Periodic accumulation of interstitial cells generates the branched architecture of *Cladonema* medusa tentacles

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The interaction between the epithelial cell layer and its underlying mesenchyme plays a critical role in shaping the tissues/organs in many triploblastic animals. In well-studied branching model animals, such as mammals and *Drosophila*, the formation of a new branch is often regulated by receptor tyrosine kinase (RTK) signaling, which is activated in the epithelium by ligands emanating from mesenchyme cells. However, it remains largely unknown how tissues/organs become branched in other non-model animals, in particular, those mesenchyme-free diploblastic animals. In this study, we investigated branch formation in the tentacles of the hydrozoan jellyfish *Cladonema pacificum* to discover a new mechanism for branching morphogenesis in animals.

We found that hydrozoan-specific pluripotent stem cells, namely interstitial cells (I-cells), play major roles in the tentacle branch formation of this species. First of all, I-cells were periodically accumulated in the future branching sites when new branches form. Secondly, the accumulated I-cells had multiple differential potentials contributing to form different cell types that constitute the branches. Finally, the I-cells remained located at the tip of the growing branches, while proliferating and leaving behind their differentiating descendant cells for the branch outgrowth. In addition, through a series of pharmacological analyses, we proposed a two-step model involving RTK signaling for the tentacle branch formation. In the first step, MEK/MAPK signaling accumulates I-cells at the future branching sites by locally enhancing I-cell proliferation. In the second step, fibroblast growth factor (FGF) signaling regulates branch elongation possibly by promoting self-renewal and differentiation of the accumulated I-cells.

Taken together, the current study revealed both similar and different mechanisms for branching morphogenesis across different animals. *C. pacificum* shares common mechanisms with other animals such as involvement of RTK signaling and branch elongation by cell proliferative activity at the tip of the growing branch. In contrast, it differs from other animals in that branching relies on pluripotent stem cells instead of the interaction between the epithelium and mesenchyme.