Screening of inhibitors for modulation of exosome release by cancer cells – Gertjan Rasschaert[1], An Hendrix[1], Olivier De Wever[1]
1: Ghent University, Ghent, Belgium

Introduction: The most life-threatening aspect of cancer is the local invasive growth and distant metastasis rather than the primary tumoritself. The latter can be considered as an endocrine organ which maintains an intense communication with its environment through delivery of surface proteins and soluble factors (growth factors and cytokines) and release of nanovesicles, called exosomes, for establishment of invasive tumor growth. The specific molecular mechanisms of exosome biogenesis and secretion are still poorly understood. However, an important role in invasive tumor growth is dedicated to the small GTPase Rab27B, a regulator of vesicle exocytosis, that delivers pro-invasive signals for increased invasiveness, tumor size and metastasis of various estrogen receptor (ER)- positive breast cancer cell lines, both in vitro and in vivo.

Methods: In vitro cancer cells, MCF-7 GFP-Rab27B cells, are used to hunt down the influence of a series of potential inhibitors of exosome secretion. V-ATPase inhibitors, bafilomycin A1 and concanamycin A, because of the demonstrated presence of V-ATPase subunits on GFP-Rab27B vesicles. Cytoskeleton inhibitors, cytochalasin D (actin) and nocodazole (microtubule), to undermine major routes of intracellular vesicle transport. PI(3)-kinase inhibitor which affects a crucial step in vesicle maturation during endocytosis, a part of exosome biogenesis. MTT assays are performed to study the influence of the inhibitors on cell proliferation. To study their effect on exosome secretion the design consists of two legs. In the morphological leg fluorescence microscopy is performed on treated cells to observe changes in peripheral location of GFP- Rab27B vesicles. In the quantitative leg nanoparticle tracking analysis is performed on isolated exosomes from conditioned medium of treated cells to study the difference in total exosome secretion.

Results: Individual reversible inhibition of V-ATPase activity and cytoskeleton morphology demonstrates that both V-ATPase and actin/microtubules control peripheral localization of Rab27B vesicles. Both classes display reversible lowering in total exosome concentration in the conditioned medium of in vitro cultured cells. PI(3)-kinase inhibitor displays an analogue but smaller effect on peripheral localization of GFP-Rab27B vesicles. In this study PI(3)-kinase inhibitor does not seem to influence total exosome concentration.

Conclusion: A protocol for testing potential inhibitors of exosome release by cancer cells is established: first a screening through the morphological leg and second the confirmation through the quantitative leg. Inhibiting V-ATPase activity and interfering cytoskeleton architecture by administration of drugs might be an effective future strategy for blocking Rab27B- dependent pro-invasive vesicle trafficking in ERα-positive breast cancer patients.

Correspondence should be addressed to: gertjan.rasschaert@ugent.be