Analyzing Signaling Dynamics in Developing Salivary Glands Using a Multiplexing Approach

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Abstract

Construction of a Tissue Microarray

Figure 5. Detection of phosphorylated signaling proteins in submandibular salivary gland epithelium

Methods

Overall Project Goal:
To develop a high-throughput in situ proteomics based assay, which categorizes and quantifies active signaling proteins, cell types, cell sub-compartments, and cell sub-compartments compositions throughout all developmental time-point of mouse salivary glands.

Overview: Using multiplexed IHC as a systems approach to profile developmental signaling pathways

Screening IHC against developmental pathways

Multiplexed IHC

Collect tissue from 12 protein development

Differential expression and signaling protein

Axial sub-cellular compartments

Results

Figure 1. Cellular segmentation

Figure 2. Cellular and sub-cellular image segmentation

Figure 6. “Virtual H&E” images are computationally constructed from immunofluorescent images

Future Directions

Web-based data viewer for SGDAtlas.albany.edu

Figure 4. Identification of expression profiles throughout developmental time-points

Figure 3. Multiplexed IHC of 12 proteins in a single tissue section

Tissue microarray is sequentially detected automated system (Image-Depot, Inc) with different proteins, and in the subcellular environment. In addition, a variety of histological stains were used to determine the expression of specific antibodies to actin, myoepithelial antigens, and cytokeratins. The process of image registration and analysis of specific signaling pathways was performed using the software ImageJ and/or extended image analysis software. The optimal dye to protein ratio for maximal fluorescent signal is determined empirically.

The salivary glands are important for generating mastication, swallowing, and specifically regulate gland morphogenesis and differentiation. Most of the signaling molecules involved in different biological pathways are expressed in specific cell types and sub-compartments. Therefore, the optimal signaling pathways can be analyzed by using multiplexed IHC to capture images sequentially detected in a single tissue section. The process of image registration and analysis of specific signaling pathways was performed using the software ImageJ and/or extended image analysis software. The optimal dye to protein ratio for maximal fluorescent signal is determined empirically.

Conclusions

- We have established a multiplexing platform that can be used as a deconvolutional method to identify specific cell types, cell sub-compartments, and signaling proteins (both total and active forms).
- We will use signaling profiles as a starting point to understand the contribution of signaling pathways controlling morphogenesis and differentiation.
- The active (phosphorylated) forms of multiple signaling proteins show distinctly regulated expression patterns within any sub-compartment and/or sub-compartments.

Acknowledgements

This research was supported by the National Institute of Dental and Craniofacial Research (Grant R01DE026570 to M.L.), National Cancer Institute (Grant R01CA128288 to M.J.G.), and National Institute of Diabetes and Digestive and Kidney Diseases (Grant U01DK090817 to M.J.G.).