

“Shocking” the System to Achieve Efficient Gene Targeting in Primary Human Airway Epithelia

The ability to knock out specific genes to gain an understanding of their function is a mainstay of modern research, and the use of technologies to achieve this goal to study pulmonary biology is no exception. Homologous recombination in embryonic stem cells, antisense oligonucleotides, sh/siRNA, and CRISPR-mediated deletions are widely known and used. Most of these methods will perform well in various model systems and cell lines, with the classic method, homologous recombination in embryonic stem cells, being highly useful in animals. However, for translational questions, interspecies and/or cell line differences often require confirmation of functional findings in primary human cells. Primary human airway cultures are a highly valued model to study pulmonary biology, and the application of robust gene knockdown technologies to these cells is a valuable method to foster our knowledge in this field. Unfortunately, as with other primary cell models, primary airway cells generally remain persistently defiant to easy genetic manipulation.

Many examples of successful gene manipulation in human airway cells can be found, including from our own laboratories. However, in practice, although a method might work well for one gene in one set of studies, it is not necessarily an indication that it will be applicable for a different gene in separate experiments. Furthermore, a method that seems to work efficiently with cells from one human donor might suddenly not work effectively when attempted in cells from another donor. This is true for all types of technologies, including the use of 1) viral vectors (lentivirus, adenovirus, and/or retroviral) to express knockdown components, such as sh/siRNAs, CRISPR, and/or anti-sense species; 2) standard lipofection methods; and 3) electroporation. An easy-to-use, robust, economical, versatile, and efficient method to knock down genes in primary human airway cells would represent an important advance in the field.

As a step forward, the article by Koh and colleagues (pp. 373–381) in this issue of the *Journal* (1) describes the optimization of a method for targeting genes in primary human bronchial epithelial cells (HBECs). They used electroporation of the necessary components for CRISPR-mediated deletion (guide RNA [gRNA] sequences and recombinant Cas9 complexes) in HBECs, which was nicely demonstrated using a well-described model of the regulation of MUC5AC (mucin 5AC) by SPDEF (SAM Pointed Domain Containing ETS Transcription Factor) (2, 3). Knockdown of *SPDEF* is predicted from the literature to dramatically reduce the upregulation of MUC5AC expression resulting from treatment of HBECs with IL-13, a central mediator of allergic asthma. Applying a variety of experimental conditions, the authors were able to demonstrate near-complete loss of *SPDEF* in HBEC cultures using their electroporation method. Targeting of *SPDEF* with this technology was accompanied by the expected loss of IL-13–mediated 1) upregulation of MUC5AC

and downregulation of MUC5B expression, 2) induction of goblet cell differentiation, and 3) impairment of mucociliary clearance.

Although the science was mostly confirmatory of previous studies regarding SPDEF-dependent function, the optimized method provides a useful paradigm for other laboratories to follow. Although the usual caveats with CRISPRs apply (e.g., gRNAs do not always work as predicted), Koh and colleagues were able to demonstrate that gRNAs shown to be efficient in relevant cell line models are also effective in HBECs after electroporation of the CRISPR/gRNA complexes (1). Prescreening of gRNA sequences is still recommended. Once a gRNA is identified, it is synthesized and simply mixed with the recombinant Cas9 to form a complex in the buffer recommended for electroporation. Varying the timing of the electroporation and the concentrations of the components altered the efficiency in predictable ways, suggesting that the degree of gene knockdown could be manipulated to the experimenter’s advantage. Hence, the method might be also useful for studies evaluating the relationship between the degree of gene expression and functional responses.

Importantly, a similar method has also recently been described by Rapiteanu and colleagues (4), indicating that this approach can be robust across laboratories; indeed, we are finding similar usefulness of the technique in our own laboratories. Nevertheless, the target gene still seems to matter, and success may remain out of reach for difficult-to-target genes whose functions are necessary to maintain the integrity of the HBECs as they differentiate in culture. The technique still has some disadvantages, including cost, and time will tell if it becomes a standard method for studying airway pathophysiology. Nonetheless, the method optimized by Koh and colleagues (1) significantly contributes to the growing arsenal of new technologies that will drive the pulmonary biology field forward. ■

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