

Youthful prospects for human stem-cell therapy

In another few decades, revised attitudes towards stem cells could lead to disease prevention and life extension

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It is the year 2053. A mere century after James Watson and Francis Crick resolved the structure of DNA, scientists at the forefront of medical research have just announced the first successful regeneration of a human heart. After re-routing the blood of Jón Sigurdsson, a terminal heart-failure patient, to an advanced cardiac assist device and removing most of the damaged organ, doctors thawed a frozen tube of Jón's personalized stem cells—established in 2013 from embryonic stem cells created through somatic nuclear transfer—and injected them into his chest. Thanks to a sophisticated cocktail of growth factors, the new stem cells target the damaged area and rapidly get to work, perfectly rebuilding a youthful heart. Several weeks later, Jón is discharged in excellent health. Regenerative medicine provided him with a new kidney ten years ago, and subsequent double knee regeneration gave him renewed mobility. Now his new heart will soon have him running a six-minute mile again. Jón Sigurdsson is 100 years old.

This scenario might sound like pure science fiction, but it could become reality a few decades from now. Stem cells have attracted huge scientific and public interest, not only because they bear the promise of miracle cures for age-related heart diseases, but also because their medical use is so appealing: stem-cell therapy could augment the human body's own regenerative capacity, which declines as we grow older. The appropriate source of cells for these therapeutic applications is hotly debated, but the technical feasibility of generating replacement tissues and organs is well

within realistic projections. Nevertheless, although the prospect of rejuvenation has captured the public imagination, the field is plagued with controversy: some of the most dramatic studies have been subsequently refuted, and heated ethical debates threaten to distort the scientific work that must be done before stem-cell therapy can become a medical reality.

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The recent explosion of information on stem cells highlights their capacity for self-renewal and their contribution in creating multiple tissue types, but has still not brought us a clear understanding of the underlying molecular biology in any system. A classic distinction has been drawn between the plasticity of stem cells in the early embryo—whose fate encompasses all cells of the organism—and the more limited potential of stem cells found in adult tissues. In addition, embryonic stem (ES) cells that are derived from humans also differ significantly from ES cells derived from mice, the main animal model for stem-cell researchers. For instance, mouse ES cells proliferate more rapidly than their human counterparts, which are difficult and slow to grow (Pera & Trounson, 2004). Furthermore, whereas many of the molecular mechanisms that underlie mouse ES-cell growth are well characterized, it is not clear if these

are shared by human ES cells (Rao, 2004). Experimentally, mouse ES cells have distinct advantages because they can be genetically manipulated and can be used in assays that cannot be performed on humans for ethical and sociopolitical reasons. However, basic scientific questions about human stem cells must be answered before we can start exploring their regenerative potential and ensure their safe use in the clinic.

Human ES cells are harvested when a fertilized egg has divided for five days to form the blastocyst—a small hollow ball of cells. As with mouse ES cells, they survive and proliferate indefinitely in tissue culture when removed from the embryo. Most human ES cells recover after freezing and thawing, and can differentiate into a variety of cell types *in vitro*. However, it is now becoming clear that not all human ES-cell lines are the same, but rather reflect the genetic diversity of the embryos from which they were derived (Rao, 2004). Recent studies have described the potential of human ES cells to differentiate into multiple lineages, giving rise to a mixture of nerves, blood, heart muscle and other cell types, and researchers are now testing the differentiation potential of a human ES-cell line using molecular markers that were originally characterized in mice. Such functional assays are needed to determine the behaviour of specific stem-cell lines in the context of ageing or diseased tissues.

Even if the available human ES-cell lines are shown to produce multiple cell types, their use in the regeneration of ageing or diseased tissues could be severely limited

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NAME SIGURDSSON, JÓN F M X N DATE OF BIRTH 02-29-1953

ES CELLS ESTABLISHED 03/31/2013

Organ	Date	Checked	Regenerated	Date	Checked	Regenerated
HEART	03-14-2018	X	CHECKED	09-09-2043	X	CHECKED
	06-26-2023	X	CHECKED	09-18-2048	X	CHECKED
	05-31-2028	X	CHECKED	05-29-2053	X	CHECKED
	05-07-2033	X	CHECKED			
	02-07-2038	X	CHECKED			
KIDNEY	03-14-2018	X	CHECKED	09-09-2043	X	CHECKED
	06-26-2023	X	CHECKED	09-18-2048	X	CHECKED
	05-31-2028	X	CHECKED	05-29-2053	X	CHECKED

by their incompatibility with the patient's immune system, which is likely to recognize the incoming cells as alien and destroy them. Nuclear transfer techniques could circumvent this problem. In this approach, otherwise known as therapeutic cloning, the introduction of a nucleus from the patient's own cell into an enucleated egg from a human donor generates an early-stage (pre-implantation) embryo, or clone. Stem cells isolated from the resulting embryo are genetically identical to the patient's original cell, but have been reprogrammed by the embryonic environment to be pluripotent and can form virtually any tissue when reintroduced into the patient (Jaenisch, 2004).

