

Specifically, Li et al. [45] found that increased expression of miR-29b is able to inhibit the invasion of trophoblasts and decreased network formation and the number of capillary tubes. They also demonstrated that miR-29b overexpression reduced trophoblast invasiveness [45]. A number of different mechanisms have been suggested as being involved in this effect of miR-29. Trophoblast invasiveness is known to depend on the production of matrix metalloproteinase-2 (MMP2), and during invasion, trophoblast cells have also been shown to express various integrins, including integrin beta 1 (ITGB1). One study found that miR-29b targets MMP2 and ITGB1 directly [45]. Interestingly, this finding is supported by the fact that the messenger RNA levels of MMP2 and ITGB1 are lower in preeclamptic placenta samples [45]. In addition, miR-29b has also been found to induce the dysregulation of signaling in trophoblast cells via the involvement of extracellular signal-regulated kinase (ERK) and focal adhesion kinase (FAK) [45]. ERK/FAK signaling is one of the most important signaling pathways controlling the expression of the growth factors that are involved in migration and the invasion of trophoblasts. Taking these findings together, they indicate that overexpression of miR-29b in preeclampsia would seem to cause dysregulation of ERK/FAK signaling pathways and this is able to suppress MMP2 and ITGβ1 production (Fig. 2). However, how miR-29b represses ERK/FAK signaling remains unknown.

Although an early study found that miR-195 is upregulated in severe preeclamptic placenta samples [73], a recent study has found that the expression of miR-195 is downregulated in both plasma [43] and placenta [56] samples from severe preeclampsia patients. A study conducted by Bai et al [56] found that downregulation of miR-195 is correlated with upregulation of activin A receptor type IIA (ActRIIA) in severe preeclamptic placenta samples compared to normal placenta samples. ActRIIA (also known as Acvr2a) is a receptor for activin A and Nodal. Both of these molecules (activin A and Nodal) play central roles in activin/Nodal signaling, a signaling system that controls the growth and differentiation of trophoblasts. miR-195 has a trophoblast invasion-promoting effect that occurs via the suppression of ActRIIA expression, which is a molecule that is important for Nodal signaling. It has also been shown that Nodal inhibits trophoblast cell invasion, whereas activin A promotes trophoblast cell invasion [56]. A previous study also revealed that an upregulation of Nodal expression occurs in preeclamptic placenta samples [78] and that it is this promotion of Nodal signaling that induced trophoblast apoptosis [79]. Therefore, it is clear that the increase in ActRIIA receptor expression caused by lowered levels of miR-195 brings about an augmentation of Nodal signaling, which in turn inhibits trophoblast invasion (Fig. 2).

Related to Nodal signaling and trophoblast regulation, another study found that miR-378a-5p is downregulated in preeclampsia and that the reduced miR-378a-5p expression leads to reduced trophoblast migration and invasion [60]. This effect is achieved via the targeting of the Nodal protein. Downregulation of miR-378a-5p increases Nodal expression, which in turn inhibits trophoblast migration and invasion. Again, Nodal is a member of the TGF-β superfamily and through activin receptor-like kinase 7 (ALK7), which is a receptor for Nodal, inhibits trophoblast cell proliferation, migration, and invasion, leading to trophoblast apoptosis [78].

In addition to the above, another miRNA, miR-376c, has also been found to be involved in trophoblast proliferation, migration, and invasion via an induction of Nodal signaling. Fu et al [50] found that miR-376c is downregulated in preeclamptic placenta samples and that this targets ALK7 and ALK5, which are molecules important to the Nodal signaling pathway. The study also confirmed that Nodal and ALK7 levels are upregulated in preeclamptic placenta samples [50]. Therefore, downregulation of miR-376c in the preeclamptic placenta results in higher ALK7 expression and this then results in the enhancement of Nodal signaling (Fig. 2).

