**Molecular Signature of the Neural Crest Invasive Front**

1. Regional differences in gene expression throughout migratory stream
   1. Introduction to cranial NCCs
   2. Image of the 8 segment isolation
   3. 77 genes used throughout in a pie chart classified by function
   4. 8 segment 24 hour clustering
   5. Heatmap of genes differentially expressed, segments 1-3 vs 6-8 showing first 3 segments distinct from rest
   6. 24 hour clustering of segments 1-3
   7. Heatmap of genes differentially expressed, segment 1 vs 2-3 showing first 1 segment is distinct
2. Confirming regional differences
   1. HCR
      1. Up in lead with HNK1 (IHC) – Images and poly-line kymograph
         1. Hand2
         2. BAMBI
         3. Glypican3
         4. FoxD3 (back)
         5. Merged image
         6. Intensity profiles
3. Schematic explaining model basics
   1. Cell-induced gradient
   2. Leader chemotaxis & sensing accuracy
   3. Followers take direction from leaders, detachment rules
   4. Domain growth, cells enter from left, first leaders, then followers, volume exclusion
4. Mathematical modeling to change the size of the lead subpopulation
   1. Schematic of how model parameters are varied (vary time after which followers enter the domain)
   2. Schematic how migration profiles are calculated as histogram over 20 simulations
   3. Compare migration profiles for fixed leader fractions, side by side or in one graph
      1. 1/16th, 1/8th, 1/4th, 1/2th, 1
5. Homogeneity of the lead subpopulation
   1. Validation of methods used for single cell analysis
      1. In vitro (LCM) compared to in vivo (LCM and FACS)
         1. PCA plot
         2. Efficiency plot
         3. Violin plots
6. Molecular profiling of trailblazers overtime
   1. Image of trailblazer dissections
   2. Single cell qPCR heatmaps
      1. HH 13 (16 hour)
      2. HH 15 (24 hour)
   3. PCA plots
      1. HH 13 (16 hour)
      2. HH 15 (24 hour)
   4. Violin plots
      1. HH 13 (16 hour)
      2. HH 15 (24 hour)
7. Quadrant profiling showing that there is a molecular profile of the trailblazers that is distinct from quadrants
   1. Image of quadrant dissections
   2. Clustering of quadrants versus the 8 segment data
   3. Clustering of the trailblazers versus the quadrants
   4. PCA of trailblazers versus quadrants
   5. Violin plots of trailblazers and quadrants
   6. Molecular profile of a trailblazer
      1. Top 25 genes expressed by trailblazers at HH 13
      2. Top 25 genes expressed by trailblazers at HH 15
      3. Consistently expressed genes by trailblazers at HH 13 and 15
      4. Top genes differentially expressed by trailblazers but not by quadrant 1
8. Transcription factor knockdown in lead NCCs (EP entire stream)
   1. Gene profiling of entire stream at 24 hrs
      1. What when up/down in lead (front genes)
      2. What is typically down in lead but now up (back genes)
   2. Statics of stream migration at 16 & 24 hrs
      1. Distance migrated
      2. Width of stream
      3. Distribution of transfected cells
      4. Neighbor relations
9. Transcription factor overexpression in trailers (EP later to hit only trailing)
   1. Statics of stream migration at 24 hrs
      1. Distance migrated
      2. Width of stream
      3. Distribution of transfected cells
      4. Neighbor relations
10. Mathematical modeling to perturb the leaders and trailers
    1. Schematic of how model parameters are varied, i.e. after some time half of new cells are leaders
    2. Comparison of migration profiles
       1. Wildtype
       2. Make half the leaders fixed as trailers
       3. Make half the trailers fixed as leaders
11. Summary/Discussion
    1. Novel methodologies/approach of
       1. 8 segment analysis
       2. Single cell analysis
       3. Rationale for approaches
    2. Relationship to other NC fronts
       1. Bronner’s work
       2. Future directions- Other migratory fronts
          1. Cranial
          2. Trunk
          3. Gut
    3. Relationship to Cancer Invasion
    4. Summary & Model
       1. We will focus on the critical role of the genes we have identified.