Stem cells: progress in research and edging towards the clinical setting

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ABSTRACT – Mouse embryonic stem cells have been shown to differentiate into a variety of tissues *in vitro* and in transplantation experiments can produce many different cell types. Multipotent stem cells in adult humans have also shown a high degree of plasticity: haemopoietic stem cells, for example, have been shown to contribute to several other tissues, such as liver. From these simple observations there has been considerable extrapolation into the use of such putative totipotent stem cells in the clinical setting, with the development of 'designer' tissue engineering, whose aim is to create large tissues or even whole organs for clinical use. In practical terms, however, there are many limitations and difficulties and clinical use has been restricted to a very few settings, eg the use of fetal cells in Parkinson's disease. Nonetheless, there is enormous potential in this area, and also in the application of embryonic or adult stem cells as carriers for gene therapy; but the limitations of such treatment, in particular the stability of manipulated cells, and the problems of ageing and Ooncogenicity, not to mention a host of ethical and regulatory issues, all need to be considered.

The report that immortalised cell lines have been established from human embryonic stem cells, coupled with other publications suggesting that adult tissue stem cells may have much wider differentiation capabilities than previously considered, has moved the field of stem cell research closer towards clinical applications[1]. Mouse cell lines with pluripotential differentiation capabilities have been available for many years, but the derivation of human stem cell lines has wider implications for studies of human cellular development and differentiation, and may create a resource for cell therapy in a variety of clinical areas.

What are stem cells?

The foundations of stem cell research and their clinical application lie in the bone marrow transplantation studies performed over 30 years ago. Mice exposed to radiation doses sufficient to ablate their haemopoietic system could be saved by subsequent infusion of marrow cells obtained from either the animal itself prior to irradiation (autologous transplant), a genetically identical animal (syngeneic transplant), or from a genetically non-identical animal (allogeneic transplant)[2]. These studies led on to the concept that the haemopoietic system depends on the maintenance of a pool of pluripotential stem cells with the capacity to divide and differentiate into cells of all lineages, including lymphoid cells, erythroblasts, megakaryocytes (platelet-forming), and myeloid cells. Therefore infusions of blood or marrow containing these stem cells should (and do) have the potential to repopulate and reconstitute the complete haemopoietic system, *providing* a suitable supportive stroma exists[2,3].

Stem cells are characterised by two fundamental features: self-renewal, as demonstrated by *in vivo* serial transplantations, and a capacity for differentiation into multiple lineages, as shown by clonal marker studies[4,5]. However, not all stem cells are the same. Between the pluripotent stem cell and its highly differentiated haemopoietic progeny, there are intermediate numbers of increasingly committed progenitor cells with progressively more limited proliferative capacity and reduced differentiation potential. Considerable work has been directed towards the isolation of stem cells at all stages of haemopoietic differentiation, largely on the basis of *in vitro* (and *in vivo*) growth characteristics and cell surface markers[5].

The differences between totipotent (or pluripotent) stem cells in the zygote or embryo, and multipotent stem cells in the adult tissues, must also be considered. Examples of these two groups of stem cells would be embryonic stem cell lines and haemopoietic stem cells, respectively. Although there is little understanding of the early events controlling organogenesis from the early embryonic stem cells and inner cell mass cells of the blastocyst, such stem cells have the capability to develop into cells of multiple tissue types. By contrast, multipotent stem cells in the adult tissues have traditionally been considered as organ- or tissue-specific stem cells. However, several recent studies have suggested that multipotent stem cells in the adult may in fact show considerably more plasticity with respect to their differentiation capability[6]. Adult human haemo-

poietic stem cells may, for example, contribute to cell lineages in other tissues such as the liver and this point will be returned to later[7].

What drives stem cells?

