Biological pathway involvement in melanoma heterogeneity and drug-induced resistance

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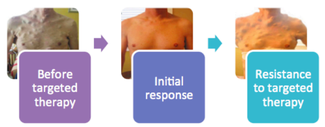
Motivation and Objectives

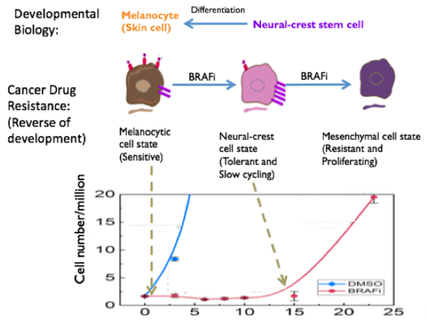
Tumors develop resistance to numerous drug therapies, and this remains a major obstacle in treating many types of non-surgical cancers. Melanoma provides a good model system for studying drug resistance in cancer due to its high propensity to incur resistance after a significant initial response to a drug. Genes that are highly expressed in melanoma cancer cells have been studied, but in order to further understand the collective function of these highly expressed genes we must analyze gene sets, or pathways. A single gene's function is rarely independent of other genes, and pathway analysis takes this into account.

Our objective is to simplify single-cell RNA sequence data to model pathways and pinpoint which unique pathways are up-regulated and down-regulated in drug resistant and nonresistant melanoma cell phenotypes. Identifying these important pathways provides a more accurate depiction of melanoma heterogeneity and informs us of the pathways that are likely to be effective targets for new drug therapies, bringing us closer to overcoming drug-induced resistance.

Background Discussion

More than half of melanoma patients have a driver mutation in the BRAF protein, and an inhibitor that targets the mutated BRAF protein leads to significant initial response in these patients. Despite this initial response, the tumors always come back, indicating that tumor cells have also developed resistance to the BRAF inhibitor.

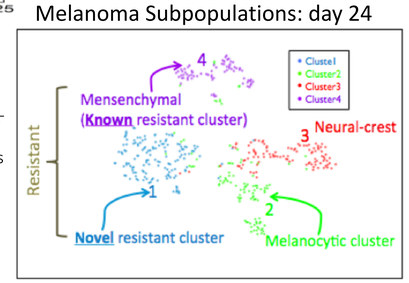




During BRAF inhibitor treatment, melanoma cells will transition from the original melanocytic, drug sensitive cancer cell phenotype towards a stem-like drug-resistant cancer cell phenotype. This process is called Melanocyte to Neural-crest Transition (MNT), and this process causes non-genetic resistance to the BRAF inhibitor drug.

Melanoma cells are not homogeneous, and we found four unique subpopulations in our day 24 melanoma cells (shown on the plot at right). Three of the four clusters are known phenotypes of melanoma cells: drug-sensitive melanocytic cells, drug tolerant neural-crest cells, and drug-resistant mesenchymal cells. Researchers also found a novel resistant cluster of cells, seen in blue on the plot at right. Identifying the important pathways in both resistant clusters, especially the previously unknown novel cluster increases our understanding of cell-state transitions.

Melanoma Subpopulations: day 24



Methods

Research Question:

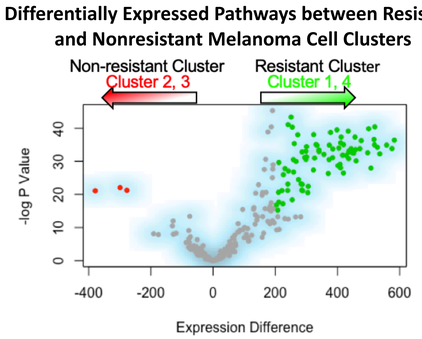
- What do the patterns of pathway expression for known resistant subpopulations of melanoma cells tell us about the cell-state transitions that lead to drug-induced resistance?

