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Repurposing A549 Adenocarcinoma Cells: New Options for Drug Discovery

According to the World Health Organization 2019 Global Health Estimates, respiratory diseases are among the leading causes of mortality worldwide. Chronic obstructive pulmonary disease, lower respiratory infections, and lung cancer are the third, fourth, and sixth most common causes of mortality, respectively (1). The cost of quality health care for respiratory diseases was a significant burden for many countries, including the United States, even before coronavirus disease (COVID-19) was declared a global pandemic (2). Despite these facts, very few new drugs have been introduced in the past few decades. Multiple factors contribute to the low rate of drug development in respiratory medicine, including poor understanding of disease's pathophysiology and the lack of good *in vivo* and *in vitro* models, which complicates identifying suitable clinical targets (3). The scientific community has taken steps to address this, creating strategies to harness the knowledge and experience of both academia and the pharmaceutical industry (3). The emergence and rapid improvement of technologies that enable simultaneous profiling of genetic, epigenetic, protein, and metabolite programs of thousands of individual cells have led to significant breakthroughs in understanding respiratory diseases, increasing the identification of candidate targets for new or repurposed drugs (4–7). The field has also experienced exponential growth in the implementation of more complex *in vitro* techniques that support the culture and expansion of several cell types, which can be used for disease modeling.

Alveolar type 2 cells (AT2) have been identified as key players in a diverse collection of lung diseases, including idiopathic pulmonary fibrosis and lung adenocarcinoma (8, 9). AT2 cells are critical for the normal function of the distal lung; they maintain homeostasis in the alveoli and participate in innate immunity by synthesizing and secreting surfactant proteins (10, 11). AT2 cells also serve as facultative progenitor cells that regenerate the lung alveoli after injury or during normal attrition (12). For years, the *in vitro* study of these cells was limited to two-dimensional cultures that failed to support their proliferation and self-renewal. Organotypic three-dimensional cultures, organ-on-a-chip, and precision-cut lung slices are now reliable tools that provide the environment required to maintain and study AT2 cells and other epithelial progenitor cells. They can be used in various settings, from understanding developmental processes to disease modeling and drug testing (13–17). These new tools offer great promise for broad applications. However, they are not well suited as high-throughput screening platforms for therapeutic discovery.

Two-dimensional cell cultures have been largely used in academic and pharmaceutical settings; these are simple, versatile, and easily reproducible methods to understand molecular mechanisms and are easily scalable to drug discovery platforms. In this issue of the *Journal*, Kanagaki and colleagues (pp. 504–514) describe how they circumvented the difficulties of maintaining primary AT2 cells in two-dimensional culture and the scalability limitations of three-dimensional cultures by genetically modifying A549 cells (18).

A549 cells are an epithelial cell line derived from lung adenocarcinoma (19). They have been used widely as a model for the study of lung cancer, and they have also been used as a model of type 2 alveolar epithelium. A549 cells express ABCA3 (ATP-binding cassette subfamily A member 3) and bear lamellar body-like structures. ABCA3 is a protein required to regulate surfactant protein homeostasis and the formation of lamellar bodies (LB), specialized secretory vesicles that store the surfactant proteins in AT2 cells (19). However, A549 cells do not express surfactant proteins. To model the LB function of AT2 cells, Kanagaki and colleagues generated stable clones of cells harboring LB-like organelles (LB cells) that express and secrete exogenous surfactant proteins. To explore how closely the engineered cells recapitulate the function of primary AT2 cells, they compared the transcriptomic profiles of LB cells and primary AT2 cells by scRNA-seq (single-cell RNA sequencing). They show that LB cells express similar amounts of ABCA3 and surfactant protein B and D compared with primary AT2 cells; interestingly, the expression of surfactant protein C, specific to AT2, was lower in LB cells. However, the expression patterns in LB cells were more similar to those in primary AT2 cells than in "wild-type" A549 cells (18). Next, they treated the LB cells with amiodarone. This antiarrhythmic drug can cause interstitial lung disease and pulmonary fibrosis by altering lipid homeostasis (20). The authors developed a high-content screening assay for LB using LB cells and used it to screen several compounds from the Kyoto University Chemistry library. This led to the identification of HPβCD as a candidate therapeutic agent for amiodarone-induced interstitial pneumonia, validating their results using induced pluripotent stem cell (iPSC)-derived alveolar organoids (18).

Alterations in lipid homeostasis and changes in surfactant proteins in AT2 cells have been associated with neonatal and adult lung disease (21). The difficulty in obtaining primary lung tissue from patients and healthy donors hinders the establishment of *ex vivo* and *in vitro* models of lung disease. In their study, Kanagaki and colleagues developed a stable cell line that recapitulates some of the central and unique functions of primary AT2 cells in a cell line that retains the high proliferative capacity of the original A549 cell line, which makes it a useful tool for the identification of new candidate molecules using high-content screening and high-throughput screening assays. However, as with all cell-based assays and, more importantly, with assays using genetically

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engineered cells, critical factors must be considered before their use in large-scale experiments. For example, transformed cell lines can exhibit different gene expression and cell cycle profiles compared with the primary cells. Depending on the particular pathway or phenotype of interest, this difference could modify the results, complicating or misleading the data's interpretation (3).

The development of a new drug from early discovery to the final product being approved by the U.S. Food and Drug Administration is a process that can take 12–15 years (22). Creating better proof-of-concept studies, from controlled and reproducible two-dimensional culture systems to more complex *in vitro* disease models, could facilitate the selection of candidate drugs and improve the process of drug discovery. The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) global pandemic created a sense of urgency in the biomedical community; strong collaborative efforts made possible the rapid generation of reliable diagnostic tests and the development of multiple vaccines. By taking advantage of the lessons learned from this health crisis and implementing the strategies that show success, we could likely improve and optimize therapeutic discovery and development. ■

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