Have you seen?

The ER under rapid fire

Benjamin M Schwenk1 & Dieter Edbauer1,2,3

Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease leading to selective death of upper and lower motoneurons. Clinically, the ALS syndrome is linked to pathogenic mutations in superoxide dismutase 1 (SOD1), though actual molecular mechanisms remain ill understood. Two papers recently published in Cell Stem Cell and Cell Reports employ syngenic, iPSC-derived cell lines of one of the most severe SOD1 mutations to report mitochondrial and ER stress as causal for perturbed electrical activity in ALS neurons (Kiskinis et al, 2014; Wainger et al, 2014).

See also: E Kiskinis et al (April 2014) & BJ Wainger et al (April 2014)

Amyotrophic lateral sclerosis (ALS) patients suffer from progressive paralysis and muscle atrophy and usually die from respiratory failure within a few years after disease onset. About 10% have a family history of ALS and there is considerable overlap with frontotemporal dementia (FTD). More than 20 ALS genes have been identified since the discovery of the first pathogenic mutation in superoxide dismutase 1 (SOD1) in 1993. Now more than 160 SOD1 mutations (mostly missense) with dominant inheritance pattern have been reported in ALS kindreds. A toxic-gain-of-function of the mutated SOD1 protein causes motoneuron degeneration (Al-Chalabi et al., 2012). Virtually all ALS patients have ubiquitin-positive cytoplasmic inclusion in motoneurons. In SOD1 mutation carriers, these inclusions also contain SOD1, in most other cases TDP-43 (Al-Chalabi et al., 2012).

A transgenic mouse overexpressing high levels of SOD1 with the G93A mutation is among the most widely used animal models for neurodegeneration, but several successful preclinical studies in these mice did not translate into clinical breakthroughs. Therefore, new model systems seem necessary to tackle this devastating disease. Two collaborative studies from the Eggan and Woolf laboratory now combined induced pluripotent stem cell (iPSC) technology, genome editing, and electrophysiology to study the impact of the very aggressive SOD1 A4V mutation in patient-derived motoneurons.

A major issue when working with patient-derived cells is high variability between individuals compared to transfected cell lines or inbred transgenic mice. To generate human isogenic controls, the authors used zinc finger nucleases to revert the SOD1 mutation in patient iPSC lines to wild-type (Fig 1A). This approach has been pioneered in the Jänsch laboratory to study pathogenic α-synuclein mutations in iPSC-derived neurons (Soldner et al., 2011) and was recently used to rescue the effects of a TAU mutation (Fong et al., 2013). While Soldner et al. analyzed copy number variation, Kiskinis et al. (2014) further raised the bar by employing whole-genome sequencing to confirm that genome editing and clonal expansion did not introduce confounding point mutations or copy number variations. Given the recent advances in genome editing with the CRISPR/Cas9 system and next-generation sequencing, this powerful approach will undoubtedly become standard especially for neurodegenerative disease, where functional effects are mostly rather subtle.

Together, both papers provide a detailed biochemical and electrophysiological analysis of SOD1 A4V motoneurons compared to isogenic controls. Motoneurons derived from the SOD1 A4V patients have a higher apoptosis rate and smaller cell somata compared to unrelated and isogenic controls, indicating that mutant SOD1 impairs neuronal health even in the absence of aggregation. In order to identify dysregulated molecular pathways, the authors used RNA sequencing in motoneurons derived from SOD1 mutant and corrected iPSCs. Bioinformatic analysis revealed upregulation of the pathways for transcription and intracellular transport in SOD1 A4V motoneurons and downregulation of mitochondrial function and protein translation. Remarkably, 80% of the validated hits were specifically dysregulated in motoneurons, but not in the parental iPSCs emphasizing the importance of models mimicking the exact disease context. Based on the downregulation of the protein translation machinery in SOD1 A4V motoneurons, the authors speculated that ER stress triggering an unfolded protein response (UPR) may cause the neurotoxicity in patients. Consistent with activation of the UPR, the transcription factors ATF4 and sXBP1 were elevated in patient-derived motoneurons. Moreover, siRNA-mediated knockdown of sXBP1 or pharmacological inhibition of ER stress with salubrinal enhanced the survival of SOD1 A4V motoneurons. Compared to other tested cell lines and tissues, wild-type motoneurons already show the highest basal levels of ER stress which is further aggravated by mutant SOD1. This finding may explain the selective vulnerability of motoneurons in SOD1 mutation carriers. Interestingly, ER stress is alleviated by blocking actions potentials with TTX. Vice versa, inhibiting ER stress with salubrinal reduces spontaneous activity in motoneurons. Thus, mutant SOD1 may trigger a vicious cycle of enhanced ER stress and hyperactivity in motoneurons.

