The contribution of macrophage migration inhibitory factor (MIF) as a modifier of familial amyotrophic lateral sclerosis (ALS)

1st supervisor: Prof. Dr. med. Susanne Petri

1. Summary

Amyotrophic lateral sclerosis (ALS) is a late-onset fatal neurodegenerative disease characterized by the loss of upper and lower motor neurons. About 20% of the familial cases of ALS are caused by dominant mutations in SOD1 gene, which encodes Cu/Zn superoxide dismutase. Interestingly, recent studies have revealed that the SOD1-mediated toxicity is also involved in sporadic ALS cases. Although the aberrant accumulation of misfolded SOD1 is tightly correlated with its neurotoxicity, the mechanism has not been clarified. Mitochondria and endoplasmic reticulum (ER) membranes have been implicated as possible targets for toxicity by several studies reporting a range of dysfunctions and the toxic binding of misfolded SOD1 to mitochondrial and ER targets. However, the mechanism by which mutant SOD1 associates with these membranes specifically from affected tissues remained unknown. It has recently been shown that a cytosolic factor in unaffected tissues is responsible for preventing the accumulation of misfolded SOD1. This factor was identified as the macrophage migration inhibitory factor (MIF). MIF, whose expression was found to be extremely low within the motor neurons, inhibits the association of mutant SOD1 with intracellular organelles and the accumulation of misfolded SOD1. In collaboration with two groups at Ben Gurion University, Israel, we aim to determine the contribution of MIF as a modifier protein in ALS. Specifically, we will determine whether upregulation of MIF in the CNS affects misfolded SOD1 accumulation and disease progression in mouse models of ALS. This will be done using a MIF transgenic mouse which is being generated and by utilizing a gene targeting approach based on peripheral administration of adeno-associated virus (AAV9) driving MIF expression in mouse models of ALS. In addition, we will determine MIF mRNA and protein expression in human CNS tissues from controls and ALS patients. Moreover, using motor neurons from different familial or sporadic ALS patient-derived induced pluripotent stem cells, we will investigate whether upregulation of MIF can rescue the human motor neurons affected by different ALS causing mutations. Finally, we will use directed evolution techniques to engineer MIF variants with enhanced stability, affinity for SOD1 mutants and biological activity in increasing the survival of motor neurons as a possible therapeutic reagent for the treatment of ALS.

Own previous work:
The supervisor Prof. Dr. Susanne Petri has long-term clinical and experimental experience related to motor neuron diseases. The lab focuses on neuropathological studies in human post mortem tissue, studies in primary motor neuron cultures and human ALS patient iPSC-derived motor neurons and preclinical trials in transgenic ALS mice.

Work plan

Aim 1: Use gene targeting as a therapeutic for ALS by peripheral administration of AAV9 (cooperation with A. Israelson, Ben Gurion University)

It will be assessed whether the administration of AAV9 driving MIF expression in a mouse model of ALS will lead to reducing misfolded SOD1 within the CNS and to slowing of ALS disease progression. Cohorts of animals carrying a mutant SOD1G93A transgene (already in our colony) will receive tail vein injections of AAV9 vector carrying MIF at 40 days of age. Control cohorts will be injected with AAV9-GFP virus, and both experimental groups will be followed and analyzed for behavior, disease onset and progression, survival, pathology and viral distribution. The treated mice will be monitored for disease progression using weekly body weight and hind limb grip strength measurements. Survival curves of the two groups will be compared to establish any effect on disease onset and progression. Survival will be
defined by the inability of mice to right themselves 20 seconds after being placed on their sides (at which point the mice will be sacrificed). We will test whether administration of AAV9 together with MIF delays the accumulation of misfolded SOD1 and the inflammatory response, which has shown to be extremely toxic to motor neurons. Furthermore, levels of astrogliosis will be evaluated in the spinal cords (cervical, thoracic, and lumbar) of all groups by analyzing GFAP, S100β, and vimentin. Additionally, MN loss will be quantified. The efficacy of AAV9-mediated increase in our candidate mRNA levels will be determined by quantitative real-time PCR assays of brain and spinal cord samples from a subset of animals from each injected group. The experiments proposed above will determine whether AAV9-mediated gene delivery following noninvasive peripheral injection can affect ALS-like disease course in an animal model of inherited ALS.

