FUScinating insights into motor neuron degeneration

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Point mutations in FUS cause amyotrophic lateral sclerosis (ALS), a devastating neurodegenerative disease—but do they do that by a loss of the protein’s normal function, or by endowing it with novel toxic functions, or both? In this issue of The EMBO Journal, Scekic-Zahirovic et al (2016) report that mutant FUS, but not the complete loss of FUS, triggers motor neuron degeneration in mice, arguing for a toxic gain-of-function mechanism.

See also: J Scekic-Zahirovic et al (May 2016)

Patients who suffer from ALS or a related disorder, frontotemporal dementia (FTD), have characteristic protein aggregates in their brains. These aggregates are found in neuronal and glial cells and contain DNA/RNA-binding proteins, most notably TDP-43 or, less frequently, FUS (Mackenzie et al, 2010). Both TDP-43 and FUS are usually located in the cell nucleus, where they regulate transcription and splicing of numerous target genes (Lagier-Tourenne et al, 2012). However, in ALS/FTD patients, TDP-43 or FUS is no longer found in the nucleus and instead accumulates in cytoplasmic protein aggregates (Mackenzie et al, 2010). Based on this neuropathology, it is thought that loss of either TDP-43 or FUS from the nucleus or aberrant function of TDP-43 or FUS in the cytoplasm, or a combination of both, is toxic to neurons (Ling et al, 2013).

To solve this interesting loss-of-function versus gain-of-function puzzle, three ALS research groups teamed up to study novel FUS mouse models that either express no FUS at all (Fus−/−) or have cytoplasmically mislocalized FUS instead of nuclear FUS (Fig 1). To generate the latter model, they genetically disrupted the protein’s nuclear localization signal (NLS), which is located at the very C-terminus and is required for proper nuclear import (Dormann et al, 2010). Interestingly, the NLS of FUS is also mutated or truncated in some ALS patients—these patients show cytosolic FUS accumulation and suffer from early onset ALS (Kwiatkowski et al, 2009; Waibel et al, 2013). Importantly, the FusANLS/ANLS mice were generated via knock-in of a floxed Stop cassette into the endogenous Fus locus—this elegant strategy not only avoids unwanted overexpression of mutant FUS, but also allows for cell type-specific reversal of the mutation by expression of Cre recombinase.

What consequences are there for mice if they lack FUS altogether or have FUS in the cytoplasm rather than in the nucleus? At first glance, Fus−/− and FusANLS/ANLS mice have remarkably similar phenotypes: Both are born alive, but are slightly smaller than normal mice and die within minutes after birth, due to respiratory problems. They also have overlapping “molecular phenotypes”, as revealed by a systematic comparison of RNA levels and alternative splicing events in Fus−/− and FusANLS/ANLS brains. Thus, deletion of the NLS causes a loss of nuclear FUS function, leading to misregulated gene expression and postnatal death.

However, this is not the whole story—a closer look revealed interesting phenotypic and molecular differences between both mice: Only FusANLS/ANLS−, but not the knock-out mice, show motor neuron apoptosis. Moreover, they show some unique expression or splicing alterations that are not seen in Fus−/− mice. Hence, cytoplasmic mislocalization of FUS does not simply cause a loss of nuclear FUS functions, but triggers additional “gain-of-function” changes. This is toxic to motor neurons, while the complete absence of FUS is not detrimental to this cell type.

Is cytosolic FUS intrinsically toxic to motor neurons, or do neighboring cells contribute to motor neuron loss in FusANLS/ANLS mice? This is an important question, as another major genetic cause of ALS, mutations in SOD1, kills motor neurons by non-cell-autonomous mechanisms (Ilieva et al, 2009). To address this question, Scekic-Zahirovic et al (2016) crossed FusANLS/ANLS mice to ChAT-Cre mice to selectively revert the mutation in motor neurons. This did not rescue the postnatal lethality, but fully rescued motor neuron loss, demonstrating that mutant FUS, in contrast to mutant SOD1, is intrinsically toxic to motor neurons.

The central finding of the present report, that mutant FUS induces cell-autonomous motor neuron loss through toxic gain of function, is in complete agreement with a recently published study that compared a series of targeted conditional FUS transgenic mice with conditional FUS knockout mice (Sharma et al, 2016). They too found that mutant cytoplasmic FUS causes age-dependent motor neuron degeneration, whereas wild-type FUS or FUS knockout leaves motor neurons unaffected.

How exactly cytoplasmic FUS kills motor neurons still remains to be unraveled. First

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hints from Scekic-Zahirovic et al (2016) indicate that it may alter the subcellular localization of FUS interaction partners and may elicit cellular stress. Another possibility is that elevated FUS in the cytosol abnormally augments the cytosolic functions of FUS in mRNA transport, stabilization, and local translation (Bowden & Dormann, 2016). Alternatively, it may drive liquid-to-solid phase transition and thus “solidification” of FUS interaction partners and may elicit cellular stress. Another possibility is that elevated FUS in the cytosol abnormally augments the cytosolic functions of FUS in mRNA transport, stabilization, and local translation (Bowden & Dormann, 2016).

Figure 1. Cytosolic mislocalization of FUS, but not loss of FUS, is toxic to motor neurons
FUS is a ubiquitously expressed DNA/RNA-binding protein that is normally localized in the nucleus, where it regulates RNA expression and splicing. FUS knockout mice (Fus\(^{-/-}\)) show loss-of-function (LOF) gene expression changes and die postnatally, but have normal motor neurons. Mice with a targeted deletion of the nuclear localization signal (Fus\(^{NLS/-}\)) have FUS in the cytosol rather than in the nucleus. This causes a nuclear LOF and postnatal death, but also gain-of-function (GOF) changes that lead to motor neuron degeneration. Motor neuron apoptosis can be rescued by motor neuron-specific reversal of the NLS deletion (CHAT-Cre Fus\(^{NLS/-}\)), demonstrating that cytosolic FUS triggers cell-autonomous motor neuron loss.

References
Liquid-to-Solid Phase Transition of the ALS Protein FUS Accelerated by Disease Mutation. Cell 162: 1066–1077