It has also been found, in relation to Nodal signaling that downregulation of miR-675 occurs in preeclamptic placenta samples compared to normal placenta samples and that miR-675 directly targets expression of the nodal modulator 1 (NOMO1) protein [55]. This study also found that the level of NOMO1 is

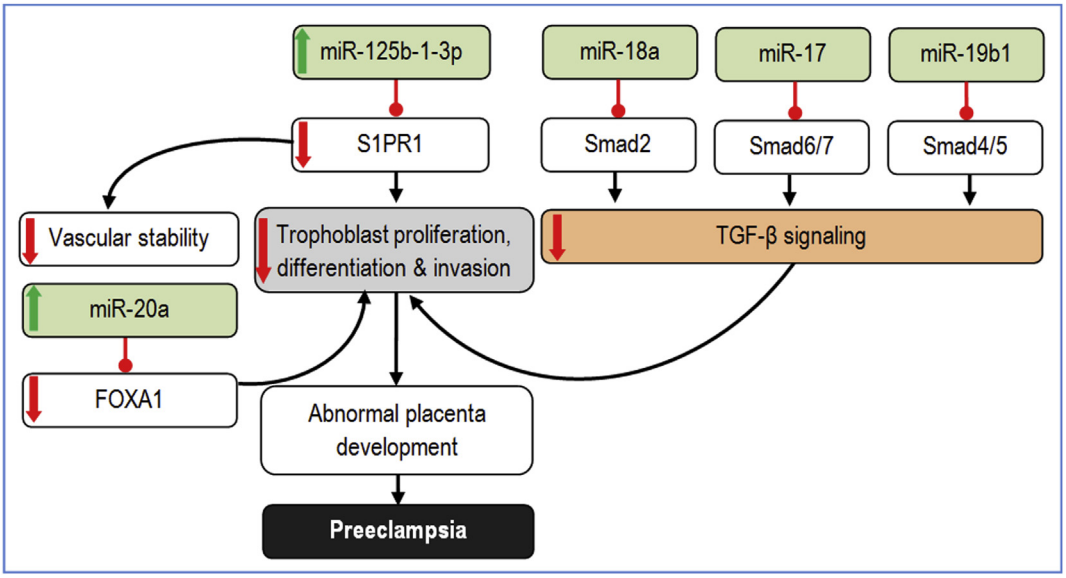

Fig. 1. A proposed scheme showing how miR-125b-1-3p, miR-20a, and the members of the miR-17-92 cluster (miR-17, miR-18a, miR-19b1) reduce trophoblast proliferation, differentiation, and invasion. Specifically, miR-125b-1-3p decreases trophoblast invasiveness by targeting S1PR1, a molecule that promotes the invasion of trophoblast cell, while miR-20a targets FOXA1, a transcription factor that has a key role in trophoblast proliferation and migration. The miR-17-92 cluster members suppress and reduce expression of various Smad proteins. Smad proteins are important mediators that are involved in the TGF-β signaling pathway, one of the most important signaling pathways associated with trophoblast growth during early gestation. The red arrows (downregulation) and green arrows (upregulation) indicate the confirmed changes in expression levels of the various miRNAs or indicated molecules during preeclampsia. FOXA1 = forkhead box protein A1; S1PR1 = sphingosine-1-phosphate receptor 1; TGFβ = transforming growth factor β. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

