Glia as primary drivers of neuropathology in TDP-43 proteinopathies

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A long-standing mystery has been understanding why neurons die in devastating neurodegenerative disorders, such as Alzheimer's disease, Parkinson disease, and amyotrophic lateral sclerosis (ALS). Abnormal intracellular protein aggregates are a hallmark feature of many neurodegenerative conditions. Based on this fundamental observation, numerous studies have attempted to shed light on the causality dilemma between protein aggregation and neurodegeneration. For many years, a neuron-centric mentality has dominated this field of research, generating hypotheses that ectopic protein aggregates within neurons act as toxic nidi, culminating in the cell-autonomous degeneration of susceptible neurons. However, this strict attention to neuronal pathology is beginning to broaden. New disease models are revealing novel mechanisms of neurodegeneration in which the glial environment, where a predisposed neuron resides, serves an equally contributory role in disease progression (1). Now, recent work published in PNAS, shows that glial cells unexpectedly can play a much more fundamental—even primary—role in driving neurodegenerative disease (2).

Seven years ago, TAR DNA-binding protein 43 (TDP-43) was identified as the major

constituent of the proteinaceous inclusions in the most predominant subtypes of ALS and fronto-temporal lobar degeneration (3). ALS is the most frequent form of motor neuron (MN) disease in which the loss of both upper and lower MNs in the brain and spinal cord invariably leads to fatal paralysis and death, and fronto-temporal lobar degeneration is currently the second leading cause of presenile dementia (4). The TDP-43 subtypes of these disorders, which are now thought to share common etiologies, are grouped under the broader classification of "TDP-43 proteinopathies." Although our clinical understanding of TDP-43 proteinopathies is based upon specific patterns of neuronal dysfunction, studies on postmortem human specimens have revealed that cytologic pathology almost universally involves glial proliferation and activation as well. Recent findings, particularly in ALS models, have shown that glial pathology may play a critical role in disease progression via secondary astrocyte and microglial activation (5), but Serio et al. (2) are unique in demonstrating the existence of a cell-autonomous pathological phenotype in human astrocytes derived from patients with ALS-causing TDP-43 mutations.

The majority of ALS cases are sporadic, and before the discovery of TDP-43, there

were no identifiable genetic leads for the most prevalent manifestation of ALS. The first clues about the pathophysiology of ALS instead arose from the study of a small number of patients who have a positive family history for the disease (~5% of all ALS cases). Unlike in sporadic ALS patients, a subtype of familial ALS cases are a result of mutations in the Cu/Zn superoxide dismutase (SOD1) protein, the first identified pathologic ALS gene (6). Before the identification of TDP-43, SOD1-mediated neurotoxicity provided the predominant genetic model for understanding ALS. Mice that ubiquitously express multiple copies of the mutant human SOD1 transgenes demonstrate numerous features of MN dysfunction and ALS pathology (7). However, a remarkable clue emerged from experiments investigating generations of chimeric mice comprised of mixtures of wild-type and SOD1 mutant cells. Mutant SOD1-expressing MNs that were surrounded by wild-type nonneuronal cells had significantly less severe pathology than the reverse scenario, in which normal MNs were confined by mutant SOD1 nonneuronal neighbors (8). These results have been corroborated in two influential *in vitro* studies in which cultured rodent mutant SOD1 astrocytes exhibited soluble toxic effects against normal MNs. In agreement with the chimeric mouse data, the presence of mutant SOD1 exclusively in MNs resulted in significantly delayed disease pathology (9, 10). Collectively, these experiments suggest a non-cell-autonomous phenomenon for SOD1-mediated ALS in which glial cell disease contributes directly to the ultimate demise of MNs.

The identification of TDP-43 instigated a barrage of studies attempting to elucidate the role it plays in neurodegeneration. TDP-43 is an RNA binding protein that contains two RNA recognition motifs, and a carboxyl-terminal glycine-rich domain in which almost all human ALS-associated mutations are located (11). Converging evidence

Fig. 1. A model of astrocyte pathology in TDP-43 proteinopathies. Astrocytes derived from human iPSCs are used to examine the cell-autonomous effects of TDP-43 mislocalization. Astrocytes that are generated from patients harboring ALS-causing mutations in the *TARDBP* gene display cytoplasmic TDP-43 inclusions, mimicking the common neuronal and glial histopathologic findings. Mutant TDP-43 patient-derived astrocytes display significantly impaired survival after 10 d.

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suggests that TDP-43 interacts with RNA by binding to mRNA and regulating splicing and turnover, in addition to regulating microRNA biogenesis. Although TDP-43 is normally largely confined to the nucleus, it can sometimes redistribute into the cytoplasm, where it resides in stress granules that play a role in the trafficking and stabilization of mRNA. When harboring ALS-linked mutations, TDP-43's carboxyl terminus, including a prion-like domain, is released by proteolysis to form the insoluble aggregates for which the TDP-43 proteinopathies are named (12).