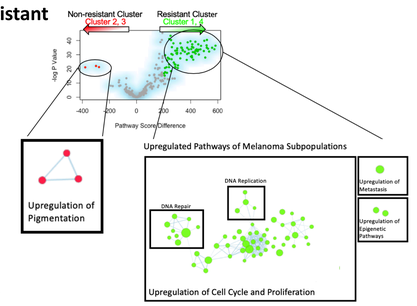
Approach:

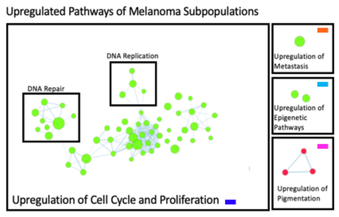
- Identify unique pathways for each subpopulation using Gene Set Expression Analysis (GSEA) and calculate the levels of pathway expression (upregulation or downregulation) for each cell using Gene Set Variation Analysis (GSVA).
- Visually represent pathway relationships using Cytoscape, an open source software platform for visualizing molecular interaction networks and biological pathways.
- Visually represent how pathway expression changes over time after exposure to a BRAF inhibitor drug with trajectory analysis of single-cell RNA sequence data combined with pathway scores obtained from GSVA analysis.

Results: Pathway Analysis of Day 24 Melanoma Cells

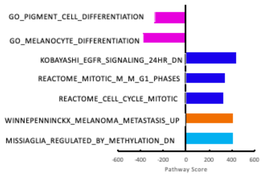
Differentially Expressed Pathways between Resistant and Nonresistant Melanoma Cell Clusters







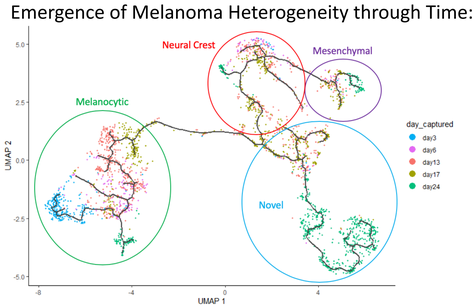
Cytoscape Model: Differentially Expressed Pathways between Resistant and Nonresistant Melanoma Cell Clusters



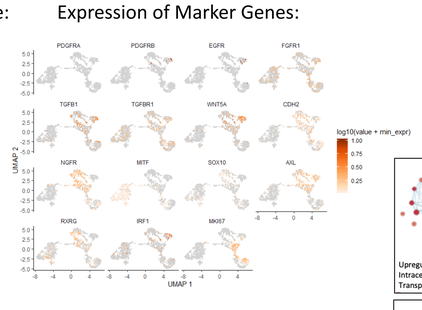
Results: Pathway Analysis of Time Series Data

- Model the emergence of heterogeneity over time in important pathways.
- How does gene expression change over time with exposure to BRAFi in these pathways?

Emergence of Melanoma Heterogeneity through Time:

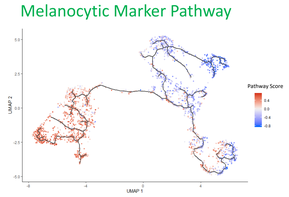


Expression of Marker Genes:

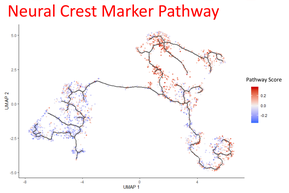


- Transpose pathway score data calculated from GSVA onto single-cell RNA sequence trajectory plot. Known patterns of pathway upregulation and downregulation are shown.