Based on these findings, Wainger et al. (2014) analyzed the excitability and membrane currents in the SOD1 A4V motoneurons. Indeed, mutant motoneurons showed more frequent spontaneous action potentials.
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Genetic repair of drugs that break the vicious cycle of ER in SOD1 A4V motoneurons suggesting that trophysiological abnormalities but also over, retigabine not only restored the electroexcitation of the membrane after an action potential. Pharmacological activation of Kv7 potassium channels with retigabine and genetically by isogenic repair of the SOD1 mutation.

Figure 1. Vicious cycle of ER stress and hyperexcitability in SOD1 A4V motoneurons.

(A) Generation of isogenic SOD1 A4V and wild-type human motoneurons: iPSCs from skin fibroblast of a SOD1 A4V ALS-patient were generated and further differentiated into motoneurons. The mutation was repaired in the iPSCs by ZFN-mediated genome editing and the SOD1 A4V and its isogenic control motoneuron line analyzed. (B) Hypothetical model of SOD1 A4V pathogenesis: SOD1 A4V mutation enhances UPR and induces ER stress. ER stress leads to hyperexcitability and hyperactivity of the motoneurons which in turn activates UPR resulting in a vicious cycle causing neuronal death. This cycle can be broken pharmacologically by inhibition of ER stress with salubrinal or activation of depolarizing Kv7 channels with retigabine and genetically by isogenic repair of the SOD1 mutation.

Both papers provide initial data suggesting that their findings in SOD1 A4V motoneurons may extend to other subforms of ALS. Wainger et al show hyperexcitation in motoneurons derived from patients with FUS mutation and C9orf72 repeat expansion using rapid multiphoton microscopy analysis, but unfortunately ER stress and UPR were not analyzed in these cells. Importantly, retigabine also blocks hyperexcitability in motoneurons with FUS or C9orf72 mutation. In contrast, Sareen et al (2013) recently reported decreased excitability in motoneurons carrying the C9orf72 repeat expansion. Thus, these promising initial findings warrant rigorous validation using isogenic cell lines and further study in other ALS models.

Previous work in N2A cells and transgenic mice has revealed signs of ER stress due to SOD1 mutant and also wild-type expression (Turner & Atkin, 2006), which could, however, been attributed to the high levels of overexpression. The new findings confirm these results for endogenous SOD1 level specifically for the mutant protein in a human motoneuron model and strongly suggest that ER stress contributes to SOD1 pathogenesis in patients. Future efforts should focus on the mechanism how ER stress induces hyperexcitability and vice versa, possibly in the absence of SOD1 aggregation. Together, the two studies provide a new model for pathogenesis of SOD1-ALS: Misfolded mutant SOD1 induces ER stress and upregulates the UPR, thus triggering hyperexcitability of motoneurons, which further enhances ER stress and thereby contributing to neurodegeneration (Fig 1B). This model not only causally links the two distinct disease-causing phenomena ER stress and hyperexcitability (Turner & Atkin, 2006; Martin et al, 2013), but further attests the Kv7 agonist retigabine as already FDA-approved compound to ameliorate disease-related symptoms in the iPSC-derived motoneurons. These novel findings highlight the value of isogenic iPSC-derived cellular models as crucial tool to characterize disease pathogenesis and screen potential drug candidates.

Conflict of interest
The authors declare that they have no conflict of interest.
References


