Manchester Institute for Collaborative Research on Ageing (MICRA)
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Project Title

Establishing a model system for monitoring correction of vascular defects in iPSC-derived vascular cells from CADASIL patients

Applicants:

Dr Tao Wang:	Division of Evolution and Genomic Medicine, School of Biological Sciences, Faculty of Biology, Medicine and Health
Dr Martin Baron	Division of Cell Matrix Biology and Regenerative Medicine, School of Biological Sciences, Faculty of Biology, Medicine and Health

Background:

CADASIL (Cerebral Autosomal Dominant Arteriopathy with Subcortical Infarcts and Leukoencephalopathy) is the most common type of genetic stroke syndrome and vascular dementia caused by the *NOTCH3* mutation. CADASIL patients usually have recurrent strokes and dementia in the late years of life with prominent pathological change of Notch3 protein accumulation in small arteries, very similar to that in the age-related neurodegenerative conditions like Alzheimer's disease, Huntington's disease and Parkinson's disease. The Notch3 accumulation highly suggests a protein trafficking defect in vascular cells. Establishing a method to visualise and track down the native Notch3 protein trafficking would have significant advantages over the conventional strategy by overexpression of the mutant proteins in cell lines.

With collaboration with Prof Sue Kimber, Dr Tao Wang's group has established an induced pluripotent stem cell (iPSC) disease model from CADASIL patients' skin biopsies, and successfully differentiated the iPSCs into endothelial cells and (ECs) and vascular smooth muscle cells (SMCs). Dr Martin Baron has strong expertise in Notch trafficking, and has experience in using fluorescent protein tagging and imaging of the highly homologous *Drosophila* Notch without affecting native protein localisation or function.

Research plan and impact:

The proposed project is to use the recent cutting edge genome editing technology, the CRISPR/Cas9 technique, to insert a green fluorescent protein (GFP) tag in the endogenous *NOTCH3* gene in the CADASIL patient-specific iPSCs, and then monitor the fate of the native Notch3 protein in the CADASIL patient-specific iPSC-derived vascular cells. Notch3 proteins undergo both ligand dependent and independent trafficking, with complicated endosomal sorting involvement. A striking pathological change in CADASIL is the extracellular domain of Notch3 (Notch3ECD) accumulation in small arteries, but it remains unknown why only Notch3ECD but not Notch3 full-length or Notch3ICD accumulates in CADASIL arteries, and at what stage the Notch3ECD is separated from Notch3ICD. Answers to these questions would help to clarify disease mechanisms and help identify drug targets with the potential to reverse the age-related disease progression. This project is to establish a model system where we can easily monitor the trafficking and fate of Notch3 in authentic wild type and patient vascular cells.

The proposed research is collaborative efforts between the applications of the patient-specific human stem cell model and the *in vivo drosophila* model in order to elucidate the molecular mechanisms of an ageing related genetic neurodegenerative disease. We anticipate that the project would uncover new insight of the age-related neurodegenerative conditions, and results will likely contribute to the understanding of the contribution of protein accumulation to the age-related neurodegenerative conditions overall.