Oxosome
The Oxford Extracellular Vesicle Group

Fourth Annual Meeting, Wednesday 23rd October 2019

Sherrington Building, Department of Physiology, Anatomy and Genetics, South Parks Road, Oxford, OX1 3QX

Organisers: **Deborah Goberdhan**, **Dave Carter**

ALL WELCOME

Talks in the Sherrington Large Lecture Theatre

Dr Cláudia Mendes (Wilson and Goberdhan Labs)
Aashika Sekar (Wilson Lab)

2.05 pm Opening comments

2.15 pm **Dr Stefan Balint** (Dustin Lab, The Kennedy Institute of Rheumatology, University of Oxford) Supramolecular attack particles - a non-vesicular ~100 nm particle mediating T cell killing

2.30 pm **Lizzie Dellar** (Carter Lab, Dept. of Biological and Medical Sciences, Oxford Brookes University/ Baena Lopez lab, Sir Dunn School of Pathology, University of Oxford) Unpacking the molecular principles facilitating RNA-loading in extracellular vesicles

2.45 pm **Dr Sascha Raschke** (Particle Metrix – technical talk) Phenotyping extracellular vesicles using tetraspanins and fluorescence-NTA

3.00 pm **Dr Shih-Jung Fan** (Goberdhan Lab, Dept. of Physiology, Anatomy and Genetics, University of Oxford) Glutamine Deprivation Regulates the Origin and Functions of Cancer Cell Exosomes

3.15 pm *Refreshments Break in the Sherrington Foyer*

Dr Genevive Melling (Carter Lab)
Dr Yvonne Couch (Buchan Lab)

4.00 pm **Dr Naveed Akbar** (Choudhury Lab, Radcliffe Dept. of Medicine, University of Oxford) Extracellular Vesicles Mediate Immune Cell Mobilisation and Transcriptional Activation Following Acute Myocardial Infarction

4.15 pm **Dr Cheng Jiang** (Tofaris Lab, Nuffield Dept. of Clinical Neurosciences, University of Oxford) Differential egress of α-synuclein and clusterin in serum neuronal exosomes precedes and predicts Parkinson's disease

4.30 pm **Dr Dimitri Aubert** (NanoFCM – technical talk) Nano-Flow Cytometry: A platform for comprehensive extracellular vesicle analysis

4.45 pm **Scott Bonner** (Wood Lab, Dept. of Paediatrics, University of Oxford) Characterisation of tumour-derived extracellular vesicle subpopulations and their role in cancer development

5.00 pm Closing comments

Wine Reception in the Sherrington Foyer

Thank you to the Department of Physiology, Anatomy and Genetics, Particle Metrix,
Evox Therapeutics Ltd, NanoFCM, iZON Science Ltd and ONI for generous sponsorship of this meeting

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Talk Summaries

Supramolecular attack particles- a non-vesicular ~100 nm particle mediating T cell killing

Dr Stefan Balint (Dustin Lab, The Kennedy Institute of Rheumatology, University of Oxford)

T cells respond to cancer-associated antigens by releasing extracellular particles that combine determinants of antigen specificity and effector function. Extracellular vesicles produced by helper T cells bud directly from the centre of the immunological synapse upon engagement of the T cell receptor. We refer to these as synaptic ectosomes and have recently shown that they contain tetraspanins as well as CD40 ligand, a protein that is involved in B-T cell communication. In contrast, detailed examination of the released particles by cytotoxic T cells identified a new type of particles that we refer to as Supra-Molecular Attack Particles (SMAPs). SMAPs are non-membranous particles formed by a glycoprotein shell and contain a core of cytotoxic proteins, chemokines and cytokines. Thus, different subsets of T cells release distinct particles which could be a powerful tool for cancer immunotherapies.

Unpacking the molecular principles facilitating RNA-loading in extracellular vesicles

Lizzie Dellar (Carter Lab, Dept. of Biological and Medical Sciences, Oxford Brookes University/ Baena Lopez lab, Sir Dunn School of Pathology, University of Oxford)

Our work makes use of Drosophila as a model system to understand the basic biological properties that facilitate the packing of RNA cargo into EVs. We have first characterised the RNA content of Drosophila S2R+-cell-derived EVs from both control and oxidative stress conditions. We then developed a bioinformatic pipeline for identification of sequence motifs and secondary structures that are enriched in EV-loaded RNAs. Using this approach, our analysis has identified several emerging properties of EV-loaded and cell-retained RNAs, findings which previous literature indicate may be evolutionary conserved. Our comparative analysis has also uncovered a subset of genes that are enriched in EVs under oxidative stress. Currently, we are validating our findings using an in vivo Cre-LoxP-based reporter system in Drosophila.

