Revista [IN]Genios, Vol. 3, Núm. 1, pp.1-7 (septiembre, 2016).
ISSN#: 2374-2747
Universidad de Puerto Rico, Río Piedras

PLACENTA-DERIVED EXOSOMES AS BIOMARKERS AND MEDIATORS OF IMMUNOREGULATION DURING PREGNANCY

Andrea Delgado Nieves
Normarie Herrera Rivera
Department of Biology
College of Natural Sciences

Recibido: 22/4/2016; Revisado: 31/7/2016; Aceptado: 20/8/2016

Abstract:
Exosomes are endosome-derived nanovesicles, which can carry different molecules to the extracellular environment and transport signals to cells in distant tissues. Recently, placental cells have been found to release exosomes to the maternal circulation during the gestation period. The concentration of placental exosomes in plasma not only varies depending on the stage of gestation but may also serve as indicators of pregnancy complications. In addition, these nanovesicles have active roles in immunity, apoptosis and vascular formation of the maternal-fetal unit. However, the biological function of placenta-derived exosomes remain to be fully-elucidated. The aim of this review is to summarize the known roles of placental exosomes in the maternal-fetal immunity and their clinical applications during the gestation period.

Keywords*:* biomarkers, exosomes, placenta, pregnancy, immunity

Resumen:
Los exosomas son nanovesículas derivadas de endosomas, las cuales cargan diferentes moléculas al ambiente extracelular y transportan señales a células de tejidos distantes. Recientemente se ha encontrado que las células de la placenta son capaces de secretar exosomas a la circulación materna durante el periodo de gestación. La concentración de exosomas en el plasma no solo varía durante la etapa de gestación sino que también puede servir como indicador de complicaciones durante el embarazo. En adición, estas nanovesículas tienen roles activos en inmunidad, muerte celular y formación vascular en la unidad maternal-fetal. Sin embargo, la función biológica de los exosomas derivados de la placenta todavía no se ha elucidado completamente. El objetivo de esta revisión de

literatura es resumir los roles conocidos de los exosomas de la placenta en la inmunidad maternal-fetal y sus aplicaciones clínicas durante el periodo de gestación.

Palabras clave*:* biomarcadores, exosomas, placenta, embarazo, inmunidad

Introduction[1]

Cell to cell communication can be mediated by the secretion of signaling molecules to the extracellular environment, direct interaction between cells or by vesicles such as exosomes (Raposo & Stoorvogel, 2013; Mincheva-Nilsson & Baranov, 2010). Exosomes are nanovesicles (50-120nm) originated by inward budding of membrane-bound intracellular compartments called endosomes, and may contain proteins, mRNAs or miRNAs (Sarker *et al.*, 2014; Chaput & Théry, 2010). The fusion of endosomes to the plasma membrane, allows the release of exosomes to fluids such as blood, urine, lymph, saliva, lacrimal and mammary gland secretions (Murray *et al.*, 2015). Through these fluids they can migrate and influence the cell behavior and physiology of distant tissues by regulating metabolism, protein translation, proliferation, apoptosis and angiogenesis (Murray *et al.*, 2015).

Recently, placenta-derived exosomes have been found to be circulating in the blood of pregnant women (Sarker *et al.,* 2014). The release of these exosomes is mediated by oxygen partial pressure and glucose concentration (Salomon *et al.,* 2013). However, the functions of placental exosomes remains to be fully-elucidated. The objective of this review is to evaluate the known roles of placental exosomes in the maternal-fetal immunity and their clinical applications during the gestation period.

Placental exosomes as biomarkers in pregnancy

Exosome levels during pregnancy

Placental cells play a crucial role in changes of metabolic, immune, and cardiovascular activities during gestation (Sarker *et al.,* 2014). Recently, Sarker *et al.* (2014) described that the concentration of exosomes in the maternal circulation increases during a normal pregnancy. They designed an experiment to determine the concentration of placenta-derived exosomes in healthy pregnant women, using Placental Alkaline Phosphatase protein (PLAP) as an indicator. Results showed that this placental exosome resident protein progressively increased during the first

trimester of the gestation period. Sabapatha, Gercel-Taylor, and Taylor (2006) also described the concentration of placenta-derived exosomes during the first trimester of pregnancy as ~20 fold higher than in the plasma of nonpregnant women. Hence, the levels of placental exosomes during the gestation period may help distinguish a healthy from a complicated pregnancy.

