NEURODEGENERATIVE DISEASE

TDP-43 shapeshifts to encipher FTD severity

TDP-43 forms cytoplasmic aggregates in degenerating neurons of frontotemporal dementia (FTD) patients. Laferrière et al. now establish that TDP-43 assemblies from distinct FTD subtypes have different structures, neurotoxicities, and seeding activities, which correlate with FTD severity. Thus, distinct pathological TDP-43 assemblies akin to prion strains might underpin distinct FTD subtypes.

Edward M. Barbieri and James Shorter

Amyotrophic lateral sclerosis (ALS) and FTD, a type of frontotemporal lobar degeneration (FTLD), are devastating and fatal neurodegenerative diseases for which there are no effective therapeutics[1]. A major hallmark of ALS and FTD is the presence of abnormally phosphorylated TDP-43 protein inclusions in the cytoplasm of degenerating neurons[1,2]. TDP-43 is an intrinsically aggregation-prone, RNA-binding protein with a C-terminal prion-like domain that usually resides inside the nucleus[1,2]. Thus, the cytoplasmic mislocalization and aggregation of TDP-43 may contribute to disease. Despite this common neuropathology, there exists a large degree of unexplained diversity in the histological and clinical presentations of patients and what appears to be a clinical spectrum of diseases ranging from ALS to FTD[1,2]. Deciphering the molecular basis for this disease heterogeneity could set the stage for personalized therapeutics aimed at specific disease subtypes.

So where does all of the disease heterogeneity come from? An answer to this puzzling question might already be suggested from pioneering advances made in infectious protein (prion) biology. Here, it is understood that different prion conformers, referred to as prion strains, can encode different phenotypes[3,4]. Likewise, for the ALS-to-FTD spectrum, it has been proposed that alternate pathological conformations of TDP-43 could elicit the various clinical presentations[5–7], but the lack of dependable methods for separating pathological assemblies of TDP-43 from physiological forms of TDP-43 has vexed researchers aiming to assess this hypothesis. In this issue of *Nature Neuroscience*, Laferrière et al. deconstruct this critical barrier and present a novel extraction method for isolating pathological TDP-43 conformers from patient brains that enables high-quality downstream analyses[8]. Using their technique, called SarkoSpin, to study brain cortical samples from over 80 patients, the authors performed a series of elegant biochemical and cell-based experiments