Phenotyping Extracellular Vesicles using Tetraspanins and fluorescence-NTA

Dr Sascha Raschke (Particle Metrix)

In addition to determining the size and concentration of extracellular vesicles (EVs), phenotyping of these particles is another important factor in EV research. With our ZetaView® QUATT, we demonstrate a 4-laser NTA instrument that uses fluorescence- (f) NTA to quantitate EVs and determine ratios in a sample of different EVs by using different surface markers. For example, CD9-, CD81- and CD63-positive EVs can be distinguished by using fluorescence-labelled antibodies in 3 of the 4 fluorescence channels. The fourth fluorescence channel was used for the membrane dye CMDR to verify the presence of membranous particles. With the ZetaView® QUATT, it is possible for the first time to quantify from a sample of diverse extracellular vesicles those EVs by surface markers that are of particular interest.

Glutamine Deprivation Regulates the Origin and Functions of Cancer Cell Exosomes

Dr Shih-Jung Fan (Goberdhan Lab, Dept. of Physiology, Anatomy and Genetics, University of Oxford)

Exosomes are generally thought to be made in late endosomal multivesicular bodies. We show that exosomes carrying unique cargos including Rab11a, are also made in recycling endosomes. Depletion of glutamine, a key metabolic nutrient, or suppression of nutrient-regulated mTORC1 signalling in cancer cells increases secretion of Rab11a-exosomes and other extracellular vesicle subtypes. These vesicles promote tumour cell turnover and blood vessel growth in xenograft mouse models. Antibodies against Amphiregulin (EGFR ligand) compromise their growth-promoting activity. We hypothesise that release of these stress-induced vesicles changes growth factor signalling in different regions of the tumour and thereby promotes tumour adaptation.

Extracellular Vesicles Mediate Immune Cell Mobilisation and Transcriptional Activation Following Acute Myocardial Infarction

Dr Naveed Akbar (Choudhury Lab, Radcliffe Dept. of Medicine, University of Oxford)

Neutrophils and monocytes are rapidly mobilised from reserve such as the spleen to peripheral blood following acute myocardial infarction. Mobilised cells undergo transcriptional activation en route to the injured myocardium and mediate further injury. We have shown that endothelial cell derived extracellular vesicles mobilise neutrophils and monocytes from the spleen and induce their transcriptional programming. Targeting neutrophil and monocyte transcriptomes in AMI with bioengineered EVs may salvage the myocardium in the immediate hours after injury.

Nano-Flow Cytometry: A Platform for Comprehensive EV Analysis

Dr Dimitri Aubert (NanoFCM)

Though of great importance, sizing, counting and molecular profiling of individual extracellular vesicles (EVs) are technically challenging due to their nanoscale particle size, minute quantity of analytes, and overall heterogeneity. NanoFCM has developed Nano-Flow Cytometry (nFCM), a technology that allows light scattering and fluorescence detection of single EVs down to 40 nm. nFCM-based approach for quantitative multiparameter analysis of EVs, which is highly desirable to decipher their biological functions and promote the development of EV-based liquid biopsy and therapeutics.

Differential egress of α-synuclein and clusterin in serum neuronal exosomes precedes and predicts Parkinson's disease

Dr Cheng Jiang (Tofaris group, Nuffield Department of Clinical Neurosciences, University of Oxford)

Among 638 individuals tested cross-sectionally, neuron-derived exosomal α-synuclein was elevated by 2-fold in prodromal and clinical Parkinson's disease but not in unrelated neurodegenerative diseases. In longitudinal serum samples, exosomal α-synuclein was stably increased with PD progression and consistent when tested across cohorts. Mean exosomal clusterin was increased in other proteinopathies but not α-synucleinopathies. Combined exosomal α-synuclein and clusterin measurement improved the predictive value of a primary α-synucleinopathy versus an alternative proteinopathy (AUC =0.98).

Characterisation of tumour-derived extracellular vesicle subpopulations and their role in cancer development

Scott Bonner (Wood Lab, Department of Pediatrics, University of Oxford)

Extracellular vesicles (EVs) represent a heterogeneous population of membrane enclosed vesicles that function as mediators of intercellular communication. In the context of tumour-derived EVs as mediators of cancer development and progression, this heterogeneity could cause certain EV subpopulations to have unique roles in the intricate biological processes underlying cancer biology. For example, we have observed that only a certain subpopulation of EVs supports ovarian cancer cell adhesion to matrixes in a CD29-dependent manner, suggesting involvement of this subpopulation in tumour metastasis. Following up on these observations, in this study we further characterised EV subpopulations from ovarian cancer cells both on a single vesicle and proteomics level. We analysed biomarker colocalization using the Nanoview ExoView R100, peptide mass fingerprints via MALDI-TOF mass spectrometry and assessed integrin beta 1 (CD29) expression on EVs using super-resolution microscopy techniques. The data gained highlights the relevance of EV heterogeneity, with particular regard to the role of EVs in the progression and development of cancer.