Editorial

Alzheimer’s disease, amyotrophic lateral sclerosis, and transgenic mice

Transgenic mouse technology has contributed much to our understanding of the function and dysfunction of the nervous system. It has helped us to model and test hypotheses relating to neurodegenerative diseases, such as Alzheimer’s disease and motor neuron disease, and has also provided insight into the molecular basis of higher brain functions such as learning and memory.

Alteration of the mouse genome is carried out by one of two methods: the first involves the addition of new genes (known as transgenes) and the second involves the modification of endogenous mouse genes. The first method usually involves microinjection of the recombinant DNA into the pronucleus of a one cell mouse embryo. The resulting manipulated embryos are reimplanted into the oviducts of a pseudopregnant female mouse and the offspring are then screened for the presence of the transgene so as to identify transgenic progeny. The frequency of production of successful transgenic progeny by this method is surprisingly good with a rate of about one in five to six offspring being transgenic.

Manipulation of endogenous mouse genes is a more complex process and involves the use of embryonic stem cells. Embryonic stem cells are derived from the inner cell mass of blastocysts, and retain the ability to differentiate into any cell type of the original mouse. Embryonic stem cells can be expanded in vitro and transfected so as to introduce new DNA. To generate transgenic mice, the modified embryonic stem cells are injected into host blastocysts and the resulting embryos are then reintroduced into the reproductive tracts of pseudopregnant females. Animals derived from these manipulated blastocysts are chimeric—that is, some of their cells are derived from the inner cell mass of the host blastocyst and some from the injected embryonic stem cells. Coat colour is often used to identify such chimeric animals. For example, embryonic stem cells derived from a white mouse might be introduced into host blastocysts from a black mouse.

The principal advantage of this route is that it allows a degree of preselection of the cells for desirable characteristics before transgenic production. This is utilised most commonly in screening for the relatively infrequent event of homologous recombination, which is when the incoming transgenic DNA has recombined with the cognate sequences residing on the host chromosome. Homologous recombination is utilised to mutate endogenous mouse genes. This approach has been successfully utilised in the creation of transgenic “knockouts”—that is, transgenic animals where a specific gene has been ablated. However, it is also possible to use this approach to introduce more subtle defects into the mouse genome.

Many transgenic animals modelling neurodegenerative and behavioural phenotypes have been created (for comprehensive reviews see Aguzzi et al and Lee et al). This editorial highlights some recent uses of transgenic mouse technology as applied to the study of motor neuron disease and Alzheimer’s disease. We have chosen to focus on these multifactorial diseases as several genetic loci have been implicated in their pathogenesis, allowing comparison of various transgenic models and approaches to be made. Furthermore, some of these transgenic lines harbouring single gene mutations relevant to Alzheimer’s disease have been crossed allowing the study of the additive effect of multiple genetic defects in development of disease.

Alzheimer’s disease

Alzheimer’s disease is the most common cause of senile dementia. Models of Alzheimer’s disease are necessary for charting the early molecular changes that lead to neurodegeneration and also for the in vivo testing of potential therapeutic strategies.

The pathology of Alzheimer’s disease involves the formation of two pathological lesions: neurofibrillary tangles and senile plaques. Neurofibrillary tangles are intracellular inclusions that comprise aggregates of paired helical filaments (PHFs). The principal component of PHFs is the microtubule associated protein tau, which in PHF is hyperphosphorylated. Senile plaques comprise areas of degenerating neurons surrounding an extracellular core of β-amyloid fibrils. β-Amyloid (Aβ protein), is a 39–43 amino acid peptide that is derived by proteolytic cleavage from its precursor, the amyloid precursor protein (APP). Animal models of Alzheimer’s disease are thus aimed at modelling these two situations.

