RESEARCH GROUP: Musculoskeletal health and neuromuscular disorders

Project Title: Identification of biomarkers to identify ALS subjects among patients affected by motor neuron disorders

Supervisor(s):
- Supervisor and chair: Stephanie Duguez, PhD
- Supervisor: William Duddy, PhD

Note: this is a ring-fenced project for newly appointed staff

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Level: PhD

Background to the project:
Amyotrophic Lateral Sclerosis (ALS) is a lethal neurodegenerative disease affecting adults (40-70 years old) characterized by a degeneration of motor neurons causing a progressive muscular atrophy\(^1,2\). Its progression is marked by major disability followed by death 3-5 years after the onset. The disease is mostly sporadic with an incidence of 2-3/100,000 inhabitants\(^3\). Diagnosis of ALS is difficult and exclusively based on clinical examination, electromyography studies, and on the exclusion of disorders mimicking ALS (e.g. adult-onset spinal muscular atrophy (SMA-IV) and SBMA)\(^4-6\). It is thus crucial to find biomarker(s) specific for ALS to improve the diagnosis and to allow neuroprotective drug treatment starting at earlier stages.

To date, ALS pathogenesis remains unknown. Studies in animal models and ALS patients show that motor neuron degeneration starts at the neuromuscular junction and that post-synaptic muscle changes may play an active role. We observed the existence of an ALS signature in muscle stem cells, as the gene expression profiles of sporadic ALS muscle cells cluster separately from the profiles of healthy and ALS-mimicking diseases\(^7\). The 30 genes most strongly contributing to this ALS-specific signature encode a number of proteins localized in secreted vesicles called exosomes – proteins that could be potential circulating biomarkers for ALS.
Objectives of the research project:
We have already confirmed that ALS muscle cells release 2-fold more exosomes than healthy controls, and that these exosomes are toxic as once added to the culture medium of healthy muscle cells or of healthy motor neurons, they induced: (1) muscle fiber atrophy, (2) cellular stress by stimulating membrane blebbing, and (3) cell death of muscle cells and motor neurons.

Working with an established collaborative network - S Knoblach (Genetic Medicine, CNMC, Washington, US), H Blasco (Universite de Tours, Tours, France) and PF Pradat (Paris Motor Neuron Disease (MND) Center, Pitie Salpetriere, Paris, France) - the student will pursue the following aims: (1) identify secreted biomarkers that distinguish ALS from other motor neuron disorders, based on our muscle transcriptomic and exosomal assays; (2) validate that the secretion of these biomarkers is specific to ALS muscle stem cells; and (3) validate secreted biomarker candidates in a moderate-sized patient cohort.

**Aim1: Identification of biomarkers that are specific to ALS.**
Characterize the composition of exosomes secreted from ALS, SBMA, SMA-III/IV and healthy human muscle cells using proteomic, transcriptomic analysis, miRNA profiling (collaboration with S. Knoblach), and metabolomics (collaboration with H Blasco). These experiments will identify candidate markers specifically secreted by ALS muscle cells. For this purpose, the student will work closely with W Duddy to analyse and identify robust biomarkers based on the omics data.

**Aim2: Validation of biomarkers specific to ALS in vitro**
Confirm by western blot or RT-q-PCR or mass spectrometry - depending on the candidates identified - the presence or the absence of the secreted candidates identified in Aim1 in the culture medium of ALS muscle cells.

**Aim3: Validation of circulating biomarkers in a cohort of 100 sporadic ALS patients.**
10 potential biomarkers identified in vitro will be validated in circulating blood samples (60 MND, of which 36 ALS, available immediately - further sample collection is ongoing and projected at >100 subjects in the coming year), using ELISA, miRNA kit, RTqPCR, or mass spectrometry (for metabolites). The candidate will then correlate biomarker levels with specific clinical patterns and genotypes. This will be done in collaboration with PF Pradat (AP-HP, Pitie Salpetriere, France), who already has a collection of blood samples and clinical data of ALS patients as well as age-and sex-matched healthy subjects and patients with pathologies that mimic ALS (PI PF Pradat, protocol NCT01984957 and protocol NCT02360891).

**Methods to be used:**

**Human muscle stem cell culture:** The myogenic cell population will be obtained from fresh muscle biopsies. The myogenic population will be enriched using a CD56 magnetic bead sorting system (MACS, Miltenyl Biotech), and the myogenicity of the cell cultures will be checked by immuno-labelling (anti-desmin,1/100). The cells will be expended in growth medium (1 vol of M199, 4 vol of DMEM, 20% FBS, Fetuin 25µg/ml, EGF 5 ng/ml, bFGF 0.5 ng/ml, Insulin 5 µg/ml) and differentiated in DMEM.

**Exosomes extraction:** Exosomes will be extracted either from the culture medium or blood samples of ALS, Healthy and MNDs muscle cells. After eliminating the cell debris and microparticles as previously described, and in order to have an optimal exosome yield, we will use the total exosome isolation reagent or plasma exosome isolation from InvitrogenTM - kit that we have already validated to extract intact cup-shaped vesicle positive for exosomal markers such as CD63 and CD82.

**RNA profiles:** The mRNA and miRNA will be extracted using the Total RNA and protein extraction kit (InvitrogenTM). The mRNA and microRNA expression profiles will be determined using Affymetrix miRNA and mRNA arrays.

**Proteome profile:** the exosome pellets will be solubilized and digested as previously described. The peptides will be analyzed by mass spectrometry using a nano HPLC system (NanoLC 2D, Eksignet, Dublin, CA) connected to a hybrid LTQ/Orbitrap instrument (Thermo Fisher Scientific, San Jose, CA).

**Metabolome profile:** the mass spectrometry method will be performed as described previously.
**Bioinformatics analyses:** Candidate biomarkers will be assessed based on multiple-testing adjusted p-values of ALS against healthy and disease controls. Systems biology tools will be used to indicate gene pathways/networks that are impacted across more than one of our omics approaches (e.g. if a transcript and its regulatory microRNAs are co-regulated), and to identify candidates that are known to interact with pathways or genes of known clinical importance to ALS.

**Validation of circulating biomarkers:** For this purpose, we will use ELISA tests (test that can be used to detect either proteins, exosomes (ExoElisaTM Plate, SBI) or miRNA (miRNA assay kit)) or RTqPCR (to quantify exosomal mRNA) or mass spectrometry (to detect and quantify the exosomal metabolites) to determine the presence and quantify the amount of candidate biomarkers in circulating blood samples of ALS patients.

**Skills required of applicant :**

- **Research and Technical Skills required:**
  - Cell culture
  - RNA and protein extractions
  - Organizational and planning skills
  - Rigorous and methodical approach to lab-work
  - Capable of both independent and team work
  - Good skills in writing and presenting data.

- **Additional preferred skills:**
  - PCR and RT-qPCR
  - ELISA Test
  - Western blots
  - Capacity to use bioinformatics tools to analyse OMICS data

**References :**

7- Duguez S. et al. EAN congress:P2149 abstract in Eur J Neurol, 2015, 22 (Suppl.1), 120-483