Unconventional secretion hijacks the conventional pathway in endothelial cells

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ABSTRACT

Endothelial dysfunction is implicated in the most life-threatening diseases, such as cardiovascular, inflammatory diseases and cancer-angiogenesis. The majority of proteins that play important role in the above pathophysiological processes are cargo molecules secreted from Weibel-Palade bodies (WPBs)[1,2], the storage secretory organelles of endothelial cells. To date, only a small number of the WPBs-released proteins have been identified.

In order to get a better understanding of the mechanisms responsible for the diversity of functions of endothelial cells, we employed a proteomic analysis of the secreted proteins in activated endothelium. For this purpose, human umbilical endothelial cells (HUVECs) were stimulated with VEGF/bFGF/ATP and the secreted material was analyzed by high resolution mass spectrometry (nLC-MS/MS). The data led to the identification of proteins that have not been linked as yet with the secretion of WPBs, among them the galectin-1 protein (Gal-1).

Given that galectin-1 is a cytoplasmic protein that is abundantly expressed in the endothelium and has been recently identified as novel partner of von Willebrand factor, the most abundant cargo of the WPBs, we sought to investigate whether it is present in the secretory WPBs of endothelial cells. Using confocal (LSCM/CLSM) and STED microscopy, as well as "internalization assays" of recombinant galectin-1 protein, we found that galectin-1 is targeted to secretory WPBs, as it is found to be co-localized with bona-fide markers of WPBs[1]. Surprisingly, galectin-1 is found only in a "rare" subpopulation of WPBs in HUVECs. Moreover the number of galectin-1 positive WPBs is increased upon stimulation of HUVECs with VEGF/bFGF/ATP.

These data provide unexpected links between unconventional protein secretion of cytoplasmic proteins (e.g. galectin-1) and exocytosis of professional secretory organelles (e.g. WPBs), implying that unconventionally secreted proteins are able to hijack the conventional pathway.

Given the variety of biological functions of galectin-1, including cell to cell or matrix interactions, intracellular signaling, proliferation, differentiation, metastasis and cancer[3], validation and functional analysis of the role of galectin-1 in WPBs is expected to reveal novel insights into its role in vascular physiology in health and disease.

REFERENCES

[1]. Rondaij MG, Bierings R, Kragt A et al. (2006). *Arterioscler Thromb Vasc Biol.*, 26(5), 1002-1007.
[2]. Goligorsky MS, Patschan D, Kuo MC. (2009). *Nature Reviews Nephrology*, 5(7),423–426.
[3]. Johannes L, Jacob R., Leffler H. (2018). *J Cell Sci.*, 131(9).