Body, heal thyself

Research on stem cells and therapeutic cloning: great hope tinged with caution

The stem-cell genie is out of the research bottle,' columnist William Safire wrote on July 16 in the New York Times editorial page, continuing, 'If we command it sensibly, this unexpected servant will help us lead much healthier, longer lives.' Safire is not alone in having these expectations. Since research using embryonic stem cells has been dragged into the limelight of public debate, its supporters have never tired of emphasising the potential medical breakthroughs that might slumber in these undifferentiated cells. But will stem cell transplants and therapeutic cloning become the new medicine of the future, and what are the prospects for tapping this spring of eternal youth, the mysterious stem cell? Since the political debate has been shaped mainly by future expectations, it is therefore appropriate and necessary to take stock of where research with embryonic stem cells is right now.

A recent symposium organised by the European Molecular Biology Laboratory

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examined the scientific and technical state of the art in stem cell research and therapeutic cloning. Prominent scientists presented the latest results in a field as beset by ethical problems as it is fast-moving.

Lovell-Badge, from the National Institute for Medical Research in London, presented a slide of a brown and white mottled mouse, a chimera made by replacing cells in a white mouse 8-cell embryo with embryonic stem (ES) cells from a brown mouse. The mouse is perfectly healthy: proof that in mice, at least, ES cells are totipotent. And they can be cryopreserved indefinitely: cells such as these have been used for 20 years and still perform, noted Lovell-Badge. *In vitro* these cells can be differentiated into cardiac and skeletal muscle, blood vessels, haematopoietic cells, insulin secreting cells and various neural cells.

As far back as 1975 scientists with foresight were considering the prospects of using human stem cells to cure disease, provided they could be isolated from embryos. Frustratingly, the choice of model was limited to the mouse. Despite numerous efforts to derive ES cells from other animals, the mouse remained the only experimental source. That is until 1998 when James Thompson at the University of Madison, Wisconsin succeeded in deriving—albeit with 25% efficiency—human ES cell lines from surplus IVF blastocysts. It is now possible to derive many of the cell types mentioned above from these cells.

But ES cells are a little maverick and cannot simply be injected into a patient ectopically, as Lovell-Badge reflected; in mice this can cause teratocarcinomas, cysts containing a bizarre combination of hair, teeth, skin and other tissues. Indeed in a Petri dish with no particular stimuli, ES cells differentiate haphazardly. It appears that unless the cells are told what to do, they follow a chaotic proliferation and differentiation pathway of their own. Give them any stimulus that causes their differentiation, however, and they no longer form cancers. For the purpose of implantation it is therefore important to select a partially differentiated cell of the type required. This is achieved by a three stage process: (i) feeding the cells growth and survival factors—to make neuronal cells, for example, one adds retinoic acid; (ii) screening the cells based on size, shape and cell-surface antigens, and sorting using a fluorescence activated cell sorter; and (iii) selecting for specific markers, e.g. drug resistance. Since the discovery that human ES cells could be isolated and cultured, *adult* stem cells have been identified in several tissues and can be cultured similarly.

But what makes a stem cell different from a somatic cell? A fundamental gap in our knowledge is which genes define the stem cell state. Clearly these must permit or encourage renewal while conferring the ability to differentiate into many cell types. The developmental *Sox* genes are promising candidates, and are expressed

in some astonishing places. As Lovell-Badge revealed, 'we have evidence to suggest that we can select stem cells from the adult spinal cord'. *Sox2* expression is seen here and in parts of the adult brain

As if that were not tantalising enough, stem cells seem to be the veritable masters of fitting in. According to Lovell-Badge, 'cells differentiate according to where you put them, not to where they came from'. Blood stem cells can become neurones, and even more astoundingly, neural stem cells can become blood. However, Lovell-Badge added with a note of caution that the regulatory regions of the *Sox* genes bear the memory of the cell's original location, even after transplantation. Therefore changing a cell's fate may not completely change the cell.

