End Stage Takes Center Stage in Pulmonary Arterial Hypertension

Until the middle of the 20th century, anatomic investigation of pulmonary hypertensive conditions (e.g., pulmonary arterial hypertension [PAH]), using tissue acquired by lung biopsy or at the time of death, was the cornerstone of a pulmonary hypertension diagnosis. In one of his final publications, Dr. Alfred P. Fishman elegantly reviewed the gradual transition of a pulmonary hypertension diagnosis based on postmortem morphology to one based on invasive hemodynamics in a living human (1). As pulmonary vascular hemodynamics gained wide attention in the 20th century, the study (and accessibility) of cardiopulmonary tissue at the time of diagnosis or death diminished significantly.

Nonetheless, pathologic descriptions of pulmonary hypertensive vascular disease have continued to evolve over the past 70 years, culminating in our current understanding of the disease as one involving pulmonary vascular remodeling of the arteries, veins, and lymphatics (see Reference 2 for a recent review of the comprehensive advances that have been made in morphologic assessment). Despite this progress, however, comprehensive molecular lung-focused insights into the pathobiology of PAH have been limited by small case numbers and technological challenges.

The term “transcriptome” refers to the total number of transcribed RNA products that are expressed from the genes of an organism (3). Many recent “omics” tools for scientific exploration have shed light on the PAH transcriptome at the lung-tissue level, facilitated unbiased discovery studies, and formed the backbone for many important molecular discoveries. The vast majority of these tools have relied on lung tissue acquired at the time of death or lung transplantation, due to the relative contraindication of a lung biopsy for the care of patients with a known PAH phenotype. Initial unbiased transcriptome studies used whole human genome array technology to study whole-lung homogenate or primary lung cells, with informative results. For example, Geraci and colleagues used this approach to examine six patients with PAH (four with idiopathic PAH [IPAH] and two with heritable PAH), and found significant differences compared with subjects with histologically normal lungs, as well as between the patients with IPAH and heritable PAH (4). In another small lung homogenate study, Rajkumar and colleagues obtained some similar results (e.g., a reduction in bone morphogenetic protein receptor II [BMPR2] expression in patients with IPAH and BMPR2 mutations) and some differences in terms of differential gene expression (5). Laumanns and colleagues subsequently used genome array technology to examine lung tissue from patients with IPAH (n = 6) undergoing lung transplantation compared with control subjects (n = 6). However, they used laser-assisted microdissection to harvest RNA from pulmonary arteries less than 500 μm in diameter, which may provide more specific information about discrete lung microenvironments (6). In addition to some overlap and some differences with prior studies, the authors’ novel findings included upregulation of the planar cell polarity pathway (a component of WNT signaling). Additional advances in unbiased studies have been made with array-based and, more recently, RNA sequencing–based approaches to determine RNA expression using isolated primary lung cells (see Reference 7 for a recent review).

Unbiased transcriptomic profiling of the lung may be approached in many ways, including assessment of lung homogenate to evaluate a given area of tissue in total, or via microdissection techniques, or through cell-sorting approaches to extract a given primary lung cell type. In this issue of the Journal, Stearman and colleagues (pp. 637–649) advance our knowledge of the lung transcriptome in PAH (8). Using specimens from the Pulmonary Hypertension Breakthrough Initiative (PHBI) study, the authors used the human HuGene1.0-ST microarray platform to study RNA expression in lung-tissue homogenate samples from several patients with PAH subtypes (n = 58) compared with control subjects (failed donor lung tissue, n = 25). Their analyses support previously published data and also provide additional insights into the PAH condition, including some stratification by PAH subtype. For example, Ingenuity Pathway Analysis highlighted several pathways that are now repeatedly implicated in PAH pathogenesis, including immunologic modulation and estrogen signaling. In addition, a dataset analysis with a newer statistical test to assess network-driven activities of biological functions, called Evaluation of Dependency DifferentialitY (EDDY), supported multiple previous findings (e.g., alterations in transforming growth factor β signaling and amino acid metabolism) and implicated new network connections and genes that are potentially relevant to PAH (9).

A major strength of this study is the large number of lung samples, which allowed the authors to carry out subtype-specific stratification, which has been challenging to do in PAH. The authors used both established and novel analytic tools, and provide an existing resource for additional analyses by other groups in the future. By adding to our knowledge of the lung ecosystem in PAH, this study both validates many previous findings and provides new clues to PAH pathobiology. Also, the authors’ findings highlight the need to look both upstream and downstream of critical genes and specific network interactions.

However, multiple caveats should be noted when considering the significance of these data, such as regarding the reliance on lung transplant–derived specimens and the exposure to polypharmacy before transplantation. Studies involving lung tissue are complicated by the fact that lung tissues are typically acquired at

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Supported by National Institutes of Health grant R01 HL 134802 (E.D.A.).
the time of death or lung transplantation and thus the tissues represent “end-stage” lungs. These lung samples are significantly modified by the disease process, PAH-directed medications, and other exposures. Thus, these tissues likely do not represent the initial stages of pathogenesis; rather, they reflect a terminal end-stage response to the pathobiology of PAH. Furthermore, the term “end-stage” more often reflects the clinical status of right-ventricular adaptation to PAH, treatment response, and other factors, as opposed to a precise metric of lung vascular pathology and injury response. Thus, it is challenging to know whether the subjects in any study of patients at lung transplantation or death are indeed at the same point in time regarding their lung vascular pathology. In addition, although it is informative and well established, microarray technology is rapidly giving way to RNA-sequencing methods, which support a deeper analysis of the transcriptome as well as additional dimensions of study, such as characterization of small RNA and detection of alternative splicing events (10). Other approaches, such as the integration of methylation analyses combined with either microarray or RNA-sequencing technology, may yield further insights from this rich resource in the future.

Although this large study of lung homogenates does advance our knowledge, future studies may benefit from attempts to use single-cell or laser-dissection approaches to help determine the vascular signature in PAH. Specifically, the expression of vascular-specific genes may be difficult to discern using lung homogenates because the vasculature represents but one of many compartments of the lung (11). Moreover, a focus solely on the pulmonary arteries may miss crucial changes in the broader vascular environment, including lung fibroblasts and inflammatory cells. As such, attempts to isolate discrete cells, or at least specific histologic compartments, may advance our understanding of the variations in changes, and response to injury, at a more discrete level in PAH. For example, in lung cancer, it has been shown that the transcriptomes of specifically chosen lung cells differ considerably from the transcriptomes of the corresponding homogenized lung tissue (12).

The current study significantly advances our understanding of PAH broadly with regard to the condition of the lung at the time of lung transplantation, verifying irregularities in many known pathways and highlighting new ones. It also highlights the tremendous benefit of multicenter tissue- and biospecimen-based programs in PAH, which often provide the framework for more extensive studies of well-phenotyped patients. We applaud the authors’ unrelenting pursuit to build the PHBI resource. Notably, without extramural financial support, research programs such as the PHBI cannot exist to support critical studies such as this one. We must continue to support the PAH community in all respects, from single-center studies to large networks, so that ground-breaking progress such as that made in this study can continue to occur.

Author disclosures are available with the text of this article at www.atsjournals.org.

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References