Unpicking neurodegeneration in a dish with human pluripotent stem cells: One cell type at a time

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Unpicking neurodegeneration in a dish with human pluripotent stem cells

One cell type at a time

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Neurodegenerative disorders such as Alzheimer disease, amyotrophic lateral sclerosis (ALS), and Parkinson disease are characterized by the selective loss of specific neuronal subtypes. While the precise mechanisms underlying neuronal dysfunction and subtype specificity remain the subject of intense study, the cellular environment in which degenerating neurons reside has been shown to be an integral part of the disease. Converging lines of evidence highlight the crucial role of glia in setting the pace of, and perhaps in some instances initiating, neurodegeneration.

ALS disease models, especially hSOD1^{G93A} transgenic mice, have been particularly influential in establishing the conceptual framework of cellular autonomy and neurodegeneration. Astrocytes, microglia, and oligodendrocytes all play different roles in neuronal death; selective lineage-specific downregulation of mutant SOD1 transgene in ALS mouse models results in altered disease onset and/or progression. The development of embryonic stem cell (ESC)-derived neural cell co-culture systems has further enabled an exploration of the role of glia on neuronal survival at the molecular level. Several groups have independently established that both rodent hSOD1^{G93A} astroglia[1,2] and astrocytes derived at postmortem from human mutant SOD1 and sporadic ALS cases are toxic to mouse ESC-derived motor neurons (MNs).

Even though mutant SOD1 cases constitute a relatively small fraction of ALS pathobiology, reactive glia are a defining feature of all neurodegenerative disorders. Astrocytes are in constant signaling exchange with the surrounding cells, acquiring a number of dynamic phenotypic changes in response to different stress stimuli broadly defined as "reactive astroglia". Against this background, several key questions emerge. These include: are there disease or mutation specific reactive properties of astrocytes?; are there aspects of reactive gliosis common to all neurodegenerative conditions?; and which, if any, of these properties are glia-intrinsic, or are they secondary to neuronal degeneration?

Dissection of these pathways requires a better understanding of glial diversity and function, a field that has been mostly left in the shadow of a neuro-centric focus on neurodegeneration. The advent of induced human pluripotent stem cells (iPSCs) now provides the opportunity to build human cellular platforms that will allow us to directly address these questions. Directed-differentiation protocols permit the generation of near-homogenous populations of functional human neurones and glia, where the effect of particular mutations and/or stressors can be studied both in isolation and in co-culture. Using patient-derived iPSCs, we and others have recently shown that a mutation in *TARDBP* encoding TAR DNA-binding protein 43 (TDP-43) associated with familial ALS underlies a cell-autonomous vulnerability both in isolated motor neurones[3,4] and astrocytes.[5] Importantly, these iPSC-derived models recapitulate key cellular and biochemical aspects of the TDP-43 proteinopathies that account for 95% of ALS and 55% of frontotemporal dementia (FTD) cases.

Is there a contribution of mutant TDP-43 astrocytes to human ALS pathology? Our in vitro co-culture experiments with real-time single-cell longitudinal survival analysis did not reveal an adverse survival effect of mutant TDP-43 astrocytes on either WT or MT human MNs, whereas hSOD1^{G93A} transgenic mouse glia were toxic to both, as predicted.[5] These contrasting findings may point to a mechanistic divergence of TDP-43 and SOD1 associated toxicity, or may simply reflect that in vitro differentiation of astrocytes from human PSCs in the absence of degenerating neurons does not capture the full spectrum of in vivo reactive properties. Indirect support of such an idea is provided by a recent study using transgenic rat models with selective neuronal lineage expression of mutant TDP-43 or fused in sarcoma (FUS), another RNA-binding protein linked to ALS and FTD, that identified lipocalin 2 as a neurotoxic factor induced in reactive astrocytes in response to neurodegeneration. This event is independent of the expression of the mutant proteins in the glial lineage.[6]

Improved understanding of the role of astrocytes in neurodegeneration requires multiple experimental—in vitro and in vivo—approaches. First, enriched in vitro astrocyte differentiation platforms from human PSCs should not only achieve the

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expression of specific lineage markers, both for glial restricted progenitors and differentiated progeny, but also include detailed functional characterization, such as glutamate uptake, induction of synaptogenesis, and propagation of calcium waves.[5] The relevance of regional heterogeneity is uncertain, as is the perennial question of whether one can ever truly replicate the complexity inherent in an aged brain in an essentially developmental in vitro system. Consequently, in vivo transplantation and/or slice culture explant studies are necessary to extend the in vitro observations of function. Second, comparative transcriptomic and proteomic approaches—ideally with reference to pathological disease samples—are necessary to delineate reactive properties of glia under different disease settings. The interplay between neuronal degeneration and glial response has recently been addressed in a genome-wide study uncovering distinct as well as shared pathways in reactive gliosis caused by inflammation, stroke, and hSOD1^{G93A} trangenic astroglial-mediated toxicity.[7]

It is now clear that glia can substantially affect the survival of neurons in disease states both by acquisition of reactive properties that are toxic and also through impairment of their normal physiological roles. Therefore, modulating astrocyte behavior by promotion of their neuroprotective functions[8] and/or limiting their neurotoxic properties offers a new therapeutic strategy for neuroprotection in neurodegenerative disorders.

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