



UMEÅ UNIVERSITET

Umeå University Medical Dissertations, New Series No 1913

Using patient-derived cell models to investigate the role of misfolded SOD₁ in ALS

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Akademisk avhandling

som med vederbörligt tillstånd av Rektor vid Umeå universitet för
avläggande av medicine doktorsexamen framläggs till offentligt
försvar i Major Groove, byggnad 6L,
torsdagen den 28 september, kl. 09:00.
Avhandlingen kommer att försvaras på engelska.

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Organization
Umeå University
Pharmacology and
Clinical Neuroscience

Document type
Doctoral thesis

Date of publication
7th September 2017

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Title
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Abstract

Protein misfolding and aggregation underlie several neurodegenerative proteinopathies including amyotrophic lateral sclerosis (ALS). *Superoxide dismutase 1 (SOD1)* was the first gene found to be associated with familial ALS. Overexpression of human mutant or wild type SOD1 in transgenic mouse models induces motor neuron (MN) degeneration and an ALS-like phenotype. *SOD1* mutations, leading to the destabilization of the SOD1 protein is associated with ALS pathogenesis. However, how misfolded SOD1 toxicity specifically affects human MNCs is not clear. The aim of this thesis was to develop patient-derived, cellular models of ALS to help understand the pathogenic mechanisms underlying *SOD1* ALS.

To understand which cellular pathways impact on the level of misfolded SOD1 in human cells, we established a model using patient-derived fibroblasts and quantified misfolded SOD1 in relation to disturbances in several ALS-related cellular pathways. Misfolded SOD1 levels did not change following reduction in autophagy, inhibition of the mitochondrial respiratory chain, or induction of endoplasmic reticulum (ER)-stress. However, inhibition of the ubiquitin-proteasome system (UPS) led to a dramatic increase in misfolded SOD1 levels. Hence, an age-related decline in proteasome activity might underlie the late-life onset that is typically seen in *SOD1* ALS.

To address whether or not SOD1 misfolding is enhanced in human MNCs, we used mixed MN/astrocyte cultures (MNCs) generated *in vitro* from patient-specific induced pluripotent stem cells (iPSCs). Levels of soluble misfolded SOD1 were increased in MNCs as well as in pure iPSC-derived astrocytes compared to other cell types, including sensory neuron cultures. Interestingly, this was the case for both mutant and wild type human SOD1, although the increase was enhanced in *SOD1* FALS MNCs. Misfolded SOD1 was also found to exist in the same form as in mouse SOD1 overexpression models and was identified as a substrate for 20S proteasome degradation. Hence, the vulnerability of motor areas to ALS could be explained by increased SOD1 misfolding, specifically in MNCs and astrocytes.

To investigate factors that might promote SOD1 misfolding, we focussed on the stability of SOD1 mediated by a crucial, stabilizing C57-C146 disulphide bond and its redox status. Formation of disulphide bond is dependent on oxidation by O₂ and catalysed by CCS. To investigate whether low O₂ tension affects the stability of SOD1 *in vitro* we cultured fibroblasts and iPSC-derived MNCs under different oxygen tensions. Low oxygen tension promoted disulphide-reduction, SOD1 misfolding and aggregation. This response was much greater in MNCs compared to fibroblasts, suggesting that MNCs may be especially sensitive to low oxygen tension and areas with low oxygen supply could serve as foci for ALS initiation.

SOD1 truncation mutations often lack C146, and cannot adopt a native fold and are rapidly degraded. We characterized soluble misfolded and aggregated SOD1 in patient-derived cells carrying a novel *SOD1* D96Mfs*8 mutation as well as in cells from an unaffected mutation carrier. The truncated protein has a C-terminal fusion of seven non-native amino acids and was found to be extremely prone to aggregation *in vitro*. Since not all mutation carriers develop ALS, our results suggested this novel mutation is associated with reduced penetrance.

In summary, patient derived cells are useful models to study factors affecting SOD1 misfolded and aggregation. We show for the first time that misfolding of a disordered and disease associated protein is enhanced in disease-related cell types. Showing that misfolded SOD1 exists in human cells in the same form as in transgenic mouse models strengthens the translatability of results obtained in the two species. Our results demonstrate disulphide-reduction and misfolding/aggregation of SOD1 and suggest that 20S proteasome could be an important therapeutic target for early stages of disease. This model provides a great opportunity to study pathogenic mechanisms of both familial and sporadic ALS in patient-derived models of ALS.

Keywords: ALS, SOD1, patient-derived models, induced pluripotent stem cells, motor neurons, astrocytes, 20S proteasome low oxygen tension, misfolded SOD1.

Language
English

ISBN
978-91-7601-759-3

ISSN
0346-6612

Number of pages
95 + 4 papers