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The Coming-of-Age of Lung Generation by Blastocyst Complementation

The respiratory epithelium is characterized by a remarkable capacity to robustly respond to injury and regenerate (1). This response is mediated by region-specific stem or progenitor cells, such as airway basal stem cells and type II alveolar epithelial cells (AEC2s), but it is blunted in chronic and/or progressive lung disease, such as chronic obstructive pulmonary disease. Hence, a variety of approaches for lung epithelial regeneration are currently under investigation from enhanced recovery of functional human cadaveric lungs for transplantation (2) to pluripotent stem cell (PSC)-based replacement therapies (3).

Blastocyst complementation is another approach for the generation of functional tissues that has been gaining traction in the last decade. Initially described in the context of lymphoid development (4), blastocyst complementation is a particular case of tissue chimerism in which the injection of PSCs in an early embryo (blastocyst stage) results in the contribution of the donor cells to various tissues and organs postnatally (5). The deletion of transcription factors important for lineage formation in recipient embryos creates a tissue- or organ-specific niche that allows the formation of the respective parenchyma predominantly from injected PSCs. Since the first groundbreaking demonstration of solid organ generation (mouse pancreas in rats) (6), studies of intraspecies and interspecies blastocyst complementation have investigated questions of developmental potency and paved the road for future generation of xenogeneic (human) organs in large animal recipients. Overcoming the numerous technical hurdles involved in interspecies blastocyst complementation will open new avenues for precision-medicine studies in chimeric animal models and curative organ-replacement therapies.

In this issue of the *Journal*, Wen and colleagues (pp. 471–483) describe the highly efficient derivation of foregut epithelia (lung and thyroid) in mice by intraspecies blastocyst complementation of *Nkx2-1*$^{-/-}$ embryos (7). Deleting the transcription factor *Nkx2-1* (NK2 homeobox 1), previously known as *Ttf-1*, to vacate the thyroid and lung niches is a judicious choice given its important role in the development of the forebrain, thyroid, and respiratory system (8). Nkx2-1^{+} lung primordial progenitors give rise to all lung epithelial lineages (9), and *Nkx2-1* embryonic deletion results in severe lung defects, such as tracheoesophageal fistula and hypoplastic, cyst-like lungs lacking most epithelial cell types (10).

In the present study, blastocyst complementation was performed by injecting GFP (green fluorescent protein)-labeled mouse embryonic stem cells (ESCs) in wild-type, *Nkx2-1*$^{+/-}$, and *Nkx2-1*$^{-/-}$ embryos (Figure 1). Although noncomplemented *Nkx2-1*$^{-/-}$ embryos displayed the aforementioned defects, *Nkx2-1*$^{-/-}$ chimeras exhibited restored lung morphogenesis and epithelial cell organization, with GFP-labeled type I alveolar epithelial cells and AEC2s lining the alveolar space and ciliated and club cells lining the conducting airways. Goblet and basal cells were also present, indicating that donor ESCs were able to contribute to major lung epithelial lineages. Morphometric and histological analysis was reinforced by single-cell RNA sequencing and flow cytometry, with the overall data strongly indicating that *1*) the vast majority of lung epithelial cells in *Nkx2-1*$^{-/-}$ chimeras were derived from donor ESCs and *2*) the donor cells were indistinguishable from endogenous cells (in *Nkx2-1*$^{+/-}$ chimeras) in terms of cell distribution, lineage-specific gene and protein expression, and ultrastructural features, such as AEC2 lamellar bodies. Interestingly, blastocyst complementation also fully restored the thyrocyte compartment of the thyroid gland, which is absent in *Nkx2-1*$^{-/-}$ embryos because of apoptosis of early thyroid progenitors (11). On the other hand, defects in dorsoventral patterning in the trachea and esophagus were not reversed, and the contribution of donor ESCs to forebrain-derived structures, such as diencephalon and corpus striatum, was minimal. Either or both of the latter observations may explain the inability of *Nkx2-1*$^{-/-}$ chimeras to survive postnatally.

