MiRNAs in the Immune Dysregulation of Preeclampsia

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The main complications causing practically 75% of all maternal deaths are severe bleeding, infections, and high blood pressure during pregnancy (preeclampsia (PE) and eclampsia). The usefulness of ncRNAs as clinical biomarkers has been explored in an extensive range of human diseases including pregnancy-related diseases such as PE. Immunological dysregulation show that the Th1/17:Th2/Treg ratio is “central and causal” to PE. However, there is evidence of the involvement of placenta-expressed miRNAs and lncRNAs in the immunological regulation of crucial processes of placenta development and function during pregnancy. Abnormal expression of these molecules is related to immune physiopathological processes that occur in PE.

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1. Introduction

For optimal cellular function, the presence of a complex network of molecular factors is imperative. These factors intricately interact, ensuring precise control over gene expression. Recent advancements in molecular biology have elucidated that gene expression regulation is not solely governed by proteins but also by non-coding RNAs (ncRNAs) [1]. Due to the remarkable progress in the realm of next-generation sequencing and bioinformatics analysis, it has become feasible to identify a multitude of novel ncRNA molecules engaged in diverse physiological processes. Among these, microRNAs (miRNAs) and long non-coding RNAs (lncRNAs) play pivotal roles in transcriptional regulation across different levels [1].

MicroRNAs are non-coding RNAs with a ~22 nucleotide length that are typically processed by RNA polymerase II/III in a post- or co-transcriptionally manner. A high proportion of identified miRNAs to date are intragenic and processed from intronic regions; nonetheless, there are a few which are transcribed from intergenic regions. In some cases, miRNA transcription results in clusters which share similar seed regions that are considered as miRNA families [2][3][4].

After processing by RNA polymerase, the first transcript consists of a hairpin structure (primary miRNA) which presents a base-paired stem structure; then, these are cleaved by a microprocessor containing Drosha (RNAase III-type) and its cofactor DGCR8, taking place in the nucleus [5]. Eventually, a hairpin structure of ~60–70 nucleotides in length, known as precursor miRNA (pre-miRNA), which contains a stem-loop structure is released

and exported from the nucleus to cytoplasm through exportin-5 (EXP 5) and Ras-related nuclear protein guanosine triphosphate (RAN-GTP) [5]. Once pre-miRNA is in the cytoplasm, its terminal loop structure is cleaved by the action of Dicer and its cofactor TRBP, a ~20–22 nucleotide miRNA duplex containing two 5′ phosphorylated sequence strands, named a miRNA guide strand and its complementary passenger strand [5]. Then, the miRNA-duplex binds to the Argonaute protein to conform RISC (RNA-induced silencing complex), and the passenger strand is degraded [5]. Finally, the miRISC complex participates in gene expression regulation through the interaction of the miRNA seed region with the target messenger RNA 3′-UTR sequence, which leads to degradation or a blocking of mRNA to inhibit its translation [6].

One of the most important functions in which miRNAs participate is gene regulation, through mediating the degradation of mRNAs [7]. Transcription and translation are also meditated by miRNAs by two main mechanisms: the canonical pathway, which has been briefly mentioned above, and exerts degradation of mRNAs by the miRNA seed sequence [6]. Nonetheless, it has been reported that approximately 60% of mRNA-miRISC interactions depend on non-canonical pathways, supporting the idea that one miRNA sequence can target a plethora of mRNAs due to no complementary union among both sequences [8]. Thus, miRNA dysregulation can affect a vast number of functions in different biological processes.

Moreover, it has been shown that circulating microRNAs have pivotal roles in basic and clinical areas, and is known that fluctuations in circulating miRNAs have been associated with pathological processes such as inflammatory diseases [9][10], cancer [11], chronic diseases [12][13], as well as PE. Several studies have focused on the identification of biomarkers for the early detection of PE; for instance, plasma exosomal miR-517-5p, miR-520a-5p, and miR-525-5p (which belong to C19MC cluster pre-eclampsia) are down-regulated during the first trimester of gestation in women affected with gestational hypertension and PE, and were reported as a biomarker with high accuracy since their expression profile can identify women at risk of later development of gestational hypertension and PE by the first trimester [14]. Recently, there have been reported a battery of microRNAs that provided possible clinical applications since they were categorized according to PE severity [15]. In severe PE patients, miR-215, miR-155, miR-650, miR-210, and miR-21 were upregulated, and miR-18a and miR-19b1 present downregulation. In mild PE patients, miR-518b and miR-29a were found upregulated, while miR-15b and miR-144 were downregulated [15].

2. MiRNAs Involved in Immune Dysregulation in Preeclampsia

Recently, it has been described that miRNAs can be wrapped in exosomes, a subtype of extracellular small vesicles with varying sizes (20–130 nm) [16], to protect them from degradation by RNases [17]. These vesicles can be secreted to the systemic circulation from the placenta, resulting in multisystemic organ damage in patients with PE [17]. For the above, it is possible to detect circulating plasma exosomes which contain miRNAs as a diagnosis technique [18]. miRNAs involved in immune dysregulation in PE are shown in **Table 1**.