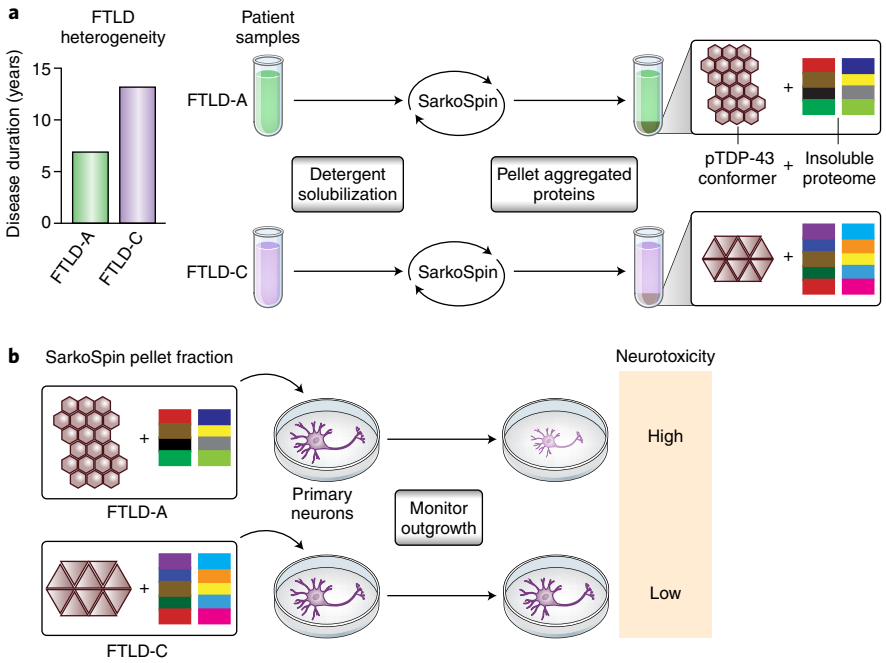

Fig. 1 | SarkoSpin enables isolation of distinct pathological TDP-43 assemblies from FTLD subtypes that capture disease subtype heterogeneity. a, FTLD subtypes A and C exhibit very different mean disease durations. Autopsy material from FTLD-A and FTLD-C patients are subjected to the SarkoSpin procedure consisting of detergent solubilization and subsequent centrifugation to separate the detergent-soluble from insoluble fractions. The pellet fractions from each subtype are enriched with hyperphosphorylated TDP-43 (pTDP-43) in different pathological conformations. Each subtype also displays a distinct panel of insoluble proteins beyond pTDP-43. Data adapted from ref. [8], Nature Publishing Group. **b**, Addition of the isolated SarkoSpin pellet material to primary neurons in culture recapitulates the relative neurotoxicities observed in patient cohorts.

with the isolated pathological assemblies of TDP-43[8]. This innovative approach revealed distinct forms of pathological TDP-43 with varied biochemical features, which manifest in distinct toxicities to neurons in culture that correlate with the FTLD subtypes whence they came[8] (Fig. 1a,b).

Pathological forms of TDP-43 accumulate in large detergent-resistant complexes with nucleic acids[2]. Thus, the authors developed a facile extraction scheme that combines detergent solubilization with nuclease treatment followed by a single centrifugation step[8]. When performed on motor and frontal cortex homogenates from controls versus FTLD subtypes, the resultant detergent-insoluble fractions contained ~0.1% and ~12% of the total TDP-43, respectively[8], suggesting that pathological forms of TDP-43 were specifically enriched[8]. Indeed, detergent-insoluble fractions from FTLD patients were enriched for hyperphosphorylated TDP-43, another indication that pathological TDP-43 conformers had been isolated[8]. But what about the vast pool of other RNA-binding

proteins containing prion-like domains with high aggregation propensity[5]? Were these also enriched in detergent-insoluble fractions from FTLD patients? Remarkably, more than 99.8% of these proteins were found in the supernatant, highlighting the specificity of the approach[8].

Armed with the newfound ability to isolate disease-associated assemblies of phosphorylated TDP-43, the authors performed a series of biochemical characterization experiments that exposed stark differences between TDP-43 forms isolated from different FTLD subtypes[8] (Fig. 1a,b). TDP-43 isolated from different FTLD subtypes showed distinct size distributions, density profiles, and alternate morphologies never before characterized[8] (Fig. 1a). By coupling SarkoSpin to liquid chromatography–mass spectrometry, they defined the specific insoluble proteome and TDP-43 ubiquitination signatures associated with the FTLD subtypes[8] (Fig. 1a). Based on these data, it is possible that the biochemical properties of different pathological forms of TDP-43 and the unique subset of associated insoluble proteins may serve to 'barcode' each disease subtype[8].

This study would be a cliffhanger without an attempt to close the causal loop between the distinct TDP-43 conformers and the heterogeneity observed in FTD subtypes. Fortunately, Polymenidou and co-workers do not disappoint[8]. They engineered a stable cell line expressing a tagged TDP-43 and challenged these cells with SarkoSpin pellets isolated from a control sample and from two FTLD subtypes (A and C) that manifest with significantly different disease durations[8]. FTLD subtype A progresses more rapidly than subtype C[8] (Fig. 1a). The rationale behind this approach is that the pathological TDP-43 assemblies might act as protein 'seeds' in much the same way that prions do, which could convert the physiological form of TDP-43 into toxic aggregated conformers[5]. Interestingly, they observed increased seeding activity and reduced viability for cells inoculated with insoluble fractions from type A but not from type C or from the control[8], which is consistent with the relative severity of disease durations in patients (Fig. 1a,b). This reduction in cell viability was accompanied by an increase in tagged TDP-43 in newly induced aggregates for type A but not in type C or in the control[8]. The authors confirmed these findings in mouse primary cortical neurons using calcein staining to quantify the number of live neurons after inoculations with different doses of SarkoSpin aggregates[8]. They also assessed neurotoxicity by measuring aggregate-induced changes in neurite length[8]. Thus, TDP-43 assemblies isolated from FTLD type A, the more severe form of disease, displayed enhanced toxicity and seeding activity in cells in culture[8] (Fig. 1a,b).