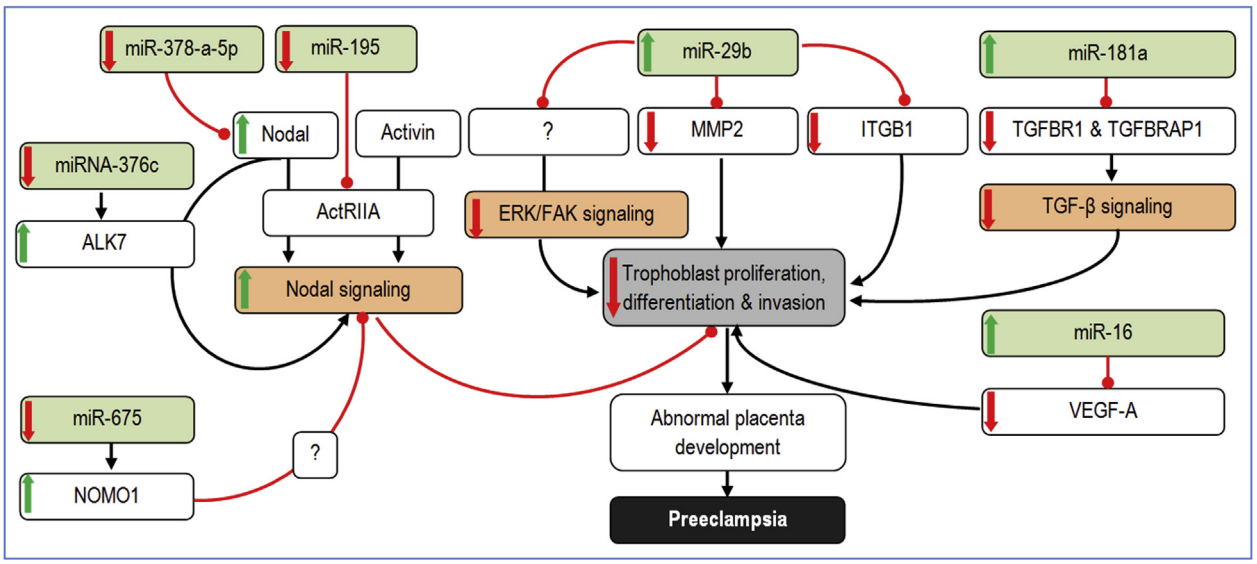

Fig. 2. A proposed mechanism model showing how miRNAs regulate trophoblast proliferation, differentiation, and invasion. Several miRNAs (miR-378-a-5p, miR-195, miRNA-376c, and miR-675) target and interact with molecules upstream of the Nodal signaling pathway. As a result, they increase Nodal signaling, a signal cascade system that inhibits proliferation, differentiation, and invasion of trophoblast cells. Specifically, miR-29b inhibits trophoblast function during early pregnancy by dysregulation of ERK/FAK signaling and inhibition of the expression of MMP2 and ITGB1 on the trophoblast cells surface. miR-181a and miR-16 dysregulate the TGF-β and VEGF signaling pathways, respectively. Both of these signaling pathways are important to trophoblast cell growth during placenta development. The red arrows (downregulation) and green arrows (upregulation) indicate the confirmed changes in the expression levels of the miRNAs or indicated molecules during preeclampsia. ActRIIA = activin A receptor type IIA; ALK7 = activin receptor-like kinase 7; ERK = extracellular signal-regulated kinase; FAK = focal adhesion kinase; ITGB1 = integrin beta 1; MMP2 = matrix metalloproteinase-2; NOMO1 = nodal modulator 1; TGFBR1 = TGF-β receptor 1; TGFBRAP1 = TGF-β receptor associated protein 1; TGFβ = transforming growth factor β, VEGF = vascular endothelial growth factor A. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

higher in preeclamptic placenta samples compared to normal placenta samples. NOMO1, a highly conserved transmembrane protein, is known to be involved in the Nodal signaling pathway. Although a previous study in zebrafish found NOMO1 to be a Nodal signaling antagonist [80], a recent study clearly demonstrated that silencing of the NOMO1 gene inhibits the expression of a variety of Nodal signaling pathway-related genes in P19 cells [33]. This result indicates that NOMO1 is important to Nodal signaling. Therefore, it would seem that the downregulation of miR-675 in preeclampsia patient samples increases the level of NOMO1, which in turn enhances Nodal signaling (Fig. 2). This then affects trophoblast proliferation, differentiation, and invasion.