The human placenta-associated miRNAs are expressed in villous trophoblasts and secreted into maternal circulation via exosomes [19]. Several exosome-derived miRNAs have been associated with PE development such as miR-31-5p, which has been proposed as a potential biomarker to evaluate preeclampsia progression [19]. Additionally, exosomal miRNAs (Exo-miRNAs) miR-483-3p, hsa-miR-1237-3p, 365b-5p, hsa-miR-155-5p, hsa-miR-200b-3p, hsa-miR-342-3p, hsa-miR-140-3p, and hsa-miR-3909 can be found in umbilical serum, offering a pattern associated with microvascular dysfunction in mothers with PE [20]. Also, miR-210 expression in serum is increased during PE pregnancy progression, being associated with the severity of this disease [21] since its up-regulation is correlated with the inhibition of migration and the invasive capability of trophoblasts, and is linked to induction of the activity of several intracellular transcription factors [22].

Interestingly, miRNA analysis employing placental tissues showed a negative correlation between miR-126 (upregulated) and VCAM-1 (downregulated), a protein expressed by placental villous trophoblasts in the PE pregnancies group, proposing that this miRNA participates in the occurrence and development of EOPE through modulation of the invasion ability of trophoblast cells [23][24]. Another miRNA associated with the pathogenesis of PE is the upregulated miR-200b-3p, which contributes to the dysregulation of cell adhesion molecules (CAMs) and tight junction via profilin 2 (PFN2) regulation in placenta tissues [25]. Also, through in vitro analysis, it has been concluded that miR-146a-5p mediates trophoblast cell proliferation and invasion by Wnt2 expression regulation since this ligand promotes migration and proliferation of trophoblast cells via triggering the Wnt/β-catenin pathway [26]. In addition, the lncRNA DANCR activates the PI3K/AKT pathway by miR-214-5p downregulation, promoting the migration and invasion of chorionic trophoblast cells in PE [27].

In an immunological context, the cytokine TNFSF15, which has been identified in blood as a possible type 1 immune response during PE [28][29], can be regulated by miR-517a/b and miR-517c in extravillous trophoblast cells (EVTs) [30]. Studies have indicated that miR-146a regulates the immune microenvironment of the placenta by TGF-β/Smad4 pathway activation, promoting inflammatory factor expression in PE patients [31]. Additionally, let-7a expression in the placenta tissue of patients with severe preeclampsia (SPE) is significantly reduced, and the mRNA and protein levels of the important inflammatory factor TNF-α are significantly increased and, hence, significantly negatively correlated [32]. Interestingly, miR-145-5p regulates TNF-α expression by its upregulation in serum and mediating trophoblast cell invasion in women with EOPE [33].

Furthermore, a negative correlation between miR-203a-3p and IL-24 has been described in extract placental mononuclear cells and serum exosomes from PE patients, indicating that miR-203a-3p plays an important anti-inflammatory role in PE pregnant women [34]. The miR-548c-5p is downregulated and protein tyrosine phosphatase receptor type O (PTPRO) is upregulated in serum exosomes and placental mononuclear cells from PE patients, establishing a negative association [34]. Also, it has been reported that miR-548c-5p inhibits inflammation by IL-12 and TNF-α downregulation and less nuclear translocation of pNF-κB in macrophages [35]. Remarkably, serum IL-10 levels are decreased in women with PE, possibly contributing to systemic inflammation and decreasing the number of circulating $CD4^{+}CD25^{+}CD127^{-}$ Tregs in women with PE. Also, placental tissues from PE patients showed a significantly decreased Foxp3 and significantly increased expression of miR-210, indicating a possibly positive co-regulation among them [36]. In vitro assays showed that under hypoxic conditions, human trophoblast cell-derived

extracellular vesicles release miR-1273d, miR-4492, and miR-4417 to target HLA-G, mediating immune- and inflammation-related pathways and, consequently, triggering the development of PE [37].

Table 1. miRNAs involved in immune dysregulation in Preeclampsia.

Molecule	Target	Function	Reference
miR-517a/b and c	TNFSF15	Type 1 immune response regulation during PE	[27]
miR-146a	SMAD4	Immune microenvironment regulation in placenta promoting inflammatory factors expression in PE patient	[28]
let-7a	TNF-α	Participate in the occurrence and development of SPE	[29]
miR-145-5p	TNF-α	Mediates trophoblast cell invasion in women with EOPE	[30]
miR-203a-3p	IL-24	Anti-inflammatory role in PE pregnant women	[31]
miR-548c-5p	PTPRO	Anti-inflammatory factor in preeclampsia	[32]
miR-210	Foxp3	Association with maternal immune tolerance of the fetus by T-cells regulation	[33]
miR-1273d, miR-4492, and miR-4417	HLA-G	Mediate immune- and inflammation-related pathways promoting the development of preeclampsia.	[34]

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