These toxicity assays provide an exciting platform in which potential therapeutic interventions can be tested, engineered, and weaponized against damaging ex vivo TDP-43 conformers[8]. For example, it will be enlightening to establish whether ataxin-2 antisense oligonucleotides[9], TDP-43 disaggregases[10,11], or poly-(ADP-ribose) polymerase (PARP) inhibitors[12,13] antagonize TDP-43 toxicity in this setting. In this way, potential therapeutics could be challenged with disease-relevant TDP-43 conformers.

Although the differences in cytotoxicity between TDP-43 conformers isolated for FTLD subtypes A and C correlate with the apparent seeding activity, this result begs the question of whether the additional panel of insoluble proteins that are unique to each subtype might also contribute to the differential toxicity (Fig. 1a,b). A strong case is presented for the TDP-43 seeding hypothesis, but effects from the rest of the insoluble proteome in the SarkoSpin fractions cannot yet be entirely ruled out. It will be interesting to determine whether SarkoSpin pellets isolated from cells exposed to pathological TDP-43 seeds consistently show the same complement of accompanying insoluble proteins beyond TDP-43 for each subtype. If true, such a finding would strongly support the case that different conformations of pathological TDP-43 can drive distinct and predictable downstream events leading to different forms of disease.

In a similar vein, the TDP-43 assemblies isolated from patients could be used to seed pure, recombinant TDP-43 to generate synthetic TDP-43 conformers devoid of other components. One could then determine whether these pure and distinct TDP-43 assemblies display properties akin to those of different prion strains and elicit different neurodegenerative phenotypes when introduced into neurons in culture or mice[3–7]. Moreover, it will be important to assess whether various TDP-43 disaggregases can dissociate the purified TDP-43 assemblies and recover functional TDP-43 from these disease structures[10,11]. These purified TDP-43 assemblies might also serve as valuable substrates to engineer and optimize protein disaggregases able to therapeutically dissociate them[11].

It will also be fascinating to compare the biochemical and structural properties of TDP-43 assemblies isolated from FTLD patients to functional TDP-43 assemblies, termed myogranules, which assemble in the cytoplasm of regenerating muscle[14]. TDP-43 myogranules appear to play a critical role in muscle regeneration after injury, but are cleared once regeneration is complete[14]. Interestingly, isolated TDP-43 myogranules display amyloid-like properties and can seed the fibrillization of recombinant TDP-43[14]. It will be important to determine what differentiates these beneficial TDP-43 assemblies from their pathological counterparts.

Perhaps the most intriguing implication of the Laferrière et al. study is the prospect that distinct TDP-43 assemblies isolated with SarkoSpin could serve as seeding material to propagate specific pathological TDP-43 conformations and disease phenotypes in neurons and even mouse models. If this possibility could be realized, it would ultimately enable robust animal models for ALS and FTD subtypes, which would be invaluable resources for basic studies and to assess therapeutic interventions. Timely work from Porta et al.[15] has now demonstrated that injection of FTLD-patient-derived brain extracts (generated using a different protocol than SarkoSpin) into mice induces de novo TDP-43 pathology that progressively spreads throughout the mouse brain in a manner characteristic of cell-to-cell propagation of self-templating conformers. Despite this seeding activity, no toxicity in cell culture or pronounced neurodegeneration in mice was reported[15]. By contrast, TDP-43 conformers isolated by Laferrière et al.[8] from FTLD subtype A were toxic to cells and neurons in culture. This difference could reflect differences in extraction protocol, study design, or both[8,15]. Nonetheless, combining SarkoSpin with subsequent injection of isolated TDP-43 assemblies into mice could enable measurement of subtype-specific spreading of toxic TDP-43 assemblies in vivo and could provide deeper insights regarding disease heterogeneity in patients.

In the future, it will be exciting to apply SarkoSpin to additional ALS and FTD subtypes to assess whether the disease spectrum between ALS and FTD can be further refined by the detection of new pathological TDP-43 conformer subtypes and their accompanying insoluble proteomes. As the diagnostic criteria and clinical presentations for these diseases becomes increasingly stratified, combining this clinical information with a higher-resolution understanding of the underlying molecular signatures and causes of each disease form could empower a more personalized approach to therapeutics in patient subpopulations. Indeed, the

pieces are now in place to understand the mechanisms by which specific TDP-43 conformers might cause neurodegeneration and how they might be therapeutically counteracted or eliminated. ❐

Edward M. Barbieri and James Shorter*
Department of Biochemistry and Biophysics, Perelman School of Medicine at the University of Pennsylvania, Philadelphia, PA, USA.
**e-mail: jshorter@pennmedicine.upenn.edu*

Published online: 17 December 2018
https://doi.org/10.1038/s41593-018-0299-6

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Competing interests

The authors declare no competing interests.

