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Synthetic recombinant cultures to investigate and control lung morphogenesis

Branched epithelial trees are a structural hallmark of many human tissues and organs and are intimately tied to physiologic function. However, the signaling network principles that drive the self-assembly of such branching structures are not clear, which hinders our understanding of tissue development and constrains potential regenerative medicine strategies. For example, in the developing mouse distal lung, many of the key signaling molecules have been identified, but the biological circuits that map these signaling pathways onto the physical topology of the lung remain unknown. Here, we take a forward-engineering approach to this problem by co-culturing embryonic mouse distal lung epithelium with synthetic mesenchymal cells with user-defined morphogen expression programs. These “synthetic recombinants” re-imagine a classical developmental biology model and combine the native branching competence of the epithelial explant with the programmability of the engineered mesenchyme.

We demonstrate that synthetic recombinants can communicate bidirectionally using the key signaling pathways mediating branching morphogenesis. Secreted FGF10 from the synthetic mesenchyme potently stimulates proliferation and chemotaxis in the explanted epithelium, replicating the inductive effect of native mesenchyme. In turn, the epithelium secretes Sonic Hedgehog, which is sensed by the synthetic mesenchyme as evidenced by an integrated reporter circuit. We employ time lapse confocal microscopy to capture real-time reciprocal signaling between the two tissue layers using a panel of fluorescent reporters, and we extract quantitative spatial gradients of reporter activation in three dimensions to understand the dynamics of epithelial-mesenchymal crosstalk.

Branching morphogenesis is thought to operate as a reaction-diffusion patterning system, and our current experiments focus on manipulating elements of the signaling network which are thought to modulate the size, shape, and frequency of airway bifurcations. For example, we currently seek to control the expression levels and diffusion rates of the key morphogens FGF10 and Hedgehog, as well as to tune the feedback network used in sensing Hedgehog. To achieve these aims, we are leveraging a combination of existing tools developed within

the community (e.g. inducible gene expression system), as well as new ones (e.g. synthetic receptors) that are tailored for this recombinant model. We expect that this set of experiments will yield new insights into the signaling algorithm underlying branching morphogenesis, as well as a potential blueprint to generate branched epithelial tissues de novo.