to understand fully the pathways that DARPP-32 is involved in before they can determine where to intervene therapeutically. 'You would have to find some way to regulate what it does, either its level of phosphorylation or some effect downstream from there,' Chipkin said.

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New rodent models gnawing at the black box of ALS

Kathryn Senior, freelance writer

Despite intensive research, potential therapies prove elusive for the fatal disease amyotrophic lateral sclerosis (ALS). To encourage aggressive research towards a cure, the ALS patients' group in the USA set up the Lou Gehrig Challenge in May 2000. One result is a new transgenic rat model of the disease, which became available to laboratories worldwide in August 2002.

David Howland of Wyeth Research (http://www.wyeth.com) has developed transgenic rats that, like the transgenic mice already used extensively in ALS research, overexpress Cu^{2+}–Zn^{2+} superoxide dismutase 1 (SOD1) [1]. Some inherited cases of human ALS are a result of defects in this gene. The transgenic rats and mice show remarkably similar signs and symptoms, making them valuable research tools.

Worldwide, about 120,000 individuals develop ALS (also called motor neuron disease or MND) each year, typically active adults between 40 and 70. Most die within 2–5 years of diagnosis. Currently just one drug, Rilutek, has FDA approval for ALS, but this extends life by an average of only three months. The new rat model should enable research to progress more quickly toward new stem cell therapies as well as new drugs, because the transgenic rat, larger and longer-lived than the mouse, should enable experimental approaches that were impossible before.

Recent progress using transgenic mice

Meanwhile, the mouse model has been providing useful insights. It figured in a study published in September 2002, which sheds light on the origins of the progressive motor-neuron degeneration typical of ALS. The results suggest that exposure to free radicals disturbs membrane lipid metabolism in motor neurons, causing cellular accumulation of ceramides and cholesterol esters. This eventually pushes the cell into apoptosis.

'By blocking sphingolipid synthesis, the first step in this altered pathway, it may be possible to prevent ceramide accumulation and so protect motor neurons against death induced by oxidative stress,' commented senior author Mark Mattson of the Gerontology Research Center at the US National Institute on Aging (http://www.grc.nia.nih.gov/).

Mattson and colleagues found that sphingomyelin, ceramides and cholesterol esters were all elevated in spinal cords from presymptomatic and symptomatic Cu^{2+}–Zn^{2+}-SOD mice, and also found evidence for the same altered lipid metabolism in spinal cords from ALS patients. Cells cultured from spinal cord tissue from these mice responded to experimentally induced oxidative stress by increasing ceramide and cholesterol ester production, sensitizing the motor neurons to apoptosis.

'This makes sense since in normal cells, ceramides play an important role in apoptosis and they have been implicated in the deaths of neurons that occur in ischemic stroke and Parkinson's disease,' Mattson declared [2].

ISP1 as a potential therapy

A drug that hinders sphingolipid production brought this abnormal lipid metabolism cascade to an abrupt halt. 'Blocking *de novo* sphingolipid synthesis using myriocin (ISP1), an inhibitor of serine palmitolytransferase (Fig. 1), prevents increased ceramide synthesis and protects cells from apoptosis,' said Mattson. Work is under way to establish whether ISP1 can delay the onset of motor neuron degeneration and extend lifespan in Cu^{2+}–Zn^{2+}-SOD mice with ALS. The team plans to test whether changes in dietary fatty acids and cholesterol affect ALS development in mice, and (collaborating with epidemiologists) whether risk of ALS is affected by blood levels of sphingolipid and cholesterol-binding proteins.

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One important issue is side effects because ISP1 can have toxic effects at high doses. 'If results continue to be promising, we will modify the structure of ISP1 to improve its ability to reach nerve cells in the spinal cord and to reduce its side effects,' reported Mattson. Even if ISP1 does not live up to expectations, simply identifying the lipid pathway identifies several genes (*SPT, SMase* and *ACAT*) that could be potential targets for gene therapy. The team also plans to explore this, using the mouse model.

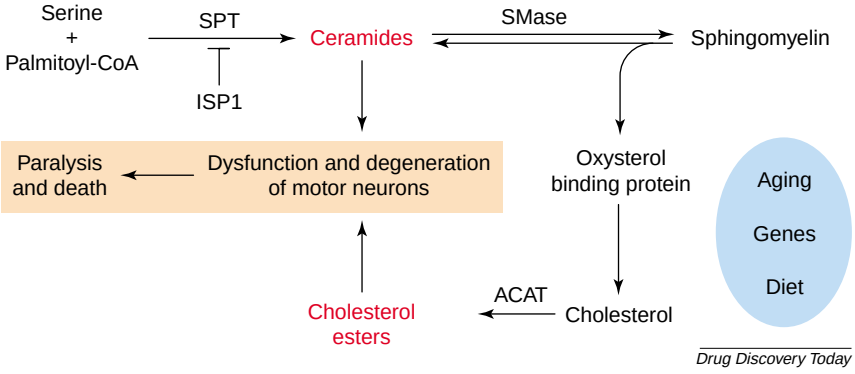

Figure 1. Metabolic pathways of sphingolipid and cholesterol metabolism and their interaction with environmental factors to cause motor-neuron cell death. The drug ISP1 blocks ceramide production by inhibiting the enzyme serine palmitoyl transferase, and thus could help reduce neurodegeneration. Abbreviations: ACAT, acyl coenzyme-A cholesterol acetyl transferase; SMase, sphingomyelinase; SPT, serine palmitoyl transferase. Figure kindly provided by Mark Mattson (Gerontology Research Center at the US National Institute on Aging; http://www.grc.nia.nih.gov/).

Not an easy task ahead

Allan Butterfield, director of the Center of Membrane Sciences at the University of Kentucky in Lexington (http://www.uky.edu/RGS/Membrane/), says the association between sphingolipid metabolism, oxidative stress and ALS is 'intriguing', in light of a solid consensus that oxidative stress plays a central role in other neurodegenerative disorders, including Alzheimer's disease, multiple sclerosis, Huntington's disease and Parkinson's disease. 'The possibility of using antioxidants is being widely investigated,' he adds. 'Our group has been looking at vitamin E, but so far, the results are mixed [3]: targeting the sphingolipid pathway would be a novel therapeutic approach.' Butterfield also urges caution not to interfere with sphingomeylin's normal, beneficial functions.

Phamacologist Elena Posse de Chaves of the University of Alberta (http://www.ualberta.ca) agrees. Before developing therapies to block ceramide production, she says, it is important to understand why some neurons react differently to ceramide. She reports that her own group has found that ceramide protects sympathetic neurons from apoptosis caused by neuronal growth factor deprivation (unpublished data).

A crucial first step in new drug development for ALS is presymptomatic diagnosis, Mattson says, because almost all mouse studies of the disease show that drugs extend survival only if treatment begins many months before the onset of symptoms. The ALS patients available for trials already have extensive motor-neuron degeneration. 'This suggests that it may be possible for drugs like ISP1 to prevent or delay the onset of ALS in at risk individuals, rather than reversing the effects of established disease,' he observed. 'So early diagnosis will be critical.'

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