



Scientists have generated nerve cells from post-mortem human tissue to demonstrate that astrocytes are toxic to neurons in both familial and sporadic forms of amyotrophic lateral sclerosis (ALS). Funded in part by the National Institute of Neurological Disorders and Stroke, the Ohio State University-led team generated astrocytes from post mortem spinal cord neural progenitor cells (NPCs). Their resulting studies indicated that a similar mechanism of astrocyte-mediated toxicity leads to motor neuron death occurs in both FALS and SALS.

Reporting on their human cell model in *Nature Biotechnology*, Arthur H. M. Burghes, M.D., Brian K. Kaspar, M.D., and colleagues says their work implicates wild-type superoxide dismutase 1 (SOD1) in neuronal toxicity, which suggests that therapies targeting SOD1 in astrocytes may be beneficial not only to FALS patients who carry SOD1 mutations, but also against SALS. Their work is described in a paper titled "Astrocytes from familial and sporadic ALS patients are toxic to motor neurons."

ALS is characterized by loss of motor neurons in the motor cortex, brain stem, and spinal cord. About 90% of cases are classified as SALS, and are not associated with any family history of the disease. The remaining 10% or so FALS cases are inherited in a dominant fashion, about 20% of which are associated with mutations in SOD1.

The toxicity of astrocytes to motor neurons in FALS has previously been demonstrated in a mouse model of the disease that overexpresses mutant SOD1, but to date has not been confirmed in humans, the Ohio researchers report. Moreover, neither cell nor animal models of SALS exist to determine whether astrocytes may be toxic to neurons in this most common form of the disease.

To test whether SALS astrocytes are similarly neurotoxic as FALS astrocytes, the researchers successfully generated astrocytes from adult NPCs isolated from postmortem lumbar spinal cord tissue from one FALS patient and seven SALS patients. Sequencing studies confirmed that the FALS patient harbored a mutation in the SOD1 gene, but the SALS patients carried no mutations in any common ALS gene loci.

The team then co-cultured the astrocytes with green fluorescent protein-tagged mouse embryonic stem cell-derived motor neurons. After 96 hours in culture, motor neurons that were cultured on top of FALS astrocytes began to degenerate, and at 120 hours the number of motor neurons present in FALS astrocyte co-cultures was reduced by 50% compared with non-ALS

astrocyte control cultures. Equivalent results were obtained with co-cultures of SALS astrocytes. In fact, the researchers note, motor neuron damage elicited by the SALS patient-derived astrocytes was indistinguishable from that elicited by FALS astrocytes, “suggesting a shared mechanism for motor neuron death in both types of co-cultures.”

Interestingly, co-culturing FALS and SALS-derived human astrocytes with GABAergic neurons didn't trigger nerve cell death, “suggesting the ALS astrocyte-derived toxicity is specific toward motor neurons.”

Similar patterns of cell death occurred when the mouse motor neurons were co-cultured with non-ALS astrocytes that overexpress mutated SOD1 transgenes, supporting previous co-culture studies with mutant SOD1 astrocytes.

Because previous work has suggested that astrocytes from the FALS SOD1 mouse model can cause motor neuron death in vitro by means of secreted factor, the Ohio researchers evaluated the effects of treating motor neurons with astrocyte-conditioned media. They found that motor neurons cultured in conditioned media from both SALS and FALS died about 50% faster than those treated with conditioned media from non-ALS control astrocytes, but weren't affected by conditioned media collected from ALS patient-derived fibroblasts. The SALS and FALS media were also nontoxic to GABAergic neurons, supporting their effects specifically on motor neurons. “These results suggest that FALS and SALS astrocytes either secrete factors that are toxic to motor neurons, or alternatively do not provide factors needed for motor neuron survival, resulting in cell death,” they write.

Genetic analysis of the FALS and SALS astrocytes derived from the different patients showed that about 35–60% of the inflammatory genes assayed were upregulated, when compared with the same genes in non-ALS control astrocytes. There was some heterogeneity between the different patient-derived astrocyte cell lines, but clustering analysis revealed a set of 22 upregulated genes encompassing chemokines, proinflammatory cytokines, and components of the complement cascade, many of which have previously been implicated in ALS. Network-based pathway analysis strongly suggested involvement of the NF- κ B signalling complex and interferon-alpha, along with pathways involving kinases such as MAPK, INK, and AKT.

“Many of these signaling networks have been previously implicated in FALS, and we now show evidence for their potential involvement in mediating inflammatory responses in SALS

astrocytes,” the authors state.

The team moved on to investigate the role of SOD1 in the neurotoxicity of astrocytes, by using short hairpin RNAs (shRNAs) to knock down SOD1 in both the FALS and SALS astrocytes. They found that knocking down mutant SOD1 in the FALS astrocytes completely reversed astrocyte-mediated motor neuron toxicity. Significantly, suppressing wild-type SOD1 in the SALS astrocytes also protected co-cultured motor neurons from astrocyte-mediated death, as long as about 50% wild-type SOD1 was suppressed.

“Our data highlight astrocytes as a noncell autonomous component in SALS and provide an in vitro model system to investigate common disease mechanisms and evaluate potential therapies for SALS and FALS,” the authors conclude. Dr. Kaspar suggests the next stage will be to further define what is happening to the astrocytes to turn them toxic. “It’s been a long road, but the hard work starts here,” he states. “We will need to confront fundamental questions about what is happening to astrocytes and how they are killing motor neurons. And the ultimate goal is to identify therapies that will translate into helping humans.”

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