One critical aspect of stem cell biology concerns the controlling factors for differentiation: more specifically, to what degree is stem cell differentiation regulated by cell-autonomous (or intrinsic) signals, or controlled by external signals? In other words, how do committed cell lineages develop from pluripotential progenitors? For example, the external signals that direct haemopoietic stem cell fate form part of the critical micro-environment. Recent work is more consistent with the hypothesis that multiple positive and negative signals received through cytokine or other receptors might stabilise a particular pathway of determination[8]. Reverse transcription PCR studies on single cells have revealed that individual haemopoietic progenitor cells co-express different lineage associated genes before commitment[9]. At the level of a single stem cell rather than a population of cells, these processes or differentiation decisions may appear 'stochastic' in nature[10].

Further understanding of the specific nature of the signals that direct expansion and early differentiation along particular differentiating cell lineages in humans will have major implications for clinical practice, and the establishment of human embryonic stem cell lines will facilitate this research[11]. Mouse embryonic stem cells can be made to differentiate into different tissues such as blood and brain *in vitro*[12]. Their pluripotentiality is also well documented by transplantation experiments, since after injection of such cells into host blastocyts and transplantation into the oviducts of pseudopregnant female mice, newborn mice develop whose tissues can be derived wholly or in part from the embryonic stem cell line. Current clinical practice involving the use of characterised lineage-restricted (as opposed to specific) growth factors in humans is most commonly exemplified by the use of recombinant haemopoietic molecules such as G-CSF, but this is predominantly aimed at progenitor cells[13], rather than pluripotential stem cells.

Do non-haemopoietic stem cells exist?

There has been progress in our understanding of stem cell development in non-haemopoietic tissues with, in some cases, partial characterisation of stem cell populations. However, it is possible that not all tissues and organ systems will be based on comparable models of stem and progenitor cells as defined in the haemopoietic system.

There have been recent reports describing the identification of candidate neural stem cells: experimental approaches have included growth characteristics using both *in vitro* and *in vivo* transplantation techniques[14]. Neural stem cells from the adult hippocampus can be expanded *in vitro* and re-implanted where they generate different types of brain cells, depending on their transplanted location; alternative locations for neural stem cells include the sub-ventricular zone[15–17]. The existence of such neural stem cells in the adult might at first appear difficult to reconcile with our understanding of this organ as a post-mitotic tissue. However, the identity of these stem cells is inferred from the methodology and further progress will depend (as for haemopoiesis) on the exact characterisation of stem cells. This requires the use of combinations of probes directed at the patterns of cell surface markers or integrin expression[18,19].

Skeletal muscle satellite stem cells have also been reported, as has the early identification of an islet cell stem cell populations[20,21]. Metaplasias in epithelial tissues are well recognised in histopathology departments, for example patches of ectopic intestinal epithelium in the stomach, which might implicate a degree of plasticity within the stem cell department, although the isolation and characterisation of these cells is not complete[22].

What do we mean by developmental history and plasticity?

Stem cells isolated in an adult may not be the same as those found in an embryo, since these cells will have a developmental history, influenced by both temporal and spatial cues during their lifetime. As an example, red blood cells contain different types of haemoglobin at fetal and adult stages of human development, but most evidence does not indicate changes in stem cell lineage to account for switches in haemoglobin type. At the molecular level, one critical issue in understanding developmental stage-specific expression of the globin genes is whether the complement of transcription or *trans*-acting factors present at the time of transcription is the sole determinant of differential gene expression. There is some evidence that this may not be the case, and that *cis*-active or intrinsic modifications may also be important as a form of epigenetic information in addition to changes in the environment of *trans*-acting factor[23,24].

At a clinical level, stem cells for haemopoietic transplantation may be derived from umbilical cord blood instead of from adult bone marrow[25]. Cord blood cells may show proliferative and other advantages over their adult counterparts, such as higher endogenous levels of telomerase. Telomerase is the enzyme responsible for synthesising telomeric repeats at the ends of chromosomes to maintain telomere length; enzyme activity may correlate with self-renewal capacity of stem cells and progenitor cells[26]. A number of intriguing questions then follow: what is the relationship between adult and embryonic stem cells? Are the former programmed differently, and is this reversible?

