The potential for stem cell therapy in cystic fibrosis

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Gene therapy by topical delivery to the airway of patients with cystic fibrosis (CF) has proven disappointing to date. Vectors are either inefficient[1] or induce an inflammatory response.[2] In addition barriers such as mucus prevent transfection of airway cells, even in relatively well patients.[3] It could be argued that even if these hurdles were overcome, this approach can at best offer a treatment rather than a 'cure' for CF. Another approach in which gene therapy may work in CF is by the utilization of stem cells, which has been successful in children with single-gene blood disorders such as adenosine deaminase (ADA) deficiency and X-linked severe combined immune deficiency disease (SCID).[4,5] This method offers a potential cure to patients with CF. We envisage that the ultimate therapy will be gene therapy to CF babies by utilizing their own stored umbilical cord blood stem cells.

In recent years there has been a great deal of interest and research into stem cells, with the hope of using such cells in tissue regeneration. Stem cells are a heterogeneous group with differing potentials for self-renewal and differentiation. Much interest in their potential for treating lung disease has come from several recent studies which have demonstrated that adult stem cells may show considerable plasticity with regard to their development potential. This may overcome some of the ethical concerns of using embryonic stem cells for research and therapy. This review will discuss basic stem cell biology and consider the evidence for plasticity in the lung and potential for future novel therapies for CF.

WHAT ARE STEM CELLS?

A stem cell is an undifferentiated cell capable of long-term self-renewal. It can divide to produce differentiated cells and functionally repopulate the tissue of origin. Stem cells have varying degrees of self-renewal and differentiation capacity. Embryonic stem (ES) cells are derived from the inner cell mass of the blastocyst and are pluripotent, i.e. they can produce all types of cells from all three embryonic germ layers: endoderm, ectoderm and mesoderm. ES cells can be cultured in an undifferentiated state indefinitely. In contrast to ES cells, adult stem cells are multipotent. They have less self-renewal capacity and their potential for differentiation is usually limited to cells of the tissue of their origin. The best-characterized adult stem cell is the haemopoietic stem cell (HSC) of the bone marrow, which can differentiate into all blood elements. These are rare cells and represent only 1 in 10^4 to 1 in 10^5 of total blood cells in the bone marrow. CD34, a surface marker, has been used to identify and isolate HSCs from bone marrow. The bone marrow also contains mesenchymal stem cells (MSCs), which have the capacity *in vitro* and *in vivo* to differentiate into osteoblasts, chondroblasts, fibroblasts and adipocytes.[6] Verfaillie's group recently identified a multipotent adult progenitor cell (MAPC) from a population co-purified with mesenchymal cells which can differentiate into most somatic cell types, including lung, when injected into an early blastocyst.[7] These cells, which can be derived from mice, rats and humans, can be grown indefinitely in culture. There are also unipotent stem cells such as epidermal stem cells, which are able to generate only one cell type. Tissue-specific stem cells are now thought to exist in most tissues; however, apart from liver and neural stem cells they have not been well characterized.

STEM CELL NICHES

Adult stem cells 'home' or migrate to specialized niches within tissues. These niches provide a protective, well-vascularized microenvironment. Intrinsic and extrinsic factors such as cytokines, growth factors, adhesion molecules and the niche itself are thought to influence the balance between stem cell self-renewal and differentiation into committed cells. Various factors have been implicated in the molecular mechanisms of homing of HSCs to the bone marrow, including the chemokine stromal cell-derived factor-1 (SDF-1) and its receptor CXCR-4.[8] A recent study has shown that SDF-1-induced stem cells home to infarcted myocardium in a rat model, and there was significantly improved left ventricular function.[9] Left ventricular remodelling describes changes in left ventricular size and contour that occur following a myocardial infarction, and the improved left ventricular function was probably a result of reverse remodelling induced by homing of progenitor bone marrow progenitor cells to the infarct region, as significant regeneration of cardiac myocytes was not found. In view of the recent plasticity studies it is interesting to

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speculate that damaged tissues may release homing signals that attract stem cells to the damaged organ. CF could be a useful potential model disease, as in most studies some sort of injury mechanism is required for homing and engraftment to occur and inflammation and tissue injury begin early in the CF airway.

