**Abstract**

microRNA (miRNA) has been focused on placental biology and pathogenesis. Chromosome 19 miRNA cluster (C19MC) miRNAs are known for placenta- and primate-specific miRNAs. C19MC miRNAs are detected in maternal plasma even as early as the first trimester. To date, there are no miRNAs to be used as biomarkers of some disease in routine practice, although a few studies suggested C19MC miRNAs as biomarkers of obstetrical diseases. C19MC miRNAs have been also reported as onco-miRs and C19MC miRNAs target multiple genes in several kinds of malignancies. Two new insights into C19MC miRNAs in obstetrics have been recently reported. First, C19MC miRNAs induced viral resistance in non-trophoblast cells by autophagy. Second, fetal oncogenesis is associated with C19MC miRNAs. To clarify the pathogenesis of an obstetrical disease, C19MC miRNAs need to be further investigated.

**INTRODUCTION**

miRNA: microRNA; RISC: RNA-Induced Silencing Complex; C19MC: Chromosome 19 miRNA cluster; C14MC: Chromosome 14 miRNA Cluster; HSD17B1: Hydroxysteroid 17-beta Dehydrogenase 1; FGR: Fetal Growth Restriction; MEK1: Mitogen-activated protein kinase 1; FGFR1: Fibroblast Growth Factor Receptor 1; ETMRs: Embryonal Tumors with Multilayered Rosettes; DNMT3B: DNA-Methyltransferase 3 Beta.

miRNA (miRNA) is a highly conserved, single-stranded, 20-23-nucleotide RNA molecule. miRNA binds to target mRNA after miRNA is endocyted by Argonaute protein to form an miRNA-complex, which is called the RNA-induced silencing complex (RISC). RISC represses or degrades the target gene expression. One important character of miRNA is: miRNA targets multiple genes, and vice versa, i.e., a gene is targeted by multiple miRNAs. Thus, miRNA, regulating the gene expression, mediates multiple biological behaviors, such as invasion [1], proliferation [2], inflammation [3], and apoptosis [4]. To date, approximately 2,500 human miRNAs have been deposited in miRBase, which constitutes a database of miRNA. New miRNAs have been rapidly reported, which are added to this database, with the database rapidly expanding.

The human placenta expresses numerous kinds of miRNA. More than 800 miRNAs are reported to express in the human placenta [5]. The expression of miRNAs in the placenta changes during pregnancy. Several miRNAs form miRNA clusters, which are localized in chromosome14q and 19q. Chromosome 19 miRNA cluster (C19MC) and the chromosome 14 miRNA cluster (C14MC) have 46 and 52 miRNAs, respectively [6]. Of those, C19MC is a primate-specific cluster, and it is predominantly expressed in the placenta [7-10]. Thus, it is reasonable to assume that C19MC miRNAs may be involved in the pathogenesis of primate-specific diseases: especially its contribution to preeclampsia has now become a matter of topic. Furthermore, C19MC miRNAs can be detected in maternal circulating blood flow from the 1st trimester [11].

miRNA, to continue to be present in the circulating blood, needs to be protected from degradation by RNAase. As described later, exosomes may contribute to it. Exosomes, approximately 50-nm-sized microvesicles, contain miRNA and miRNAs, and thus miRNAs, being present in exosome without direct contact with RNAase, remains un-degraded. Multiple exosomes are secreted from trophoblasts, which are the functional cells of the placenta. Circulating C19MC miRNAs are candidate markers of obstetrical/placental diseases because, as described, they are solely derived from the placenta. In addition, recent papers revealed that C19MC miRNAs were also associated with new roles such as fetal carcinogenesis, pluripotency, and viral resistance [12-14]. In this paper, miRNAs in the placenta are reviewed, with special reference to C19MC miRNAs.

**C19MC miRNAs**

The human placenta expresses 3 miRNA clusters: C19MC, C14MC, and miR-371-3 clusters. Of the 3 miRNA clusters, C19MC...
and miR-371-3 clusters have orthologs only with orangutan, gorilla, and chimpanzee [6]. The miR-371-3 cluster is composed of only miR-371, miR-372, and miR-373, whereas C19MC, located in human chromosome 19 (19q13.42), includes 46 miRNA transcribed from the -100-kb region [7,15]. The methylation of C19MC is regulated, namely, the (epigenetic) methylation patterns of the GC-rich promoter region in C19MC alter the expression of the region [16,17]. Experimentally, C19MC miRNAs are activated by DNA methylation inhibitors, indicating that the region is under epigenetic control [16,18,19]. It was also reported that C19MC miRNAs are onco-miR. A target gene of each C19MC miRNA has been clarified mainly in terms of oncology (Table 1).

