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Function of Surface-Associated Protein and DNA on Extracellular Vesicles

Akademisk avhandling

Som för avläggande av medicine doktorsexamen vid Sahlgrenska akademin, Göteborgs universitet kommer att offentligen försvaras i hörsal Europa, Wallenberg Conference Center, A, Medicinaregatan 20, Göteborg,
Fredag, 1st Juni 2018, kl.13:00
Av

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Fakultetsopponent:
Professor Randy Wayne Schekman
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This thesis is based on the following studies, referred to in the text by their roman numerals.

I. Importance of exosome depletion protocols to eliminate functional and RNA-containing extracellular vesicles from fetal bovine serum. Shelke GV, Lässer C, Gho YS, Lötvall J. *Journal of Extracellular Vesicles* (30th Sept 2014). DOI: 10.3402/jev.v3.24783

II. Regulation of human mesenchymal stem cell function by mast cell exosome surface TGFβ-1 - role of endosomal retention. Shelke GV#, Yanan Y#, Jang SC, Lässer C, Wennmalm S, Hoffmann HJ, Nilsson J, Li L, Gho YS, Lötvall J. (# Equal Contribution)
(*Submitted*)

III. Epithelial mesenchymal transition induced in respiratory epithelial cells by mast cell extracellular vesicles. Shelke GV, Yanan Y, Brismar H, Lässer C, Lötvall J
(*In Manuscript*)

IV. Human mast cells release extracellular vesicle-associated DNA. Shelke GV#, Jang SC, Yanan Y, Lässer L, Lötvall J (# Corresponding Author)
Matters (21th Feb 2016). DOI: 10.19185/matters.201602000034

V. Extracellular vesicle-associated DNA is present on both the inside and the surface of vesicles and has a possible role in the activation of STING-associated pathways in recipient cells. Lázaro-Ibáñez E, Shelke GV, Crescitelli R, Jang SC, Cvjetkovic A, Garcia A, Lässer C, Lötvall J.
(*In Manuscript*)

SAHLGRENSKA AKADEMIN

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Function of Surface-Associated Protein and DNA on Extracellular Vesicles

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Abstract

Extracellular vesicles (EVs), including exosomes, are nano-sized, lipid bilayer-enclosed vesicles that are released into the extracellular environment from by almost all cells. EVs contain biomolecules, such as proteins, lipids, and nucleic acids, and they are suggested to play vital roles in cellular communication. In addition, they are used as biomarkers and have therapeutic applications. The goal of this Ph.D. thesis was to define the localization of EV-associated cargo (particularly proteins and DNA) and to determine the role of EVs in regulating biological processes. We addressed these questions by using mast cell-derived EVs (exosomes) and determining their effects on signaling pathways in primary human mesenchymal stem cells, epithelial cells, and monocytes. We have made three important discoveries.

First, we showed that the protein cargo, TGFβ-1, present on the surface of EVs derived from mast cells, activated a migratory phenotype in primary human MSCs. The major form of TGFβ-1 was inactive and was associated with heparan sulfate proteoglycans. Moreover, these EVs enhanced the immunosuppressive phenotype of MSCs in a mouse model of allergic airway inflammation. EVs activated prolonged and efficient TGFβ-signaling and were retained in the endosomal compartments of MSCs during this period. Furthermore, based on the protein expression and the morphological features that were induced in lung epithelial cells, we also concluded that the epithelial-to-mesenchymal transition could be induced by these EVs. Additionally, we found that these EVs could activate the phosphorylation of proteins that are involved in EMT. **Second**, we showed that the surface of EVs is associated with extracellular-DNA that induced the aggregation of EVs. Additionally, DNA was also present on the inside of EVs. The DNA on both the inside and outside of the EVs consisted of both mitochondrial and nuclear DNA. In this study, we were able to separate the EVs based on their density, followed by detection of the DNA that was associated with the EVs. The EV-associated DNA was able to initiate the activation of innate immune signaling by phosphorylation of interferon regulatory factor-3 in monocytes. **Third,** we evaluated and found that 18 hour is more efficient than 1.5 hours of ultracentrifugation in depleting EV-associated RNA (as well as DNA) from fetal bovine serum prior to its use in cell culture media.

We conclude that mast cell-derived EVs harbors bioactive molecules (e.g., TGFβ-1 and DNA) on their surfaces. These EVs can affect MSCs by regulating the immune environment of the lung during inflammation. Some portion of the secreted TGFβ-1 is inactive and is attached to the surface of EVs. This might target the EVs to the acidifying compartment of early/late endosomes and lead to the activation of TGFβ-1 along with the uptake of the EVs. Additionally, EVs also carry DNA. Most of the DNA molecules were present on the surface of the EVs and were able to activate the DNA sensors in recipient cells. Thus, EVs assist in the uptake of DNA into the cytoplasm of the recipient cell, and this mechanism has implications in autoimmune disease and in the maintenance of inflammation.

Keywords: Extracellular Vesicles, Exosomes, Mast cell, Mesenchymal stem cells, TGFβ-1, Endosomes, Epithelia-to-mesenchymal transition, Extracellular DNA, IRF-3

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