Contrary to our previous understanding, recent reports have suggested that stem cells derived from adult tissues may have much wider differentiation capabilities[27]. Mouse cells derived from the adult, as well as the embryonic brain, may give rise to many types of progeny, including haemopoietic cells[28]. Conversely, other transplantation studies in mice have provided evidence that haemopoietic cells can differentiate into cells that express neural characteristics[29]; they may also be able to differentiate into liver cells[7,30]. Stem cells from human adult bone marrow may also form a variety of tissue cells including bone, cartilage and fat cells[31]. Indeed, it has been suggested that adult stem cells from one tissue or organ can be induced to

differentiate into cells of cell types that differ from their tissue origin[5,27]. The underlying mechanisms to account for this degree of plasticity in adult cells have not been clarified, and more information is needed about the identity of the so-called 'transdifferentiating' stem cells. However, if the existence of rare pluripotent stem cells in adult tissues is confirmed there could be many potential applications in medical practice.

Stem cell uses in the clinical setting

Table 1 summarises some of the existing and developing clinical indications for stem cells. Tissue transplantation is an expanding field, reflecting mounting demand within orthopaedics, plastic surgery, cardiovascular surgery and other branches of medicine. Although some tissues can be obtained from living donors, predominantly patients undergoing hip replacement, the only source of other tissues such as corneas, tendons and skin is from cadaver donors[32]. This field therefore lends itself to the application of stem cell research, in particular the growing field of 'designer' tissue engineering, with the aim of creating large tissues and even whole organs for human transplantation[33]. Some of these developing techniques currently rely on matrices to support tissue formation, but in other cases stem cells alone could be the starting material.

As an example, liver transplantation is the therapy of choice for selected patients. This organ is characterised by a high capacity for self-regeneration, and small oval cells in the liver have been described, which appear to have the capacity to turn into either mature liver cells or bile ducts *in vitro*[34]. Further isolation of liver stem cell populations, followed by *in vitro* expansion, could be used as a form of transplantation therapy for syndromes of liver failure. An alternative approach might involve using 'transdifferentiating' stem cells from other adult tissues, and as an example, haemopoietic stem cells have been reported to correct an animal model of severe liver failure (hereditary tyrosinaemia)[30].

To mention three further examples:

- mouse embryonic stem cells may form nerve cells that appear able partially to restore spinal cord function in a rat model[35]
- stem cell therapy using human bone-marrow stem cell populations (with presumptive endothelial precursors) may have a role in promoting revascularisation in rat models of myocardial function[36]
- pluripotent stem cells isolated from adult pancreatic ducts may produce islet cells, and reverse diabetes in a diabetic mouse model[37].

Clinical cell therapy has begun in humans, using transplanted fetal cells in Parkinson's disease, but many difficulties still need to be overcome before this technology can be widely applied[38,39]. Other limitations to the use of stem cells for therapeutic purposes clearly exist, including questions about the long-term stability of the differentiated state in these manipulated cell populations, potential problems of ageing and oncogenicity related to multiple cell cycling, and ethical/regulatory issues.

Immunological effects related to stem cell transplants

Stem cell transplants may also have a role in clinical situations in which the primary aim is not replacement therapy but an immunological effector function (Table 1). Haemopoietic stem cell transplants differ fundamentally from those of solid organs, in which the graft contains only limited numbers of lymphoid effector cells that do not regenerate to constitute a new immune

Table 1. Indications for stem cells and transplantation: present → future

To replace absent or non-functioning cells or tissue, and for cell replacement therapy

- neural cells → degenerative brain disorders, Parkinson's disease
- immune system → severe combined immunodeficiency
- tissue engineering → skin, bone, tendons, designer tissues
- muscle cells → muscular dystrophy
- liver → liver failure
- islet cells → diabetes
- haemopoiesis → haemoglobinopathies, aplastic anaemia
- blood cells synthesising missing enzymes in the host → mucopolysaccharidoses (α-iduronidase: Hurler disease)

To generate a specific immunological action in the host
(usually in the context of haemopoietic stem cell transplantation)

- induction of specific transplantation tolerance → organ transplantation
- graft versus leukaemia effect → malignant disease, leukaemia
- graft versus virus effect → EBV lymphoproliferative disorders
- replace inappropriately functioning cells of the immune system → severe autoimmune disease

