Lactate-starved neurons in ALS

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Summary and comment on a recent *Nature* article entitled 'Oligodendroglia metabolically support axons and contribute to neurodegeneration' (Lee et al., 2012).

Central nervous system (CNS) function in vertebrates requires extensive myelination of axons by oligodendrocytes. Similarly, peripheral nervous system (PNS) function requires myelination by Schwann cells. Myelination, which involves lamellar cytoplasmic extensions of glia, allows neurons to conduct rapid action potentials, transduce signals efficiently across long distances and use space ergonomically (Baumann and Pham-Dinh, 2001).

Interactions between myelinating glia and axons are important for neuronal health, and demyelination is a feature of several disorders. For example, multiple sclerosis (MS) is thought to be caused by autoimmune-mediated degradation of myelin (Matusevicius et al., 1999; Allegretta et al., 1990). In other demyelinating axonopathies, such as Charcot-Marie Tooth disease type 1 (CMT1), axonal injury is length dependent, in that it is worse distally than proximally with respect to the cell body, suggesting that altered transport of some factor contributes to neurodegeneration (Garbern et al., 2002). However, in the motor neuron disorder amyotrophic lateral sclerosis (ALS), how pathology is related to myelination is not well understood (Lee et al., 2012), although interactions between oligodendrocytes and axons seem to be important. One recent study suggests that, in a mouse model of ALS, precursor cells differentiate into oligodendrocytes at a rate up to 20-fold higher than in normal mice, suggesting that rapid turnover of oligodendrocytes contributes to disease-associated gliosis, exhausts resources and hastens neurodegeneration (Kang et al., 2010).

Other studies have shown that axon degeneration is not always linked with altered myelination, suggesting that other interactions between oligodendrocytes and neurons mediate axon survival. For example, shiverer mice, with a mutation in the myelin basic protein gene (*Mbp*), lose myelin wrap density (Rosenbluth, 1980) but display no axonal damage (Griffiths et al., 1998). Conversely, mice and patients with mutations in the major myelin protein, proteolipid protein-1 (PLP1), exhibit neurodegeneration without demyelination (Garbern et al., 2002; Griffiths et al., 1998). One hypothesis proposes that long axonal tracts might depend on glial cells for the demanding metabolic costs of rapidly conducting action potentials (Nave, 2010).

In a recent *Nature* article, Lee et al. sought to determine how oligodendrocytes metabolically support axons by focusing on lactate delivered via monocarboxylate transporter-1 (MCT1) (Lee et al., 2012). Lactate is a glycolytic product known to support cellular metabolism, and lactate-shuttle hypotheses propose that lactate is transported between cell types (such as glia-neuron) based on redox changes and energetic demands (Gladden, 2001). The transport of lactate is facilitated by MCTs, which comprise a family of 14 transporters that distribute monocarboxylates across membranes (Halestrap and Price, 1999). Among the MCT family, MCT1-MCT4 are proton linked, and MCT1 has the widest expression pattern, being found in muscles, the gastrointestinal tract and the CNS (Draoui and Feron, 2011; Lee et al., 2012).

Lee et al. note that traditional methods for determining expression profiles of MCT1 have been limited by the lack of specific antibodies (Lee et al., 2012). To circumvent this limitation, the authors generated bacterial artificial chromosome (BAC) transgenic mice expressing a tdTomato fluorescent reporter for in vivo analysis of *MCT1* mRNA (see supplemental data figure 1 in Lee et al., 2012). By crossing tdTomato MCT1 transgenic mice with strains carrying GFP reporters for MOBP (an oligodendrocyte-specific marker) or GLT1 (an astrocyte-specific marker), the authors could observe expression in the CNS. These crosses revealed that MCT1 is not detectably expressed by astrocytes but is highly expressed in oligodendrocytes. Importantly, oligodendrocytes express high levels of MCT1 compared with its expression in other cell types of the CNS.

To address the possibility that MCT1-expressing oligodendrocytes have important metabolic roles for associated axons, the authors tested the consequences of MCT1 downregulation in organotypic spinal cord cultures generated from post-natal mice (figure 2 in Lee et al., 2012). Disrupting MCT1 function by two distinct methods indicated that the expression of this transporter was important for neuronal survival. First, cultured cells were exposed to *MCT1* antisense oligonucleotides. After 3 weeks of treatment, more than 30% of neurons had died compared with control cultures. Second, cultured cells treated with an MCT1-specific inhibitor for 3 weeks resulted in comparable levels of cell death. Notably, oligodendrocytes were not adversely affected by MCT1 downregulation.

