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Epigenetics and microRNAs in Preeclampsia

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Abstract

Strong evidence suggests a potential link among epigenetics, microRNAs (miRNAs), and pregnancy complications. Much research still needs to be carried out to determine whether epigenetic factors are predictive in the pathogenesis of preeclampsia (PE), a life-threatening disease during pregnancy. Recently, the importance of maternal epigenetic features, including DNA methylation, histone modifications, epigenetically regulated miRNA, and the effect of imprinted or non-imprinted genes on trophoblast growth, invasion, as well as fetal development and hypertension in pregnancy, has been demonstrated in a series of articles. This article discusses the current evidence of this complicated network of miRNA and epigenetic factors as potential mechanisms that may underlie the theories of disease for PE. Translating these basic epigenetic findings to clinical practice could potentially serve as prognostic biomarkers for diagnosis in its early stages and could help in the development of prophylactic strategies.

Keywords

epigenetics; preeclampsia; pregnancy; microRNA; DNA methylation; imprinted genes

INTRODUCTION

Preeclampsia (PE) is a leading cause of maternal and fetal mortality and morbidity in both well-resourced and under-resourced settings (1,2). Preeclampsia usually develops after the 20th week (late 2nd or 3rd trimester) of pregnancy and in some cases during the postpartum period (3,4). It is a rapidly progressive condition characterized by high blood pressure and the presence of protein in the urine (5). It affects at least 5–8% of all pregnancies worldwide (15–18% in developing countries), and approximately 75 000 mothers and 500 000 babies die every year because of the complications of PE (<http://www.preeclampsia.org/pdf/ar2006.pdf>). In addition to new-onset hypertension and proteinuria, heartburn, headache, seizures, hemolysis, elevated liver function tests, low platelets, and epigastric pain are not uncommon in PE (6), and several theories (e.g., immunological, placental ischemia, and genetic) have been described to explain its pathogenesis, but thus far no unifying characteristic accounts for the vast numbers of risk factors associated with this disease (7). Although the underlying causes of PE are many, it is

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generally agreed that one of the origins of PE is the placenta itself, given that the only cure for PE is the delivery of the placenta, after which the symptoms regress rapidly (8,9).

The placenta is of ultimate importance for intrauterine fetal development and growth. Deregulation of placentation can lead to adverse outcomes for the mother and fetus, including gestational trophoblastic disease, fetal growth retardation, and definitely PE (8,10–13). Wang et al. (14) suggested that early gamete alterations (of epigenetic origin) may be adequate to induce placental defects. For example, the clinical complications of PE may originate from defective trophoblast invasion and aberrant placental formation resulting in placental insufficiency, due to inadequate remodeling of the maternal vasculature in early pregnancy (15,16). Two concepts are mostly accepted: hypertension results either from defects in fetal or placental tissue itself or from a maladapted maternal response to pregnancy. Most genetic studies of PE have focused on maternal susceptibility and have tried to identify genetic aberrations in the mother/fetus (17–19). A recent genetic analysis study (20) suggests that dysregulation of complementary regulatory proteins [membrane cofactor protein, complement factor I, and complement factor H (CFH)] is involved in the development of PE. Among these proteins, CFH has been shown to be regulated by microRNA (miRNA) 146 in the brain (21,22). Further studies are needed to confirm any epigenetic link to this finding.

Evidence from emerging research has shown that environmental factors can cause epigenetic marks in DNA and proteins that may be associated with increased susceptibility to several diseases (23,24). Epigenetics is the study of heritable changes in gene function that occur without a change in the DNA sequence. These modifications generally turn genes on or off, allowing or preventing the gene from being used to make a protein. On the other hand, miRNAs undergo the same epigenetic regulatory laws like any other protein-coding gene. Moreover, a specific group of miRNAs (defined as epi-miRNAs) can directly target effectors of the epigenetic machinery [such as DNA methyltransferases, histone deacetylases (HDACs), and polycomb repressive complex genes] and indirectly affect gene expression controlled by epigenetic factors. The result of this epigenetic–miRNA interaction is a new layer of complexity in gene regulation, whose comprehension opens new avenues to understanding human disease and perhaps achieving new treatments. How these epigenetic signatures contribute to maternal disease risk and whether their pattern can be used to predict susceptibility to serious disorders of pregnancy is still unknown. The mother's epigenome has a unique history that may be useful to interrogate in order to understand her susceptibility to PE and detect the disease at an early stage.