WHAT IS THE LUNG STEM CELL?

The lung is usually able to repair and restore itself to normal homeostasis after injury, which suggests that it retains regenerative capacity.[10] A single 'lung stem cell' has not been isolated and probably does not exist, which reflects the complex composition of the lung. It is more likely that there are regional stem cells or progenitors that respond to local needs. The lung is made up of a conduction airway and gas-exchange system with many different cell types including ciliated, mucous, basal, Clara, type I pneumocytes, type II pneumocytes and pulmonary neuroendocrine cells (PNECs). Human studies of tracheal and bronchial specimens using immunohistochemical staining of Ki-67, a marker of proliferation, have found a range of potential cells which can repopulate the injured lung, classically the basal and mucous secretory cells of the trachea, the Clara cells of the bronchiole and, from animal studies, the type II pneumocyte of the alveolus.[11–13] This range has been extended to include the mucus-gland duct cells of the trachea and bronchus from animal experiments.[14]

Experiments to elucidate possible lung stem cell candidates are difficult because of the slow turnover of such cells, and therefore most animal studies use an injury model which promotes cell turnover. Studies also use labels such as [^{3}H]thymidine or bromodeoxyuridine (BrdU) which mark cells that retain the label for an extended period due to slow turnover, so-called label-retaining cells (LRCs), which are thought to represent possible stem cells. Borthwick *et al*. injected mice with BrdU intraperitoneally and found that mice receiving weekly epithelial damage from intratracheal detergent or inhaled sulphur dioxide had LRCs that were able to repopulate the tracheal surface after injury. In the lower trachea the LRCs were found at intercartilage zones of the surface epithelium. They found that most LRCs in the upper trachea were in the tracheal gland ducts.[14] This is a promising finding in relation to CF as the *cystic fibrosis transmembrane conductance regulator* (*CFTR*) gene is predominantly expressed in the submucosal glands,[15] which are inaccessible by topical gene therapy but may be accessible by targeting these lung stem cells. Further studies are required to ultimately identify cell surface markers of lung stem cells.

ARE HSCs CAPABLE OF PLASTICITY?

The huge recent interest in stem cells has come about because of the cloning of the sheep Dolly[16] and other studies, which have challenged the dogma that adult stem cells are tissue-restricted in their differentiation ability. Numerous studies have now shown that stem cells isolated from one tissue can differentiate into a variety of unrelated cell types, and even cross lineages. There is no official definition of plasticity, but it is normally defined as a committed adult stem cell that can differentiate into cells of a different tissue under the correct conditions. To demonstrate this studies need to fulfil the following suggested criteria: cells should be prospectively isolated at the single-cell level and differentiate into cell types of the tissue of origin and into at least one cell type of a different tissue; they must show *in vivo* evidence of functional differentiation into the cell types of origin and at least one cell type other than the tissue of origin.[17] However, most studies have not fulfilled these criteria.

Studies have shown that bone marrow harbours cells that have the capacity to differentiate into cells of neuronal, endothelial, epithelial and muscular phenotype.[18–20] Others have reported neural stem cells that have generated blood[21] and skeletal muscle.[22] Most of these studies have only shown evidence of cell morphology and phenotype, and have not demonstrated that the cells were functional. Lagasse *et al*. were the first group to provide evidence that haemopoietic stem cells could generate hepatocytes that were functional *in vivo*. Normal male donor HSCs were transplanted into female mice with hereditary tyrosinaemia. Without transplant the mice died of liver failure, but with bone marrow transplant liver function was restored and up to 50% of hepatocytes were shown to be of donor origin at 6 months.[23] This study illustrates that for robust engraftment to occur, injury is probably required. Although further research is required to understand the complex mechanisms involved in transdifferentiation, it is becoming more acceptable that adult stem cells are capable of such plasticity.

IS PLASTICITY REAL OR IS IT JUST FUSION?

