ALS Model Glia Can Mediate Toxicity to Motor Neurons Derived from Human Embryonic Stem Cells

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In this issue of *Cell Stem Cell*, Di Giorgio et al. (2008) and Marchetto et al. (2008) culture motor neurons derived from human embryonic stem cells with astrocytes expressing mutant SOD1. In these human ALS models, motor neurons are selectively destroyed by mutant astrocyte-secreted factors, and potential neuroprotective pathways are revealed.

Amyotrophic Lateral Sclerosis (ALS) is a fatal disease, characterized by the degeneration of somatic motor neurons in the spinal cord, brain stem, and cortex. ALS is dominantly inherited in approximately 10% of patients (familial ALS, fALS), but in 90% of patients, there is no apparent genetic linkage (sporadic ALS). Approximately 20% of fALS cases have been linked to mutations in the gene encoding Cu/Zn superoxide dismutase 1 (SOD1). SOD1 converts naturally occurring but harmful superoxide radicals to molecular oxygen and hydrogen peroxide. Mutant SOD1 animal models have provided abundant information regarding the possible mechanisms of disease. Overexpression of mutant forms of human SOD1 in rodents results in ALS-like motor neuron disease, indicating that a toxic gain of function rather than a loss of enzymatic activity is the likely cause of fALS. Further analyses of such transgenic animals suggest that disease initiation is caused by direct damage of motor neurons (cell autonomous) while progression is dependent on nonneuronal cells, such as astrocytes and microglia (non-cell autonomous) (Boillee et al., 2006). Several drug therapies have exhibited benefical effects in SOD1 transgenic models; however, the same candidates have not been successful in human clinical trials, indicating that current ALS rodent models provide only a limited insight into the pathogenesis of human ALS. Thus, developing humanized ALS models is a critical step toward identifying successful therapeutic alternatives.

In this issue of *Cell Stem Cell*, two groups, Di Giorgio et al. (2008) and Marchetto et al. (2008), show that human embryonic stem cell-derived motor neurons are sensitive to toxic effects of mutant SOD1 overexpressing glia, whereas exposure to wild-type SOD1 glia left motor neurons unharmed. In both studies, the Hb9 promoter was used to specifically label motor neurons by eGFP expression. While Di Giorgio and colleagues made a stable Hb9-eGFP hESC line, Marchetto et al. utilized a lenti-Hb9-eGFP construct to label motor neurons in differentiated hESC cultures. About 50 percent of human motor neurons were lost when co-cultured either with primary human astrocytes infected with a lenti mutant SOD1 construct for 4 weeks (Marchetto et al., 2008) or mutant SOD1-overexpressing rodent astrocytes or their conditioned media for 10–20 days (Di Giorgio et al., 2008). Factors responsible for the toxicity of ALS model glia included prostaglandins (Di Giorgio et al., 2008) and proinflammatory cytokines (Marchetto et al., 2008).

Overexpression of different SOD1 point mutations in glia induced similar levels of motor neuron loss in both studies. The degenerative effect appeared specific to glia since mutant SOD1 overexpressing fibroblasts were not harmful to motor neurons, confirming previous findings utilizing mouse motor neurons (Di Giorgio et al., 2007; Nagai et al., 2007). Both studies also show that interneurons remained unaffected, suggesting that the toxic effects elicited by astrocytes are relatively target cell-specific. The observed selective toxicity raises some interesting questions for future investigation. Specifically, while motor neuron death is the prominent feature in ALS, spinal interneurons also degenerate during disease progression. In fact, some evidence suggests that a majority of ventro-lateral and medio-lateral spinal interneurons degenerate in mutant SOD1 mouse models prior to onset of motor neuron loss (Martin et al., 2007) and activation of glia (Tu et al., 1996). Thus, if mutant SOD1-activated astrocytes are not responsible for the early degeneration of these interneurons, what other cell types participate in their demise and are cell autonomous or non-cell autonomous events required?