Another miRNA that has an important role in trophoblast invasion is miR-181a. Studies have found that the expression of miR-181a is upregulated in plasma [63] and placenta [33,73] samples from severe preeclampsia patients as well as in MSCs derived from the umbilical cord and decidua of preeclampsia patients [58]. Liu et al [58] demonstrated that overexpression of miR-181a results in a downregulation of the mRNA and protein expression of TGF-β receptor 1 (TGFBR1) and TGF-β receptor associated protein 1 (TGFBRAP1); therefore, higher levels of miR0181a will block activation of the TGF-β signaling pathway (Fig. 2). Both TGFBR1 and TGFBRAP1 are direct targets of miR-181a. In turn, TGF-β signaling affects a wide range of cellular processes, including proliferation, migration, differentiation, and apoptosis. Therefore, upregulation of miR-181a in preeclampsia decreases TGF-β signaling and this should lead to dysregulation of trophoblast proliferation, migration, and invasion. Apart from TGF-β, another study found that vascular endothelial growth factor A (VEGF-A) was involved in trophoblast migration, in tube formation, and in network formation (angiogenesis); furthermore, it was identified that VEGF-A was one of the targets of miR-16 [53]. Studies of severe preeclampsia pregnancies have consistently found that the expression of miR-16 is upregulated in placenta samples [33,73] and in the MSCs of decidual tissue [37,53]. Wang et al. [53] confirmed that overexpression of miR-16 is able to reduce the protein levels of VEGF-A in preeclampsia samples.

Several studies have revealed that expression of miR-210 increased in preeclampsia patients and upregulation of miR-210 affects trophoblast migration and invasion [32,33,39,40,43,46,54,63,65–67,71]. It is generally accepted that expressions of various miRNAs, including miR-210, are able to be upregulated by activation of Toll-like receptor (TLR), which includes activation of variousTLR3 downstream pathways, such as via hypoxia-inducible factor 1α and via tumor necrosis factor α (TNF-α) (Fig. 3). These findings are also supported by an animal model study [81]. Taken together, these findings indicate that miR-210 is likely to contribute to preeclampsia pathogenesis via several different regulatory mechanisms.

Firstly, miR-210 targets signal transducer and activator of transcription 6 (STAT6). STAT6 plays a central role in interleukin-4 (IL-4)-mediated biological responses, including being responsible for the antiapoptotic activity of IL-4. One study found that upregulation of miR-210 decreases STAT6/IL-4 and these changes may contribute to the inflammatory state that is evident in preeclampsia patients; this state may partly contribute to the development of preeclampsia (Fig. 3) [81]. IL-4 is regulated by STAT6 and increases during a normotensive pregnancy [81]. However, in a preeclampsia pregnancy, the levels of IL-10 are significantly lower than those of a normal pregnancy [82]. In addition, one study using a preeclampsia mouse model found that the level of IL-4 failed to increase when the serum of preeclampsia mice was examined [83].

Secondly, miR-210 overexpression is able to reduce trophoblast invasion in an ERK and mitogen-activated protein kinase (MAPK) dependent manner [46]. The MAPK pathway is known to participate in trophoblast invasion during normal pregnancies and the ERK signaling pathway is important to the regulation of inflammation-associated trophoblast invasion [46]. Therefore, by

targeting a molecule upstream of these signaling pathways, miR-210 is able to inhibit trophoblast cell invasion during preeclampsia (Fig. 3). The exact molecule remains unknown; however, Anton et al [46] suggest that miR-210 may function upstream of the MAPK signaling pathway.

Thirdly, a previous study has indicated the involvement of miR-210 in the shallow trophoblast invasion of the preeclamptic placenta and that this occurs via targeting potassium (K^+) channel modulatory factor 1 gene (KCMF1) (Fig. 3) [40]. KCMF1 enhances the proliferation, migration, and invasion of epithelial cancers [84]. A study has found that KCMF1 expression is significantly lower in the placenta of preeclampsia women compared to the normal placenta of normal women and that these levels are inversely correlated with the level of miR-210 [40]. Luo et al. [40] speculated that the miR-210–KCMF1 pathway is involved in immune maladaptation at the fetal–maternal interface during early gestation.

Fourthly, studies have revealed that miR-210 targets Ephrin-A3 (EFNA3) (Fig. 3) [67,85]. EFNA3 is a member of the ephrin ligand family and functions during the development of diverse organ systems being involved in cell migration. EFNA1 expression is limited to the invasive trophoblast lineage during pregnancy and therefore this receptor ligand system would seem to be responsible for interstitial invasion and endovascular invasion during placentation [67].