Given the current problems with controlling the proliferation and differentiation of cultured ES cells—which might cause tumours in patients as they do in some animal models (Hochedlinger & Jaenisch, 2003)—nuclear transfer seems to be the most promising method. During the past few years, animal experiments have validated this strategy as an effective and safe method to generate personalized, immunocompatible human stem cells for therapeutic tissue replacement. For example, partially differentiated cloned cells can be transplanted back into the hearts of nucleus donors without destruction by the immune system (Lanza *et al.*, 2004). Other

experiments in which cloned cells were introduced into normal blastocysts have resulted in perfectly healthy animals, so it appears that, at least for therapeutic applications, cloned stem cells are equivalent to normal ES cells. Growing Jón a new heart from personalized ES cells may thus not be as far off as we think.

Scientists have already taken nuclear transfer one critical step further as a proof of principle. When transplanted into the uterus of a foster female, an embryo harbouring a transferred adult nucleus has the potential to develop into an offspring that is a genetic clone of the adult donor cell, in a process known as 'reproductive cloning'. Although this demonstrates the promise of nuclear transfer, it also reveals the problems that may arise. Stem-cell lines generated with nuclei from older donors have accumulated mutations that could render them ineffective, or worse, prone to cancerous growth. Although the untimely death of Dolly, the first cloned sheep, was attributed to unrelated causes, many of the rare animal offspring from other reproductive cloning experiments share common abnormalities, such as fetal overgrowth and renal, cardiovascular and pulmonary defects (Rhind *et al.*, 2003).

Furthermore, the cloning procedure itself is inefficient and error-prone, and enormous difficulties have been encountered in generating a single reproductive offspring, which has taken years of effort in accomplished laboratories. The main problem is the relatively inefficient reprogramming of the donor nucleus back to a pluripotent state. It is also possible that the development of a cloned embryo after implantation does not select for functional cells, which allows defective cells to proliferate and pass on developmental defects or phenotypic abnormalities to the resulting animal. Indeed, recent gene-expression analyses indicate that many genes are not correctly regulated in cloned mouse pups. As these mice age, they develop severe pathological alterations in multiple organs and major metabolic disturbances that are not apparent at younger ages (Jaenisch, 2004).

If ... defective nuclei from senescent tissue were used to generate personalized stem cells for therapy, they could cause more harm than good

Reproductive cloning is not envisioned in humans, but the lessons learned from cloned animals may be important for therapeutic applications of nuclear transfer. Large deletions involving millions of base pairs have been found in ageing post-mitotic tissues, such as the heart (Vijg, 2004), thus removing large numbers of genes, which leads to cellular degeneration. If such defective nuclei from senescent tissue were used to generate personalized stem cells for therapy, they could cause more harm than good. Moreover, nuclei from patients with inherited diseases, such as haemophilia or muscular dystrophy, may first need to be manipulated to correct the genetic defect before they can be used in clinical settings.

Such a transfer, with subsequent manipulation of genes in human ES cells using human viral vectors and other techniques, could be used on aged nuclei to avoid creating stem cells with dangerous mutations. Any strategy for introducing genetic changes must be applied with care, however, due to the possibility of these genes randomly integrating into the host genome, causing even more serious mutations. To circumvent this danger, techniques for gene-specific modifications that are routinely performed in mouse ES cells have recently been applied to human ES cells, thereby providing the opportunity to correct genetic mutations in stem cells derived from nuclear transfer before administering them to patients.

In parallel with studies on ES cells, a concerted search for similar adult stem-cell lineages has yielded a flood of recent publications. These challenge the classical concept that stem cells in the adult are present in only a few locations, such as the skin or bone marrow, and are committed to differentiate into the tissue in which they reside. Nevertheless, rigorous criteria are required to distinguish an adult stem cell from partially committed cells with limited potential. True stem cells are self-renewing during the lifetime of an organism and they undergo asymmetric division, so that one daughter cell maintains the stem-cell lineage while the other daughter cell matures into a specialized cell type. The criteria for defining stem cells in the adult are still difficult to satisfy experimentally. There is no predictable location for stem cells in most adult tissues, and we still have only limited tools for identifying them.