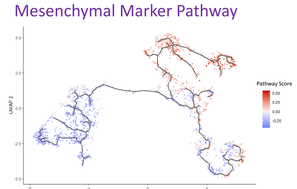
GO_MELANOCYTE_DIFFERENTIATION:
Melanocytic Marker Pathway



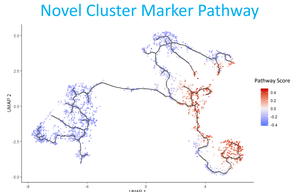
LEE_NEURAL_CREST_STEM_CELL_DN:
Neural Crest Marker Pathway



HALLMARK_EPITHELIAL_MESENCHYMAL_TRANSITION:
Mesenchymal Marker Pathway

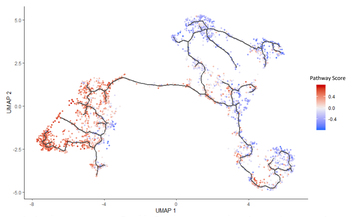


REACTOME_CELL_CYCLE
Novel Cluster Marker Pathway

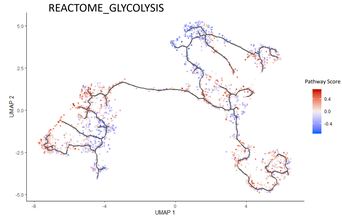


Pathway Scores of Notable Pathways Transposed onto Single-cell RNA-seq Trajectory

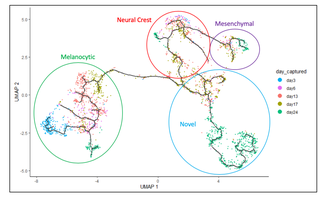
GO_OXIDATIVE_PHOSPHORYLATION



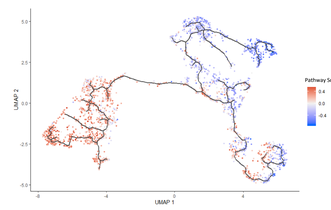
REACTOME_GLYCOLYSIS



Melanoma Cell Subpopulation Locations:



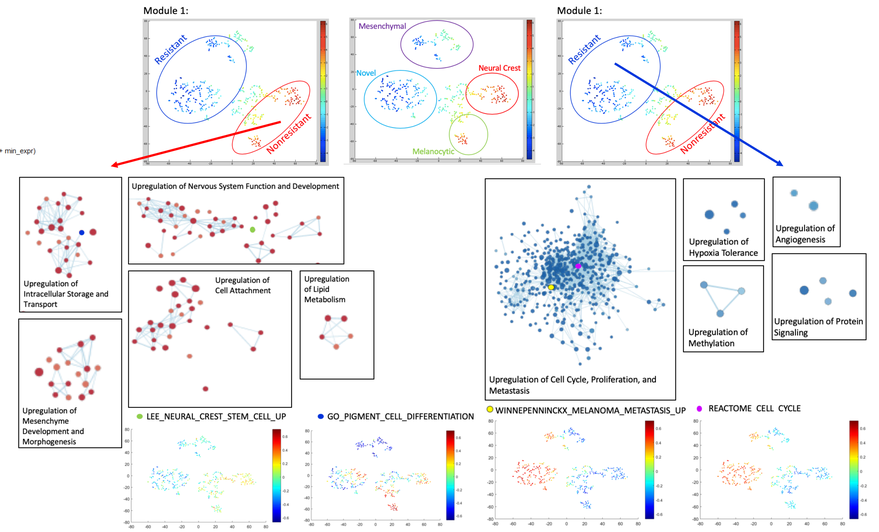
GO_PIGMENT_CELL_DIFFERENTIATION



Future Direction

Surprisal Analysis:

- We are planning to use surprisal analysis, a method derived from physical chemistry, to cluster our cells and find new modules of coregulated genes that differentiate our novel cluster from the other resistant and nonresistant subpopulations.
- We will use Cytoscape to visually represent pathways of interest
- We will first use our day 24 melanoma cells and then extend our analysis to our time series data.



Acknowledgements

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References

Su Y, *et al.* (2017) Single-cell analysis resolves the cell state transition and signaling dynamics associated with melanoma drug-induced resistance. *PNAS*..
Wagle N, *et al.* (2011) Dissecting therapeutic resistance to RAF inhibition in melanoma by tumor genomic profiling. *J Clin Oncol.*