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Structural Investigation of SOD1 aggregates in ALS

Identification of prion strains using anti-peptide antibodies

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Akademisk avhandling

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Abstract
Amyotrophic Lateral Sclerosis (ALS) is a fatal neurodegenerative syndrome characterized by progressive degeneration of motor neurons that result in muscle wasting. The symptoms advance gradually to paralysis and eventually death. Most patients suffer from sporadic ALS (sALS) but 10% report a familial predisposition. Mutations in the gene encoding superoxide dismutase-1 (*SOD1*) were the first identified cause of ALS. The disease mechanism is debated but there is a consensus that mutations in this protein confer a cytotoxic gain of function. SOD1 aggregates in motor neurons are hallmarks of ALS both in patients and in transgenic mouse models expressing a mutated form of human SOD1 (hSOD1). Recently, our group showed that SOD1 aggregates are present also in sALS patients, thus indicating a broader involvement of this protein in ALS. Misfolding and aggregation of SOD1 are difficult to study *in vivo* since aggregate concentration in the central nervous system (CNS) is exceedingly low. The aim of this thesis was to find a method circumventing this problem to investigate the hSOD1 aggregate structure, distribution and spread in ALS disease.

Many studies provide circumstantial evidence that the wild-type hSOD1 protein can be neurotoxic. We developed the first homozygous mouse model that highly overexpresses the wild-type enzyme. These mice developed an ALS-like syndrome and become terminally ill after around 370 days. Motor neuron loss and SOD1 aggregate accumulation in the CNS were observed. This lends further support to the hypothesis of a more general involvement of SOD1 in human disease.

A panel of polyclonal antibodies covering 90% of the SOD1 protein was developed by our laboratory. These antibodies were shown to be highly specific for misfolded SOD1. Aggregated hSOD1 was purified from the CNS of terminally ill hSOD1 mice. Disordered segments in aggregated hSOD1 could be identified with these antibodies. Two aggregate strains with different structural architectures, molecular properties, and growth kinetics, were found using this novel method. The strains, denoted A and B, were also associated with different disease progression. Aggregates formed *in vitro* were structurally different from these strains. The results gave rise to questions about aggregate development and possible prion-like spread. To investigate this, inoculations of purified strain A and B hSOD1 seeds was performed in lumbar spinal cords of 100-day old mice carrying a $hSOD1^{G85R}$ mutation. Mice seeded with A or B aggregates developed premature signs of ALS and became terminally ill 200 days earlier than mice inoculated with control preparation. Interestingly, a templated spread of aggregates along the neuraxis was concomitantly observed, with strain A and B provoking the buildup of their respective hSOD1 aggregate structure. The phenotypes initiated by the A and B strains differed regarding progression rates, distribution, end-stage aggregate levels, and histopathology.

To further establish the importance of hSOD1 aggregates in human disease, purification and inoculation of aggregate seeds from spinal cords of ALS patients and mice carrying the $hSOD1^{G127X}$ mutation were performed. Inoculation of both human and mouse seeds as described above, induced strain A aggregation and premature fatal ALS-like disease.

In conclusion, the data presented in this thesis provide a new, straightforward method for characterization of aggregate strains in ALS, and plausibly also in other neurodegenerative diseases. Two different prion strains of hSOD1 aggregates were identified in mice that resulted in ALS-like disease. Emerging data suggest that prion-like growth and spread of hSOD1 aggregation could be the primary pathogenic mechanism not only in hSOD1 transgenic models, but also in human ALS.

Keywords
ALS, SOD1, prion, motor neuron disease, neurodegeneration, strain, seeding, protein aggregation, transgenic mice, peptide antibodies

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