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Intersection of Gene Therapy and Progenitor Cell Biology in the Lung

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Gene transfer to airway and alveolar epithelial cells *in vivo* continues to offer hope as a therapeutic approach for genetic diseases such as cystic fibrosis (CF), as a pharmaceutical delivery technique for acute and acquired lung injuries, and as a powerful research tool. However, successful and sustained gene expression in the epithelium has often been limited by factors associated with effective vector delivery, particularly in injured or diseased lungs; innate inflammatory and immune responses to vectors; and longevity of gene expression.¹ One hypothetical means of overcoming the last limitation would be to target endogenous progenitor cells in the lung, cells that would subsequently provide sustained and widespread gene expression in the daughter cells arising from the progenitor-cell populations. However, specific targeting of endogenous airway progenitor cells has not previously been described.

It is in this context that the timely and important work of Liu and colleagues in this issue of *Molecular Therapy* elegantly demonstrates that recombinant adeno-associated virus (AAV) vectors, notably AAV1 and AAV5, seem to preferentially transduce progenitor cells in the lower airways in mice.² This exciting and new finding provides an important platform for specifically targeting airway progenitor cells and raises hope that, once duplicated in humans, this approach may

someday be used in clinical gene therapy attempts in CF and other lung diseases.

Endogenous tissue stem cells are undifferentiated cells that have been identified in nearly all tissues, including lung, and are thought to contribute to tissue maintenance and repair. These rare, highly specialized cells are often localized to specialized niches within each tissue and not only can self-renew, although they usually cycle infrequently, but also can give rise to more daughter cells known as progenitor cells or transit-amplifying cells. Both stem and progenitor cells may give rise to the more specialized, or differentiated, cells of the organ.³⁻⁵ The focus in lung has been predominantly on epithelial progenitor cells, but increasing evidence indicates the potential existence of vascular and mesenchymal progenitor cell populations as well. Moreover, because the lung is a complex organ, several airway epithelial stem and progenitor-cell hierarchies have been identified along the tracheobronchial tree in mouse models (**Figure 1**).³⁻⁵ In trachea and large airways, a subpopulation of basal epithelial cells that express cytokeratins 5 and 14 has been implicated.⁶⁻⁸ Aquaporin-3 has also been identified as a possible marker of human fetal airway progenitor cells.⁹ In lower airways in mice, Clara cells exhibit characteristics of transit-amplifying cells following injury to differentiated ciliated airway epithelial cells. However, unlike transit-amplifying cells in tissues with higher rates of epithelial turnover, such as intestine, Clara cells show a low proliferative frequency in the steady state, are broadly distributed throughout the bronchiolar epithelium, and contribute to the specialized tissue function. In more distal airways, toxin (i.e., naphthalene)-resistant variant Clara

cells have been identified as having stem cell functions and have been termed bronchiolar stem cells.^{4,10} Naphthalene-resistant cells are also located within discrete microenvironments within bronchioles that include the neuroepithelial body and bronchioalveolar duct junction (BADJ).^{4,11}

Another population of naphthalene-resistant cells that stain both for Clara cell secretory protein (CCSP) and for pro-surfactant protein C, termed bronchioalveolar stem cells (BASCs), has also been described at the BADJ in mice.¹² It is possible that toxin-resistant Clara cells, BASCs, and other cells may represent different interpretations of the same cell population(s); this both highlights the need for rigorous methods of lineage tracing and further underscores the importance of the *in vivo* microenvironment in cell behavior. Most recently, another population of putative progenitor cells expressing CCSP, stem cell antigen 1, stage-specific embryonic antigen 1, and the embryonic stem cell marker Oct-4 have been identified in neonatal mice.¹³ These cells were able to form epithelial colonies and differentiate into both type 1 and type 2 alveolar epithelial cells. Interestingly, these cells were susceptible to infection with the severe acute respiratory syndrome (SARS) virus, raising the possibility that endogenous lung progenitor cells may be specific disease targets. The possibility remains that other endogenous stem or progenitor populations exist, and there is much room for additional information on the regulatory mechanisms and pathways that have been elucidated in other epithelial progenitor cell populations (reviewed in refs. 3-5, 14). The human correlates of the endogenous airway progenitor populations described in mice are less well understood. Defining human airway progenitor populations is a critical step and the focus of intense research activity.^{3-5,14}