A critical question is whether the cytoplasmic accumulation of TDP-43 is itself cytotoxic, or instead whether its mislocalization creates a relative nuclear deficiency of the protein that is detrimental to cell survival. Growing evidence suggests that cytoplasmic TDP-43 accumulation in the face of adequate nuclear levels is sufficient to cause cytotoxicity in a yeast proteinopathy model (13), as well as in isolated rodent MNs (14). The suggestion that cytoplasmic accumulation of TDP-43 could be directly cytotoxic to MNs raises the question: Does the same phenomenon hold true in the neighboring glial cells where these aggregates are also found (15)?

This is the question that Serio et al. (2) have now tackled in elegant experiments. The authors first established a robust platform to study astrocyte-neuronal interactions. Whereas most previous studies of neurodegenerative disease using patient-derived, induced pluripotent stem cells (iPSC) have involved transforming iPSCs into human neurons; Serio et al. instead use the iPSCs to generate human astrocytes. Taking advantage of proglial signaling pathways, the authors were able to produce significantly purified (>90%) iPSC astroglial populations from patients with specific TDP-43 mutations (M337V). The authors next characterize how the expression of mutant TDP-43 affects the cellular phenotype. As expected from histopathologic evidence, M337V astrocytes accumulated 30% more cytoplasmic TDP-43 than control astrocytes (Fig. 1). Additionally, control astrocytes that were transfected with mutant TDP-43 displayed similar levels of cytoplasmic accumulation, indicating that the specific TDP-43 mutation was directly responsible for the observed subcellular mislocalization.

To assess what longitudinal effects cytoplasmic mutant TDP-43 accumulation may have on cell viability, the authors adapted a live fluorescence microscopy approach in which cell death could be easily measured by a loss of constitutively active EGFP fluorescence. This method provides a useful

technique for analyzing large quantities of cells longitudinally. Using this method, the authors describe a 223% increase in the risk of cell death for M337V astrocytes compared with controls. This cumulative risk was only partially blocked by caspase inhibitors, suggesting that nonapoptotic pathways may be contributing to the increased susceptibility of M337V astrocytes. These inflammatory manifestations of cell death could help to explain the in vivo toxic effects of primary astrocyte disease upon neuronal cell health. One potential mechanism could involve the observation that activated and dying astrocytes release inflammatory cytokines that play a critical role in the recruitment and activation of microglia.

Collectively, these findings have major implications for understanding how perturbed neuron–glial interactions may lead to neurodegeneration. The authors provide evidence that cytoplasmic TDP-43 accumulation can cause cell-autonomous glial pathology and may directly contribute to the initiation of glial activation, rather than this glial activation being a secondary effect of a primary neuronal pathology. The fact that the authors did not observe any non-cell-autonomous glial effects in their platform is an intriguing diversion from the numerous past results in SOD1 models and from recent observations of non-cell-autonomous toxicity in human sporadic ALS neural stem cell-derived astrocytes (16). This discrepancy may be because of distinctly separate disease pathways in TDP-43 and SOD1 models, or may result from the fact that in vitro differentiation in the absence of degenerating neurons may not entirely capture the reactive qualities of in vivo astrocytes.

As further evidence accumulates regarding the role that activated astrocytes and microglia play in the progression of neurodegeneration (17), it is now imperative for us in future work to tease out the mechanisms that initiate detrimental gliosis and to better understand its functional role. This work by Serio et al. (2) raises compelling questions about how primary astrocyte pathology may play a causal role in TDP-43 proteinopathies. Does the prevention of TDP-43 accumulation in astrocytes via RNAi or knockdown rescue glial pathology, or has an irreversible activated phenotype already been established? How does local astrocyte degeneration affect the surrounding neuronal environment? An intriguing possibility, consistent with the findings of Serio et al., is that astrocyte death is directly responsible or at least contributory to the subsequent death of neurons by the loss of a critical astrocyte-secreted neurotrophic signal. Gliosis could conceivably represent replacement of one neurotrophic population of astrocytes by another astrocyte population that lacks this neurotrophic capacity.

With the advance of methodologies for generating human neurons and glia from patient-derived iPSCs, we now have new tools to ask provocative questions about neuron–glial interactions. The answers to these questions may clarify the therapeutic window for potential disease-modifying agents, and could reveal new candidate pathways in the progression of TDP-43 proteinopathies. To achieve these strides forward, however, it is becoming increasingly apparent that we must continue to broaden and redefine our approach toward neurodegenerative disease to include the critical contributions of our nonneuronal neighbors.

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