An appreciation of adult stem-cell plasticity initially grew from observations of human bone marrow transplants after donor cells were found in various tissues of the recipient

Searches for adult stem cells have relied on information derived primarily from studies of the bone marrow, the source of the body's blood supply (Shizuru *et al*, 2005). In mouse bone marrow, stem cells are as rare as 1 in 10,000, and may be even less common in humans; however, these special cells proliferate constantly to renew circulating blood. Bone-marrow-derived mesenchymal stem cells readily proliferate in culture, which makes them attractive candidates for therapy, whereas haematopoietic stem cells display additional characteristic morphologies and cell surface markers that allow them to be labelled and tracked in the bloodstream to target tissues, or to be isolated and cultured *in vitro*.

An appreciation of adult stem-cell plasticity initially grew from observations of human bone marrow transplants after donor cells were found in various tissues of the recipient. Since then, accounts of the repopulation of adult organs by bone-marrow-derived stem cells have flooded the literature. These studies suggest that, under the right conditions, certain stem cells derived from the bone marrow can contribute to virtually any part of the body. Whereas bone marrow is our richest source of stem-cell populations, it now appears likely that pluripotent stem cells also exist in specialized niches within many other adult tissues, and that these common progenitor cells, with properties not unlike ES cells, are able to regenerate and repair tissues throughout the body (McKay, 2004). Neural stem cells can differentiate into neurons, astrocytes and oligodendrocytes and functionally reconstitute at least some compartments of the brain, such as the dopamine-producing cells in the *substantia nigra*, or the myelin sheets of neurons. Other mesenchymal stem cells, termed marrow stromal cells, differentiate into osteocytes, adipocytes, chondrocytes, skeletal myocytes and smooth muscle myocytes. A specific kind of stem cell called a mesangioblast has recently been found embedded in the endothelial lining of certain blood vessels, and its ready proliferation and plasticity make it an attractive candidate for therapy. The reported ability of

mesangioblasts to significantly improve muscular function in a dystrophic mouse model is another exciting example of what adult stem cells might do in the clinic (Cossu & Bianco, 2003). However, these results must be interpreted with care. For all their amazing abilities, stem cells found in tissues such as skeletal muscle or fat may be nothing more than the descendants of circulating bone-marrow stem cells, which are far more prevalent and easier to harvest from patients. More research is needed to determine to what extent the tissue niche in which a stem cell resides affects its subsequent expansion or differentiation potential.

Although studies that tout the proliferative properties of adult stem cells are often cited by opponents to ES-cell research, the field is fraught with controversy. Prominent scientists have claimed that adult haematopoietic stem cells do not normally adopt the phenotype of other cells, citing studies that failed to detect descendants of a single labelled stem cell in other tissues. The extent to which adult stem cells fuse with other cell types is also variable: fusion is the most prevalent source of hepatocytes in the liver, and may also be commonplace in the injured heart (Dimmeler *et al*, 2005). These findings offer an alternative explanation for the presumed transdifferentiation of adult stem cells in a new environment. It is even possible that fusion with a circulating stem cell may actually rescue a damaged or ageing tissue cell. These uncertainties underscore our limited understanding of regenerative biology and the necessity for more research into the fundamental mechanisms by which our bodies heal themselves.

It has recently become clear that deficiencies in these healing processes are associated not only with cancer and/or ageing but also with defective stem cells that have DNA repair abnormalities. New studies on mice suggest that the age-related decline of regenerative potential is affected by systemic factors that change with age, but that this can be reversed by modulating the signalling pathways that are critical for the activation of tissue-specific progenitor cells (Conboy *et al*, 2005). When such cells from an old animal are introduced into a young animal, the new environment re-activates these cells and promotes their successful participation in the regeneration process. Conversely, in an old animal, young progenitor cells either fail to promote, or are

inhibited from promoting, tissue repair. Although these age-dependent factors have yet to be identified, these results relay the hopeful message that progenitor cells retain much of their intrinsic regenerative potential even when old.

Future studies will pinpoint the repair processes that enhance stem-cell survival and influence the stem-cell failures that are associated with ageing. Better understanding of other cellular responses, such as cell cycle checkpoint control, mutagenesis and apoptosis in response to DNA damage, will enable us to identify more specific targets for the development of stem-cell protectants that prolong stem-cell regeneration and increase stem-cell pools.

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The biggest technical hurdle to the clinical application of stem cells is still the small number of cells that can be isolated from any adult tissue. The recent successful propagation of several adult human stem-cell types (Lanza & Rosenthal, 2004) suggests that expansion in culture may be the answer. However, these cells are often slow and labour-intensive to grow. Once cells are in the culture dish, unknown extrinsic and intrinsic factors may control cell fate. Extensive culture of human adult stem cells may also subtly change their intrinsic properties and render them unfit for restoring injured or diseased tissues in patients. We do not yet have the magic cocktail of factors that might make growing Jón an entirely new heart possible 50 years from now.

