Study of early changes in structure and composition of neuromuscular junctions in different muscle fiber types during amyotrophic lateral sclerosis

Sujet proposé par

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M2 Research project title

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Keywords

neuromuscular junction, motor neuron, myofiber, MuSK/LRP4, amyotrophic lateral sclerosis

Description of the project

Amyotrophic lateral sclerosis (ALS) is the major adult-onset motor neuron (MN) disease, characterized by the progressive loss of MNs. Patients develop extensive muscle atrophy and weakness leading to paralysis. Death usually occurs by respiratory failure typically within 2-5 years after symptoms onset. ALS has long been viewed as a “dying forward” MN disease where the disruption of the neuromuscular junction (NMJ), the synapse between a MN and a skeletal muscle fiber, and muscle changes were almost exclusively interpreted as a secondary consequence of the progressive loss of MNs. More recently, increasing evidence has suggested that a "dying back" process, where degeneration starts distally at the nerve end and NMJ, and progresses toward the MN cell body, may operate in ALS. In support of this “dying back” hypothesis, pathological changes occur at NMJs and muscles, at very early stages of the disease, prior to MNs degeneration and onset of clinical symptoms. Another hallmark of ALS is that fast-fatigable MNs innervating a subtype of fast-twitch muscle fibers are most vulnerable and degenerate first, whereas fast fatigue-resistant and slow MNs degenerate at later disease stages. However, to date, the structural and molecular changes that occur at the NMJs during the presymptomatic phase, in particular at early stages before the first signs of MN denervation, and their evolution with time, have not been consistently examined and are still poorly known. More, it is still unknown whether the differential vulnerability of fast and slow MNs is due to differential changes in the structure and molecular composition of fast and slow NMJs. Thus, to better understand the mechanisms underlying NMJ pathology and their relationship to the differential vulnerability among MN subpopulations we propose in the present project to undertake a systematic comparative analysis of the changes in NMJ structure and composition of fast and slow myofibers throughout the presymptomatic phase of ALS. This should help to identify molecules involved in NMJ function and stability that could serve as early biomarkers or molecular targets for new therapeutic treatments. We will use the SODG93A ALS mouse model, that expresses mutated human SOD1 (superoxide dismutase-1), crossed with a rainbow transgenic line that allows to visualize the different myofiber types of a muscle according to their fluorescent color. The aims will be to compare at different presymptomatic ages between WT and SODG93A mice: the structure of NMJs on different fast and slow myofibers and the expression of key synaptic proteins, such as the MuSK/LRP4 (Muscle specific kinase/Low-density lipoprotein receptor-related protein 4) muscle receptor complex, for NMJ stability in fast and slow NMJs. According to the progress of the work, the student may also participate to the transcriptomic analysis of subsynaptic nuclei from different fast and slow muscles using single-nucleus RNA sequencing, in collaboration with the team head by Pascal Maire (Institut Cochin).

Methods and techniques

For this project the student will have to perform muscle dissection, cryosections and laser microdissection of neuromuscular junctions and use molecular biology and biochemistry techniques as well as immunohistochemistry and confocal microscopy for neuromuscular junction analysis.

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