There has been much debate as to whether plasticity is a real phenomenon or whether it could be the result of other mechanisms such as cell fusion or transfer of apoptotic DNA fragments. Fusion occurs when two cells fuse and form a cell with two nuclei. In two studies, bone marrow and neural cells have been shown to fuse spontaneously with ES cells *in vitro* to form tetraploid cells, which subsequently adopt the phenotype of the recipient cells.[24,25] Both studies found cell fusion at the frequency of 1 in 10^4 to 1 in 10^5. However, the frequency of cell fusion *in vivo* is thought to be very low and is unlikely to account for all the plasticity results that have been shown. It may be that, *in vivo*, cells fuse only under very selective conditions. An example of this selection pressure has been shown with the fatal metabolic liver disease tyrosinaemia type I mouse model.

One study found that hepatocytes expressed both donor and host genes, consistent with polyploid genome formation by fusion of host and donor cells.[26] Unfortunately, most studies showing evidence of plasticity have not actually looked for evidence of fusion, including those studies on the lung. They do highlight the need in future studies for genetic analysis of cells that are thought to have undergone transdifferentiation.

EVIDENCE OF PLASTICITY IN THE LUNG

There is growing evidence to suggest that a sub-group of HSCs can differentiate into lung cells. Krause *et al.* demonstrated evidence of multi-organ, multi-lineage engraftment by a single transplanted bone-marrow-derived stem cell.[19] A population enriched for long-term repopulating cells was obtained by transplanting male, lineage-depleted, labelled bone marrow cells into lethally irradiated female recipients. Cells that homed to the bone marrow after 48 h were infused into secondary female recipients at a dilution that ensured each animal received only a single male cell. At 11 months post-transplant donor cells, identified by the Y-chromosome, expressing the epithelial marker cytokeratin, were identified in the oesophagus, stomach, bowel, bile duct, skin and lung. Surprisingly up to 20% of cytokeratin and surfactant B expressing type II pneumocytes and less than 4% of bronchial epithelial cells were Y-chromosome positive. This high engraftment was attributed to the lung damage caused by irradiation and presented the first evidence for the potential of peripheral stem cells to become lung cells.[19] Very different findings were reported by Kotton *et al.*, who injected MSCs into recipient mice 5 days after intratracheal bleomycin, a chemical which causes alveolar damage. Type I pneumocytes of donor origin were found between 1 and 30 days after injection;[27] however, no type II pneumocytes were found at any stage, which is interesting as type II pneumocytes are believed to be the precursor of type I pneumocytes as previously described. The differences between these papers could be a result of the different types of stem cells and/or the different injury mechanisms used. A recent study in which MSCs from male bleomycin-resistant mice were injected into female bleomycin-sensitive mice found that male DNA as quantified by real-time polymerase chain reaction (PCR) accounted for approximately 2.21×10^{-5} per cent of the total lung DNA in the control-treated mice. This was increased 23-fold in the animals exposed to bleomycin prior to MSC transplantation. Most of these cells were found to be type II pneumocytes. It was also found that MSC administration after exposure to bleomycin reduced the degree of bleomycin-induced inflammation and collagen deposition within the lung tissue.[28]

Studies from sex-mismatched bone marrow or lung transplants provide a unique opportunity to look for evidence of engraftment in the human lung. There have been two such human lung studies published; one study showed evidence of donor-derived cells in the lung following HSC transplantation. This studied lung specimens from a retrospective cohort of female allogeneic HSC transplant recipients who received stem cells from male donors, and showed that 2.5–8% lung epithelial cells and 37.5–42.3% lung endothelial cells were donor-derived.[28] Another study looked at archived tissue biopsies from several human lung allografts and showed evidence of recipient-derived cells in the bronchial epithelium, type II pneumocytes and seromucous glands. They also showed that epithelial structures that displayed evidence of chronic injury such as squamous metaplasia showed an increased degree of engraftment (24% versus 9.4%).[29] Thus, the evidence to date suggests that engraftment mostly occurs in the alveoli and epithelial engraftment is quite low. The relevance to CF is that lung disease primarily affects the conducting airway rather than the alveoli and it is likely that the epithelial cells of the conducting airway will need to be corrected. It is unknown whether restoration of the chloride channel alone will be sufficient to have an effect on respiratory disease or whether there is a need for correction of the sodium defect. Reassuringly, Johnson *et al.* demonstrated restoration of chloride secretion in a CF epithelial sheet with the correction of as few as 6–10% of CF cells,[30] similar to the number of cells seen to engraft in the airway by stem cells.