Endogenous progenitor cells may also be attractive candidates for targeting with gene transfer vectors that provide sustained expression. Using adult transgenic Rosa26-Flox/LacZ reporter mice, Liu and colleagues demonstrated that airway-based administration of recombinant rAAV1 and rAAV5 Cre

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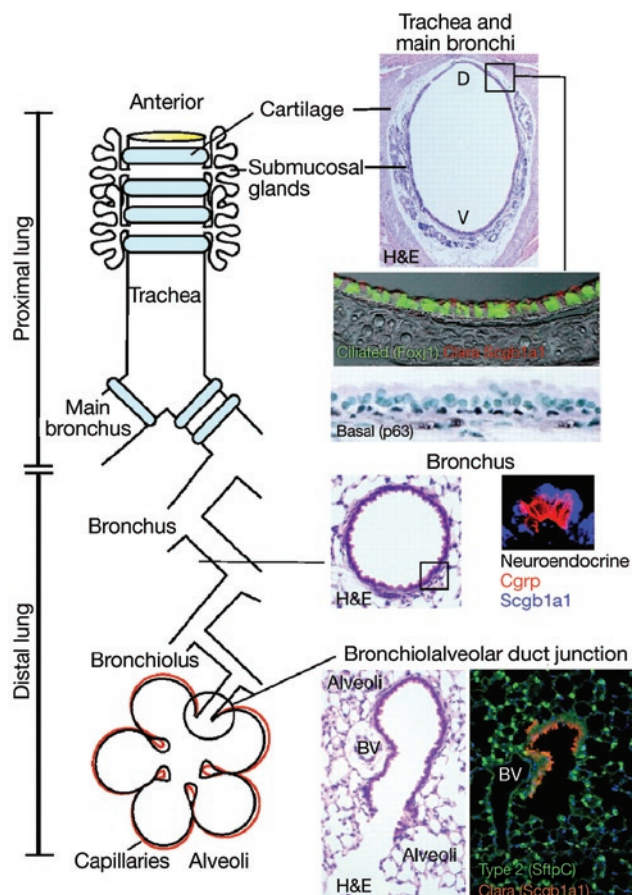


Figure 1 Schematic of endogenous progenitor cells in mouse lung. BV, blood vessel; D, dorsal; H&E, hematoxylin and eosin; V, ventral. (Adapted with permission from ref. 3.; <http://dev.biologists.org/cgi/content/full/133/13/2455>.)

vectors preferentially transduced type 2 alveolar epithelial cells and cells in the conducting but not larger airways.² Notably, the number of β -gal-expressing conducting airway cells, predominantly Clara cells, and overall amount of β -gal activity in lung homogenates steadily increased over a 6-month period, reaching, respectively for rAAV1 Cre and rAAV5 Cre, 3% and 5% of total activity measured in positive control Rosa26-LacZ reporter mice—despite the absence of detectable Cre in Clara cells. Speculating that this might result in part from rAAV-mediated transduction of airway progenitor cells, the investigators administered naphthalene to the Rosa26-Flox/LacZ mice to selectively deplete Clara cells but leave in place toxin-resistant variant Clara cells that might serve as precursors. Administration of either rAAV1 Cre or rAAV5 Cre along with bromodeoxyuridine (BRDU) identified both LacZ-positive

and LacZ-negative label-retaining cells in bronchiolar airways, at BADJs, and in some type 2 alveolar epithelial cells.

An important point is that LacZ-positive label-retaining cells in bronchiolar airways were found whether vector was given before or after naphthalene and BRDU administration. Further immunostaining demonstrated that a subset of LacZ-positive label-retaining cells in the bronchiolar airways and BADJs stained for CCSP and that these infrequent cells were associated with large transgene-expressing patches of cells, consistent with clonal expansion of the rAAV-transduced stem/progenitor cells in the regenerating airway epithelium. Indeed, analysis of mice 1 month after infection demonstrated larger LacZ-positive cell clusters, again suggestive of clonal expansion. Moreover, primary cultures of epithelial cells obtained from extralobar bronchi from rAAV1 Cre- and rAAV5

Cre-transduced Rosa26-Flox/LacZ mice demonstrated a six- to sevenfold increase in colony-forming efficiency when cultured at the air-liquid interface. These results suggest that rAAV1 and rAAV5 selectively transduce airway epithelial cells *in vivo* with higher proliferative capacity. Notably, comparable results were not found with rAAV2 vectors.

