Preeclampsia and MicroRNAs

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Abstract

Preeclampsia is a critical gestational condition that threatens the life of both mother and child. One of the most serious aspects of preeclampsia hampering both clinical management and scientific understanding is that there are, as yet, no early warning signs or risk markers. The discovery of microRNAs (miRNAs), tiny post-transcriptional regulators of gene expression, offers potentially fertile ground for developing such markers. The current state of knowledge about miRNAs in preeclampsia is presented along with information regarding miRNA detection in peripheral fluids that could lead to minimally invasive risk assessment.

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Introduction

Hypertension complicates up to 10% of all pregnancies worldwide. In the United States, preeclampsia affects 5-7% of all pregnancies, approximately 300,000 pregnancies a year, yet it disproportionately represents 15% of all maternal-fetal morbidity and mortality. Preeclampsia is known to cause immediate maternal-fetal morbidities such as growth restriction, oligohydramnios, fetal death, maternal seizures, stroke, cerebrovascular hemorrhage, and maternal death.1 Mothers with a history of preeclampsia are at increased risk of future cardiac disease including myocardial infarction and stroke.2,3,4 Children born from preeclamptic pregnancies are also at increased risk of stroke,5 epilepsy,6 and metabolic, nutritional and blood disease7 in later childhood or as an adult. Clearly, preeclampsia has immediate and long term effects on both the fetus and mother. However, its pathogenesis is poorly understood.

The two-stage etiologic model has been the prevailing view of the pathogenesis of preeclampsia. The first stage is the existence of poor placentation exhibited by poor trophoblastic invasion, poor remodeling of spiral arteries, and increased placental necrosis. Despite the many mechanisms that may produce the preeclamptic phenotype,
data demonstrate that the placenta plays the central role in the development of preeclampsia. In preeclampsia, there is abnormal cytotrophoblast differentiation and apoptosis, incomplete spiral artery invasion, and decreased blood flow to and from the placenta. Trophoblast necrosis releases cell fragments into the maternal bloodstream which trigger a systemic immunologic response and placental oxidative stress. As seen in Figure 1, Redman and Sargent have recently updated this 2 stage model to include an important etiologic component: poor immunoregulation that involves a dysregulation of regulatory T cells, IDO, and dendritic cells. Multiple processes involving placental dysregulation, endothelial cell dysfunction, immunology, oxidative stress, altered vascular biology, and angiogenesis make finding a singular cause of preeclampsia nearly impossible. As preeclampsia is a disease resulting from multiple pathways, the development of a predictive model and the search for a therapeutic pathway for preeclampsia may need to come from the regulation of these multiple pathways.

MicroRNAs and Human Disease

MicroRNAs, as a group, represent a level of regulation that may address all these multiple pathways at once and differentially throughout pregnancy. MicroRNAs (miRNAs) are 21-23nt long regulatory RNAs first identified in Caenorhabditis elegans in 1993. Since their discovery, miRNAs have been found in nearly every eukaryotic species and have been shown to play critical roles in gene regulation in both normal and pathologic cellular
Preeclampsia and MicroRNAs

The majority of primary miRNA transcripts (pri-miRNAs) are initiated by RNA polymerase II. These transcripts can be from a few hundred to several thousand nucleotides in length and are produced from both inter-genic regions and introns. Pri-miRNAs contain within them one or more shorter sequences that form a characteristic thermodynamically stable hairpin structure. These hairpins in the nucleus induce the formation of an RNA-protein complex composed of the hairpin and RNase III endonuclease DROSHA along with its protein partner DGCR8. The latter protein binds the pre-miRNA hairpin and DROSHA excises it from the pri-miRNA transcript. Nuclear processing of these miRNAs involves multiple enzymes and proteins including Exportin 5, which binds the hairpin and transports it out of the nucleus. In the cytoplasm, the pre-miRNA interacts the RNase III endonuclease DICER which binds to the hairpin along with its partner protein TRBP (TAR RNA binding protein). This RNA-protein complex allows DICER to cleave the hairpin in a precise manner to produce a 21-23nt double-stranded RNA composed of the mature miRNA effector sequence and its complement. Finally, this dsRNA is processed by an Argonaute protein, AGO2, which is a primary component of the ribonucleoprotein complex called RISC (RNA induced silencing complex). The mature miRNA strand is selected in RISC and transported to the target messenger RNA (mRNA) where it binds in an antisense orientation to a target sequence in the 3’ untranslated region of the message. Once bound, the miRNA:mRNA complex will either participate in mRNA degradation or translational repression of the message. MicroRNA regulation of gene expression is often highly specific in terms of tissue and/or developmental stage or pathology.

Although the mechanisms of these regulatory processes are not fully delineated, their functional importance is evidenced by the fact that miRNAs have been found in nearly every eukaryotic species. As of Release 18 (November, 2011) of the miRNA repository miRBase, there are nearly 2,000 miRNA loci in the human genome. The vast majority of these has been discovered in just the past few years by deep sequencing of specialized tissue targets and are only expressed as a few copies in one tissue type or pathology. The one human disease in which extensive miRNA expression studies have been carried out is cancer. More than two-thirds of the current miRNA literature is composed of miRNA studies of cancers. In the field of obstetrics and gynecology, the vast majority of the miRNA literature is addressed to cancers and, in particular, ovarian cancers. However, there have been a number of miRNA expression studies of uterine cancers of which two of the most extensive have been carried out by us. With regard to other OB/GYN pathologic processes, the literature is less extensive. Among these, preeclampsia has received the most attention, but even this literature is small.