CURRENT EPIGENETIC LANDSCAPE IN PE

In recent years, there has been a rapid expansion of new research in the field of epigenetics and fetal and maternal biology (25–27). Major areas of epigenetics include DNA methylation, histone modifications, and genomic imprinting (28,29). Given a fixed genotype, epigenetics can confer a degree of phenotypic plasticity due to stress, nutrition, or any other environmental change, allowing the fetus to respond to the environment and change its gene expression accordingly. Moreover, epigenetic alterations, if they occur in the gametes, may be heritable and can have phenotypic consequences in the next generation (30). An emerging concept within this field suggests the possibility that epigenetic dysregulation prior to conception (31) or during pregnancy might increase PE susceptibility. Regarding pregnancy, there is evidence showing a role for epigenetics not only in placental morphology but also in placental development and functioning (32). Proper epigenetic regulation of imprinted genes as well as non-imprinted genes is crucial in the developing placenta. Environmental factors (e.g., ethanol, oxygen tension, and assisted reproduction technologies) can disturb placental epigenetics and therewith placental physiology, with

possible consequences for maternal morbidity, fetal development, and complications (33,34).

Although the precise mechanism is still unknown, epigenetic features within the placenta have been implicated in the pathogenesis of PE (32,34). Mammalian placentas share a basic common function in modulating exchange at the fetomaternal edge, but the human placenta is unique in structure and function (e.g., primary interstitial implantation in a simplex uterus, the absence of yolk sac placentation, and the occurrence of an allantoic stalk rather than an allantoic sac) compared with placenta from other species (35). An examination of human placentation is therefore critical to understand its unique function and potential role in PE. On the other hand, animal or cell studies aid the discovery of new mechanisms, and drug treatment can help advance the understanding of the disease. For example, the general disruption of DNA methylation using drug treatment has been shown to disrupt placental development and also inhibit invasiveness in trophoblast cell models or in rat placenta (36,37).

Interestingly, investigations have shown the presence of fetal cells in the maternal circulation (38). From there, several studies investigated a possible correlation between the circulating fetal DNA concentrations and pregnancy-associated complications such as PE, fetal trisomies, and preterm labor (39–44). From an epigenetic point of view, biomarkers from the inherent placental dysfunction or the by-products of placental trophoblastic apoptosis may lead to improvements in the prediction of high-risk pregnancies. Therefore, epigenetically modified circulating cell-free nucleic acids in plasma and serum could be novel biomarkers with promising noninvasive clinical applications in the diagnosis of pregnancy complications.

EPIGENETIC CHANGES IN NON-IMPRINTED GENES

Chelbi and Vaiman (45) suggest that an abnormal methylation pattern may be an important mechanism underlying PE. In PE, cytotrophoblasts fail to invade deeply spiraled uterine arteries (46–48). This shallow invasion leads to ischemic lesions and hypoxia (49,50). The Chelbi group has confirmed that *SER-PING1*, *SERPINE1*, and *SERPINE2*, members of the serine protease inhibitor (*SERPIN*) gene family, are induced by hypoxia and often modified in terms of expression in placentas from PE pregnancies. Chelbi et al. (51) showed that the promoter of the serine protease inhibitor, *SERPINA3*, is hypomethylated in PE placentas compared with normal placentas. In general, the serine protease family regulates several molecular pathways such as inflammation, coagulation, and complement activation, and these cascades are affected in placental disease (52). In addition, hypomethylated cytosine guanine islands (CpGs) were situated at putative binding sites for developmental and stress response (hypoxia and inflammation) factors. This hypomethylated pattern might contribute to reduce the chromatin accessible to transcription factors. Several studies reported that the absence of CpG methylation at the hypoxia-inducible factor (HIF)-1 α binding sites is required to allow gene transcriptional induction (53,54). Thus, demethylation may facilitate interaction of this transcription factor with the *SER-PINA3* promoter in the hypoxic environment generated in PE. This result was confirmed by Yuen et al. (55) in a large genome-wide study. They showed that 34 loci were hypomethylated in the early-onset PE placentas, whereas only 5 differentially methylated loci were found in the intrauterine growth restricted (IUGR) placentas. No loci with altered methylation were found in the placentas of late-onset PE. The DNA methylation differences of CpGs in *CAPG*, *GLI2*, *KRT13*, and tissue inhibitor of metalloproteinases 3 (*TIMP3*) have been confirmed in an independent study. Among these four genes, *TIMP3* showed the greatest degree of hypomethylation in early-onset PE compared with control placentas, and in addition, its role in PE is much clearer than the other genes. *TIMP3* regulates a wide range of physiological

