

Stem Cells in CNS Repair

There is no question that stem cells are an enormously hot topic. You can be sure something is afoot when stem cell scientists become US Presidential Special Advisors, report to House of Lords Select Committees, are interviewed on Newsnight, and appear in Doonsbury cartoons. So, what are the prospects that stem cells will have any influence on clinical neuroscience? In this short review, I will identify what seem to me the four most immediate areas of impact.

Endogenous stem cells

Stem cells are usually defined as having two seminal properties: the capacity for extended self-replacement, and multipotentiality (defined in the nervous system as the potential to generate neurons, astrocytes, and oligodendrocytes). The mammalian nervous system is poor at cell regeneration, so the first question is do endogenous neural stem cells (NSCs) exist? If adult brain tissue is cultured under non-adherent conditions and high concentrations of growth factors, then stem cells indeed emerge to give rise to 'neurospheres'—aggregates of expanding brain tissue.²⁹ In vivo, this stem cell activity is concentrated in two specific regions: the granule layer of the hippocampus and the subependymal layer, areas rich in adult neurogenic activity.¹⁹ Remarkably, at these in vivo sites the stem cells themselves turn out to be 'specialised' astrocytes.⁸ So, if the brain does have stem cells, why does it not repair itself more successfully? Of course, 'lower' vertebrates do repair damaged neural tissue: it is just we mammals that do so poorly. Is that because of a dearth of resident stem cells, or have we lost the repair mechanisms?

Probably, it is the former. Nakitomi et al (2002) infused FGF2 and EGF (the growth factors that expand neurospheres) into mouse hippocampus following ischaemic brain injury and replaced a substantial proportion of lost CA1 pyramidal neurons, far more than seen in controls.²² This implied that if stem cells can be expanded in vivo then they can indeed home to sites of cell loss and differentiate appropriately. One therapeutic avenue, therefore, would be to drive this process.

NSC Engraftment

If there are not enough endogenous cells, can we graft more? Cells from human 'neurospheres' have been grafted into animal models of disease. They differentiate to an extent,^{9,11} but reports of functional effects have been few and inconsistent.^{15,23} Another strategy is to use conditional-immortalisation to generate lines of NSCs in culture. NSCs from aborted fetal tissue can be engineered to express an immortalising oncogene (typically SV40 T or c-myc). Such cells expand almost infinitely in culture, without their multipotentially being compromised.^{3,10} So when the oncogene is turned off (using an engineered molecular switch), the cells still differentiate as neurons, astrocytes, and oligodendrocytes. Much evidence suggests that immortalised NSC lines grafted into the damaged brain can replace lost neurons and glia, inhibit further neurodegeneration, and bring about a degree of functional improvement.²⁷ There are now reports of human lines, grown to clinical grade under GLP conditions that would be suitable for clinical studies.²⁶ Evidence suggests these lines are safe and have efficacy in animal models of stroke and are suitable for clinical trials. These may prove to be the earliest NSCs to provide proof-of-concept for this approach to neurodegeneration.¹

ES Cells

Embryonic stem cells (ES cells) justifiably get the lion's share of stem cell publicity because they have the greatest

clinical potential, but they also have the most profound ethical and technical concerns. Because they are derived from blastocytes (cells of the pre-implantation embryo) they are not just multipotential but pluripotent; that is, they can generate all the cell types in the body. Their potential in regenerative medicine is therefore enormous, but the problems are commensurately large.

- ES cells can be tumorigenic when undifferentiated.⁵
- Their wide potential requires precisely controlled differentiation, otherwise they could generate inappropriate cell types following engraftment.
- Human ES cells will be difficult to grow to GLP because they require 'feeder cells' and cannot be grown in defined conditions.⁶

There has been startling progress in generating neural cells from mouse ES (mES) cells. For example:

- Wichterle et al (2002) manipulated mES cells to generate progenitor cells, which generated motor neurons following engraftment into a chick embryo.³²
- Kim et al (2002) and Björklund et al (2002) both engrafted mES cells into a rat model of Parkinson's Disease and generated graft-derived dopaminergic neurons and improvement of motor dysfunction.^{4,12}

Nonetheless, human (hES) cells will be required for clinical studies, and progress here is more modest. Such cells have been shown to engraft neonatal or adult rodents,^{21,28,31,33} but there are few reports yet of functional improvement following engraftment with hES cells.²⁵ Clinical trials with hES-derived NSCs are probably still a little way off.

Non-CNS stem cells

Several other stem cell types have been engrafted into the damaged CNS of experimental animals in order to evaluate their potential for repair. They include

- Olfactory ensheathing cells
- Haematological Stem Cells (HSC: Bone marrow- or Umbilical Cord Blood-Derived)
- Mesodermal Stem Cells (MSC).

The target indications have been equally diverse including:

- Stroke
- Spinal Cord injury
- Traumatic Brain injury
- Multiple Sclerosis
- Batten's Disease.

There are too many individual studies to discuss in this short article, but a common thread links many of the studies. The conventional view of adult (or fetal) stem cells was that they were tissue restricted: ie. NSCs give nervous tissue; HSCs give blood cells; etc. This view was challenged by studies indicating that tissue-specific stem cells could transdifferentiate into progeny from a different lineage. To cite just one pivotal study, Mezey et al (2000) presented *prime facia* evidence that bone marrow infused into mice differentiated into neurons and glia: the so called, 'Blood into Brain' discovery.¹⁸ The number of 'transdifferentiating' cells are low, however, and might be better explained by fusion of host and grafted cells.¹⁷ None the less, the findings have proven sufficiently robust to maintain interest, and both HSCs (from bone marrow and umbilical cord) and MSCs are actively being studied at this time for their potential to repair CNS damage.

Several reports suggest that infusion of these stem cell types brings about functional improvement in animal models of disease, for example in stroke.¹³ The question



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that arises, however, is whether efficacy is linked to transdifferentiation. The case against is that functional recovery can be observed where little neural differentiation can be seen, and is often quite fast in comparison to cell replacement. Optimal recovery is observed in most laboratories when the stem cells are administered within a few hours post-lesion, suggesting a neuroprotective effect. They appear to induce structural change in the host brain rather than replacing lost cells, the 'classic' stem cell mode of repair. So while this approach is clearly 'cellular therapy' it might not be 'stem cell therapy' in the pure sense. Then again, NSCs in the studies cited above might also be working through means other than cell replacement: studies with both conditionally-immortalised NSCs and neurospheres suggest that they too repair where they do not replace.^{15,20} Again, the evidence is that they have a neurotrophic or 'plasticity-inducing' effect on the host tissue.²⁴

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