To generate a supply of cells for gene therapy

- cancer
- monogenetic diseases
- HIV
- adenosine deaminase deficiency

system. These immune mediated functions depend on the proliferating lymphoid cells derived from donor stem cells and may be harnessed for additional therapeutic effects, which include seeking out and destroying remaining tumour cells (graft versus leukaemia effect) or host cells affected by persisting viral infections such as Epstein Barr virus (EBV)[40–42]. The identity of the subsets of lymphocytes that are the mediators of the graft versus leukaemia (GVL) effect, but crucially not the graft versus host (GVH) effect, is the subject of ongoing research, with the potential aim of separating out these lymphocyte fractions for use as a form of 'adoptive cellular immunotherapy' in the controlled management of leukaemic relapse at a molecular level.

Autologous or allogeneic stem cells for particular organ systems might in the future be used in place of organ or tissue transplants. The short-term success rates in solid organ transplantation are good, but long-term graft survival is less favourable; therefore, attaining long-term donor-specific tolerance to prevent immunological rejection remains a major issue. One well-documented effect of haemopoietic stem cell transplantation is the induction of transplantation tolerance to organs and tissues of the same donor, and therefore transplantation using well-defined populations of haemopoietic stem cells might be used to generate immunological tolerance for solid organ or tissue engineered transplants[5,43]. Autoimmune diseases in humans have established associations with HLA types, and haemopoietic stem cell transplants might also have a role to play in the treatment of selected cases of these diseases.

Gene therapy and stem cells

A final area where stem cell research will undoubtedly influence clinical practice is gene therapy. Embryonic and adult stem cell lines will be resources for research aimed at studying the expression of genes inserted into human cells during development and differentiation. It may be possible to replace defective genes in human diseases by modifying the genetic makeup in stem cells before transplantation back into the patient. Gene therapy has been reported for immunodeficiencies, initially adenosine deaminase deficiency, but will become more relevant for other monogenetic disorders in the future, such as haemophilia or cystic fibrosis[44,45]. In the case of gene therapy for haemoglobin disorders, a major group of diseases worldwide, progress has been hindered by difficulties of vector design, gene transfer efficiency and problems related to vector silencing and position effect variegation[46]. For many inherited diseases in which gene therapy is planned, a number of issues remain, including the identification of *all* gene regulatory sequences and the degree to which *tight* regulation of the transgene in the stem cells is necessary[24].

However, the potential for gene therapy extends further than a form of replacement therapy. Genes could be inserted into stem cells of a patient infected with the human immunodeficiency virus, with the intention of biochemically blocking viral replication[47]. Cancer remains the disease most frequently targeted by current gene therapy trials; a number of approaches have been reported, including the delivery of suicide or 'Trojan horse' vectors into tumour cells[48,49]. Stem cells could be targeted with drug- and/or radiation-resistant genes such as BCL-2 and the multidrug resistance gene (MDR-1; P-glycoprotein) that provide relative chemo- and radio-therapy resistance, or be used to enhance the immune response to tumours by supplying T-cell receptor repertoires that are involved in the recognition of specific cancer peptides in the context of their HLA class molecules[50–53]. Gene transfer of MHC genes across species to induce tolerance to xenogeneic solid organs is another area of development, which may become more relevant should stem cells from other animal species be identified as a resource for engineered tissues.

Key Points

- **Mouse embryonic stem cells may differentiate into a variety of tissues in vitro**
- **Adult human haematopoietic stem cells may develop into other tissues such as liver**
- **Stem cell differentiation is influenced by the local micro-environment**
- **Stem cells may be used to create large tissues or potentially whole organs**
- **Stem cells may be used as carriers to replace defective genes in human tissues by transplantation**
- **Regulatory restrictions and ethical issues are as important as the potential therapeutic value**

What are the ethical and regulatory issues?

There is insufficient space in this article to review the ethical issues relating to the derivation of cell lines from early embryos. Regulatory restrictions related to these ethical issues will also be important considerations for this field of stem cell research. The potential benefits of a better knowledge of cellular differentiation and the medical applications must be weighed against our understanding of the value and status of the human embryo[54].

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