Elucidating the role of oligodendrocytes in amyotrophic lateral sclerosis by the use of induced pluripotent stem cells

Acronym: ALS_OL

Principal Investigator: Catherine Verfaillie

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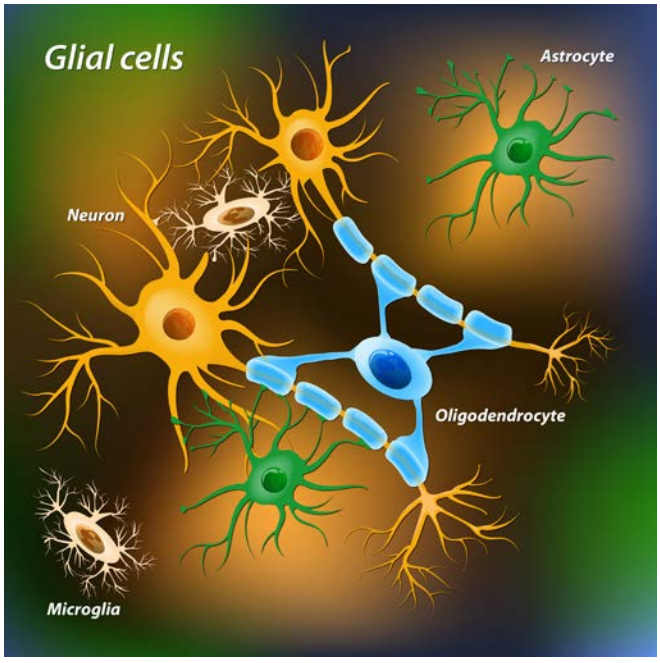
Duration: two years

Summary of the research project

Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease characterized by loss of both upper and lower motor neurons which give electrical signals to the muscle. Consequently, muscles are not properly innervated which causes shrinkage of the muscle and muscle weakness. Most ALS patients die within 5 years from diagnosis due to failure of the respiratory muscles (1). Most ALS cases are sporadic (90%), while 10% of the ALS patients have a familial form of the disease and carry disease-causing mutations. Important mutations contributing to the disease are found in the genes superoxide dismutase 1 (*SOD1*), fused in sarcoma (*FUS*), TAR DNA-binding protein 43 (*TDP43*) and *C9ORF72* (2). These ALS-associated mutations provide a starting point for investigating possible pathogenic pathways causing degeneration which might also contribute to the sporadic form of ALS, which could then yield innovative ways for treatment of the disease.

The main target cells in ALS are motor neurons, as the mutated genes cause oxidative stress and dysfunction of mitochondria, alter RNA metabolism, cause increased electrical activity, defects in transport along the axon, and formation of protein aggregates; all processes that contribute to the degeneration of these neurons (3). However, abnormalities in neuron-supporting cells, such as astrocytes and microglia, also contribute to motor neuron degeneration. *SOD1* mutated astrocytes for example, lose their protective and supportive effect towards motor neurons. Likewise microglia, the immune cells of the central nervous system, can be chronically activated during ALS stress which is toxic for motor neurons (4). Recently, defects in another neuron-supportive cell, the **oligodendrocyte**, was found to contribute to ALS disease. For instance, the same aggregates found in neurons have also been found in oligodendrocytes in the spinal cord of ALS patients (5). Oligodendrocytes isolate motor neurons via myelination to allow fast



conduction of the electrical signal along motor neurons. Moreover, they metabolically support motor neurons by providing lactate as important energy source.

The exact role of mutant oligodendrocytes in ALS is not yet fully understood. Although it is hypothesized that ALS-linked mutations make oligodendrocytes more vulnerable to oxidative stress. This initial oligodendrocyte damage is then intensified by the damaging environment created by activated astrocytes/microglia and degenerating motor neurons. In this way, a vicious cycle is created because degenerating MNs are damaging to oligodendrocytes while damaged oligodendrocytes are detrimental for MNs. As a compensatory mechanism, oligodendroglial precursor cells become activated and start proliferating to replace the lost oligodendrocytes. However, this damaging environment impairs the differentiation capacity of oligodendroglial precursor cells resulting in dysfunctional and immature oligodendrocytes (6).

To gain more insights into the pathological role of oligodendrocytes in ALS, the use of induced pluripotent stem cells (iPSCs) is a very good tool, as these pluripotent stem cells can be generated from ALS patients and subsequently differentiated towards oligodendrocytes *in vitro*. In 2016, Ferraiulolo *et al* generated via this route oligodendrocytes from sporadic ALS patients as well as familial cases with mutated *C9ORF72*, *TDP43*, *SOD1* and factor-induced gene 4 (*FIG4*). These ALS-associated oligodendrocytes induced motor neuron death by decreased lactate release and cell-cell interactions (7). The Verfaillie lab has generated a method to differentiate iPSC to 100% pure oligodendrocytes within 20 days, which is substantially faster and more robust than published so-far (Garcia-Leon, et al., under revision). Using this protocol, we want to investigate more thoroughly the role of ALS oligodendrocytes and their contribution to the MN degenerative pathology.