Placental exosomes as predictors of pregnancy complications

Abnormalities in the levels or in the composition and molecular cargo of placenta-derived exosomes in the maternal plasma are associated with pregnancy complications such as preeclampsia, gestational diabetes mellitus, and pre-term birth (Murray *et al.*, 2015; Salomon *et al*., 2014). Decreased levels of placental exosomes have been found in the plasma of pregnant women who had pre-term birth when compared to the plasma levels of women who had normal deliveries (Murray *et al.*, 2015). Conversely, an increase in placental exosomes has been shown in gestational diabetes mellitus (Salomon et al., 2015). In addition to exosome levels in complicated pregnancies, Vargas *et al.* (2014) demonstrated that the protein syncytin-2 was decreased in the exosomes of women with preeclampsia during their second and third trimester. Therefore, placental exosome levels, as well as their molecular composition in the plasma of pregnant women, may be important in predicting different complications during gestation.

Role of placental exosomes in the maternal immune system

Exosomes secreted by placental cells carry membrane proteins, which can influence the maternal immune state (Mincheva-Nilsson & Baranov, 2012). Sabapatha *et al.* (2006) performed a study where they assessed the role of these exosomes in T cell regulation by evaluating the expression of CD3-*zeta,* JAK3, and SOCS-2. CD3-*zeta* promotes T-cell proliferation and cytokine production, whereas JAK3 is involved in the signaling pathway of the activation and functionality of T regulatory and natural killer cells (NK). SOCS-2 has a role in the suppression of cytokine signaling. After exposing T lymphocytes to placenta-derived exosomes, the expression of JAK3 and CD3-*zeta* was down regulated, while SOCS-2 expression was increased. Thus, placenta-derived exosomes inhibited lymphocyte activation, further suppressing the maternal immune system.
Recently, Hedlund *et al*. (2009) showed that the human placenta could also regulate the maternal immune system by the release of NKG2D ligands, such as ULBP1-5 and MIC proteins, via exosomes. These proteins were able to suppress the cytotoxicity of different immune cells by down-regulating the NKG2D receptor, *in vitro*. In addition, several studies have demonstrated that placental

exosomes also carry Fas ligand (FasL), TRAIL, and PD-L1 proteins that signal apoptosis in activated immune cells and peripheral blood mononuclear cells through their membrane receptors (Sabapatha *et al.*, 2006; Stenqvist, Nagaeva, Baranov, and Mincheva-Nilsson, 2013). Furthermore, Kshiragar *et al.* (2012) showed that placenta-derived exosomes are capable of transport other proteins, such as B7-H1, B7-H3, and HLA-G5. Although their mechanism has not yet been elucidated, the study suggests that these latter proteins could play a role in the interaction of the receptor-ligand between T cells and antigen presenting cells. Taken together, these findings propose that the human placenta is capable of conveying protection to the fetus. This response is mediated by the suppression of the maternal immune cells via protein-carrying placental exosomes.

Conclusion

Placenta-derived exosomes can modulate the maternal immune system during the gestation period. These nanovesicles may carry various types of proteins that can cause a decrease in T-cell proliferation and activation, suppression of cytokine signaling and can furthermore induce apoptosis. The actions of these molecules towards the maternal immune cells all contribute to the protection of the fetus. Monitoring the levels and establishing a normal concentration range of these exosomes in the maternal plasma may have clinical applications. Deviations from these levels can signal an overly suppressed immune system, which may increase the susceptibility of pathogenic invasion during pregnancy. Also, alterations from the normal levels could indicate if there is a risk of a maternal immune response against the fetus.

Placenta-derived exosomal levels can be detected by minimally invasive or non-invasive techniques to potentially diagnose other risks during gestation. However, methods by which placental exosomes are isolated and characterized require optimization, since current procedures lack specificity for these exosomes. The existing protocols include differential centrifugation, size exclusion chromatography, flow cytometry using beads, and solid phase sedimentation which can isolate heterogeneous vesicle populations rather than exosomes alone (Murray *et al*., 2015). Therefore, characterization of vesicular size and composition should be enhanced by other methods for successful segregation and further use of placental exosomes. The improvement of these techniques could increase the possible early diagnosis of conditions related to dysfunctional placenta. In addition, new roles of placenta-derived exosomes in maternal-fetal communication may perhaps be elucidated.

Bibliography

Chaput, N., & Thery, C. Exosomes: Immune Properties and Potential. *Semin Immunopathol*, 2011, 33(5), 419-440. Digital http://link.springer.com/article/10.1007%2Fs00281-010-0233-9

Hedlund, M., Stenqvist, A.-C., Nagaeva, O., Kjellberg, L., Wulff, M., Baranov, V., et al. Human Placenta Expresses and Secretes NKG2D Ligands via Exosomes that Down-modulate the Cognate Receptor Expression: Evidence for Immunosupressive Function. *The Journal of Immunology*. 2009, 183(1), 340-351. Digital http://www.jimmunol.org/content/183/1/340.long