Consistent with previous studies on mouse ALS model systems (Nagai et al., 2007; Pehar et al., 2004), both Di Giorgio et al. and Marchetto et al. found that the toxicity of mutant SOD1 overexpressing glia was mediated by factors released into the cell culture media. In an effort to isolate the soluble mediators responsible, Di Giorgio and colleagues used microarrays to identify genes that were uniquely transcribed in astrocytes overexpressing mutant SOD1. One of the results from the screen, prostaglandin D2 (PGD2), could by itself cause motor neuron loss at levels similar to that

induced by coculture with SOD1 overexpressing astrocytes. Interestingly, blocking the PDG2 receptor significantly rescued motor neurons plated on mutant SOD1 overexpressing astrocytes. However, the rescue was modest, indicating that mutant astrocyte-induced degeneration of motor neurons is mediated only in part by secreted PGD2. Inhibition of cyclooxygenase 2 (Cox-2), the enzyme involved in prostaglandin synthesis, has been shown to delay onset of disease in ALS mouse models. However, as discussed above, a clinical trial failed to show benefit for patients with manifest ALS, so therefore, blocking prostaglandins alone is likely not sufficient for significant clinical benefits, at least in cases in which disease progression has been initiated. Marchetto and colleagues observed that the cultured astrocytes activated an inflammatory response when expressing mutant SOD1, concomitant with increased production of reactive oxygen species (ROS) and proinflammatory cytokines such as nitric oxide enzyme (iNOS), chromogranin A, Cystatin C, and Nox2. Blocking ROS production using the Nox2 inhibitor apocynin rescued human motor neurons from mutant SOD1-toxicity, confirming recent data generated from mutant mouse model experiments (Harraz et al., 2008). Nox2 has also been shown to be upregulated in the spinal cord of sporadic ALS patients, indicating that Nox2 could be a general therapeutic drug target for ALS patients.

Several compounds tested in mutant SOD1 animal models have succeeded in delaying onset of disease, but very few molecules, including IGF-I and VEGF, have increased life span of the animals when delivered after onset of symptoms. Substances that delay or prevent disease onset could, thus, potentially be of clinical benefit provided that preclinical ALS biomarkers can be identified. However, given the current lack of such presymptomatic markers, substances that can increase the life-span of already affected individuals represent a higher priority for clinical development. Culture-based systems of human stem cells and their derivatives, such as those developed by Di Giorgio et al. and Marchetto et al., could facilitate the discovery of such neuroprotective compounds with potential clinical relevance. Notably, overexpression of mutant SOD1 activates multiple detrimental biochemical pathways, such as the NADPH oxidase (Nox) cascade and its resulting downstream overproduction of superoxide (Harraz et al., 2008). Mutants also exhibit reduced degradation of misfolded proteins, which may result in ER stress and concomitant activation of ASK1 and apoptosis (Nishitoh et al., 2008). Combined, these results underscore that many toxic pathways may need to be targeted in concert to effectively protect the functionality of motor neurons. Furthermore, it is not known whether glial cells in patients elicit the same toxicity as the ALS model astrocytes, as the latter commonly harbor multiple copies of mutant SOD1. An analysis of induced pluripotent stem cell (iPSC)-derived glial cells from patients with mutant SOD1-induced ALS (Dimos et al., 2008) could possibly reveal whether a single gene copy renders human glial cells as toxic as those harboring multiple mutant SOD1 copies. Derivation of glial cells from patients with sporadic ALS could reveal if the toxicity induced by this population is consistent across different forms of ALS or dependent on the particular cause(s) of the disease. Finally, the ESC-based systems described in these two studies could be used to investigate the cell autonomous events in motor neurons that appear to initiate their degeneration and that render mutant SOD1-containing motor neurons more vulnerable to the toxic effects of mutant SOD1 glia than wild-type motor neurons (Di Giorgio et al., 2007).

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