miRNAs as biomarkers in obstetrical/placental diseases

The expressions of circulating miRNAs change in the

<table>
<thead>
<tr>
<th>miRNA</th>
<th>Cell</th>
<th>Target</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>miR-498</td>
<td>HEK293, H5V-1 infected body cavity-based lymphoma</td>
<td>KSHV replication and transcription activator</td>
<td>Yan (2013) [31]</td>
</tr>
<tr>
<td>miR-512(-3p,-5p)</td>
<td>BeWo</td>
<td>CD44</td>
<td>Rutnam (2012) [34]</td>
</tr>
<tr>
<td>miR-515(-3p,-5p)</td>
<td>Breast cancer cell line</td>
<td>CD44</td>
<td>Chen (2010) [35]</td>
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<tr>
<td>miR-516a(-3p,-5p)</td>
<td>Gastric cancer cell line, HEK-293</td>
<td>1,25-Dihydroxyvitamin D3</td>
<td>Kaslappan (2012) [32]</td>
</tr>
<tr>
<td>miR-517a(-3p)</td>
<td>Liver cancer cell line (Y8103)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>miR-517c(-3p)</td>
<td>HEK293</td>
<td></td>
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</tr>
<tr>
<td>miR-518b</td>
<td>NT2/D1, HEK-293</td>
<td></td>
<td>Kushwaha (2014) [43]</td>
</tr>
<tr>
<td>miR-518c(-3p,-5p)</td>
<td>Esophageal cancer cell line</td>
<td>rap1b</td>
<td>Zhang (2012) [44]</td>
</tr>
<tr>
<td>miR-519b(-3p,-5p)</td>
<td>Breast cancer cell line</td>
<td>NK1</td>
<td>Navarro (2012) [45]</td>
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<tr>
<td>miR-519c(-3p,-5p)</td>
<td>Gastric cancer cell line, C19MC</td>
<td>HIF1a</td>
<td>Cha (2010) [46]</td>
</tr>
<tr>
<td>miR-519d(-3p,-5p)</td>
<td>Hepatocellular carcinoma cell line</td>
<td>PTEN, AKT3, CDKN1A/p21</td>
<td>Fornari (2012) [47]</td>
</tr>
<tr>
<td>miR-520a(-3p,-5p)</td>
<td>Hepatocellular carcinoma cell line (QGY-7703)</td>
<td>MKN67</td>
<td>Hou (2011) [48]</td>
</tr>
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<td>miR-520b</td>
<td>Hepatocellular carcinoma cell line</td>
<td>MEK2, cyclinD1</td>
<td>Zhang (2012) [51]</td>
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<td>miR-520c(-3p,-5p)</td>
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<td>eIF4G</td>
<td>Maxan-Marmarz (2014) [55]</td>
</tr>
<tr>
<td>miR-520d(-3p,-5p)</td>
<td>Breast cancer cell line</td>
<td>Glypican-3</td>
<td>Miao (2013) [56]</td>
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<tr>
<td>miR-520e</td>
<td>Hepatocellular carcinoma cell line</td>
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<td>miR-520f</td>
<td>Gastric cancer cell line (MKN45)</td>
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<td>Shen (2014) [60]</td>
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<td>miR-522(-3p,-5p)</td>
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<td>miR-524(-3p,-5p)</td>
<td>Globlastoma cell line (LN229)</td>
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<tr>
<td>miR-525(-3p,-5p)</td>
<td>Vascular endothelial cell line</td>
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KSHV: Kaposi’s sarcoma-associated herpes virus; HSV: Herpes simplex virus; c-FLIP: FLICE-like inhibitory protein; SK-1: Sphingosine kinase 1; SULF1: KLK10: Kallikrein-related peptidase 10; Extracellular sulfatase 1; TNIP1: TNFAIP3 interacting protein 1; FOXN1: Forkhead box protein A1; PIK3CA: PIK3CA: Phosphoinositide 3 kinase; HBXIP: Hepatitis B X-interacting protein; MICA: MHC class I-related chain A; eIF4GII: Eukaryotic translation initiation factor 4, gamma; HDAC1: Histone deacetylase 1
maternal blood during pregnancy [11]. Several studies have reported the relationships between circulating miRNAs and obstetrical and/or placental diseases [20–23]. Apart from C19MC miRNAs, miR-210 has been one of the most well-known miRNAs, and is associated with hypoxia. A recent study involved high-throughput sequencing and quantitative PCR-based array of normal and preeclamptic placentas [24]. As a result, miR-210 was up-regulated in both analyses. Furthermore, the study revealed that miR-210 targeted hydroxysteroid17-β dehydrogenase 1 (HSD17B1). HSD17B1 was down-regulated in preeclamptic maternal serum even before the appearance of clinical manifestation of preeclampsia in a prospective cohort study. miR-210 also targets HIF-1α, which is known as a hypoxia-associated gene [25]. miR-424 has also attracted attention recently. miRNA expressions at pre-labor and delivery were analyzed [26]. From pre-labor toward delivery, miR-210, -424, -199a, and -20b increased by 4.2-fold 2.7-fold 2.6-fold and 2.3-fold, respectively. Furthermore, hypoxia-related miRNAs (miR-424, -21, -199a, and -20b), which are associated with HIF-1α, significantly increased in pregnancies complicated by severe FGR [26]. Indeed, miR-424 and miR-21 were up-regulated in cases of absent diastolic umbilical arterial flow (a sign of placental insufficiency) compared with those showing normal flow [26]. Another study revealed that miR-210 expression in placental tissue with FGR is significantly increased, whereas mitogen-activated protein kinase 1 (MEK1) and fibroblast growth factor receptor 1 (FGFR1) are inversely decreased [27]. Another author proved that MEK1 and FGFR1 are target genes of miR-424 [28]. Taken together, miR-210 and miR-424 seem to be induced in placental hypoxia.