The current study, together with two recent studies that used similar strategies to vacate the pulmonary niche (12, 13), firmly establishes blastocyst complementation as a viable research strategy for lung developmental studies, disease modeling, and, in the long term, generation of xenogeneic, transplantable human lungs. One can envision future mechanistic studies of pathways with putative roles in lung epithelial specification or progenitor expansion and differentiation using a conditional gene ablation strategy within the

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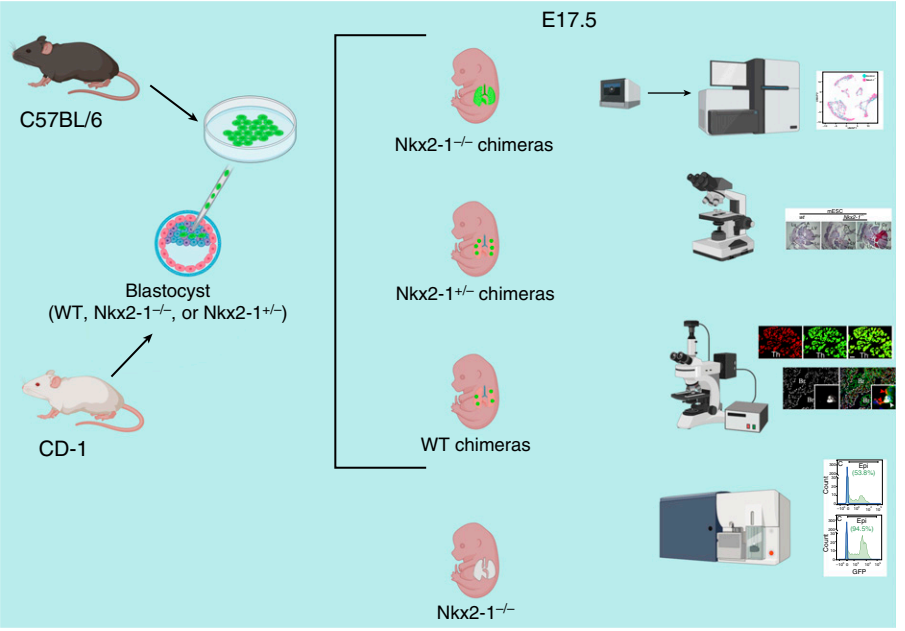

Figure 1. Schematic of methodology and major findings in the study by Wen and colleagues (7). E17.5 = Embryonic Day 17.5; WT = wild-type.

anterior foregut endoderm (12). In addition, patient-specific induced PSCs (e.g., from patients with pulmonary arterial hypertension) can be used in the creation of interspecies chimeras for human disease modeling in laboratory animals.

Interspecies blastocyst complementation in the lung will bring about an entirely different host of ethical and scientific problems, especially in chimeras containing human-derived material (14). Salient questions of organ size control, efficiency of donor cell engraftment, and functionality of the chimeric organ will need to be systematically addressed for this approach to be successfully used in basic research and organ manufacturing. Most importantly, it is unlikely that *Nkx2-1* deletion will be an ethically acceptable strategy in creation of animal–human chimeras, as Nkx2-1^{+} forebrain progenitors give rise to neurons within structures such as the striatum, cerebral cortex, and pituitary (15). A region-targeted approach, such as conceptus complementation, may be preferable in this context for the formation of human lungs or thyroid in such chimeras.

Overall, the study by Wen and colleagues provides compelling proof of principle as to the possibility of efficient lung and thyroid epithelial reconstitution after blastocyst complementation in mice. Future studies will most certainly delve into the complexities of interspecies chimeras and establish whether this approach will find its place in the armamentarium of lung regenerative medicine. ■

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Laertis Ikonomou, Ph.D.
Department of Oral Biology
University at Buffalo
Buffalo, New York

ORCID ID: 0000-0003-0993-6713 (L.I.).

