Modeling ALS with patient-derived pluripotent stem cells

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Grant: € 60 000

Amyotrophic Lateral Sclerosis (ALS) is one of the most devastating diseases in neurology. The majority of ALS cases has no obvious family history and is referred to as sporadic ALS while a small percentage of patients have a family history of ALS, referred to as familial ALS. Most of our understanding of the molecular causes of ALS has come from genetics studies in patients with familial ALS, which have led to the identification of twelve loci and eight genes for this form of the disease. Sporadic ALS is considered a complex multifactorial disease with interplay of multiple susceptibility or disease modifying genes and a variety of environmental risks affecting susceptibility and its clinical expression. In sporadic ALS a number of susceptibility genes have been identified using the candidate and genome-wide approaches. Yet the molecular mechanisms that trigger disease onset and progression remain unknown, largely due to the lack of tractable model systems for the study of the disease using human primary cells.

The similar clinical and pathological presentations of familial and sporadic ALS imply common pathways leading to motor neuron degeneration. ALS associated genes suggest an interplay of distinct pathways in disease development: axonal transport (*KIFAP3*, *DCTN1, UNC13A* and *SOD1*), RNA metabolism (*FUS, TDP-43, ELP3, SMN* and *ANG*), oxidative stress (*SOD1* and *HFE)* and angiogenesis (*VEGF* and *ANG*). Environmental or lifestyle factors may include smoking, premorbid excessive physical activity, and exposure to heavy metals, formaldehyde and electromagnetic fields. How these factors and pathways interact, and whether these pathways are truly causal to ALS, remains to be elucidated.

An important breakthrough in our understanding of ALS pathogenesis was the identification of the 43 kDa TAR DNA-binding protein (TDP-43) as a major component of ubiquitinated protein aggregates found in 95% of patients with ALS. TDP-43 immunoreactive inclusions are observed in the cytoplasm and nucleus of both neurons and glial cells. Interestingly, mutations in the gene encoding for TDP-43 cause ALS in a minority of FALS patients. With the exception of patients with familial ALS caused by *SOD1* and *FUS* mutations, TDP-43 inclusions are now recognized as a common characteristic of sporadic and familial ALS patients, and provide thereby potentially an exciting link between the pathophysiology of sporadic and familial ALS.

Most research effort in elucidating the molecular basis of ALS has been put in studying mice carrying mutations in the SOD1 gene (found in 20% of the FALS patients = 1% of ALS patients). However, SOD1 mutations have not been shown to play a major role in sporadic ALS. Thus additional pathways must exist that lead to the same clinical symptoms. A lack of tractable experimental tools, primarily our inability to study patient motor neurons in vitro, has severely hampered the exploration of the molecular basis of ALS. However, new developments in stem cell research offer an exciting novel approach to study this disease.

In our proposal we plan to use a novel approach using stem cells to model the disease in the laboratory. New technologies now allow the generation of stem cells from patient's fibroblasts, generating stem cell lines that carry the genetic footprint of motor neuron diseases such as ALS. Until recently, working with human stem cells posed a real challenge since, unlike mouse stem cells, human cells are not very amendable to genetic manipulation. Our lab has recently made an important improvement on the method of creating so-called human induced pluripotent stem cells (iPS cells), which greatly facilitates transgenesis in human stem cells . Pluripotent stem cells have the ability to give rise to any cell type in the human body, including neurons and glia. Pluripotent stem cells allow us for the first time to make neural tissue in vitro, thereby providing an unobstructed view at the molecular pathways that underlie ALS.

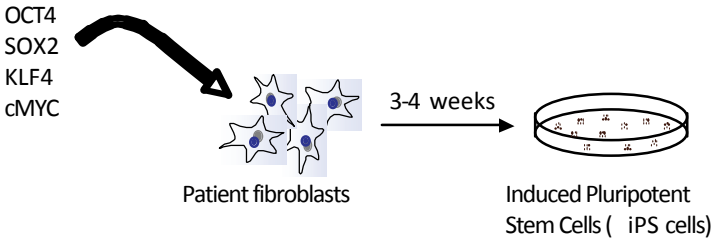

Figure 1: Schematic representation of the iPS procedure
Skin fibroblasts from healthy control- or patient donors are transduced with lentiviral vectors expressing the four reprogramming factors OCT4, SOX2, KLF4 and cMYC. After 3-4 weeks of culture in stem cell medium stem, induced pluripotent stem cell colonies appear that can be propagated and have the ability to differentiate into all somatic cell types including (motor) neurons and glia.

Furthermore, it was recently demonstrated that pluripotent stem cells can be derived from mere skin cells, called **i**nduced **p**luripotent **s**tem cells, or iPS cells (Figure 1). Since patient-derived iPS cells carry the genetic traits of ALS, they can be used to derive neurons and glia in vitro that display the degenerative defects observed in the disease.

Using this method we plan to generate iPS cell lines from patients with familial- or sporadic ALS. Pluripotent stem cells allow the generation of unlimited quantities of these cells and offer for the first time a tractable tool for the investigation of ALS. In addition, the derivation of iPS cells from ALS patients now enables us to make pluripotent stem cell lines that carry the genetic predisposition for the disease and enable us to test the causal relationship between various molecular pathways and ALS motor neuron degeneration. Finally, the above method for the genetic manipulation of human pluripotent stem cells we recently developed, uniquely allows us to generate reporter cell lines for the isolation of motor neurons and glial cells from differentiating stem cells as well as to interrogate how changes in gene expression, or specific mutations lead to the motor neuron death observed in patients with ALS.

The Department of Neurology of the UMC Utrecht has been active in genetics research in ALS and has established a unique national ALS database and biobank containing cells from more than 1,500 patients. This biobank includes fibroblast cell lines of more than 150 patients with various genetic backgrounds, which are already consented an approved for research, providing us with an unprecedented toolbox of diverse patient samples to model and explore the disease. The combined expertise of the cell biobank

at the Utrecht Medical Centre and the new technologies developed at our labs at Harvard University and now transferred to the Hubrecht Institute in Utrecht, allow the derivation of ALS patient-specific iPS cell lines, a promising and essential new approach to model this disease in a laboratory setting. In addition, our recent findings with human iPS cells uniquely facilitate the generation of specific genetic mutations in these human stem cell lines.

The results of these experiments may yield new insights in the molecular pathways that underlie ALS disease progression and potentially will form the initial basis to perform high throughput small molecule- and siRNA screens to identify new targets for disease intervention

TEAM

This project is a new collaboration that uniquely combines the expertise of Leonard van den Berg (University Medical Center, Utrecht, The Netherlands, ALS, genetics), Niels Geijsen (Hubrecht Institute, Utrecht, The Netherlands and Harvard Stem Cell Institute, Boston, MA, USA, iPS cells), and Jeroen Pasterkamp (University Medical Center, Utrecht, The Netherlands, HT screening, Molecular Neurobiology). The combined expertise provides a unique platform for the study of ALS and will ultimately lead to the development of new therapeutic targets for this devastating fatal disease.