processes such as cell growth, invasion, migration transformation, and apoptosis and is suggested to be important for implantation and decidualization by regulating trophoblast invasion (56). Interestingly, *TIMP3* is highly expressed in the PE placenta (57). Generally, methylation at CpG islands in a gene promoter favors transcriptional repression (58). Hence, increased *TIMP3* expression may reduce the invasiveness of the trophoblast during placental development, which then leads to placental hypoperfusion in early-onset PE. This has also been shown by Godbole et al. (59), who concluded that when the trophoblast gets more invasive, there is reduced *TIMP3* expression. *TIMP3* could inhibit angiogenesis by blocking the vascular endothelial growth factor (VEGF) from binding its receptor (60), a well-known defect found in the trophoblast of PE pregnancies. In PE, the placenta increases the release of two main anti-angiogenic factors sFlt1 and sEng, which neutralize the pro-angiogenic proteins placental growth factor (PlGF), VEGF, and transforming growth factor- β (61,62). Blockade of both PlGF and VEGF is required to produce PE-like changes (endothelial dysfunction, and so on) in pregnant rats (62), and clinical studies have also confirmed the link between high concentrations of sFlt1 and sEng and PE patients (63–65). Although there is no evidence of epigenetic regulation of sFlt1 and sEng, scientists have recently shown the epigenetic influence on VEGF (66,67).

Furthermore, elevated concentrations of RASSF1A, which can damage the placenta and its vascular endothelium, and the *SERPINB5* gene with placenta-specific methylation patterns, have been demonstrated in PE maternal plasma (68–70). These genes may serve as potential predictors of PE in the future. By focusing on placenta-specific methylation markers, early prediction of PE could be achieved by measuring placental DNA concentrations in maternal plasma, as indicated by several studies (68,69,71,72).

Recent studies have shown elevated plasma homocysteine concentrations in PE (73–75). Kulkarni et al. (74) have also shown increased homocysteine and global DNA methylation levels in the PE group (term and preterm PE) when compared with the normotensive group. This study also showed a positive association between global DNA methylation and systolic/diastolic blood pressure in the term PE group. Interestingly, another study indicates that supplementation of folic acid early in the second trimester is associated with decreased plasma homocysteine and reduced risk of PE, implying that genomic methylation may be required in this process (76). Yet another group showed an opposite methylation alteration in the cullin-7 (*CUL7*) promoter region in PE (hypermethylation) and hypomethylation in idiopathic IUGR, compared with controls (77). *CUL7* might play an important role in PE, given that targeted disruption of the *Cul7* gene in mice results in abnormal vascular morphogenesis with defects in the differentiation of the trophoblast lineage and an abnormal vascular structure, IUGR, and lethality immediately after birth (78). These studies provide novel insights into the role of epigenetic regulation of placental genes at the onset of PE. Therefore, a search for abnormal methylation (hypo/hyper) patterns would be a reasonable approach to finding new markers involved in PE, with a view to prophesying and deciphering the pathogenesis of PE.

