SOD1 misfolding and aggregation in ALS

In the light of conformation-specific antibodies

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Akademisk avhandling

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The aim of this thesis was to use antibodies as tools to study the role of disordered and aggregated SOD1 species in ALS. These antibodies recognize epitopes exposed in disordered SOD1 species and hence, discriminate between natively folded SOD1 and the disordered or misfolded protein.

SOD1 is expressed in all cell types, but aggregates of misfolded SOD1 are predominantly found in motor neurons and associated glial cells in the spinal cord of ALS patients. To understand why misfolded SOD1 targets the motor system, we used ELISA and immunocapture methods to quantify soluble SOD1 species in patient-derived cell models of ALS. The highest levels of soluble disordered SOD1 were detected in induced pluripotent stem cell (iPSC)-derived motor neuron and astrocytes cultures (MNACs) compared to fibroblasts, iPSCs and sensory neuron cultures. These results suggest that the selective vulnerability of motor areas to SOD1-ALS could derive from an enhanced burden of disordered SOD1.

To understand factors that might promote SOD1 unfolding, we focussed on the disulfide bond that is required for the stability of natively folded SOD1. Formation of the bond is oxygen-dependent and reduction of the bond promotes SOD1 unfolding. We studied the stability of SOD1 in patient-derived cells exposed to lowered oxygen tensions. This induced increases in disulfide-reduced, disordered mutant and wild-type SOD1. The response was time- and concentration-dependent and more pronounced in MNACs, where even increased aggregation of mutant SOD1 was observed. These results are consistent with the enhanced vulnerability of the motor system in ALS and suggest that conditions causing impaired oxygen perfusion could contribute to the initiation and progression of the disease.

Inclusions containing aggregated misfolded wild-type SOD1 have been found in sporadic ALS (sALS) patients without SOD1 mutations and those carrying mutations in genes other than SOD1. However, other groups have reported contrasting results and the contribution of misfolded wild-type SOD1 to ALS pathology is controversial. Guidelines for preservation, storage, and analysis of tissues under standardized conditions would facilitate the comparison of results between different laboratories. We established an optimized immunohistochemistry protocol to detect misfolded wild-type SOD1 in paraffin-embedded spinal cord samples from sALS patients. We also developed a method to immunocapture disordered SOD1 from frozen post-mortem tissue. High, but variable, levels of disordered SOD1 were detected in spinal cords from sALS patients. Our data support a possible pathological role of misfolded wild-type SOD1 in sALS.

Recent evidence suggests that SOD1 aggregates can induce templated aggregation of disordered SOD1 and spread from cell-to-cell via a prion-like mechanism. To test if antibodies could block this process in vivo, we conducted an immunotherapy study in a model of prion-like spread, where SOD1 aggregate seeds are inoculated into the lumbar spinal cord of SOD1G85R transgenic mice and lead to accelerated disease onset and progression. Novel monoclonal antibodies (mAb) against disordered domains of SOD1 aggregates were developed and validated for their reactivity to disordered and aggregated SOD1 species in vitro and in vivo. Immunotherapy using a mAb against the C-terminal end of SOD1 attenuated the onset and progression of prion-like SOD1 spread. However, no effect was seen on onset, duration or progression of the underlying disease. This suggests that, under the conditions tested, immunotherapy against disordered domains of SOD1 does not affect intracellular aggregation and additional strategies might be needed to reduce intracellular accumulation of misfolded SOD1 aggregation.

In conclusion, we show that conformation-specific antibodies are powerful tools to investigate disordered and potentially pathogenic species of SOD1 in various biochemical, cellular and in vivo contexts. The development of the novel immunocapture strategy could facilitate future research on characterizing SOD1 aggregates from mouse tissues, patient-derived cells or post-mortem tissues with the goal of determining their role in ALS disease pathogenesis.

**Keywords**
ALS, antibodies, SOD1, disordered SOD1, patient-derived models, low oxygen tension, Immunotherapy