Lastly, miR-210 also targets genes that are crucial to the cell cycle, such as E2F transcription factor, fibroblast growth factor receptor-like protein, and homeobox A1 protein and such targeting results in an inhibition of cell growth [40]. Therefore, it is clear that miR-210, by targeting several essential biology molecules, is able to repress trophoblast proliferation, migration, and invasion. As a consequence, these effects will lead to abnormal placenta development, which is the major causal factor of preeclampsia.

Another miRNA that is consistently overexpressed in preeclampsia is miR-155 [32,42,69,72] and studies have indicated that miR-155 is important to the regulation of trophoblast invasion [72,86]. miR-155, a common target of a large range of inflammatory mediators, is able to be upregulated by a range of inflammatory factors including interferon β, lipopolysaccharide, TNF-α, and IL-1β; this occurs via the TLR [87].

The role of miR-155 in trophoblast proliferation, differentiation, and invasion involves targeting of at least three main molecules (Fig. 4). One study found that miR-155 is able to directly target cyclin D1 and that overexpression of miR-155 reduces the level of cyclin D1 protein [86]. Cyclin D1, by facilitating the activation of E2F-responsive genes, is important during cell growth, which includes the promotion of cell migration and the decreasing of cell adhesion. Cyclin D1 is expressed in cytotrophoblast and extravillous trophoblast cells in the placenta during normal pregnancy. One study has found that the level of cyclin D1 is reduced in preeclamptic placentas [88]. These findings indicate that cyclin D1 has important roles to play in the regulation of trophoblast proliferation and migration. In addition, cyclin D1 also interacts with p27, a cyclin-dependent kinase inhibitor that plays an important role in the regulation G1/S progression. Specifically, p27 is a cell cycle inhibitor protein. Interestingly, overexpression of miR-155, a condition that is found in preeclampsia, causes a decrease in cyclin D1 expression and an increase of p27 expression; this subsequently

