Microplate-based 3D Culture System to Screen for Hedgehog Pathway Gene: Environment Interactions that Cause Orofacial Clefting

Brian Johnson
Biomedical Engineering
University of Wisconsin
Conflict of Interest

Brian Johnson owns equity in Onexio Biosystems. A company that develops solutions for high-throughput toxicity testing.
Gene-environment interactions in OFCs

• Most birth defects are thought to result from a complex interaction of genetic and environmental factors

• OFC etiology
  • Several OFC genes have been identified but these do not act with Mendelian inheritance
  • Environmental factors have also been associated that elevated risk ratio

• Prevention strategies for OFCs have not been developed because our understanding of causative factors is inadequate
Epithelial-mesenchymal interactions drive outgrowth of the facial processes

- The tissues that form the upper lip and palate are primarily comprised of **surface epithelium** covering **cranial neural crest-derived mesenchyme**
- Outgrowth of the tissues that form the upper lip and palate is dependent upon dynamic molecular cross talk between the epithelium and mesenchyme
- Multiple pathways involved
  - WNT
  - BMP
  - FGF
  - Hedgehog (Hh)
    - SHH from the surface epithelium activates pathway activity in the adjacent mesenchyme
Genetic and environmental disruption of Hh signaling

- Mutations in core Hh pathway genes (e.g., SHH, GLI2, and PTC1) as well as putative downstream targets (e.g., FOXF2) have been linked by case studies to syndromic and non-syndromic OFCs.

- The pathway is inherently sensitive to small molecule modulation by environmental compounds:
  - Cyclopamine-like dietary alkaloids
  - Natural and synthetic pharmaceuticals
  - A common pesticide synergist

Peukert et al.
Building a Microphysiological Model for Hh Pathway Antagonism Leading to OFCs

The AOP guides design ensuring incorporation of key molecular, cellular and tissue level components and endpoints

- Epithelial:Mesenchymal organization
- Directed Hh gradient and outgrowth
- Tissue fusion
- Low/Medium throughput compatible
  - Fluorescent and Luminescent readouts
  - Format/Material (PDMS, PS, etc.)
  - Compatible with high content imaging
  - Chemical sequestration (PS)
Microphysiological Model Design

- Epithelial:Mesenchymal organization
- Directed Hh gradient and outgrowth
- Tissue fusion
- Low/Medium throughput compatible
- Fluorescent and Luminescent readouts
- Compatible with high content imaging
- Chemical sequestration (PS)
Exogenous and Endogenous Hh Pathway Response

- Live cell luciferase activity.
  - Red Brightfield
  - Green Luminescence
- Exogenous Shh activates Gli driven luciferase in 3T3 cells.
- Endogenous SHH secreted by overlaid GMSM-K cells activates Gli driven luciferase in 3T3 cells.
- Combination indicates max responsiveness
Antagonism of Endogenous Hh Pathway Response

- Hh antagonists Vismodegib, Cyclopamine and Piperonyl Butoxide.
- Microtissues dosed for 3 days show dose response.
- Plates fully recovered Hh activity after treatment.
- Microtissues re-dosed in reverse at 21 days show similar dose-response curves
Matched High-Content Imaging

Vital Fluorescence

Vital Luciferase

Fixed Ki67
Antagonism of Endogenous Hh Pathway Activity

Hedgehog gradients drive developmental patterning throughout the body (shown - hind limb bud E10.5)

Yina Li et al. PNAS 2006;103:6548-6553
The AOP guides design ensuring incorporation of key molecular, cellular and tissue level components and endpoints

- Epithelial:Mesenchymal organization
- Directed Hh gradient and outgrowth
- Tissue fusion.....TBD
- Low/Medium throughput compatible
  - Fluorescent and Luminescent readouts
  - Format/Material (PDMS, PS, etc.)
  - Compatible with high content imaging
  - Chemical sequestration (PS)
Acknowledgments

• MMB lab
  • Ross Vitek & Pete Geiger

• Lipinski Lab
  • Rob Lipinski, Dustin Fink, Hannah Chung

• Murphy Lab
  • Bill Murphy, Angie Xie, Bill Daly

• Funding
  • Molecular and Cellular Mechanisms of Tumor Development T32 CA157322 to BPJ, NIH Biotechnology Training Program NIGMS 5 T32-GM08349 to A.W.X. and the National Science Foundation DGE-1256259 to A.W.X. 5R00DE022101-04 to RJL from the NIH/NIDCR and EPA -Science to Achieve Results (STAR) Center 835737
  • RO3, K99 pending