use of a brain-specific promoter [6]. In the primate experiments, the exogenous genes were on plasmids under the influence of a widely expressed SV40 promoter.

However, the fact that the exogenous genes do not integrate into the genome, and thus their expression is short lived, could limit the therapeutic applications of this synthetic vector. 'If you're trying to treat an inherited disease, you're looking for permanent expression,' said Wolfe. Transient expression might be preferable for some diseases, and it also might be possible to deliver the genes using a vector that results in permanent expression, he added.

Promise in rats

Pardridge's team has already demonstrated they can use this delivery system to get a pharmacological effect in rodents. A plasmid that expresses antisense mRNA to the human epidermal growth factor receptor injected intravenously in a PIL led to 100% increase of survival time in mice with brain cancer [3]. In addition, the effect of a neurotoxin that causes Parkinson's like symptoms in rats was reversed by using PIL to deliver the affected enzyme, tyrosine hydroxylase [4]. 'We showed that gene therapy not only normalizes the biochemistry but also eliminates the motor abnormality of the disease.'

The team is currently working to develop a brain-specific promoter for Parkinson's disease and also to use an RNA interference-based approach in the mouse brain cancer model: the combination of gene therapy and RNAi removes aberrant expression of the gene that is specific to the cancer, however, according to Pardridge, the problems preventing this from happening are 'delivery, delivery and delivery!'

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Gene therapy success for Alzheimer's?

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Using gene therapy, the levels of a protein implicated in Alzheimer's disease have been dramatically reduced in mice. Researchers from the Salk Institute (http://www.salk.edu) and the University of California, San Diego (UCSD; http://www.ucsd.edu) have introduced the gene for neprilysin into the brains of transgenic mice, reducing levels of amyloid protein by up to 50% [1]. Their findings offer hope to the millions of Alzheimer's sufferers, and their families, around the world.

More common than you might think

Alzheimer's disease (AD) is the most common form of neurodegenerative disease in people over 65 years. It is estimated that >15 million people are affected by the disease worldwide and almost half of those over 85 years show signs of the disease [2]. The cognitive areas of the brain are the first to be affected, leading, amongst other things, to memory loss and behavioural abnormalities. The disease then spreads to the parts of the brain that control movement, and the patient requires constant care. Eventually, the loss of brain function becomes so severe that it can be the primary cause of death. As the average lifespan of people in the Western world increases, so too will the numbers of people affected by neurodegenerative illnesses. Needless to say, a tremendous amount of time, effort and money has been poured into finding a means to cure or prevent the disease.

A complicated aetiology

Despite great strides in AD research, the precise mechanisms that lead to the disease are not fully understood and many genetic, cellular and molecular irregularities are implicated. One of the most established factors, however, is a build-up of amyloid proteins around brain cells to form harmful plaques. Mutations in the amyloid-precursor protein (APP) gene can result in increased production of $A\beta_{1-42}$, the form of amyloid protein that clusters into the harmful plaques. However, in rarer forms of AD, other factors might be contributory. For example, amyloid build up might be caused by a decreased $A\beta_{1-42}$ clearance, or from a shift in the balance of production and degradation.

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Neprilysin: a new hope

Researchers have recently taken an interest in the role that the neprilysin protein has in amyloid regulation. This 97 kDa protein has been identified as a major extracellular Aβ-degrading enzyme in the brain [3]. When the neprilysin gene is knocked out, Aβ levels in the brains of mice increase. This, coupled with various other lines of evidence, has pointed to a key role for neprilysin in the clearance of Aβ. However, no *in vivo* study had directly proved this.

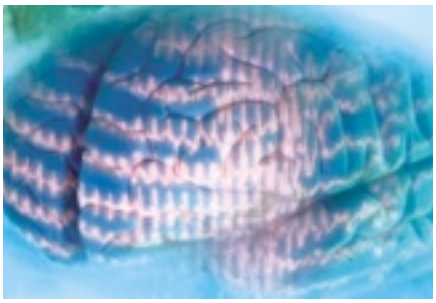
Building on this work, researchers led by Inder Verma and Fred Gage of the Salk Institute have performed the first *in vivo* studies to determine the precise role that neprilysin has in AD. To model the disease, transgenic mice whose brains expressed the human form of the Aβ protein were used. A modified lentiviral vector was then employed to deliver the gene for neprilysin into neurons of the frontal cortex and hippocampus by direct injection. Lentiviruses are particularly well suited to this work because their efficiency had been previously demonstrated in the CNS and they are non-cytotoxic. In this case, the researchers employed a version of the HIV virus that lacked the genes required for infection. After one month, the brain cells of the mice were analyzed for neprilysin expression and the presence of $A\beta_{1-42}$. Immunohistochemistry revealed that neprilysin expression was concentrated at the injection site, thus reducing the chances of side effects. The levels of amyloid in transfected mice were half those of control mice who lacked the neprilysin gene, and treatment eliminated the degeneration caused by the build-up of Aβ. For the first time, therefore, it had been shown that neprilysin reduces Aβ levels in animals.

This is not the only time that gene therapy for Alzheimer's disease has been attempted. In 2002, clinical trials began on a gene-therapy approach to delivering nervous system growth factors to the brain, in an attempt to reduce cholinergic neuron degeneration in AD sufferers [4]. This study, however, used *ex vivo* gene therapy, whereby cells were modified to produce growth factors outside of the body and then surgically implanted in the brain. Such a technique has several advantages, notably cell-type selectivity, immunocompatability and the safety of not using virus particles. However, there are also several potential problems, including the invasiveness of surgery and the possibility that immortal cell lines could induce tumour growth.

Could it work in humans?

The new *in vivo* gene therapy study from Salk hopes to circumvent some of these problems. Robert Marr, a member of the Salk team, explained the potential benefits: 'This treatment involves augmentation of a natural process that controls β-amyloid levels. Therefore, it is hoped that it will be better tolerated. Furthermore, this gene transfer approach allows for localized delivery of the therapeutic agent, reducing the opportunities to develop systemic side effects.'

It is too early to speculate on when clinical trials might begin in humans. Mice do not suffer from AD, so the work thus far is a model only. No data have been gathered on potential side effects but studies are set to continue in



mice. In around six month's time, the team hope to have completed behavioural experiments in mice to see if the therapy results in cognitive improvement. They are also investigating the role of neprilysin at different stages of the disease.

A note of caution was sounded by Mark Tuszynski of the UCSD, one of the researchers heading the current clinical trials of the *ex vivo* technique. There is still the question of whether a region as large as the human cortex, one of the areas of the brain most affected by AD, could be practically targeted with an approach such as this.

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