NEUROMODULATION

LC modulation of sensory feature selectivity

The locus coeruleus is known to be an essential source of neuromodulation that influences sensory processing, including the enhancement of feature selectivity associated with attentional focus. A new study shows that the primary sensory thalamus encompasses one circuit that underlies this enhancement.

Tingting Zhou and Michael M Halassa

Behavioral states, such as arousal, locomotion and attention, exert heavy influences on sensory processing and perception[1–4]. Imagine that you are at a seminar: your ability to discern the details of the ongoing presentation may fluctuate quite a bit depending on changes in your internal state. How are sensory systems informed of changes in internal state? It is well known that neurons in the locus coeruleus (LC) can reflect changes in attentional state[5,6] and that their output broadcast as neuromodulatory norepinephrine signals influences sensory processing[7,8]. Among changes in sensory processing is enhancement of the feature selectivity of incoming signals, a key ingredient of selective attention and perceptual decision-making[9,10]. Because feature enhancement can occur at multiple levels of the sensory processing hierarchy, such as brainstem, thalamus and cortex[11,12], it has been unclear exactly where the attention-enhancing effects of the LC are realized.

In this issue of *Nature Neuroscience*, Rodenkirch et al.[13] use the rat somatosensory system to address this critical issue. Using a variety of experimental and modeling approaches, they show that the effect of LC activation on the enhanced feature selectivity of neurons in the ventral posteromedial (VPm) thalamus is intrinsic to the thalamus, as it is neither observed in presynaptic inputs from the principal trigeminal nucleus (PrV) nor impacted by inactivating cortical feedback (Fig. 1).

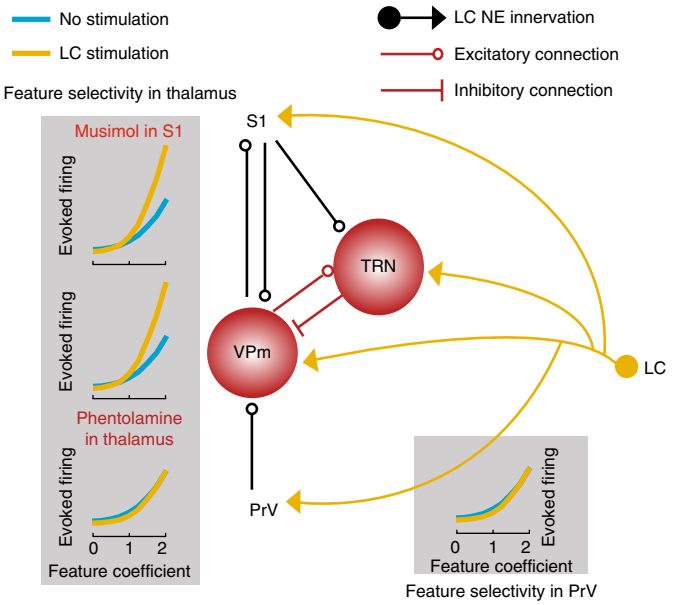

Fig. 1 | LC modulation of sensory thalamic feature selectivity. LC activation enhancement of sensory feature selectivity of VPm neurons is not inherited from its subcortical inputs, as such effects are not observed in PrV neurons. These effects are also independent of cortical inputs, as muscimol infusion into primary somatosensory cortex (S1) does not attenuate them. The effects are direct, as localized infusion of the α-adrenergic receptor antagonist phentolamine eliminates feature enhancement following LC stimulation. NE, norepinephrin.

Rodenkirch et al.[13] started their investigation by recording VPm neural responses to white Gaussian noise whisker deflections, allowing them to precisely determine the feature selectivity of each neuron on the basis of a linear–nonlinear Poisson (LNP) model of stimulus-evoked spikes. Using either electrical or optogenetic stimulation, they found that VPm responses to the preferred sensory feature were