Some forms of Alzheimer’s disease are familial and are inherited in a dominant fashion. Mutations in three different loci have so far been found to be the causative genetic lesions in these familial forms of Alzheimer’s disease. These are in the APP gene on chromosome 21 and the presenilin 1 and 2 (PS1 and PS2) genes on chromosomes 14 and 1 respectively. There is evidence that mutations in the APP gene predispose to increased secretion of Aβ protein or to increased secretion of the longer, more amyloidogenic, Aβ 1–42/43 rather than Aβ 1–39 isoforms. Cellular studies have also shown that patients harbouring PS-1 mutations have increased production of the longer forms of Aβ protein. Thus these APP and PS-1 mutations seem to be causative in the amyloid pathology of Alzheimer’s disease.
Attempts have been made to model Alzheimer’s disease. The first of note involved overexpression of a familial Alzheimer’s disease mutant  APP minigene containing a valine to phenylalanine substitution at position 717. APP expression was under the control of the platelet derived growth factor (PDGF-β) promoter. High amounts of transgenic APP were produced in the brains of these animals and high level production of Aβ protein was noted. Characterisation of these transgenic lines disclosed that although there was no obvious phenotype at 6–9 months, the animals developed Aβ protein deposits in the hippocampus, corpus callosum, and cerebral cortex. The numbers of these Aβ protein deposits increased with the age of the animal. Most interestingly, the deposits were stained with thioflavin S and were birefringent with Congo red, two classic stains for senile plaques in Alzheimer’s disease. Neuritic pathology was also seen around the Aβ protein deposits although no neurofibrillary tangle pathology has been described to date. This mouse has recently been crossed to a transgenic mouse overexpressing transforming growth factor-β1 (TGF-β1). TGF-β1 is thought to play an important part in brain response to injury and increased concentrations have been detected in the CNS of patients with Alzheimer’s disease. The bigenic progeny of this cross had accelerated deposition of Aβ protein in both cerebrovascular and neuronal structures, compared with singly transgenic litter mates carrying either parental genotype. This finding provides further support for the role inflammatory mediators may have in the pathogenesis of neurodegenerative disease.

A second APP transgenic mouse model of interest involved overexpression of a familial Alzheimer’s disease mutant APP containing Lys670–Asn and Met671–Leu substitutions. Expression of the mutant APP was under the control of the hamster prion protein gene regulatory elements. Both Aβ 1–40 and Aβ 1–43 production is increased in the brain of these animals and deposition of Aβ protein in typical amyloid plaques was seen in animals of about 12 months old. Behavioural testing of these mice in the Morris water maze, which enables analysis of spatial memory function, disclosed that the animals had impaired memory function and that the loss of memory correlated with the degree of pathology.

A more recent transgenic mouse expressing the carboxy-terminal 104 amino acids of APP—which includes the Aβ protein sequence—under the control of a neurofilament gene promoter has also shown development of extracellular β-amyloid plaques together with cell loss in the CA1 region of the hippocampus. Adult mice at 4 to 5 months of age exhibited spatial learning defects in the Morris water maze. These mice also exhibited a defect in maintenance of long term potentiation (LTP).

Although no neurofibrillary tangle pathology was seen in any of the above models, these mice show that introducing either the amyloidogenic portion of wild type APP or a mutant APP known to cause Alzheimer’s disease in some families, can induce typical amyloid pathology and loss of memory function.

Some groups have attempted to model Alzheimer’s disease by constructing PS1 transgenic mice. Duff et al have made transgenic mice over expressing human wild type PS1 and the PS1 mutants Met146–Val and Met146–Leu under the control of the PDGF promoter. Other PS1 transgenic mice have also been created in which the wild type and mutant PS1 cDNA is driven by the mouse prion promoter. In each case the wild type and Alzheimer’s disease mutant PS1 proteins encoded by the transgenes have been shown to be correctly processed.

As discussed earlier, there is evidence that Alzheimer’s disease mutations in PS1 lead to increased production of the longer, amyloidogenic forms of Aβ peptides. For this reason all of the early studies carried out on these presenilin transgenic mice have concentrated on looking for alterations in processing of endogenous APP. However, more recent studies have taken this further by examining the effect of Alzheimer’s disease PS1 mutants on APP processing in double transgenic mice. These double transgenic mice express wild type or mutant human PS1 along with wild type human APP 695 or a chimeric mouse/human APP cDNA which incorporates a double mutation at position 670–671.

In each case studied to date it was found that overexpression of wild type human PS1 did not significantly increase the amounts of Aβ1–42 in the brains of these mice. By contrast, expression of the mutant PS1 cDNAs led to a roughly two to threefold increase in Aβ1–42 detected in the brains of transgenic mice, and also greatly accelerated the deposition of Aβ protein in the cortex and hippocampi of these bigenic mice relative to mice expressing the APP mutation alone. These studies show an in vivo link between mutant presenilin, APP processing, and Aβ protein production.

Wong et al have performed targeted disruption of the mouse PS1 gene by homologous recombination in embryonic stem cells. In this approach exon 4 of the endogenous PS1 gene is replaced by a neomycin resistance gene. PS1 homozygous null mice did not survive beyond day 1 after birth and exhibited a severe perturbation in the development of the axial skeleton. Interestingly, the defects bore a striking similarity to those in mice in which the genes encoding Notch1 or delta-like protein 1 (Dll1) had been knocked out. Notch is a receptor involved in cell fate determination and Dll 1 is one of its ligands. Further analyses of the PS1 knockout embryos disclosed that they also had markedly reduced mRNA for Notch1 and Dll1. These results suggest that PS1 is required to regulate spatial and temporal expression of Notch1 and Dll1 in the developing embryo. To a certain extent this is supported by studies in Caenorhabditis elegans that suggest that Sel-12 (a C elegans homologue of PS1) facilitates Notch function. An additional PS1 knockout mouse confirms the above findings and in addition, neurons cultured from these mice display abnormal APP processing, implying a role for PS1 in γ-secretase mediated proteolytic cleavage of the C-terminal transmembrane fragments of APP.

