The Pathogenesis of Spinal and Bulbar Muscular Atrophy

https://neurodegenerationresearch.eu/survey/the-pathogenesis-of-spinal-and-bulbar-muscular-atrophy/

Name of Fellow

Dr H Devine

Institution
Funder

MRC

Contact information of fellow
Country

United Kingdom

Title of project/programme

The Pathogenesis of Spinal and Bulbar Muscular Atrophy

Source of funding information

MRC

Total sum awarded (Euro)

€ 310,702

Start date of award

01/04/16

Total duration of award in years

3.8

The project/programme is most relevant to:

Spinal muscular atrophy (SMA)

Keywords

Induced pluripotent stem cells | Motor neuron | Neurodegeneration | Spinal Bulbar Muscular Atrophy

Research Abstract

All the experimental protocols described are established in my host labs 0-18m: Characterise

iPSCMNs Initially 2 patient, 2 healthy control lines. More lines are available Differentiate MN: Spinal: Developmentally rationalised cues used including retinoic acid (RA) and Sonic Hedghog (SHH). Bulbar: WNT agonist combined with RA for rostrocaudal patterning. MN creation: Immunocytochemistry (HB9, ISL1, SMI32, ChAT), electrophysiology SBMA: karyotype analysis, CAG using Sanger sequencing, expression and localisation of AR Cell death: cell counts +/- dihydrotestosterone by immunofluorescent staining using Dapi, B-III tubulin and actived caspase 3 (quantified with Western Blot) Axonal transport: Live cell imaging and a fluorescent atoxic tetanus toxin fragment for retrograde AT, Mitotracker for anterograde/retrograde mitochondrial AT Protein mishandling: WB and immunostaining for components of heat shock response (HSR) at baseline and following exposure to cell stressors ER stress: Changes in cytosolic Ca2+ levels using ER stressors (thapsigargin, ionomycin) and fura-2. Markers (PDI, CHOP, BiP ATF4) with WB and immunostaining Mitochondria: Live cell imaging with fluorophores (TMRM, FCCP, Rhod 5N, Indo-1 AM) to measure: membrane potential, redox state, and Ca2+ levels. Electron microscopy to assess morphology 0-24m: Isogenic controls CRISPR spacers to replace expanded CAG repeat with normal length sequence in a patient line and insert an expanded repeat into a control line. Confirm genetic correction with PCR 18- 36m: Astrocyte-neuronal interaction Differentiate astrocytes: Precursors cycle in FGF2 for >80d then terminal differentiation with BMP4 and LIF Phenotype: Reactive state using process length, GDNF and GFAP upregulation. Co-cultures of iPSC-astrocytes and MNs, to examine Ca2+ regulation and mitochondria function 28-36m: Therapeutics Test potential therapeutics which may ameliorate MN dysfunction as described in the experiments above

Types:
Fellowships

Member States:
United Kingdom

Diseases:
Spinal muscular atrophy (SMA)

Years:
2016

Database Categories:
N/A

Database Tags:
N/A