Despite the anticipated problems in expanding and maintaining stem cells in culture, a small number of stem cells may be all that is necessary to induce the regeneration of injured or diseased organs. This is best illustrated by the power of adult stem cells to overcome the limited restorative capacity in the adult heart, which is attributed to the loss of cardiomyocyte versatility soon after birth. Surprising results have been obtained in clinical trials to improve the recovery of heart attack patients using several types of patient progenitor cells. These cells were isolated from skeletal muscle, bone marrow or

vascular tissue and were administered either by direct cardiac injection or by introduction through the circulation (Dimmeler *et al*, 2005). In these studies, the number of foreign cells that lodged in the injured hearts was far too low to physically replace the damaged cardiac tissue, but they presumably proceeded to the wound and instructed revascularization in the damaged area as well as reconstitution of the heart muscle itself. Increasing evidence suggests that stem cells, similar to metastatic tumour cells, use common chemo-attractive mechanisms that guide them to damaged zones, where they induce regeneration in surrounding tissues. Circulating blood cells are also known to secrete survival molecules or other growth factors that promote local regeneration at sites of injury. Activated progenitor cells also may help repair damaged tissue by dissolving scar tissue and reconstructing appropriate matrix scaffolds that provide niches for new cells to inhabit (Sussman & Anversa, 2004). If stem cells can act as instructors in the healing process, we may not need many of them to change the face of regenerative medicine.

How close are we to cell therapy, if the experts themselves cannot agree on definitions or converge on common standards? Despite promising animal trials, how do we know that human ES cells will integrate into the patient's tissue in sufficient numbers and, once engrafted, take over or at least promote the appropriate physiological functions? And if the adult human body already has stem cells that are truly pluripotent, why is the response to ageing or injury in many tissues so inefficient under normal circumstances?

Stem-cell research has raised as many new questions as it has answered, but this should not deter further study. Early tests of human adult stem cells in the treatment of cardiovascular disease and pancreatic insulin-producing cell replacement are encouraging, and will certainly be the foundation for more extensive testing in the near future. Given the promising preclinical evidence, therapeutic trials using ES cells in neurodegenerative disease are probably imminent. Improved protocols for selecting, purifying and screening stem cells from any source will enable scientists to generate differentiated cell phenotypes from ES cells. Subpopulations of patients' own adult stem cells may be identified that match or surpass ES cells in their growth and engraftment properties. However, we still do not have

sufficient information to identify the stages of stem-cell differentiation that are optimal to ensure their survival and function as an integral part of the host tissue.

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Nuclear transfer, or therapeutic cloning, in combination with the broad differentiation potential of ES cells, is potentially too valuable not to be pursued in humans. As the process seems to select for functional cells, the ethical concerns associated with reproductive cloning are not expected to impede the application of this technique for treatment of human diseases. Because nuclei from older adult cells may have inherited or accumulated mutations that predispose them to senescence or cancer, more research is needed to identify these problems before re-introducing cloned cells to a new and potentially dangerous milieu. In this regard, the application of genetic engineering to the repair of mutations in stem cells extends the utility of these cells beyond their use in regenerative medicine.

In the future, direct reprogramming of the adult genome through a combination of chemical and genetic means may allow the creation of stem cells from the patient's own differentiated tissues. Although the intrinsic plasticity of adult stem cells remains hotly contested, a deeper understanding of the egg's extraordinary nuclear reprogramming capacity may lead to the identification of molecules and mechanisms for facilitating the latent pluripotency of virtually any cell. Reaching this objective would eliminate the intermediate stage of the pre-implantation human embryo, thereby defusing the current ethical objections to such research. Nevertheless, further study is imperative to improve our understanding of the molecular events that take place during nuclear reprogramming, in order to develop these potential new therapies.

To some readers, the confusion and controversy surrounding stem-cell research might provoke a sense of *déjà vu*. Thirty years have passed since the uproar over a different kind of cloning. Back then, similar issues were raised over the safety of recombinant DNA, and reasonable concerns about the

future of genetic research were distorted by media hype that invoked Andromeda strain scenarios (Crichton, 1969). A national moratorium paralysed or severely impeded research on recombinant DNA for several years, forcing researchers abroad to carry out experiments that we now know to be benign. Molecular cloning is now accepted for what it is: an extraordinarily powerful tool that, by and large, has been of more help than harm. Although the spectre of malevolent genetic engineering still looms, practicality has won out over moral protestation. Today, we would be hard pressed to deprive a diabetic patient of recombinant human insulin, or a needy child of growth hormone, merely on the basis of the ethical objections that the production of these molecules originally raised. In another few decades, similar attitudes toward stem cells are likely to prevail and aid patients like Jón Sigurdsson, as we harness our expanded understanding of their role in human biology and their potential to cure or prevent disease and prolong life.

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