MicroRNAs and the Development of Preeclampsia

To date, there have been four surveys of miRNA expression in preeclampsia (Table 1). Taking these four surveys...
together, a total of 67 different human miRNAs have been shown to be significantly dysregulated in preeclampsia as compared with controls. Among these, only three miRNAs (miR-181a, miR-195, and miR-584) are reported in two of the four studies and only one, miR-210, is found in three of the four studies. Replication of increased miR-210 expression in preeclampsia is important as it is known to be the most hypoxia-inducible microRNA. In addition, there are contradictory data concerning expression of some miRNAs. For example, Hu et al concluded that miR-195 is upregulated in placenta from severe preeclamptic pregnancies. In contrast, Zhu et al demonstrate that miR-195 is downregulated.

### Table 1: Summary of the Literature of Differentially Expressed microRNA in Preeclampsia versus Control Placentas: qRT-PCR=quantitative reverse-transcriptase polymerase chain reaction

<table>
<thead>
<tr>
<th>Citation</th>
<th>Method</th>
<th>Upregulated</th>
<th>Downregulated</th>
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<tbody>
<tr>
<td>Pineles et al. Am J Obstetric Gynecol 2007 (31)</td>
<td>qRT-PCR of placenta</td>
<td>210, 155, 181b, 182, 200b, 154, 183</td>
<td>N/A</td>
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<tr>
<td>Hu et al. Clin Chem Lab Med 2009 (29)</td>
<td>miR microarray and qRT-PCR of severe PE placenta</td>
<td>16, 29b, 195, 26b, 181a, 335, 222</td>
<td>N/A</td>
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<tr>
<td>Zhu et al. Am J Obstet Gynecol 2009 (30)</td>
<td>miR microarray and qRT-PCR of severe PE placenta</td>
<td>181a, 584, 30a-3p, 210, 152, 517, 518b, 519e, 638, 296, 362</td>
<td>101, 10b, 218, 590, 204, 32, 126, 18a, 19a, 411, 377, 154, 625, 144, 195, 150, 1, 18b, 363, 342-3p, 450, 223, 374</td>
</tr>
<tr>
<td>Enquobahrie et al. Am J Obstet Gynecol 2010 (32)</td>
<td>miR microarray and qRT-PCR of placenta</td>
<td>210</td>
<td>328, 584, 139-5p, 500, 1247, 340-5p, 1</td>
</tr>
<tr>
<td>Mayor-Lynn et al. Repro Sci 2010 (33)</td>
<td>miR microarray and qRT-PCR of placenta</td>
<td>493</td>
<td>15b, 181a, 210, 483-5p</td>
</tr>
<tr>
<td>Zhang et al. Am J Obstet Gynecol 2010 (34)</td>
<td>qRT-PCR mechanism paper: miR-155 downregulates CYR61 gene involved in trophoblast function and angiogenic cells.</td>
<td>N/A</td>
<td>N/A</td>
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It is important to note that, though there is ample evidence that miRNAs are dysregulated in preeclampsia, these studies were all carried out using RNA from placental tissues obtained after the patients had delivered. Methodologically, this clearly limits the use of miRNAs as a predictive tool. As obtaining placental tissue biopsies during gestation is neither feasible nor ethical, predictive miRNA assays must be done on RNA from a different source. Recently, Wu et al. showed that miRNA dysregulation can be detected in plasma drawn from women at 37 to 40 weeks gestation. Their study confirmed the
results of previous studies of placental tissues by detecting significant dysregulation of four miRNAs, miR-130a, miR-144, miR-181a, and miR-574-5p compared with plasma RNA from controls. They discovered a further eleven significantly dysregulated miRNAs not previously reported in other preeclampsia studies. This demonstrates that potentially meaningful miRNA expression changes can be detected in plasma samples. Having the ability to assay miRNA expression in a peripheral fluid opens the opportunity to carry out longitudinal cohort studies of preeclampsia using a minimally invasive sampling technique.

First stimulated by an observation that very small microvesicular bodies shed by cultured human tumor cells into the media displayed molecular markers characteristic of the tumor plasma membranes themselves, Douglas Taylor and colleagues have shown that miRNAs, along with a specific range of mRNAs, that are shed by cancer cells into the blood stream are encapsulated within the structures called exosomes. Importantly, these studies showed that the principle sources of RNA-containing exosomes are placenta and solid tumors. Indeed, the most prolific exosome-producing tissue is the placenta. Several studies have shown that miRNAs in placental-derived exosomes can be reliably assayed in plasma and that they appear to participate in numerous crucial functions, including those related to immune suppression and angiogenesis. With respect to the reliability of these plasma-based miRNA assays, we recently determined expression of 377 human miRNAs in both placental tissue and plasma, albeit in a single individual. However, of the 315 miRNAs expressed in placenta, we found that 286 (91%) were also detectable in plasma. Moreover, there was a highly significant correlation in expression levels of miRNAs (r = 0.6, p < .001, df = 284).

Conclusion

Preeclampsia is a serious and life-threatening gestational condition whose etiology is still not well understood. In addition to its pathology, both the diagnosis and clinical management of preeclampsia are subjects of debate and controversy. One of the most important aspects impeding advances in understanding preeclampsia is that the majority of studies of the condition are carried out only after clinical signs such as progressive proteinuria and hypertension are clearly manifest. Thus, identification of a minimally invasive marker or markers that correlate with risk will be a major advance informing both clinical management and studies of pathogenesis. The discovery of miRNAs, gene expression regulators reliably detectable in both tissue and peripheral fluids, offers a potentially fertile field for identifying such markers.

References


