ImmunoTools IT-Box-139 Award 2013

Roberta Freezor

PhD Supervisor: Dr. Sheelagh Heugh

London Metropolitan University
Faculty of Life Sciences
166-220 Holloway Road
London N7 8DB

The clinical importance of microvesicles as diseases biomarkers - developing a reference range for microvesicles in healthy volunteers, to determine the significance of change in disease.

Microvesicles (MV's), including exosomes and ectosomes are membrane-coated vesicles shed in body fluids (especially blood) and are released from virtually all cells types, including erythrocytes, lymphocytes and platelets. They are characterized by antigenic markers exposed on their surface, which depend on parent cell origin, and vary in size, biochemical composition, and biological effects. The release of MV's is a complex process (cell apoptosis/activation), not fully understood; nevertheless MV's share common formation by budding from the cell membrane, whether on the cell surface or from a vesicular compartment inside the cell. Once released, it is speculated that MV's act as a form of long-distance cell-cell communicator through interaction with surrounding cells, delivering molecular signals in the form of lipids, nucleic acids, and functional transmembrane proteins from the parent cell; accumulating in internal endocytic/phagocytic compartments. This involves cell surface binding via specific receptors, internalization by endocytosis or micropinocytosis, and/or fusion with the recipient cell plasma membrane.

The clinical relevance of MV's remains theoretical because there is a lack of a robust reference range of MV levels, despite links connecting increased MV levels in a variety of disease states, including Heparin-induced Thrombocytopenia, Arterial Thrombosis, Sickle Cell Anaemia, Nonvalvular Atrial Fibrillation, Uremia, Thalassaemia, Paroxysmal Nocturnal Haemoglobinuria,, Haemolytic Anaemia and Hypercoagulability, Pulmonary Arterial Hypertension, as well as to *in vivo* coagulation, fibrinolysis and endothelial activation. Research to ascertain their relevance is urgent and forms the basis of this study, in order to contribute to ongoing research to establish the significance of its existence in modern medicine, focusing on the biological effects of MV release levels.

The ImmunoTools *IT-Box-139* (100 antibodies), will provide a valuable cocktail of specific fluorescent labelling antibodies, that allows differentiation between different cell type derived MV's, which are naturally constituted within human blood plasma samples and saliva, with the intention to produce a "normal" reference range of the release levels of MV's. Using flow cytometer and Lumascope antibodies from the *IT-Box-139* will elucidate the specificity of originating cell source. Annexin V has proven an excellent

immune label for PS on MV's, it is hoped some of the labels chosen below may extend this research. Once an appropriate cocktail of labels is produced this study will be extended to investigate a large group of healthy subjects, to ascertain normal MV release levels ("normal" reference range), and investigate the differentiation with the number of MV's released during disease stages, reflecting a balance between generation and clearance; so the differences can be accurately investigated.

ImmunoTools *IT-Box-139.3* for **Roberta Freezor** includes 100 antibodies

FITC - conjugated anti-human CD1a, CD2, CD3, CD4, CD5, CD6, CD7, CD8, CD9, CD11a, CD11b, CD14, CD15, CD16, CD18, CD19, CD21, CD25, CD29, CD36, CD41a, CD43, CD45, CD45RA, CD46, CD52, CD53, CD54, CD58, CD62p, CD63, CD69, CD71, CD80, CD86, CD95, CD235a, HLA-ABC, HLA-DR, IL-6, Control-IgG1, Control-IgG2a, Control-IgG2b, Annexin V

PE - conjugated anti-human CD2, CD3, CD4, CD8, CD11b, CD14, CD15, CD18, CD19, CD20, CD21, CD22, CD27, CD33, CD34, CD37, CD38, CD40, CD42b, CD45, CD45RB, CD50, CD72, CD95, CD105, CD147, CD177, Control-IgG1, Control-IgG2a, Control-IgG2b, Annexin V

PE/Dy647 -tandem conjugated anti-human CD45

APC -conjugated anti-human CD3, CD4, CD7, CD8, CD10, CD11c, CD14, CD16, CD19, CD27, CD37, CD40, CD44, CD56, CD59, CD61, CD62L, CD62P, CD69, IL-6, Control-IgG1, Control-IgG2a, Control-IgG2b, Annexin V DETAILS

plus CD35 FITC, CD31 PE, CD61 PE