These exciting findings support the possibility of selectively or preferentially transducing stem/progenitor cell populations in the lung. Although much further investigation is necessary, this provides a new potential therapeutic approach for diseases affecting airway and alveolar epithelium. For example, airway epithelium in CF patients contains primitive cuboidal cells that express primitive cell markers, including thyroid transcription factor and cytokeratin 7 (ref. 15). CF transmembrane conductance regulator knockout mice also contain fewer pulmonary neuroendocrine cells during embryonic development but increased numbers of these cells postnatally.¹⁶ This suggests that endogenous progenitor cell pathways in CF lungs may be altered and are potentially amenable to selective transduction by rAAV or other vectors. Whether other lung diseases prove amenable to these approaches remains to be determined. Furthermore, despite a growing understanding of the identities and roles of airway endogenous lung stem/progenitor cells in mice, there is little comparable information available for the human lung. Nonetheless, the union of gene and cell therapies holds promise for lung diseases.

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Casting Doubt on the Safety of “Off-the-shelf” Mesenchymal Stem Cells for Cell Therapy

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Mesenchymal stem cells (MSCs) show promise for gene delivery to treat various diseases such as anemia and stroke, as well as other oncological and neural disorders.^{1,2} In this issue of *Molecular Therapy*, Campeau *et al.*³ report on an experimental model that compares the responses of allogeneic and syngeneic hosts to the transfer of erythropoietin (EPO)-expressing MSCs. The studies were based on the premise that EPO could be delivered using genetically modified MSCs to treat anemia or myocardial infarction. The treatment of anemia with expanded autologous MSCs seems plausible, in that the chronic nature of anemia is compatible with the time needed to expand bone marrow-derived MSCs to sufficient

numbers. However, in the case of acute disorders such as myocardial infarction and stroke,^{4,5} gene delivery interventions would have to be immediate, thereby eliminating autologous gene-modified MSCs as an option. Because MSCs have been reported to suppress allogeneic responses, in particular graft-versus-host disease,^{6–8} “off-the-shelf” sources of such cells have been proposed to treat various clinical disorders that require intervention at early time points.

Allogeneic MSCs are already being evaluated in the clinic to treat graft-versus-host responses and other autoimmune disorders. These treatments are based on the immunosuppressive properties of MSCs. MSCs, as third-party cells in the allogeneic hematopoietic stem cell transplantation setting, can function as immunosuppressive cells. Similar immunosuppression would not be relevant in autologous transplantation where rejection would not be a problem. Despite this promise of MSCs as third-party cells, this type of application is different from the delivery of MSCs to an allogeneic host to

deliver a therapeutic gene, such as EPO. Because the delivery of allogeneic MSCs has been reported only relatively recently, it is difficult to verify their safety in allogeneic hosts, such as their use as cellular vectors for gene delivery. Thus, the safety of the use of MSCs for therapeutic gene transfer remains to be established.

Campeau *et al.* now report unexpected phenotypic and immunological effects following the delivery of allogeneic MSCs engineered to express EPO into mice of different haplotypes: BALB/c and C57BL/6 (Figure 1). MSCs from C57BL/6 mice were engineered to express EPO using a retroviral system. The engineered cells were then injected subcutaneously into healthy syngeneic C57BL/6 mice and allogeneic BALB/c mice. In both cases there were transient increases in hematocrit. Although the baseline level of EPO was maintained following cell transfer in the syngeneic transplants, hematocrit levels soon decreased below baseline levels in the allogeneic transplants. The allogeneic mice showed rapid increases in antibodies against EPO (anti-EPO), whose levels were sustained for at least 7 weeks. In contrast, there was a gradual increase in anti-EPO levels in the syngeneic animals. At week 12 the significant differences in hematocrit levels between the two strains of mice could not be explained by differences in anti-EPO levels. The authors further explored this paradox by determining whether there were differences between the neutralizing abilities of the anti-EPOs in the two strains of mice. Using an EPO-responsive cell line, the authors compared the neutralization properties of anti-EPO in the sera of both strains of treated mice. Although anti-EPO from the allogeneic sera was able to neutralize EPO, the sera from the syngeneic sera showed only partial neutralization, suggesting differences in the avidity of the antibodies.

To further understand the mechanism by which EPO expression induced anti-EPO in the allogeneic mice, the authors analyzed the MSCs for cytokine secretion. The major upregulated cytokine in the EPO-engineered MSCs, C-C motif chemokine 2, did not show evidence of involvement in the anti-EPO response, suggesting other mechanisms and/or involvement of other cytokines.

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