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When "AMBITION" Isn't Good Enough: Risk Status and Dual Oral Therapy in Pulmonary Arterial Hypertension

In 2015, the AMBITION (The Ambrisentan and Tadalafil in Patients with Pulmonary Arterial Hypertension) trial permanently altered the landscape of pulmonary arterial hypertension (PAH) therapy by demonstrating conclusively the efficacy of upfront dual oral therapy (1). However, despite examining multiple primary and secondary clinical endpoints, AMBITION did not include any cardiopulmonary hemodynamic metrics as an endpoint (1). In this issue of the *Journal*, Badagliacca and colleagues (pp. 484–492) retrospectively examined the effect of initial dual oral therapy with an ERA (endothelin receptor antagonist) and PDE5i (phosphdiesterase type 5 inhibitor) (predominantly ambrisentan and tadalafil) on pulmonary vascular resistance (PVR) and risk-assessment score in 181 patients newly diagnosed with PAH (2). Risk-assessment scores were calculated using the simplified European Society of Cardiology/European Respiratory Society (ESC/ERS) guidelines score and the REVEAL 2.0 risk-assessment tool (3, 4). Superficially, their results support the conclusions published in the AMBITION trial—therapy was well tolerated, and, on average, patients demonstrated significant improvements in World Health Organization functional class, 6-minute-walk distance, PVR (−40.4%), mean pulmonary artery pressure (mPAP), right atrial pressure, cardiac index (CI), and several echocardiographic parameters; moreover, the magnitude of the decrease in PVR did correlate with outcome (1, 2). In addition, the authors found that starting in low-risk status was associated with maintaining low-risk status on dual oral therapy at follow-up (19/27 remained low risk by ESC/ERS, 11/19 by REVEAL 2.0) (2).

However, on closer examination, the picture is far less rosy. Only a minority of patients actually achieved low-risk status at follow-up: 43.1% by ESC/ERS and even fewer by REVEAL 2.0 (34.8%). Furthermore, only ∼50% of patients at intermediate risk on presentation improved to low-risk status, and none of the high-risk patients improved to low risk at follow-up, with almost 50% remaining high risk. Only 7.7% normalized their PVR, whereas 10.5% demonstrated progression despite therapy. Notably, several factors were associated with poor PVR response, including age >60, male sex, baseline mPAP >48 mm Hg with low CI, and an elevated right ventricle (RV) to left ventricle (LV) ratio (RV/LV) with low tricuspid annular plane systolic excursion (TAPSE) by echocardiography (2).

The conclusion is unmistakable: In a disconcerting majority of patients, dual oral therapy is simply not good enough. In fact, even in the original AMBITION trial, only a minority of patients (39%) demonstrated a "satisfactory clinical response" at 6 months (1) despite being on dual oral therapy under optimal conditions. Furthermore, it has been well demonstrated that persistence of high-risk status is associated with poor outcomes (5). Yet despite this, current recommendations from the World Symposium on Pulmonary Hypertension 2018 recommend an initial trial of dual oral therapy for non–high-risk patients, with transition to triple combination therapy in intermediate- or high-risk patients on follow-up (3). However, there are a number of studies demonstrating persistently poor outcomes even if parenteral therapy is employed as the rescue maneuver (6, 7). As a result, it has been suggested that this approach may be too little too late (8). The results of the current study support this conclusion, demonstrating in a "real-world" setting that for the overwhelming majority of patients not deemed low risk at initiation of therapy, dual oral therapy is inadequate as an initial treatment strategy.

There is a sound physiologic rationale for this conclusion. As has been repeatedly shown, RV dysfunction is a strong predictor of outcomes in PAH (9). PVR is a surrogate measure of RV afterload in PAH. It is therefore not surprising that in the present study, PVR reduction was tightly associated with improvement in risk status (2). As such, adequate upfront reduction of PVR should be the primary goal of initial therapy. Though dual oral therapy is clearly beneficial, it just does not achieve timely or sufficient reduction of PVR in the majority of patients.

This conclusion leaves us seeking more aggressive upfront treatment strategies. Although triple oral add-on therapy has been employed with incremental benefit in prevalent patients (10), in the recently concluded TRITON (The Efficacy and Safety of Initial Triple versus Initial Dual Oral Combination Therapy in Patients with Newly Diagnosed Pulmonary Arterial Hypertension) trial investigating triple upfront combination oral therapy, all primary and secondary endpoints (similar to those in this study) were negative (11). In contrast, in two small, uncontrolled studies with different—albeit much sicker—incident cohorts, triple upfront therapy regimens with a systemic prostanoid had robust hemodynamic and clinical effects (12, 13). In addition to the

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