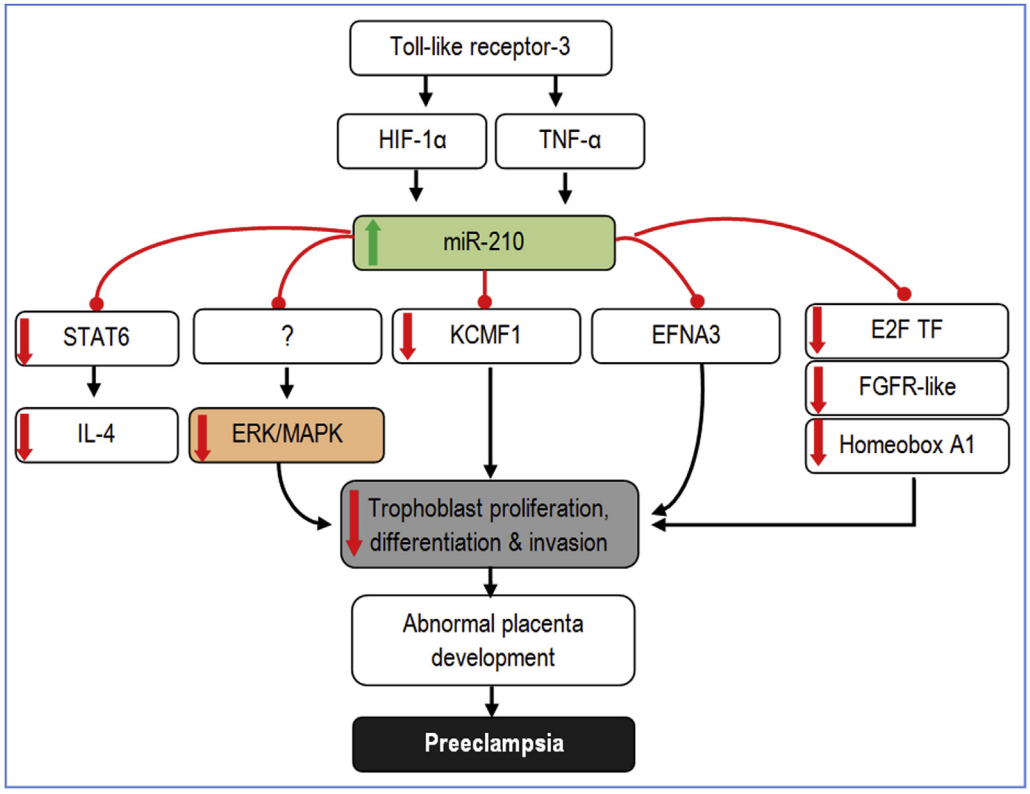

Fig. 3. A proposed mechanism model of miR-210 regulation of trophoblast growth. Specifically, miR-210 inhibits trophoblast proliferation, differentiation, and invasion by inhibiting various molecule targets including STAT6, KCMF1, EFNA3, E2F, FGFR-like protein, and homeobox A1 protein. In addition, miR-210 also dysregulates the ERK and MAPK signaling pathways. The red arrows (downregulation) and the green arrows (upregulation) indicate the confirmed changes in expression level of the miRNAs or indicated molecules during preeclampsia. EFNA3 = ephrin-A3; ERK = extracellular signal-regulated kinase; FGFR = fibroblast growth factor receptor; HIF-1α = hypoxia-inducible factor 1α; IL-4 = interleukin 4; KCMF1 = potassium channel modulatory factor 1; MAPK = mitogen-activated protein kinase; STAT6 = signal transducer and activator of transcription 6; TNF-α = tumor necrosis factor α. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

leads to cell cycle arrest at G1 and an inhibition of trophoblast proliferation (Fig. 4).

Furthermore, another study found that the role of miR-155 in trophoblast proliferation, invasion, and migration is achieved via the targeting of the cysteine-rich angiogenic inducer 61 (CYR61) gene and the VEGF gene [72]. CYR61 is strongly expressed in the placenta during normal pregnancy and is involved in angiogenesis as well as the migration properties of trophoblast cells during placentation. VEGF is pro-angiogenesis factor that is essential for trophoblast invasion. In addition, VEGF is also essential for endothelial cells stabilization, angiogenesis, and the control of vascular pressure [89]. A study has confirmed that overexpression of miR-155 brings about a downregulation of CYR61 and VEGF and that this interferes with trophoblast migration [72].

4.2. The roles of miRNAs on trophoblast apoptosis

In addition to the inhibition of trophoblast proliferation, differentiation, and migration, miRNAs are also able to interfere with placenta development by inducing trophoblast apoptosis. For example, overexpression of miR-29b promotes trophoblast apoptosis by targeting myeloid cell leukemia 1 (MCL1) [45], whereas downregulation of miR-378a-5p [60] and miR-376c [50] promotes trophoblast apoptosis via regulation of the Nodal signaling pathway.

Studies have found higher levels of expression of miR-29b in decidual MSCs [37] and placenta samples [57,73] obtained from women with preeclampsia, when these are compared to similar samples obtained from women undergoing a normal pregnancy. Furthermore, overexpression of miR-29b has been shown to promote the apoptosis of trophoblast cells and this occurs via the downregulation of MCL1 expression, an antiapoptotic member of the Bcl-2 family (Fig. 5). Another study confirmed that the level of expression of MCL1 mRNA is lower in preeclamptic placenta compared with control placenta [45].

A study by Luo et al [60] found that miR-378a-5p is downregulated in placenta from women undergoing a preeclampsia pregnancy and this reduction in miR-378a-5p expression leads to excessive apoptosis of trophoblast cells, thereby contributing to preeclampsia. Another study found that miR-378a-5p is able to target Nodal, and the effect of miR-378a-5p on trophoblast apoptosis is partially mediated by an increase in Nodal signaling (Fig. 5) [78].