**Aim 2: To determine MIF mRNA and protein expression in human CNS tissues**

For the experiments with human post mortem tissue, tissue from ALS patients with a diagnosis of probable or definite ALS according to the El Escorial criteria will be used, after having obtained informed consent by the patient or his/ her relatives as approved by the ethics committee of Hannover Medical School. Control tissue (post mortem brain and spinal cord specimens) has been and will be obtained at autopsies of patients with no evidence of neurologic or psychiatric disease, again after informed consent. To better understand whether changes in MIF mRNA and protein expression also play a role in human sporadic ALS, human post mortem spinal cord and motor cortex tissue from ALS patients without a family history for ALS or any known gene mutation will be analyzed as previously described. Immunohistochemical analyses will study the distribution and cell specific expression patterns of MIF in neuronal and non-neuronal cells. Its mRNA and protein expression will be determined by qRT-PCR and Western Blot analysis.

**Aim 3. To determine whether MIF can rescue motor neurons from ALS patient-derived induced pluripotent stem cells (iPSC)**

We have already analyzed various ALS patient-derived iPSC cell lines with mutations in the SOD1 and FUS genes in detail showing a hypoexcitability phenotype which could be reversed by the potassium channel blocker 4-aminopyridine (4AP) (Naujock et al., 2015). The effects of MIF overexpression in ALS patient-derived iPSC carrying mutation in the SOD1, but also the FUS, TARDBP and C9ORF72 genes as well as in respective isogenic control lines and healthy control iPSCs. ALS-specific MNs will be differentiated starting from the expandable population of iPSC-derived smNPC and compared to healthy control cell lines. To ensure successful maturation of MNs, a detailed characterization using immunocytochemistry and quantitative real-time PCR will be performed. MIF mRNA expression levels in the different ALS and control iPSC-derived motor neurons will be quantified by real-time PCR.

iPSC-derived ALS and control motor neurons as well as healthy control derived cells and isogenic controls will be transfected using the AAV9-MIF construct from A. Israelson’s group. Mitochondrial and cell viability will be assessed by MTT and LDH assays. The impact of MIF overexpression on caspase activation and mRNA expression of unfolded protein response (UPR) markers will be investigated by immunocytochemistry and quantitative real-time PCR. The impact of MIF overexpression on misfolded SOD1 accumulation will be analyzed in mutant SOD1-iPSC derived motor neurons. It will also be assessed whether increased MIF levels can influence ER mitochondria calcium cycle (ERMCC) dynamics using fluorescent calcium imaging.

**Methods:** Induced pluripotent stem cell proliferation and differentiation, calcium imaging, immunocytochemistry, fluorescence microscopy, real-time PCR, in vitro studies in animal models of ALS, behavioural tests
References:


2. In welchem der drei wissenschaftlichen Schwerpunkte des ZSN ist das Projekt lokalisiert?

3 = Störungen motorischer Systeme: Modelle und Klinik

3. Mit welchen anderen ZSN-Gruppen wird im Projekt kooperiert?

AG Prof. F. Wegner (Klinik für Neurologie)
AGs Prof. C. Grothe u. Prof. P. Claus (Institut für Neuroanatomie)

4. Wenn Sie bereits Bewerbungen für dieses Projekt erhalten haben, geben Sie bitte die Namen der Bewerber an.

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5. Weitere Kooperationspartner, die nicht im ZSN sind.

Prof. Dr. Alexander Storch und Dr. Andreas Hermann, Neurologische Klinik, TU Dresden
Dr. Adrian Israelson und Prof. Amir Aharoni, Ben Gurion University, Israel

6. Finanzierung von Projekt und Bewerber: Förderung durch die German Israeli Foundation (GIF) ab 1/2019, vorher/nachher: Mittel der AG Petri