EPIGENETIC ALTERATIONS IN IMPRINTED GENES

Genomic imprinting is a gamete-specific epigenetic modification. In somatic cells, imprinting causes differential expression of the two parental alleles. In addition to histone acetylation and methylation, DNA methylation is a key molecular mechanism of imprinting (79–81). The differences in the methylation of parental alleles are initiated in the embryonic germ cell. These are heritable after fertilization, maintained during development and adulthood, and normally play important roles in growth regulation during embryonic and postnatal development. It is believed that genomic imprinting may play a critical role in placental biology, as alterations to these imprints have been linked to severe placental

pathologies (34,82,83). Marie van Dijk and colleagues report evidence for imprinting in PE and the role of the gene *STOX1* in the molecular pathogenesis of this disease. The authors identified five different missense mutations in *STOX1* that cosegregate with the maternal PE phenotype in addition to a differentially methylated region in intron 1 of the *STOX1* gene. The mother develops hypertension only when the fetus inherits the maternal loss-of-function mutation (84,85).

Cyclin/cyclin-dependent kinase (CDK) complexes are imprinted genes in both humans and mice (86,87). Loss of imprinting of the *Cdkn1c* gene in a mouse model showed the characteristics of PE, including hypertension and proteinuria (88). Another imprinted gene, *H19*, is highly expressed in the human embryonic tissue, regulating the growth and development of the embryo and the differentiation of placental cytotrophoblasts as well as the process of embryonic development in animals and humans (89–91). Yu et al. (92) have suggested that the loss of the *H19* gene imprinting in the placental tissues of PE patients may be associated with severe hypertension, contributing to the pathogenic process of PE. It has also been shown that downregulation of *H19* expression is associated with the development of choriocarcinoma and its highly invasive capacity (93). Gao et al. (94) have shown that global DNA methylation and DNA methylation of the *H19* promoter region were significantly higher in the early-onset PE placentas than in the normal controls. On the other hand, Bourque et al. (95) reported hypomethylation at the *H19/IGF2* imprinting control region in IUGR-associated placentas, but did not find altered methylation of *CDKN1C* or other imprinted genes in IUGR or PE. The discrepancy may be due to the different ways of grouping samples. Hence, we need meticulously designed large-scale investigations in this area.

HISTONE MODIFICATIONS IN PE

Another epigenetic regulatory mechanism involves the acetylation, methylation, phosphorylation, and ubiquitination of histones, leading to regulation of gene expression. These modifications are mainly focused on trophoblast biology and differentiation, as well as on placental immunology. Kimura et al. (96) investigated H3 and H4 acetylation and H3K4 methylation within and flanking the human growth hormone multi-gene cluster in human placenta and suggested that these modifications play a key role in placental gene activation. Morris et al. (97) assessed the kinetics of transcription factor assembly and histone modifications that occur during gamma interferon (IFN- γ) induction of the *CIITA* gene (master regulator of major histocompatibility complex class II) expression in the human placental cell line. Chuang et al. (98) suggest that trophoblastic fusion in the placental morphogenesis depends on the regulation of GCMa activity by histone acetyltransferase and HDAC. GCMa plays a role in regulating syncytin (a placental protein that mediates trophoblastic fusion). In addition, H3 arginine methylation predisposes blastomeres to contribute to the pluripotent cells of the inner cell mass, which appears to require higher global levels of H3 arginine methylation (99). Although histone modification has been investigated in the placenta, more research is needed to explore histone modifications in the PE patient.

INVOLVEMENT OF miRNA IN PE

In addition to DNA methylation and histone modifications researched in placentas of mothers with and without PE, the expression patterns of miRNAs in placentas with distinct pathologies have recently been published. Several miRNAs are expressed abundantly in the human placenta (100). Recent evidence has shown that miRNAs could also play important roles in controlling DNA methylation and histone modifications (101). On the other hand, an exciting new discovery by Saito et al. (102) has shown that DNA methylation and histone

modifications can affect the miRNA expression. The miRNAs are short (22 nucleotides long) and are found in almost all eukaryotic cells. These are post-transcriptional regulators that bind to complementary sequences on target messenger RNA transcripts, usually resulting in translational repression and gene silencing. An alteration in miRNA expression in PE suggests the downregulation/upregulation of potential target genes, which may contribute to the pathology of PE.