If neurons are dependent on oligodendrocytes for energetic demands, the authors reasoned that neurons lacking lactate support would be especially sensitive to glucose deprivation. Initially, both untreated and MCT1-inhibitor-treated cell populations were resistant to glucose deprivation. However, after more than 2 hours of glucose deprivation, MCT1-inhibitor-treated cells underwent cell death in a manner that could be rescued by lactate.

Thus far, in vitro evidence suggested that oligodendrocyte MCT1 function was important for neuronal survival. Next, the investigators set out to test the findings in vivo. They used lentiviral cytomegalovirus in mice to knock down expression of MCT1 (figure 3 in Lee et al., 2012). Compared with control treatments, the consequences of this knockdown included axonal swellings expressing the neurofilament marker SMI32, microglial activation and widespread motoneurodegeneration.

Next, in vivo experiments were carried out with mice lacking endogenous expression of MCT1. A homozygous ($Mct1^{-/-}$) genotype was embryonic lethal, but heterozygous

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(*Mct1*$^{-/+}$) animals developed normally. To determine the consequences of loss of MCT1 expression, distinct oligodendrocyte-associated axonal populations were observed in the CNS and in the optic nerve, which metabolically relies on lactate produced by glia (Tekkök et al., 2005). In the brain and spinal cord, heterozygous animals displayed an axonopathy characterized by axonal swellings and motor neuron loss, without obvious changes to myelination (figure 4 and supplemental data figure 9 in Lee et al., 2012). Similarly, in the optic nerve, a small (~1.75%) but significant population of axons demonstrated axonal swellings in the absence of altered oligodendrocyte morphology or myelination. These findings demonstrate the importance of the MCT1 transporter in vivo and highlight oligodendrocytes as a supporter of CNS axons.

At this point, non-autonomous contributions to the oligodendrocyte-axon system could not be discounted because MCT1 is also expressed in other cell types (albeit at low levels). To address this, MCT1 expression was specifically knocked down in oligodendrocytes by two distinct methods (figure 5 in Lee et al., 2012). First, the authors engineered a lentivirus construct expressing shRNA for *MCT1* driven by the myelin basic protein (MBP) promoter of oligodendrocytes. When the optic nerve was injected with this lentivirus, SMI32-associated axonal swellings similar to those induced by the lentiviral treatments described above were observed. Second, MCT1 knockdown was carried out specifically in PLP1-expressing oligodendrocytes of the corpus callosum using a Cre-*loxP*-regulated transgene expressing *MCT1* RNAi (Ventura et al., 2004). This treatment induced SMI32-positive axonal swellings similar to those observed in the first method (supplemental data figure 14 in Lee et al., 2012).

In vivo disruption of MCT1 genetically or by lentiviral knockdown specifically in oligodendrocytes produced a neuropathy characterized by swellings of myelinated axons without damaging the oligodendrocytes themselves. Studies of SOD1 transgenic mice, an established model of ALS, have observed a similar phenotype as well as indications of rapid oligodendrocyte turnover (Kang et al., 2010). To determine whether MCT1 expression is altered in ALS pathology, MCT protein expression was measured in brain samples from individuals with ALS. The investigators found that MCT1 expression was reduced by more than 50% in the motor cortex, but not in the frontal lobe (which is unaffected in ALS) (figure 6 in Lee et al., 2012). When SOD1 (G93A) transgenic mice were crossed onto BAC tdTomato MCT1 mice, a marked reduction in MCT1, similar to the reduction observed in ALS brain samples, was observed. Thus, MCT1 is reduced in brain samples from individuals with ALS and in mouse models of ALS, indicating that impaired lactate transport by oligodendrocytes in the CNS is a feature of the disorder.

In summary, Lee et al. report that oligodendroglia, the main myelinating glial cell type of the CNS, metabolically support axons via the lactate transporter MCT1 (Lee et al., 2012). Glia have important roles in sustaining neuronal function, including glutamate cycling and transport of glycolytic substrates to axons during periods of intense activity (Chih et al., 2001). Evidence gathered in the paper by Lee et al. supports the conclusion that lactate transport by oligodendrocytes is important for axonal health, especially during increased activity such as under exogenous application of glutamate (supplemental data figure 8 in Lee et al., 2012). In vivo, disrupting MCT1 expression resulted in neurodegeneration without damaging oligodendrocytes. In vitro, lactate treatment rescued neurodegeneration caused by MCT1 deficiency, demonstrating that lactate can metabolically support axons and prevent neurodegeneration.

Importantly, Lee et al. found that reduced MCT1 expression occurs in ALS (Lee et al., 2012), a disorder characterized by progressive muscle paralysis. It seems likely that axons are chronically limited in their energetic needs during the course of ALS progression. However, it is not known whether reduced MCT1 expression is a result of specific underlying causes of ALS. Regardless, the findings of Lee et al. provide crucial insights into understanding glia as important determinants of motor neuron health (Lee et al., 2012).

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