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Stem cells and cystic fibrosis

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Abstract

Although cystic fibrosis at first sight appears to be one of the most obvious human diseases to treat with gene therapy, since it is caused by a single-gene defect and the main affected organ is the lung which is relatively easily accessible, clinical results have thus far been disappointingly limited. At least one cause for this lack of success is the failure to permanently correct the gene defect in addition to the rapid turnover of lung epithelial cells. Alternative approaches therefore involve the search for and use of stem cell populations. This review presents an overview of recent attempts to identify lung- or bone marrow-derived populations of stem cells or progenitor cells and to apply such cells, heterologous or gene-corrected autologous, to colonize the airways while differentiating into functional respiratory columnar epithelial cells. The most successful approaches thus far appear to be obtained with bone marrow-derived cells such as mesenchymal stem cells, although the transdifferentiation rate thus far has been limited to below the 1% level. As an alternative the proven multipotent nature of bronchioalveolar stem cells isolated from lung tissue may provide another promising approach for successful stem cell therapy.

Keywords: Cystic fibrosis; Hematopoietic stem cells; Mesenchymal stem cells; Lung

Contents

The ultimate goal of gene therapy of cystic fibrosis (CF) is to permanently correct the genetic defect in the target cellular compartment. This could conceivably be achieved by gene transfer into the "stem" cell compartment of the respiratory epithelium. Although the identification of a resident pulmonary multipotent stem cell still remains to be accomplished, it is clear that local stem or precursor cells contribute to the repopulation of the injured epithelium in different anatomical regions of the airways [1].

While repair and regeneration are naturally achieved in mitotic cellular compartments, like the skin, it has long been a paradigm in medical science that it is not possible to achieve this in so-called post-mitotic tissues, such as brain and heart. Recently however, this concept was challenged by the observation that these two organs contain multipotent adult stem cells [2,3]. The role of these resident stem cells in cardiac and neuronal regeneration, either occurring spontaneously or as a result of damage (i.e. in the case of a myocardial infarction) is currently being studied.

Stem cell homing and engraftment into different organs and new tissue formation are the major goals of regenerative medicine. Thus far this has been accomplished in the heart and the nervous system applying various sources of fetal and adult stem cells [4]. Interestingly, hematopoietic stem cells (HSCs) show developmental plasticity typical for embryonic stem cells. It has been demonstrated that HSCs

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are able to transdifferentiate into epithelial cells. Grafting of bone marrow-derived cells (BMDCs) or enriched HSCs in liver, lung, gut and skin epithelia has been detected after transplantation of BMDCs or enriched HSCs into irradiated recipient animals [5].

There are several stem cell niches that play a crucial role in the renewal of the epithelial layers in the rodent lung. Reynolds et al. demonstrated that progenitors of bronchial Clara cells are essential for maintaining bronchial, bronchiolar, and alveolar epithelia [6]. Hong et al. identified a population of basal cells that differentiate into Clara cells in the proximal airway epithelium [7]. Stem cells residing at the bronchioalveolar duct junctions were first described by Giangreco et al. [8]. The multipotent nature of these lung stem cells, called bronchioalveolar stem cells (BASCs), was also reported by Kim et al. who demonstrated that single BASCs give rise to colonies containing Clara cells and alveolar type I and II cells [9].

There are a number of reasons why CF should be an ideal disease for the application of gene therapy. First of all, it is caused by a single-gene defect. Secondly, the main pathology is in the lung, an organ which is relatively easily accessible for treatment. In addition it offers a therapeutic window for treatment since CF patients demonstrate an almost normal phenotype at birth. Finally, it has been suggested that restoration of CFTR function to a level of only 5–10% of normal will suffice to adequately reverse the chloride transport dysfunction [10,11] and give complete recovery of the intestinal disease in CF mice [12]. However, despite these encouraging features, clinical trials of gene therapy in CF patients have only resulted in inconsistent and low levels of vector-specific CFTR expression and at best minor functional changes towards normality [13].

