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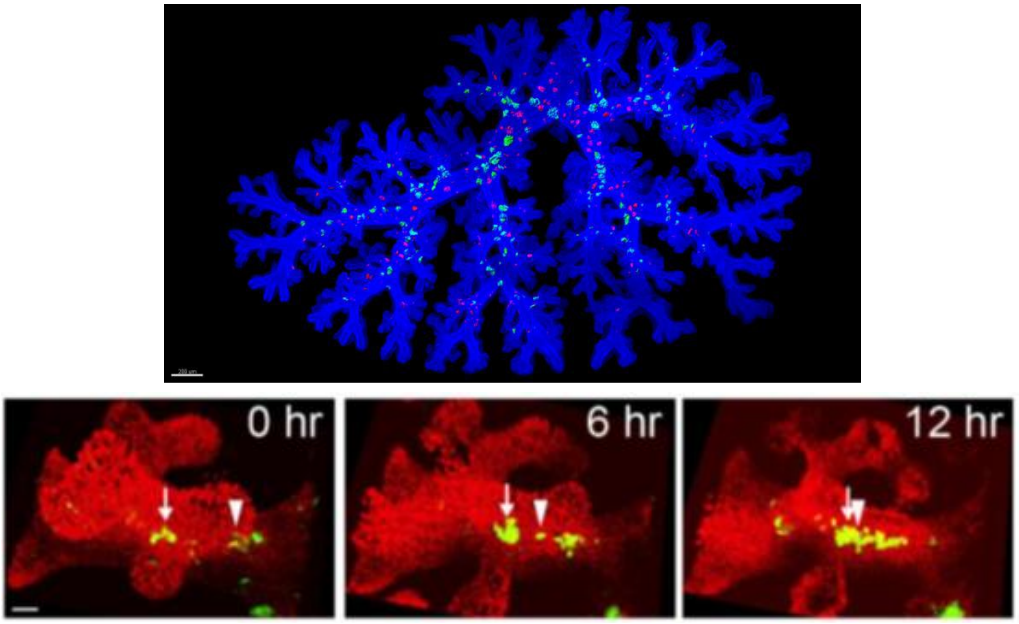
Pulmonary neuroendocrine cell clusters at airway branches

January 29, 2016– The cellular composition of respiratory epithelium varies in different sections of this highly branching organ system. The airway, for example, contains numerous ciliary cells capable of sweeping out foreign material, while Club cells that secrete surfactant protecting the airway surface dominate. A third type, neuroendocrine (NE) cells, has been found to aggregate at branch points in the airway in many different species, but the developmental process responsible for this localization has remained unknown.

A new study by research scientist Masafumi Noguchi and others in the Laboratory for Lung Development (Mitsuru Morimoto, Team Leader) uses single cell-resolution 3D time-lapse imaging of an entire lung lobe to show how NE cells cluster at airway bifurcation points in the mouse lung. They also find that Notch signaling plays a critical role in inhibiting NE cell differentiation, thus limiting the numbers of such cells. This work was published in the December issue of *Cell Reports*.



(Top) Localization of NE cell clusters in 3D airway. Clusters at branch points shown in green, those at other sites in red. (Bottom) NE cell (yellow) migration in developing airway epithelium (red). Initially isolated cell (arrowhead) converges toward bifurcation point (arrow).

In order to overcome the historical challenge of observing minute changes in the developing lung over time, the team first generated a mouse in which epithelial cell nuclei and NE cells were labeled with different genetic fluorescent reporters. They next used two-photon microscopy to visualize whole-mount preparations of cleared fetal lung at high precision and tissue depth, and used these images to generate a 3D model of the entire airway, making it possible to analyze the sizes and spatial relationships of NE cell clusters, which they found indeed form following a consistent pattern.

Previous works by the Morimoto team and other groups have shown that Notch-Hes1 signaling is involved in NE cell cluster formation, so in the new study they used a *Hes1* knockout mouse to investigate how this would affect the differentiation of emerging NE cells. In wildtype embryos, NE cells appear as solitary cells throughout the airway, not just at branch points, in a salt-and-pepper distribution from about day 13.5, but in the *Hes1* knockout the number of NE cells clearly increased, with greater numbers of neighboring cells undergoing NE cell differentiation, and much larger clusters of NE cells at bifurcation points by day 16.5. This suggests that the Notch signaling pathway plays a role in suppressing the NE cell differentiation of adjacent cells as a means of restricting the NE cell numbers, and with the ultimate effect of limiting NE cell clusters to appropriate sizes.

What then leads differentiated NE cells to aggregate at airway branches? Using a 4D live imaging setup, Noguchi visualized changes in cultured fetal lung lobes for up to 15 hours, and was surprised to find

that the NE cells appear to be moving autonomously toward branch points, crawling through the epithelium and taking a direct path to a distal bifurcation, an unusual example of independent epithelial cell migration in morphogenesis.

"So we know that NE cells cluster at branches, but the next question is, what controls their migration to such points. I think this would require at least three factors – a chemoattractant to draw cells from distant sites, a second to anchor them at branch points, and a third to bind similar cells to each other," says Morimoto. "If we can gain a better understanding of this process, I think it may also shed light on the mechanisms at work in the metastasis of small cell lung cancer as well."