Finally, Fu et al. [50] found an miRNA that seemed to be involved in trophoblast apoptosis; this was miR-376c and this miRNA is downregulated in both the placenta and plasma from preeclampsia patients. Furthermore, there is a positive association with

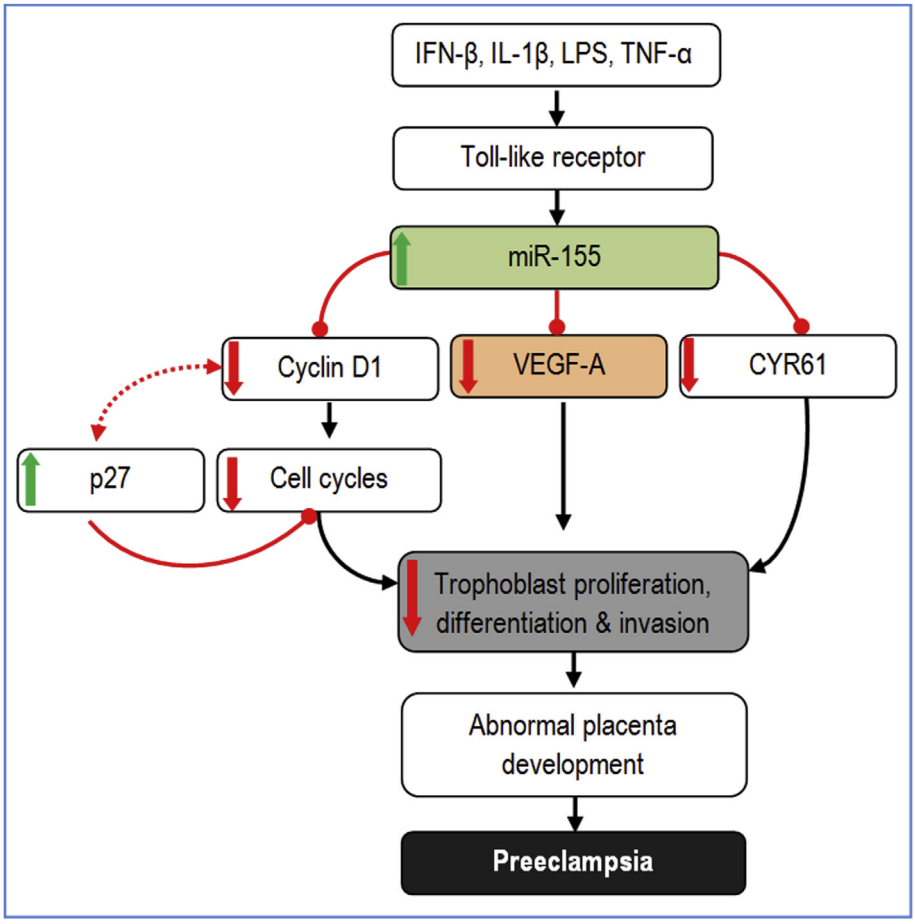

Fig. 4. A proposed mechanism model of miR-155 regulation of trophoblast proliferation, differentiation and invasion. Specifically, miR-210 inhibits trophoblast proliferation, invasion, and migration mainly by suppressing the expression of cyclin D1, VEGF-A, and CYR61. Cyclin D1 is important to the trophoblast cell cycle, while CYR61 and VEGF are involved in the angiogenesis and migration properties of trophoblast cells during placentation when there is a normal pregnancy. The red arrows (downregulation) and the green arrows (upregulation) indicate the confirmed changes in expression level of the miRNAs or indicated molecules during preeclampsia. CYR61 = cysteine-rich angiogenic inducer 61; IL-1β = interleukin 1β; LPS = lipopolysaccharide; p27 = atypical tumor suppressor that regulates G0 to S phase transitions; TNF-α = tumor necrosis factor α; VEGF-A = vascular endothelial growth factor A. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