Future studies on the PS1 knockout mice should shed light on the ability of Alzheimer’s disease mutant PS1 to complement the effect of the wild type PS1 knockout. This may help us to understand how Alzheimer’s disease mutations affect PS1 function.

Motor neuron disease

Amyotrophic lateral sclerosis is the most common of the human motor neuron diseases and results in the selective degeneration of the motor neurons of the motor cortex, brain stem, and spinal cord. Selective atrophy of the muscles supplied by these neurons follows, ultimately resulting in death, usually from respiratory insufficiency. Pathological examination of the spinal cords from affected people often shows the presence of accumulations of neurofilaments in the cell bodies and proximal axons of motor neurons.

Five to 10% of amyotrophic lateral sclerosis is familial and in 20% of these familial cases, the causative genetic lesions are found in the gene encoding copper-zinc superoxide dismutase-1 (SOD-1) on chromosome 21. The human SOD-1 gene codes for a 153 amino acid metalloenzyme that catalyses the conversion of O2 to O2- and H2O2.
Several transgenic mouse lines expressing familial amyotrophic lateral sclerosis mutant SOD-1 genes have now been created. Many of these mice display motor neuron degeneration and these animals have greatly assisted our knowledge of pathogenic mechanisms in amyotrophic lateral sclerosis.

The first of these mouse models, developed by Gurney et al., analysed the effects of two human SOD-1 mutations, an Ala1-Val substitution (A4V) and a Gly5-Ala substitution (G93A). Mice from one of the G93A lines (G1) developed a stereotyped syndrome suggestive of motor neuron disease with hind limb weakness evident at three to four months of age. Other clinical features included impaired grooming, decreased stride length, tremor of the hind limbs when suspended by the tail, and frequent extensor spasms of the hind limbs. Pathological examination of these mice showed a loss of large myelinated axons from the ventral motor roots and from the intramuscular nerves with consequent reinnervation of muscle fibres by primary nodal sprouts. The surviving motor neurons in spinal cord sections showed neurofilament accumulations typical of those seen in human cases of amyotrophic lateral sclerosis. Several other transgenic mice expressing further mutant SOD-1 mutations have now been described. These include the G37R, G85R, and G86R mutants. All of these mice develop motor neuron degeneration and are valuable models of amyotrophic lateral sclerosis. However, transgenic mice expressing wild type SOD-1, even to levels higher than some of the mutant SOD-1 mice, do not develop motor neuron disease. Furthermore, transgenic mice in which the SOD-1 gene has been ablated do not develop the disease. Such findings strongly suggest that the amyotrophic lateral sclerosis SOD-1 mutants have acquired a toxic property that is harmful to motor neurons.

The nature of this toxic property is not known. One suggestion is that the amyotrophic lateral sclerosis SOD-1 mutants act as peroxidases to catalyse the formation of damaging hydroxyl radicals. A second possibility is that mutations cause the enzyme to unfold slightly allowing increasing access of peroxynitrite to copper at the active site. The nitronium-like intermediates formed could nitrate tyrosine residues and thereby damage proteins. Indeed, a recent publication has shown raised concentrations of free 3-nitrotyrosine in spinal cords from G37R mutant SOD-1 mice relative to control mice. A third possibility is that mutant SOD-1 might influence phosphorylation and signal transduction as wild type SOD-1 seems to protect calcineurin from inactivation. Mice transgenic for G85R SOD-1 develop pathogenic changes in astrocytes before motor neuron pathology. In particular, expression of the glial glutamate transporter (GLT-1) is decreased. This finding supports the hypothesis that glutamate mediated motor neuron injury is secondary to a defect in astrocyte handling of glutamate. Finally, there is evidence that the SOD-1 mutants might cause motor neuron cell death by apoptotic mechanisms. None of the above hypotheses are mutually exclusive and they may act collectively leading to a final common pathogenic pathway. However, there is little experimental evidence that any of these proposed mechanisms cause amyotrophic lateral sclerosis.