With regard to C19MC miRNAs in maternal blood, C19MC miRNAs are also involved in obstetrical/placental diseases (Table 2). Some studies showed that C19MC miRNAs changed in obstetrical/placental diseases, such as preeclampsia and fetal growth restriction (Table 2). However, there is no evidence to support using circulating miRNAs as diagnostic markers. For instance, miR-154 in placental tissue is up-regulated in preeclampsia with small-for-gestational age [29]. On the other hand, another author showed that miR-154 is down-regulated in the preeclamptic placenta [30]. We assume that one of the key points for these discrepancies may be the different in miRNA measurement, i.e., the standardization of miRNA for miRNA. To standardize miRNAs, several internal controls such as RNU6, RNU44, S18, miR-16, and let-7d are usually employed. However, it remains unknown whether these internal controls are validated in maternal plasma.

New insights into C19MC miRNAs

Recently, two novel insights into C19MC miRNAs were published. Firstly, a study showed that C19MC miRNAs induced viral resistance in non-trophoblast cells by autophagy [13]. The authors focused on cultures supernatants of full-term trophoblasts. The efficacy of virus infection significantly decreased in non-trophoblastic cell lines using only the trophoblast-derived supernatant. The supernatant includes numerous exosome-containing C19MC miRNAs. Then, the authors hypothesized that the recipient cells gained viral resistance by taking in exosome-containing C19MC miRNAs. To clarify the mechanism, they focused on autophagy, which induces the degradation of viral invaders. They discovered that autophagosomes were significantly present in recipient cells supplemented with conditioned medium (containing primary trophoblast-derived exosomes) compared with exosome-depleted conditioned medium, which was confirmed by electron micrographs. Furthermore, they suppressed autophagy by treatment with 3-methyladenine, an inhibitor of autophagosome formation, and siBeclin 1, a key factor in autophagic induction.

Secondly, fetal oncogenesis is associated with C19MC miRNAs [14]. C19MC miRNAs may be associated with human primate evolution [19]. Of those, miR-498 expression can be detected in the fetal brain at 20 weeks [17]. Kleinman et al. focused on embryonal tumors with multilayered rosettes (ETMRs), which is a disease associated with a poor prognosis. ETMR is known for its characteristic DNA methylation and high-level expression of C19MC miRNAs. They conducted copy number variation analysis of exome sequencing in 12 ETMR samples. Surprisingly, C19MC miRNAs were highly expressed by fusing TTYH1 in all the samples. The fusion of C19MC miRNAs and TTYH1 increases brain-specific DNA-methyltransferase 3 beta (DNMT3B), which is expressed only in the first weeks of neural tube development in normal fetal growth. The C19MC miRNAs targeted RBL2, which is a suppressor of DNMT3B. Thus, the authors concluded that DNMT3B overexpression by suppressing RBL2 might alter DNA methylation and the programming of neural tube development.

CONCLUSION

C19MC miRNAs are primate- and organ-specific, as described. There are strong reasons to use C19MC miRNAs as novel biomarkers in obstetrics, although several technical problems need to overcome. Also, clarifying the roles of C19MC miRNAs may be a key to the pathologic elucidation of obstetrical diseases including fetal abnormalities. Target miRNAs targeted by C19MC miRNA have been poorly validated. Targeting C19MC miRNAs can lead to new diagnostic and/or therapeutic options.

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