Dichotomous Branching of Human Fetal Lung Demonstrated with Light Sheet Fluorescence Microscopy

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Figure 1. Light sheet fluorescence microscopy images of the anterior (A) and hilar (B) surfaces of a 9.5-week human fetal lung stained with anti–basic fibroblast growth factor. Bronchi and dichotomous branching morphogenesis of preacinar airways are appreciable at subcellular resolution using this technique.

Video 1. A 360° view of reconstructed light sheet fluorescence microscopy images of a 9.5-week human fetal lung stained with anti–basic fibroblast growth factor. Anterior and hilar lung surfaces are both visible. Bronchi and dichotomous branching morphogenesis of preacinar airways are appreciable at subcellular resolution using this technique.

Between the 7th and 16th weeks of development, corresponding to the pseudoglandular phase of lung development, the conducting airways of the human fetal lung are formed by generations of repeated dichotomous branching (1). This proliferation and differentiation stems from interactions between lung epithelium and its surrounding mesenchyme (2) (Figure 1 and Video 1).

The depicted human fetal lung was collected with institutional review board approval after elective dilation and curettage. Fetal age was estimated to be 9.5 weeks by heel-toe length. It was fixed in 4% paraformaldehyde, embedded in acrylamide hydrogel matrix, and rendered transparent with the passive clarity technique (3). The specimen was immersed in anti–basic fibroblast growth factor, a marker for developing airway epithelial cells (4), followed by fluorophore-conjugated secondary antibody. It was embedded in low-melting point agarose, equilibrated to $n = 1.46$ in refractory index matching solution, and imaged on a Lightsheet Z.1 microscope (Carl Zeiss AG, Oberkochen, Germany). Three-dimensional models were constructed using Imaris (Bitplane AG, Zurich, Switzerland).

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The uncompressed video is accessible from this article's supplementary material page.

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The imaged fetal lung demonstrates primary, secondary, and tertiary bronchi with clear dichotomous branching of pseudoglandular epithelial tubes. Lungs of this age should continue to branch for several more weeks of development, establishing the mature number and distribution of preacinar airways (5). ■

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

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