Editorial

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Regenerative Medicine

Fixing stem cells via genome editing: hope for cystic fibrosis?

"Transplantation of lung stem/progenitor cells represents a potential therapeutic approach for cystic fibrosis."

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Transplantation of lung stem/progenitor cells represents a potential therapeutic approach for a variety of inherited monogenic lung diseases including cystic fibrosis (CF). The primary defect in CF, an autosomal recessive disorder, is the regulation of epithelial chloride transport by a chloride channel protein encoded by the *CFTR* gene [1,2].

One potential therapeutic approach would first involve generation of patient-specific induced pluripotent stem cells (iPSCs) from skin or blood cells of affected patients. Utilizing site-specific gene editing, the disease-causing *CFTR* mutation would then be corrected in the endogenous, chromosomal DNA sequence. Site-specifically editing the endogenous *CFTR* gene should, in principle, provide for sustained, non-silenced *CFTR* gene expression at levels appropriate for CFTR functional restoration. Furthermore, editing and correction at the chromosomal DNA level in stem cells will ensure that the correction is permanently encoded and long lasting in the cells derived from the corrected iPSCs. Finally, a differentiation approach would be employed to obtain populations of the relevant lung stem/progenitor cells from the corrected iPSCs for the purpose of transplantation [2].

The generation of iPSCs from CF patients has been reported previously [3–6], with subsequent differentiation into epithelial cells. The design and assessment of *CFTR*-specific nucleases also has been reported previously, including repair of the mutant *CFTR* gene [6–10]. There are now several classes of sequence-specific nucleases available (ZFNs, TALENs, CRISPR/Cas9, Meganucleases) for DNA sequence-specific gene editing.

In principle, depending upon the specific application, gene editing approaches may utilize either homology directed repair (HDR) or nonhomologous end joining (NHEJ). Following introduction of a sequence-specific dsDNA break, HDR utilizes homology sequences (e.g., flanking a transgene in a donor construct or present in a ssDNA oligo) to facilitate accurate sequence-specific targeted transgene integration or sequence substitution. HDR requires cells to be in cycle and is most active at the G2 stage. NHEJ, active in both cycling and noncycling cells, is an error prone process that, following cleavage of dsDNA, seeks to rejoin the two separated ends of dsDNA. This rejoining is frequently associated with small insertions/deletions/duplications at the original site of cleavage. Since resident stem cells in many tissues are largely quiescent, unless they can somehow be triggered or stimulated to proliferate, this noncycling state would largely render them resistant to HDR.

We recently published the correction of the mutant *CFTR* locus in CF iPSCs using ZFN-based genome editing [2]. This correction, first demonstrated at the genomic DNA level in the treated iPSCs, was shown to result in expression of corrected CFTR mRNA and protein in iPSC-derived lung epithelium. Most importantly, using Ussing

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chamber assays, we demonstrated restoration of CFTR chloride channel function in the iPSC derived lung epithelial cells. Significantly, we demonstrated that this correction was indeed precise – with extensive analysis (comparative genome hybridization, whole exome sequencing and whole genome sequencing analysis at predicted potential ZFN off-target sites) revealing no evidence for ZFN-mediated introduction of off-target mutations in corrected iPSC clones [2].

Now that sequence-specific *CFTR* gene correction in iPSCs has been demonstrated, a necessary next step will be deriving from the corrected iPSCs the lung stem/progenitor cells appropriate for transplantation. There is still much to be learned about turn over of cells in the human airway, as well as the identity of potential stem/progenitor cells responsible for its maintenance. But there is an emerging consensus that the pseudostratified epithelial tissue of the human proximal airway contains basal cells capable both of self-renewing cell division as well as differentiation to other specialized cells that are present – namely ciliated cells and secretory cells. As such, the proximal airway basal cells serve as a major class of stem/progenitor cells within the proximal airway [11,12]. Thus, there is a strong rationale to develop differentiation protocols appropriate for derivation of proximal airway basal cells from corrected CF iPSCs.

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Much of what we know of human basal cells has come from well-differentiated airway epithelial cultures, which employ air–liquid interface culturing to differentiate airway epithelial cells into functional airway cells, such as ciliated and secretory cells [13]. Recent advances in culture techniques, such as 'conditional reprogramming' with ROCK inhibitors and irradiated fibroblast feeders [14] or cloning of airway epithelium basal stem cells [15] have optimized the survival and significant expansion of the key stem cell population that gives rise to these airway epithelial cell cultures, the primary airway basal stem cells. This ability to directly obtain from CF patients an expandable population of primary airway basal stem cells potentially offers an alternative to the iPSC derivation strategy outlined above. Namely, there is reason to also consider future autologous cell therapeutic approaches for CF in which patient-specific airway basal cells are site-specifically corrected in the *CFTR* gene and subsequently transplanted into the lungs of the affected patient.

Clearly, even if patient-specific corrected basal stem cells (either iPSC-derived or derived and expanded from primary tissue) can be generated in sufficient number, there still remain significant technical and clinical issues to be resolved. For example, will some type of preconditioning of the airway be required in order to create 'space' for the transplanted basal stem cells to take up residence? It is likely that such questions will require extensive evaluation in relevant CF animal models such as the CF pig, ferret, or mouse.

It is possible to also conceive of *CFTR* gene editing strategies that are focused directly on *in vivo* correction of the CF airway. Again the basal cells, due to their self-renewing capacity, are presumed to be at least one of the preferred target cells for long-term efficacious *CFTR* gene editing. Although basal cells are presumed to be largely quiescent under normal conditions, it has been reported that CF airways, relative to normal airways, have significantly increased percentage of basal cells in cycle – perhaps in response to continuous damage to and inflammation of the airway [16–18]. If true, this property of CF-patient basal cells would improve their ability to carry out HDR.

Genetic analysis of *CFTR* gene mutations in CF patients has identified approximately 2000 disease-causing mutations. What is necessary for CF is a universal tool kit of gene editing reagents able to correct the vast majority of *CFTR* mutations. Whether this ultimately calls for a safe-harbor or endogenous knock-in approach remains to be seen [19].

Finally, we note that although most efforts today are focused on lung, in principle, the *CFTR* editing approaches to be developed may be relevant for editing of mutant *CFTR* cells in other affected organ systems: for example, intestine [7] and pancreas. There clearly remains much to be explored regarding optimal methods of *CFTR* gene editing, identification of appropriate stem cell populations for transplantation, and preclinical evaluation. Nonetheless, it is hoped that a safe and efficacious gene editing therapeutic may be developed and eventually made available for the benefit of CF patients.

Financial & competing interests disclosure

BR Davis, Associate Professor and an employee of the University of Texas Health Science Center at Houston, is co-inventor of patent application #20130145485 Methods and Compositions for Alteration of a Cystic Fibrosis Transmembrane Conductance Regulator (*CFTR*) Gene and is currently a scientific collaborator of Sangamo BioSciences. The author has no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

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