DYSREGULATED PATHWAYS IN SPINOCEREBELLAR ATAXIA TYPE 2 AND ATAXIA TELANGIECTASIA

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Cerebellar ataxias are a group of neurodegenerative disorders primarily affecting the cerebellum. Although causative mutations in several genes have been identified there is currently no cure for ataxias.

The first part of this dissertation is focused on Spinocerebellar ataxia type 2 (SCA2). SCA2 is a dominant ataxia caused by repeat expansion mutations in the *ATXN2* gene, which encodes the protein Ataxin2 (ATXN2). A polyglutamine (polyQ) tract consisting of CAG repeats interrupted by CAA was identified at exon 1 of *ATXN2*. Healthy individuals have between 22 and 23 glutamines, while expansions longer than 33 CAG repeats cause SCA2. The most noticeable symptom that SCA2 patients show is ataxic gait; however, they also show cerebellar dysarthria, dysdiadochokinesia, and ocular dysmetria caused by the progressive cerebellar degeneration.

To model the SCA2 disease, we generated a new mouse model where 100 CAG repeats were introduced in the mouse *Atxn2* gene via homologous recombination. The characterization of this mouse model, *Atxn2*-CAG100-KIN, demonstrated that it reproduces the symptomatology observed in SCA2 patients. These animals showed significant loss of weight over time, brain atrophy, and motor deficits.

In addition, ATXN2 intermediate expansions have been linked to the pathology of Amyotrophic lateral sclerosis (ALS) as a risk factor. ALS is a fatal neurodegenerative disease where the motor neurons in the brain and spinal cord degenerate. A hallmark of ALS is the presence of TDP43-positive inclusions in neurons and glia. Further studies of *post mortem* spinal cord samples from SCA2 patients showed severe and widespread neurodegeneration of the central somatosensory system. Therefore, it was of interest to further investigate the pathology affection of this tissue in the *Atxn2*-CAG100-KIN line and the

relationship between ATXN2 and TDP43. The characterization of the spinal cord pathology via protein quantification, transcript quantification, and immunohistochemistry showed a preferential affection of RNA binding proteins (RBP) in the spinal cord rather than the cerebellum. The ALS-linked factors TDP43 and TIA1 showed time-dependent co-aggregation with ATXN2 in spinal cord sections together with an increase of CASP3 levels. Therefore, this mouse model can help develop new therapies and evaluate their effect in differently affected areas.

A transcriptome data set from *Atxn2*-CAG100-KIN spinal cord samples at the final disease stage of this mouse model showed a strong up-regulation of RNA toxicity-, immune- and lysosome-implicated factors. These data pointed to a pathological reactivation of the synaptic pruning and phagocytosis in microglia. ATXN2-positive aggregates were found in microglia from spinal cord sections of 14-month-old *Atxn2*-CAG100-KIN via immunohistochemistry. The characterization of microglial response and the potentially deleterious effects of the expanded ATXN2 in this cell type could lead to therapies to improve patients' living standards or delay the symptoms' onset.

The second part of this thesis was focused on an autosomal recessive form of cerebellar ataxia, Ataxia Telangiectasia (A-T), with childhood onset. A-T patients show severe cerebellar atrophy manifesting as ataxia when the child starts to walk. The genetic cause of A-T is loss-of-function-mutations in the Ataxia Telangiectasia Mutated gene (*ATM*). ATM is a kinase involved in DNA damage response, oxidative stress, insulin resistance, autophagy via mTOR signaling, and synaptic function.

Working with proteome data from cerebrospinal fluid of 12 A-T patients and 12 healthy controls, we aimed to define novel biomarkers that would allow following the neurodegeneration in extracellular fluid. Additional validation efforts with ~2-month-old *Atm*-knock-out (*Atm*-/-) cerebellar samples helped us to define a scenario were the deficit of vesicle-associated ATM alters the secretion of ApoB, reelin, and glutamate. As extracellular factors, apolipoproteins and their cargo such as vitamin E may be useful for neuroprotective interventions.