STEM CELLS AND POTENTIAL CLINICAL USES

HSCs have been used successfully for many years to treat haemopoietic disorders and rescue patients undergoing chemotherapy for malignancies. There have now been some successful clinical trials which have corrected SCID by *ex vivo* gene therapy.[4,5] In one study, CD34-positive bone marrow cells from five boys with X-linked SCID were transduced *ex vivo* with the use of a defective retroviral vector. These patients have developed mature, functional T-cells and no longer need intravenous immunoglobulin. Unfortunately there have been some major setbacks in the French trial, where two patients have developed a leukaemia-type illness.[31] Retroviral vectors are thought to insert themselves at random positions in the host genome and therefore insertional mutagenesis is a potential risk of any retroviral gene therapy; however, this has previously been considered as a very low risk in humans. In the first patient to develop a leukaemic illness, a proviral integration site was found on the short arm of chromosome 11 within the LMO-2 locus. Aberrant expression of LMO-2 has been reported in acute lymphoblastic leukaemia. It may be that the primary disease

X-linked SCID may predispose to the development of leukaemia by influencing retroviral vector integration near LMO-2. There have been many other studies using retroviral gene therapy without evidence of such problems.

So what about stem cell therapy and CF? Gene therapy for CF has been disappointing thus far. Basic questions remain unanswered. It is unknown which cell type should be targeted; current topical therapy targets the abundant surface epithelium, but it is the submucosal glands which express the highest CFTR in the lung. Do we just need to correct the chloride defect or do we also need to correct the sodium defect? How often would topical application be needed? Stem cell therapy could overcome some of these problems but by no means all. We have seen that stem cells in the lung may be present in the mucosal glands and could be targeted. If only 6–10% of cells need to be corrected for the chloride defect to be reversed then engraftment rates from various studies of up to 20% in the alveoli and 8% in the epithelium could be adequate. However, if the sodium defect needs to be corrected also, then nearly 100% of cells need to be non-CF and engraftment would need to be substantially increased.

CF is a potential model disease for stem cell therapy because of the continuing lung inflammation and infection leading to damage, which could promote engraftment of stem cells. It is likely that infants and children with CF would be the best candidates for this sort of therapy, when they have faster cell turnover and before they have established lung damage.

FUTURE RESEARCH

Before stem cell therapy may be used to treat CF and other lung diseases, many questions need to be answered:

- Which type of stem cell should be used: HSC or lung-specific stem cells?
- How do stem cells migrate to the lung?
- How should stem cells be delivered: intravenously or locally to the site of injury?
- What is the effect of the age of the patient?
- What injury mechanisms are required to allow lineage switching?
- What are the possible long-term effects as regards oncogenicity and ageing with using genetically manipulated stem cells?
- Will patients need to be preconditioned before stem cell transplant?
- Most importantly, is this treatment safe?

CONCLUSIONS

Stem cell research provides us with great potential but currently there are more questions than answers. Further studies are required to confirm that adult stem cells do have a greater differentiation potential and that a single stem cell is capable of creating not just a few cells but a robust population of functional cells. Recent discoveries allow us to consider how stem cells may be used to treat many human diseases and there is much excitement regarding the potential for gene and stem cell therapy in CF. Until this becomes a reality it must be remembered that the increase in median survival of CF patients has resulted from good nutrition, aggressive antibiotic use, meticulous physiotherapy and increased understanding of the disease. This must be borne in mind when families ask, 'How long until stem cell therapy is available, doctor?'

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