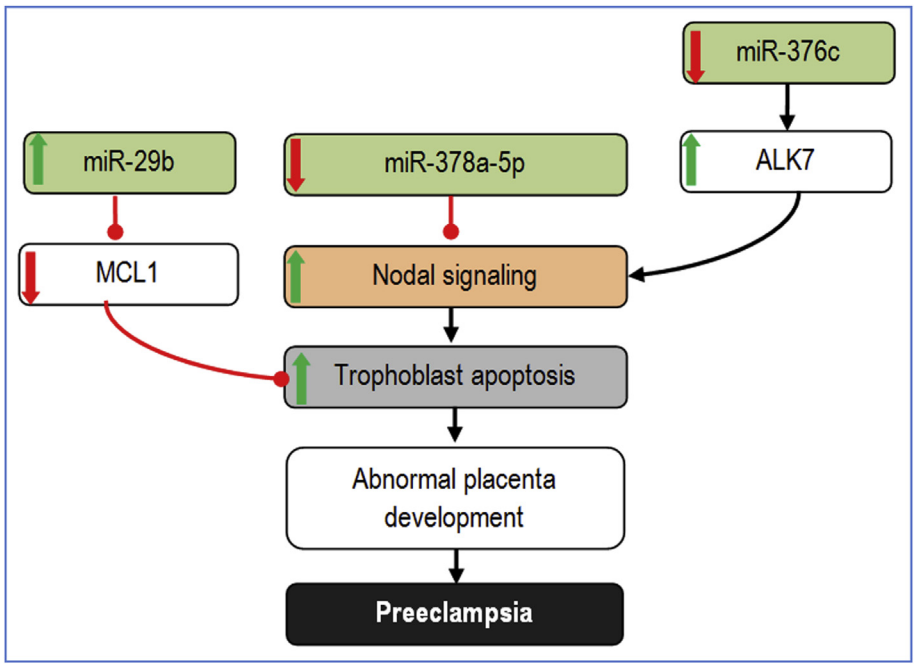

Fig. 5. A proposed mechanism model involving miR-29b, miR-378a-5p, and miR-376c showing how they affect the induction of trophoblast apoptosis. miR-29b targets antiapoptotic MCL1, which leads to increased trophoblast apoptosis directly. Furthermore, miR-378a-5p and miR-376c increase trophoblast apoptosis by upregulating the Nodal signaling pathway, an important signaling for trophoblast apoptosis. The red arrows (downregulation) and the green arrows (upregulation) indicate the confirmed changes in expression level of the miRNAs or indicated molecules during preeclampsia. ALK7 = activin receptor-like kinase 7 (a receptor for Nodal); MCL1 = myeloid cell leukemia 1. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

trophoblast apoptosis level. Specifically, miR-376c targets the ALK7 gene, which is important to Nodal signaling (Fig. 5). Downregulation of miR-376c in the preeclamptic placenta results in higher levels of ALK7 and this enhances Nodal signaling. The elevation in Nodal signaling in the preeclamptic placenta then induces excessive apoptosis [50,79].

5. Conclusion

Dysregulation of trophoblast proliferation, differentiation, and invasion during early pregnancy is one of the major ways that pathogenesis occurs during preeclampsia. Several miRNAs have been confirmed to be involved in this process. Overexpression of miR-125b-1-3p, miR-20a, miR-29b, miR-181a, miR-16, miR-210, and miR-155 together with downregulation of miR-17, miR-19b1, miR-195, miR-378a-5p, miR-376c, and miR-675 have been confirmed in plasma and/or placenta from preeclampsia pregnancy patients. Both of these changes repress the proliferation, differentiation, and invasion of trophoblast cells. In addition, the upregulation of miR-29b and the downregulation of miR-378a-5p and miR-376c have been shown to increase trophoblast cell apoptosis. To achieve this, these miRNAs dysregulate TGF-β and VEGF, as well as the Nodal, MAPK, ERK, and FAK signaling pathways and these changes interfere with the expression of various integrin molecules, a number of invasiveness enzymes such as MMP2, various transcription factors (E2F, FOXA1), a number of cell cycle regulators (cyclin D1, p27), various angiogenic inducers (CYR61), and a range of antiapoptotic proteins (MCL1).

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