Abstracts of the 2009th Maruyama Memorial Lectures of the 78th Annual Meeting of the Medical Association of Nippon Medical School

Date: September 4, 2010　Place: Nippon Medical School

Abstracts of the 2009th Maruyama Memorial Research Fund Prize Memorial Lecture (1)

Placental Exosome-Assoicated MicroRNAs in Normal Pregnancy and Preeclampsia

Osamu Ishibashi[1], Md. M. Ali[1], Toshiyuki Takeshita[2] and Toshihiro Takizawa[1]

[1]Division of Molecular Medicine and Anatomy, Graduate School of Medicine, Nippon Medical School
[2]Division of Reproductive Medicine, Perinatology, and Gynecologic Oncology, Graduate School of Medicine, Nippon Medical School

Preeclampsia is a condition of pregnancy characterized by hypertension and proteinurea, which occurs in 3% to 5% of pregnancies worldwide and occasionally threatens the lives of mothers, fetuses, and neonates. Although the exact cause of preeclampsia is unknown, an imbalance between maternal type I helper T (Th1) cells and type II helper T (Th2) cells might be involved. In the maternal decidua and peripheral blood, Th2 cells are more numerous than Th1 in normal pregnancy but not in preeclamptic pregnancy[1].

MicroRNAs (miRNAs), single-stranded noncoding RNAs approximately 22 nucleotides in length, are involved in posttranscriptional regulation of gene expression. Accumulating evidence indicates that miRNAs are associated with a variety of pathophysiological processes[2]. In addition, miRNAs have been investigated clinically to develop diagnostic and prognostic tools[3,4]. Recently, we have identified human placenta-specific miRNAs by means of small RNA library sequencing[5]. Most of these placenta-specific miRNAs are located on the miRNA cluster on chromosome 19 (C19MC). Interestingly, we have also found that some miRNAs, including the placenta-specific miRNAs, are upregulated in preeclampsia placentas (unpublished observations).

Several recent studies have suggested that miRNAs can be detected in samples of serum and plasma[6,7]. Consistent with these studies, we have reported that placenta-specific miRNAs are abundant in the plasma of pregnant women and that the miRNAs are rapidly cleared from the plasma after delivery[5]. *In situ* hybridization has revealed that placenta-specific miRNAs are expressed in the syncytiotrophoblast in human placental chorionic villi. Furthermore, by using trophoblastic BeWo cells in culture, we have demonstrated that miRNAs are extracellularly released via exosomes. These results indicate that miRNAs secreted extracellularly from the syncytiotrophoblast via exosomes enter the maternal circulation. However, little is known about the role of placental exosome-associated miRNAs in maternal cells and tissues during pregnancy.

Exosomes are involved in cell-cell communication[8]. Thus, we have hypothesized that miRNAs secreted from the syncytiotrophoblast via exosomes are incorporated by lymphocytes and are involved in the regulation of the Th1/Th2 balance. To test this hypothesis, we isolated CD63 (a representative marker of exosomes)-expressing

exosomes from the supernatant of BeWo cells by means of immunoprecipitation. We confirmed that the exosomes contained miRNAs, including placenta-specific miRNAs (e.g., *miR-517a*). Then, human T cell lymphoblast-like Jurkat cells were exposed to the BeWo-derived exosomes. Interestingly, placenta-specific miRNAs were detected in the exosome-treated Jurkat cells, indicating that BeWo-derived miRNAs can be transferred to Jurkat cells in an exosome-mediated manner.

To our knowledge, no previous study has so referred to placental exosome-associated miRNAs in the communication between trophoblasts and T cells. We are investigating the functional role of placenta-specific miRNAs in T cells. We believe our miRNA study will contribute to the progress of reproductive immunology and perinatology.

References

1. Saito S, Shiozaki A, Sasaki Y, Nakashima A, Shima T, Ito M: Regulatory T cells and regulatory natural killer (NK) cells play important roles in feto-maternal tolerance. Semin Immunopathol 2007; 29: 115–122.
2. Ambros V: The functions of animal microRNAs. Nature 2004; 431: 350–355.
3. Rossbach M: Small non-coding RNAs as novel therapeutics. Curr Mol Med 2010; 10: 361–368.
4. Bader AG, Brown D, Winkler M: The promise of microRNA replacement therapy. Cancer Res 2010; 70: 7027–7030.
5. Luo SS, Ishibashi O, Ishikawa G, et al.: Human villous trophoblasts express and secrete placenta-specific microRNAs into maternal circulation via exosomes. Biol Reprod 2009; 81: 717–729.
6. Kosaka N, Iguchi H, Ochiya T: Circulating microRNA in body fluid: a new potential biomarker for cancer diagnosis and prognosis. Cancer Sci 2010; 101: 2087–2092.
7. Ferracin M, Veronese A, Negrini M: Micromarkers: miRNAs in cancer diagnosis and prognosis. Expert Rev Mol Diagn 2010; 10: 297–308.
8. Camussi G, Deregibus MC, Bruno S, Cantaluppi V, Biancone L: Exosomes/microvesicles as a mechanism of cell-to-cell communication. Kidney Int 2010; 78: 838–848.