The reprogramming of the cell nucleus that occurs when a cell is driven backwards to a multipotent or totipotent state is shrouded in mystery. From this it follows that adult stem cells would be more safely used to repair the tissues from which they are derived, rather than used as a universally applicable therapy. ES cells taken from the 8-cell stage of a blastula, on the other hand, have no differentiation history and could be used universally.

Adult stem cells for re-transplantation seem, therefore, a second best compared with ES cells, especially as the adult stem cells in many tissues have not yet been found. Furthermore, they can be very dif-

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ficult to culture in comparison with ES cells. There is evidence that more differentiated adult cells can be de-differentiated and reprogrammed to show stem cell-like properties, but this is a difficult procedure, and the cells often have low proliferative capacity. Notwithstanding the present pitfalls, Lovell-Badge envisages a day when banks of immunologically diverse ES cell lines for transplantation are

available. As a pessimistic estimate, he thinks that perhaps 1000 cell lines would be necessary in order to overcome rejection by correctly type matching donor and recipient.

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This might not be necessary if therapeutic cloning becomes feasible. As Alan Colman, CEO of PPI Therapeutics, Roslin, Edinburgh explained, this would remove the immunological problems inherent in exogenous stem cell transplantation because the clonal cells would be derived by nuclear transfer from one of the patient's own cells to a donor oocyte. But as enthusiastic as Colman may have been about the numerous cloned animals his company has produced, when it came to the application of the technique in humans he was arguably the most sober speaker of the session. Colman reported results that, societal objections aside, rule out the notion of human reproductive cloning, and set a cautious note for the future of therapeutic cloning. For a start, the efficiency of somatic nuclear transfer is unacceptably low for human use: for cows, the most successful case, the number of live births per reconstructed embryo is between 1.1 and 10.6%; in mice it is a mere 0.4–3.1%. Clearly something is going very wrong with these cells. This is partly natural; a significant proportion of naturally created embryos do not survive because of chromosomal abnormalities at the structural or DNA level. But postnatal mortality is also unacceptably high: half of the cows die within 3 weeks of birth. This certainly is not natural, and adds weight to the suspicion that the nucleus is probably not fully reprogrammed after all.

That reprogramming of a transferred nucleus occurs at all is little short of a miracle, and so by definition scientists barely understand it, and even less are able to control it. Even if an apparently healthy animal is produced, it may only be a case of waiting until the time-bomb of an unstable nucleus sets in motion a chain of catastrophic events unleashing cancer or apoptosis. Again, this is a risk researchers could never take with humans. In

essence, there is no way to validate a good embryo short of waiting to see if it dies.

What Colman described as a 'very inefficient technique' exploits phenomena that are also very poorly understood, if at all. At present scientists can only surmise that cloning inefficiencies are due to imprinting defects (chromosomes can 'remember' the cell they came from in the pattern of gene activation or repression), time in culture, inadequate reprogramming and chromosomal changes (telomere length, methylation patterns etc.). Indeed, there is already evidence that imprinting can vary in otherwise identical clonally derived cultured cells, and that DNA methylation patterns are faulty in reconstructed embryos. It appears that in the context of cloning no one even understands the behaviour of telomeres; in cloned sheep the copy number is decreased, while in cows researchers argue as to whether the number is unchanged or even increased.

Therapeutically cloned cells may, therefore, not make their way into patients as soon as some think. Stem cell therapy, in contrast, has already been accomplished, though ironically not many people know it. As Colman put it 'we talk about stem cell therapy as if it is a technique that doesn't yet exist, but bone marrow transplants are an example'. Even more revolutionary is the dawning realisation that in cancer patients who have suffered tissue damage as a result of chemotherapy or radiotherapy, stem cells from the subsequent bone marrow graft have very likely infiltrated and repaired

Mouse Biology Programme at the EMBL outstation in Monterotondo, Italy. Noted for her radical ideas on the reconstructive powers of stem cells, to her it really is a case of 'body heal thyself'. Whereas others are examining the prospects of extracting stem cells from a host, culturing and reinjecting them into the same or a foreign recipient, Rosenthal is on the hunt for the secret behind the regenerative capacity of naturally occurring endogenous circulating and resident stem cells.