Kshirsagar, S. K., Alam, S. M., Jasti, S., Hodes, H., Nauser, T., Gilliam, M., et al. Immunomodulatory Molecules are Released from the First Trimester and Term Placenta via Exosomes. *Placenta*. 2012, 33(12), 982-990. Digital http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3534832/

Mincheva-Nilsson, L., & Baranov, V. The Role of Placental Exosomes in Reproduction. *American Journal of Reproductive Immunology*. 2010, 63(6), 520-533. Digital http://onlinelibrary.wiley.com/doi/10.1111/j.1600-0897.2010.00822.x/full

Mincheva-Nilsson, L., & Baranov, V. Placenta-Derived Exosomes and Their Role in the Immune Protection of the Fetus (Chapter 12). In Jing Zheng (Editor), *Recent Advances in Research on the Human Placenta*. Rijeka, Croatia: InTech Open Science Publishers, 2012, 243-260. Digital http://www.intechopen.com/books/recent-advances-in-research-on-the-human-placenta/placental-exosomes-in-human-pregnancy-and-their-role-in-immune-protection-of-the-fetus

Murray, M. D., Hassendrini, P. N., Miharu, K., Yong, K. Q., Duncombre, G., Illanes, S. E., et al. Placental Exosomes in Normal and Complicated Pregnancy. *American Journal of Obstetrics & Gynecology*. 2015, 213(4-supplement), S173-S181. Digital http://www.ajog.org/article/S0002-9378(15)00717-6/pdf

Raposo, G., Stoorvogel, W. Extracellular Vesicles: Exosomes, Microvesicles, and

Friends. *The Journal of Cell Biology*, 2013, 373-383. 2013, 200(4), 373-383. Digital http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3575529/

Sabapatha, A., Gervel-Taylor, C., & Taylor, D. D. Specific Isolation of Placenta-Derived Exosomes from the Circulation of Pregnant Women and their Immunoregulatory Consequences. *American Journal of Reproductive Immunology*. 2006, 56(5-6), 345-355. Digital http://biblioteca.uprrp.edu:2081/ehost/pdfviewer/pdfviewer?vid=2&sid=147a071a-7175-4872-bd64-41f0648d4a22%40sessionmgr4006&hid=4207

Salomon, C., Scholz-Romero, K., Kobayashi, M., Smith, M., Duncombe, G., Hanes, S., et al. Oxygen Tension Regulates Glucose-induced Biogenesis and Release of Different Subpopulation of Exosome Vesicles from Trophoblast Cells: Adgestational Age Profile of Placental Exosomes in Maternal Plasma with Gestational Diabetes Mellitus. *Placenta*. 2015, 36(4), p. 488. Digital http://www.placentajournal.org/article/S0143-4004(15)00517-2/pdf

Salomon, C., Sobrevia, L., Ashman, K., Illanes, S. E., Mitchell, M. D., & Rice, g. E. The Role of Placental Exosomes in Gestational Diabetes Mellitus. (Chapter 3) In Luis Sobrevia (Editor), *Gestational Diabetes: Causes, Diagnosis and Treatment*. Rijeka, Croatia: InTech Open Science Publishers. 2013, 29-47. Digital http://www.intechopen.com/books/gestational-diabetes-causes-diagnosis-and-treatment/the-role-of-placental-exosomes-in-gestational-diabetes-mellitus

Sarker, S., Scholz-Romero, K., Perez, A., Illanes, S. E., Mitchell, M. D., Rice, G. E., et al. Placenta-derived Exosomes Continuously Increase in Maternal Circulation Over the First Trimester of Pregnancy. *Journal of Translational Medicine*. 2014, 12(204), 1-19. Digital http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4283151/pdf/12967_2014_Article_1915.pdf

Stenqvist, A. C., Nagaeva, O., Baranov, V. & Mincheva-Nilsson, L. Exosomes Secreted by Human Placenta Carry Functional Fas Ligand and TRAIL Molecules and Convey Apoptosis in Activated Immune Cells, Suggesting Exosomemediated Immune Privilege of the Fetus. *The Journal of Immunology*. 2013, 191(11), 5515-5523. Digital

http://www.jimmunol.org/content/191/11/5515.long

Vargas, A., Zhou, S., Ethier-Chiasson, M., Flipo, D., Lafond, J., Gilbert, C., & Barbeau, B. Syncytin Proteins Incorporated in Placenta Exosomes are Important for Cell Uptake and Show Variation in Serum Exosomes from Patients with Preeclampsia. *The FASEB Journal*. 2014, 28(8), 3703-3719. Digital http://www.fasebj.org/content/28/8/3703.full.pdf+html

Notes

[1] The authors would like to express our profound gratitude to Dr. Rosaura Ramírez in the Biology Department (UPR-RP's College of Natural Sciences), who encourage us to design and pursue the library research and writing strategy on this review article.