Live imaging of early oral epithelium reveals epithelial migration of tooth progenitor cells

Prochazka J., Prochazkova M., Spoutil F., Hoch R., Shimogori T., Wittmann T., Klein O. D.

1Department of Orofacial Sciences and Program in Craniofacial and Mesenchymal Biology, University of California, San Francisco, CA, USA, 2Department of Anthropology and Human Genetics, Faculty of Science, Charles University in Prague, Czech Republic, 3Institute of Experimental Medicine AS CR, Prague, Czech Republic, 4Department of Psychiatry, Nina Ireland Laboratory of Developmental Neurobiology, University of California, San Francisco, CA, USA, 5RIKEN Brain Science Institute, Laboratory for Molecular Mechanisms of Thalamus Development, 2-1 Hirosawa Wako, Saitama 351-0198, Japan, 6Department of Cell and Tissue Biology, University of California, San Francisco, CA, USA, 7Department of Pediatrics and Institute for Human Genetics, University of California, San Francisco, San Francisco, CA

Email of the presenting author: jan.prochazka@ucsf.edu

Live imaging of embryonic tissues provides unique tool to underpin cellular and molecular mechanisms of morphogenesis and organ formation. In our system we used early embryonic mandibles explanted at embryonic day 11 into organ culture. This approach enabled us to perform long time-lapse confocal microscopy imaging of early oral epithelium for up to 48 hours. The speed of scanning and length and intensity of tissue exposure to excitation lasers are critical parameters to avoid photo-bleaching of fluorescence reporters, as well as to keep the tissue healthy, and to ensure that assessed developmental processes are comparable with development in vivo. To accomplish that, we established imaging setup for organ culture with use of spinning disc confocal microscope. Spinning disk imaging technology provides unique combination of sufficient speed and resolution for 48h long time-lapse imaging with minimal photo-toxicity for tissue. The use of described live-imaging microscopy setup allowed us to examine the cellular mechanisms of tooth development initiation. We identified a migratory population of Fgf8-expressing epithelial cells in the embryonic mandible which provides most of the epithelial cells required for development of future molar tooth. This population migrates anteriorly towards Shh-expressing epithelial cells at the site where tooth placode will initiate. Furthermore, inhibition of Fgf and Shh signaling disrupted the oriented migration of cells, leading to failure of tooth development. The time-lapse data allowed us to perform statistic analysis of cell movement in time and evaluate the differences in cell behavior under Fgf and Shh signaling compromising conditions compared to normal development. Our results demonstrate the importance of collective epithelial cell migration in the initiation of tooth development and provide the first example of such cell behavior during mammalian organ formation, suggesting that epithelial migration might be more frequent mechanism of morphogenesis also in other organ systems and proper live-imaging is only direct way to analyze this phenomenon.