Rosenthal's research, carried out at the Harvard University Medical School, builds on the observation that in a number of tissues there are resident stem cells, or cells that can be stimulated to become stem cells if need be. Skeletal muscle is well known for its regenerative and hypertrophic capacity. Injured muscle can regain much of its original bulk, and body builders are the somewhat gruesome proof that when required, muscles can dramatically increase the number of fibres they contain. These responses, as Rosenthal explained, appear to be due to 'satellite' cells that naturally reside in muscles. Microinjuries that occur to muscle fibres during strenuous exercise can stimulate these cells to enter a stem cell state, proliferate, and thus repair and supplement the existing fibres. Insulin-like growth factor 1 (IGF-1), a polypeptide hormone, is the active molecule released when these giant multinucleate cells are damaged. IGF-1 regulates growth and differentiation of many tissues, but as Rosenthal noted, administering IGF-1 systematically can cause uncontrolled neoplasia and cancer.

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the damage to some extent. There are, therefore, people walking around today with graft-derived cells in their brains, livers, kidneys and who knows which other organs.

Indeed, most recent research in mice points out that the body's own stem cells might have more healing power than assumed. For researchers are now developing mouse models of stem cell-dependent repair processes, notably for myocardial infarction. Prominent among these researchers is Nadia Rosenthal, the recently appointed Coordinator of the

Expressed in muscle under the control of a post-mitotic promoter, however, IGF-1 can turn a normal mouse into Mighty Mouse, as Rosenthal observed. These mutant mice do not even need to go to the gym to pump iron; they develop 20–30% more muscle without exercising, and maintain it even into old age. This promising line of research may one day cure human muscle wastage in old age, and help muscular dystrophy sufferers. Indeed, Rosenthal finds that when Mighty Mouse is crossed with a muscular dystrophy mouse, the resulting offspring have

strengthened and thickened muscles compared with their unfortunate parent.

If the muscle of Mighty Mouse is injured by toxin treatment 'we see an enormous regenerative response' said Rosenthal. In this case, something quite extraordinary is happening: bone marrow cells are migrating to the site of damage and repairing it. In a normal mouse, even a damaged heart can be repaired by injecting bone marrow-derived stem cells. In sections of the damaged area, stem cells can be seen to have infiltrated the infarct and are busy proliferating and differentiating into cardiac muscle. Conventional wisdom had all but ruled out the possibility that the cardiac muscle could be repaired. Scarring of tissue, the natural but less than useful response, prevents stem cell infiltration, but if the stem cells can access the site sufficiently quickly, they can substantially inhibit scar

tissue formation. As to how revascularisation of the reorganising area occurs, Rosenthal's conviction is that the stem cells stimulate muscle cells to redifferentiate into vascular epithelium.

These tantalising findings have sparked the belief in Rosenthal that the circulating

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and resident stem cells, something that we all have in low numbers, can be guided to the site of reconstruction and stimulated to proliferate there. The observation that metastatic cancer cells lodge in some places and not others may be the dark side of the normal process that she is looking for. The challenge now is to learn what the body already does, and then

'tweak' it to steer stem cells to a desired target.

If Rosenthal is right, and the body is a 'transparent sponge, through which [stem cells] are coursing', and if stem cells can carry characteristics that predict where they will end up, we may indeed see the day when we can ask the body to heal itself.

Andrew Moore

The meeting 'Stem cell research and therapeutic cloning: scientific and social implications' was organised by the Science and Society Committee of the European Molecular Biology Laboratory (EMBL), Chairman Dr Halldór Stefánsson, and took place on 16 June, 2001 at EMBL, Heidelberg. E-mail: andrew.moore@embo.org

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