Use of primary conjugated antibodies to model the heterogeneity of GBM’s

Ryan Gensler
NSSSP 2019
### 3 Models for GBM

<table>
<thead>
<tr>
<th>Model Type</th>
<th>Xenografts</th>
<th>Cell Lines</th>
<th>Organoids</th>
</tr>
</thead>
</table>
| Pros       | • Heterogeneity  
            • 3D morphology  
            • In vivo       | • Cheap        
            • Grow quickly | • Heterogeneity  
            • Cheap        
            • 3D morphology  
            • Act as patient avatars |
| Cons       | • Expensive  
            • Slow tumor growth | • Lack heterogeneity  
            • In vitro           | • In vitro          
            • Not yet verified   |
What/How are organoids grown?

- **Organoid Morphology:**
  - 3D
  - Populated by neuronal, glial, and NSCs
  - Organized tissue with temporal patterning

- Start from ESCs and correct factors cause neural differentiation

- At day 30, inject tumor cells into the organoid

- Day 60-90: Organoid sectioned, stained, imaged

Lancaster and Knoblich, Nat Protoc 2014
Aim of Project

- Create primary conjugated antibodies

- Use these 1º conjugated antibodies to avoid the lengthy TSA-IHC protocol

- Co-stain tissue

- Use these co-stains to understand the heterogeneity of the GBMs
  > I.e. what proteins GBMs are expressing for a specific patient tumor

- Big Picture: Prove that GBM injected tumors cells are initiating GBM growth in organoid models

- Compare organoid models to heterogeneity of the patient tumor
Methods

Unconjugated 1º antibodies

Conjugation dyes
15 minutes
Mistakes

Conjugated 1º antibodies
(Sox 2, Ki 67, Vimentin, Ptprz 1, Hop X, olig 1)

Test for optimal antibody to solvent concentration

Find optimal concentrations

Select random slide for testing

30-50 slides of sectioned tissue stored at -80ºC

Cold cryostat
18 uM
Box of slides

Conjugation dyes

HOPX

Vimentin

Ptprz1

CT19-27 w/o pdgf

PTPRZ1

DAPI

GFP

CT19-27 w/o pdgf

Dapi

Vimentin
Methods

- Overnight with only one unconjugated 1º antibody
  - 1º now conj. with fluorescent 2º antibody
    - Signal from 2º antibody is now strengthened
      - Easy to see under scope

  - Final Product ready for imaging

- Overnight with multiple conj. 1º antibodies
  - Co-staining via conj. 1º antibodies
    - Final Product ready for imaging
      - Provides info on tumor heterogeneity
Proving Tumor Cells are in Organoids

- Validated staining protocol
- Stained for all tumor cells (SOX2)
- Would expect organoids to be sox 2 and ptprz1, but not GFP
- Next step: Compare heterogeneity patient tumor to organoid via scRNA-seq
Comparing sc-RNAseq to Stains

Umap graph shows clustering of similar cells.

Heat map showing groups of cells that express similar genes.
Future Ventures

Goals:

1. Improve allograft injection method of organoid with human GBM cells
2. Use FISH to ultimately identify human tissue in organoids
3. Use scRNA-Seq to sequence organoids to prove model viability
4. Continue to use staining method to visualize heterogeneity of GBMs embedded in organoids

Figure by Anoop Patel
Acknowledgements

• Richard Ellenbogen, MD
• Sandra Ellenbogen, RN
• Anoop Patel, MD, PhD
• Eric Holland, MD, PhD
• Sam Emerson, MD, PhD
• Siobhan Pattwell, PhD
• Frank Szulzewsky, PhD
• PJ Cimino, MD, PhD
• Alysha Herich, BS, Intern

Grant Title: Summer Research Experience in Translational Neuroscience and Neurological Surgery

Grant Number: 5R25NS095377-04