Recent research has shown differential expression of various miRNAs in different pathological placentas (103–107), which may provide useful clues for the pathogenesis of PE. The differential expression of specific placental miRNAs (e.g., miR-210) was found to be upregulated in PE placentas compared with normal placentas (104,105). However, Zhu et al. (108) have shown overexpression of miR-210 in severe PE pregnancies but underexpression of miR-210 in mild PE (mPE) placentas. Interestingly, miR-210 targets HIF-1 α (a potential marker of hypoxia) (109), which might lead to hypoxic conditions in PE. In some studies, the HIF protein levels are significantly elevated in the PE placenta (110,111). Furthermore, the restriction of placental perfusion in several animal species has resulted in a PE-like illness (112), and experimental hypoxia is also correlated with PE features (113). These data lend credence to the fact that placental hypoxia may contribute to the pathogenesis of PE and lead to the syndrome. Zhang et al. (114) have also shown that hypoxia-induced NF- κ B p50 could regulate the miR-210 expression. Zhu's group argued that the increase in miR-210 may indicate the high degree of hypoxia in severe PE placentas, whereas the decrease of miR-210 in mPE placentas may be a compensatory mechanism in the pregnancies with mPE. On the other hand, Mayor-Lynn et al. (106) showed no miR-210 alterations between PE and control placentas (elective cesarean section without labor). Nevertheless, miR-210 was significantly downregulated in preterm patients. Although these studies indicate the potential involvement of this specific miRNA in PE, further studies are needed with an adequate number of specimens to resolve the discrepancy. A recent in vitro study suggested that miR-155 may inhibit the migration of HTR-8/SVneo cells and promote syncytialization of the cells, thus potentially contributing to the development of severe PE (115). One study reported for the first time that overexpression of miR-155 contributes to PE development by targeting and downregulating the angiogenic regulating factor CYR61, leading to pathological alterations (116). Recent studies have shown important roles of miRNA in angiogenesis (117). Interestingly, miR-182 has been shown as a probable angiogenesis regulator in PE (107). In cancer biology, miR-125b has been shown to regulate PlGF (118), an important factor in PE as discussed earlier.

miR-155 also regulates angiotensin II type 1 receptor expression in umbilical vein endothelial cells from women with severe PE (119). The renin–angiotensin–aldosterone (RAS) axis is involved in the renal control of salt and water, and all the components are found in placenta and severely compromised in hypertensive pregnancies (120). The RAS system is dysregulated in PE (121); a recent finding has also shown mutation and low activity in the aldosterone gene in PE patients (122). Although there is still no evidence of epigenetical regulation in the aldosterone gene, the RAS system has been shown to be regulated by epigenetics or miRNA in different tissues or under different conditions (123,124).

The understanding of the role of miRNA in the placenta and its association with PE is still in its early phases, but it is certainly an attractive and promising field of study. Further studies are needed to unravel the functions of the differentially expressed miRNAs in PE, potentially in maternal blood in high-risk women.

CONCLUSIONS

Although there is considerable evidence of an epigenetic and miRNA background and all these possibilities are intuitively attractive, there is more research needed to prove that PE is an epigenetic disease, but nonetheless, like many multifactorial conditions, it does seem to have epigenetic components. Epigenetics and miRNA are two important subjects that warrant significant growth in their fields in the near future to verify the importance of this regulatory circuit in this complex disease. It is evident from family and epidemiological studies that PE arises from a complex interplay among several factors, but the exact inheritance pattern is still unknown. Large-scale studies, genome-wide scanning, and family-based association studies plus more mechanistic studies are necessary to solve the puzzle of this disease. With epigenetic marks being reversible, we can hope that epigenetic-targeted therapies may eventually be able to reduce the morbidity and mortality of this devastating disorder worldwide.

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