RCoA Research, Education & Travel Grants 2015

Award: The Ernest Leach Fund

Applicant: Dr James Bowness

Project Title: The utility of exosomes as a biomarker in N-Methyl-D-Aspartate (NMDA) receptor encephalitis

Project Description:

N-methyl-D-aspartate (NMDA) receptor encephalitis was first described in 2007 (Dalmau et al, 2007). It is hypothesised that IgG autoantibodies to NMDA receptor NR1/2 subunits stimulate an autoimmune neuroinflammatory state (Dalmau et al, 2007; Dalmau et al, 2008). Patients present insidiously, with changes in behaviour, but subsequently develop psychotic features and movement disorders. Hypoventilation and coma can necessitate critical care support. The aetiopathology is poorly understood; greater understanding has potential to aid in diagnosis, monitoring and therapy.

Exosomes are lipid-bilayer enclosed nanoparticles shed by many cell types (Thery et al, 2009; Colombo, Raposo & Thery, 2014), containing nucleic acids and proteins from the host cell (Colombo, Raposo & Thery, 2014; Gupta & Pulliam, 2014). A growing body of evidence implies roles in immune modulation (Kim et al, 2005; Thery, Ostrowski & Segure, 2009), malignant processes (Skog et al, 2008; Kosaka et al, 2013) and as biomarkers of disease (Skog et al, 2008, Gupta & Pullman, 2014). Our initial results demonstrate a differing nanoparticle population in NMDA receptor encephalitis patient serum, as compared to that from healthy controls. Pathological serum appears to contain six to seven-times the number of exosome-sized particles, with a markedly different size distribution. They contain well-recognised exosome markers and the NMDA receptor. We have also induced a change in the exosome population produced by mouse fibroblasts, containing the NMDA receptor, by exposing them to pathological serum.

Project

We plan to assess the utility of exosomes as a biomarker of this disease. Using Nanosight Tracking Analysis, we plan to determine if the serum exosome population changes correlate with antibody load and disease severity. Electron microscopy and immunoprecipitation of these exosomes, with antibodies to known markers, will further delineate this population. The colocalisation of NMDA receptors with exosomal markers will be identified with immunoprecipitation, immunofluorescence and western blotting.

Using pathological serum to induce exosome production by fluorescently-labelled mouse fibroblasts that express NMDA receptor, spectrophotometry will investigate the changing exosome profile in response to NMDA receptor antibody.

Exosomes from pathological serum will be incubated with mouse fibroblasts which do not contain the NMDA receptor. These cells will subsequently be interrogated to determine the presence of NMDA receptor in/on them to investigate the protein-donating role of these exosomes.

Timescale

This work will be undertaken over the coming academic year (2015 - 2016) and may involve the participation of an intercalating BMSc student. We plan to present our results at the International Society for Extracellular Vesicles Annual Congress (Rotterdam, May 2016). The data will be submitted for publication in a peer-reviewed journal.