An alternative approach to cure CF is stem cell therapy, which would require heterologous or gene corrected autologous stem cells to colonize the airways, proliferate there and differentiate into columnar cells covering a sufficient part of the airway epithelium. Recent data indicate that bone marrow-derived stem cells are able to contribute to non-hematopoietic tissues, including epithelial lineages. Importantly, it has been reported that circulating bone marrow-derived stem cells are preferentially home to the damaged respiratory epithelium undergoing regeneration [14,15]. These observations are now being implemented in the development of experimental treatments of CF. In this context, two important studies have been published recently. Loi et al. [16] determined whether transplantation of adult marrow cells containing the gene for wild type Cftr might result in functional Cftr expression in lung epithelium. The authors transplanted two populations of bone marrow-derived cells (cultured stromal marrow cells and total bone marrow cells) containing the wild type Cftr gene into transgenic Cftr knockout (KO) mice. Administration of plastic adherent stromal cells to naive non-irradiated mice resulted in the engraftment of donor-derived airway epithelial cells, although at very small numbers (approximately 0.025%). In contrast, no donor-derived airway epithelial cells were detected in irradiated mice treated with total marrow cells. Cftr mRNA and protein could only be detected in the lungs of Cftr KO recipients treated with isolated adherent bone marrow stromal cells. However, the total number of chimeric lung epithelial cells exhibiting Cftr expression was small (0.01%) and unlikely to affect overall Cftr-dependent chloride transport and other functions in airway epithelium, as we have pointed out before.

Most recently, Bruscia et al. [17] demonstrated that the transplantation of GFP+/BMDCs of CFTR+/+ mice into irradiated CFTR-null mice resulted in a partial restoration of CFTR activity of gastrointestinal and nasal epithelium (Bruscia E., personal communication). Most of the CFTR−/− mice had functional CFTR channels in the rectum and nose by 5 months. These results correlated with the presence of CFTR-expressing donor derived (GFP+) columnar epithelial cells in the small intestine (with a frequency of <1%).

Very promising results have been obtained recently by Wang et al. [18]. In their experimental set-up mesenchymal stem cells (MSCs) obtained from bone marrow of healthy volunteers were mixed with airway epithelial cells (AECs) and grown in air–liquid interface cultures on semi-permeable filters. Almost 10% of the MSCs were induced in vitro to acquire an epithelial phenotype, as judged by the expression of cytokeratin 18 and occludin. Moreover, MSCs obtained from CF patients corrected ex vivo with a CFTR-encoding retrovirus and mixed with CF AECs resulted in partial resumption of CFTR-mediated chloride current.

The in vivo experiments have suggested that the capacity of transdifferentiation of HSCs into respiratory epithelial cells is limited, i.e. only few percentages of cells showed the cytokeratin pattern of expression (up to 0.6%) [19]. This may be not sufficient to obtain a therapeutic effect, as it was suggested by the work of Loi et al. [16]. However, a partial restoration of CFTR activity was obtained in organs (rectum, nose) easily investigated by the potential difference technique [17], even though the protocol employed in this last study (total body irradiation prior to bone marrow transplantation) might not be applicable to CF patients.

It is conceivable that the injury models used to date [14,15,20] are not appropriate to elicit a damage localised in the anatomical target region for CF gene therapy, i.e. the bronchi/bronchioli, indicating a need to exploit alternative injury models. In addition, more insight into the molecular mechanisms governing recruitment and phenotypic conversion of bone marrow-derived cells is needed in order to carry this approach to clinical feasibility.

The results published by Wang et al. [18] and Loi et al. [16] strongly suggest that the population of bone marrow cells relevant for repopulating the lung epithelium may be found in the plastic adherent stromal cell compartment. Yet, mesenchymal stem cells are not without drawbacks, e.g. they are limited in numbers and lack well-defined markers required for their purification [5]. One approach to

overcome these limitations is to consider alternative sources of stem cells capable of repopulating damaged respiratory epithelium. Recently, it has been shown that embryonic stem cells can differentiate into a phenotypically mature respiratory epithelium [21].

Other stem cell candidates considered suitable for cell therapy of CF are the BASCs mentioned earlier. Importantly, the use of Sca-1- and CD34-positive surface staining, together with exclusion of hematopoietic and endothelial lineage, will allow the isolation of BASCs by means of fluorescence activated cell sorting (FACS) [9]. In addition, it has been demonstrated that cultured BASCs can proliferate, are able to self-renew and are multipotent in clonal assays.

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