First, we want to characterize iPSC-derived oligodendrocytes carrying ALS-linked mutations to evaluate abnormalities in protein aggregation, electrophysiology and metabolism. Importantly, *FUS* mutated oligodendrocytes will be included as this ALS-causing mutation was not addressed by Ferraiuolo *et al*. Second, toxic and/or non-supportive effects of *FUS* mutated oligodendrocytes towards motor neurons will be investigated via co-culture experiments. Lastly to get a complete overview of possible pathways involved in oligodendrocyte dysfunction in ALS, the transcriptome of ALS-mutated oligodendrocytes will be mapped via RNA sequencing. After validating the most important deregulated transcriptional networks, new targets might be identified that can be modified by specific drugs to interfere with oligodendrocyte-based toxicity for motor neurons in ALS.

References

1. Salameh, J.S. *et al*. Amyotrophic lateral sclerosis: review. *Semin Neurol*. **35**, 469-476 (2015).
2. Renton, A.E. *et al*. State of play in amyotrophic lateral sclerosis genetics. *Nat Neurosci.* **17**, 17-23 (2014).

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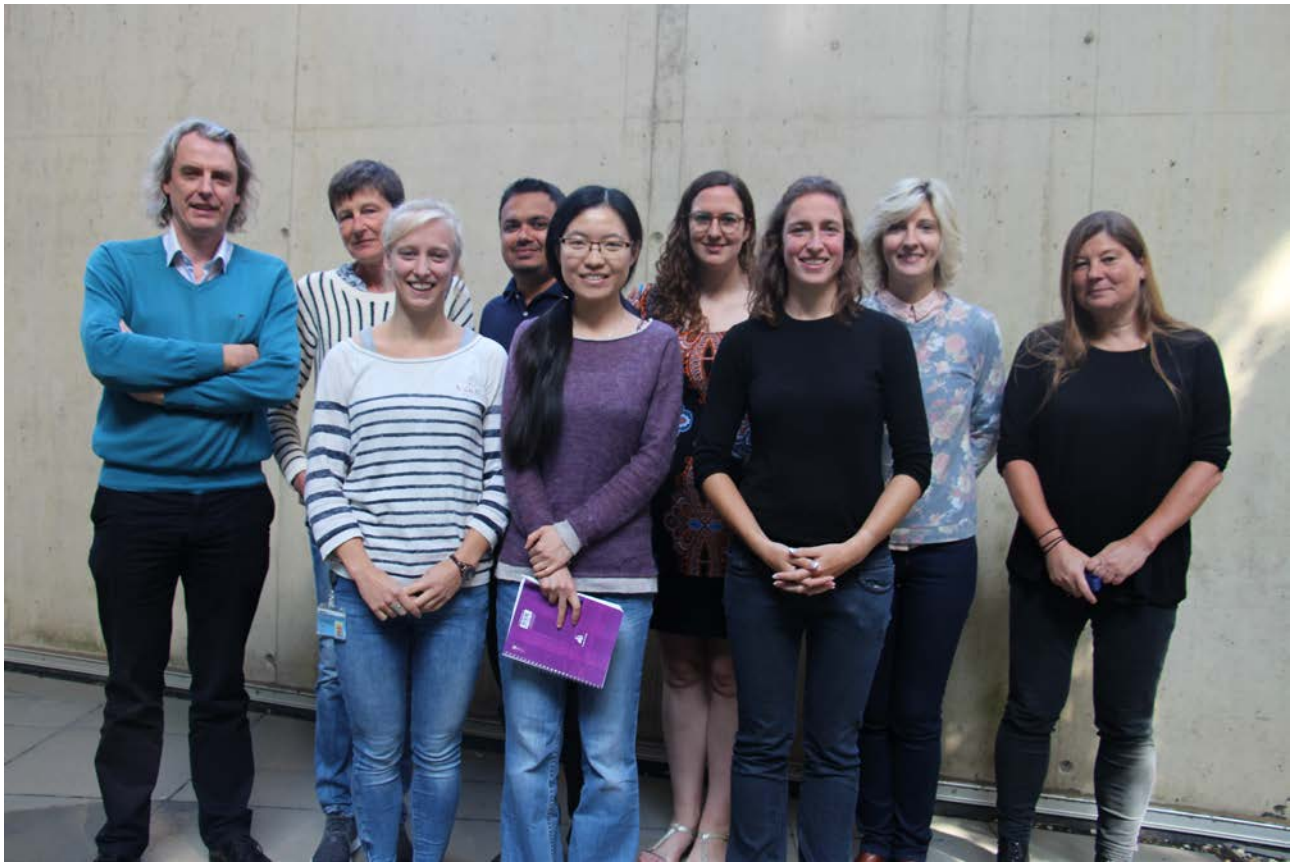
3. Shaw, P.J. Molecular and cellular pathways of neurodegeneration in motor neurone disease. *J Neurol Neurosurg Psychiatry* **76**, 1046-1057 (2005).
4. Lee, J. *et al*. Astrocytes and Microglia as Non-cell Autonomous Players in the Pathogenesis of ALS. *Exp Neurobiol* **25**, 233-240 (2016).
5. Seilhean, D. *et al*. Accumulation of TDP-43 and alpha-actin in an amyotrophic lateral sclerosis patient with the K17I ANG mutation. *Acta Neuropathol* **118**, 561-573, (2009).
6. Nonneman, A., Robberecht, W. & Van Den Bosch, L. The role of oligodendroglial dysfunction in amyotrophic lateral sclerosis. *Neurodegener Dis Manag* **4**, 223-239, (2014).
7. Ferraiuolo, L. *et al*. Oligodendrocytes contribute to motor neuron death in ALS via SOD1-dependent mechanism. *Proc Natl Acad Sci U S A* **113**, E6496-E6505, (2016).



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