One of the most valuable properties of the mutant SOD-1 transgenic mice is that they allow early pathogenic changes in the disease to be charted. Indeed, such studies have provided evidence that the different ALS SOD-1 mutants might induce disease via different aetiologies. For example, pathology in the G37R mutant SOD-1 involves the presence of membrane bound vacuoles in axons and dendrites that seem to be derived from mitochondria. Thus mitochondrial degeneration might be a key pathogenic event associated with the G37R mutant SOD-1.

Conversely the earliest pathology seen in transgenic mice expressing the G85R mutant SOD-1 involved damage to astrocytes. It has been suggested that this might lead to increased concentrations of extracellular glutamate, which in turn induce excitotoxic damage to motor neurons. Thus different ALS SOD-1 mutants might induce motor neuron disease by different primary mechanisms.

The use of transgenic mice in the testing of therapeutic reagents has been elegantly demonstrated in a study in which riluzole, gabapentin, and vitamin E were given therapeutically to transgenic SOD-1 mice. Glutamate has been linked with pathology in ALS through various studies and the use of riluzole, a glutamate antagonist, in patients has resulted in a modest improvement in survival. SOD-1 transgenic mice fed riluzole showed an increase in survival time of 13–15 days over controls, an improvement of about 11%, which closely mirrors that seen in clinical studies. Similar use of vitamin E and another glutamate antagonist, gabapentin, in this mouse model, showed that the first slowed onset of clinical disease by 12 to 15 days, and the second slowed mortality by 8 days. Taken together, this suggests that the first step in development of ALS pathology in this model may involve lipid peroxidation; hence the beneficial effect of vitamin E on onset of disease, which may be followed by glutamate mediated excitotoxicity.

Whether the neurofilament accumulation seen in the motor neurons of patients with ALS has a primary role in the pathogenesis of motor neuron disease, or is simply an epiphenomenon, has long been a subject of discussion. Strong evidence that neurofilament metabolism is central to the pathogenic process of amyotrophic lateral sclerosis has come from studies of transgenic mice expressing neurofilament proteins. Transgenic mice overexpressing either the murine neurofilament light subunit (NFL) or the human neurofilament heavy subunit (NFH) have both developed a clinical phenotype similar to motor neuron disease. Pathological examination of motor neurons from both mouse models showed the accumulation of perikaryal neurofilaments, as in human cases of sporadic and familial amyotrophic lateral sclerosis. However, despite the muscle atrophy seen in these mice, motor neuron loss was not as marked as that seen in human amyotrophic lateral sclerosis.

A closer pathological correlate of human amyotrophic lateral sclerosis is seen in the model developed by Lee et al. In this transgenic model, codon 394 of mouse NFL was mutated introducing a leucine to proline substitution that disrupts neurofilament assembly. The resulting mice had abnormal gait and weakness of all limbs from as early as three weeks after birth. Pathological examination disclosed loss of lumbar and cervical motor neurons with the presence of neurofilament accumulations.

Recently, we and others have described deletions in the NFH gene that are associated with sporadic cases of amyotrophic lateral sclerosis. These deletions involve changes to the side arm domain of NFH that involve a highly phosphorylated region of the protein. Such findings reinforce the hypothesis that neurofilament mismetabolism is part of the disease process in amyotrophic lateral sclerosis. Indeed the prominent accumulations of neurofilaments seen in the G93A SOD-1 transgenic mice show that there has to be some link between at least certain of the amyotrophic lateral sclerosis SOD-1 mutants and neurofilament metabolism. This hypothesis is further supported by a recent paper from Zhang et al who have shown a reduction both in axonal levels of neurofilament proteins and in orthograde slow axonal transport of neurofilaments and other cytoskeletal proteins in ventral root axons of G93A SOD-1 transgenic mice.
Conclusions
The examples referred to above illustrate the power of the transgenic technique in understanding and unravelling the pathophysiology of complex neurological and behavioural phenotypes. Transgenic modelling of disease gives the potential to study and dissect out the pathway from genetic mutation to abnormal protein and thence pathology. This may allow the identification of additional targets in the disease pathway for therapeutic intervention. Furthermore, crossing different transgenic lines allows the study of cumulative effects of different genetic lesions within an individual organism. This may be of importance in the study of disorders where mutations in genetic loci have been implicated in pathogenesis. Although the resulting animal models are not always a perfect replica of the disease process (for example, the lack of neurofibrillary tangles in the mouse models of Alzheimer’s disease) and given that mouse anatomy and physiology may never be considered wholly analogous to humans, the transgenic approach does give an unparalleled insight into examining some of the processes leading to the disease. As we have seen from work on the SOD-1 mouse, creation of an adequate animal model of a disease may pave the way to testing of novel pharmacological agents; currently these agents are largely disease modifying in nature, but the understanding of the genes underlying disease processes may herald a new era of gene modifying drug therapy.

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