A new ASCL1+ subtype, A2P, resembles a plastic, transit-amplifying compartment of SCLC tumors

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ABSTRACT

A recent consensus review in the SCLC field has identified **four main subtypes** each driven by master transcription factors regulating cell identity. Here, we provide evidence that the **SCLC-A subtype should be further subdivided** into two groups, which we refer to as A and A2P ("A2 provisional"). Hierarchical clustering of transcriptomic profiles provides evidence for the A2P subgroup classification, which may be **driven by alternate transcription factors such as ELF3 and NR0B1.** Differential gene expression identified enrichment in the **immune/inflammatory response** and drug catabolism gene ontology (GO) terms in the A2P subtype, suggesting these cells may more easily adapt to external perturbations such as therapy. Accordingly, A2P cells show exceptional **insensitivity to a broad range of drugs** in the FDA-approved oncology panel, in particular Aurora Kinase and mTOR inhibitors. Using a gene signature derived from cell line transcriptomic data, we found evidence of the A2P subtype in single-cell RNAseq data from human and mouse tumors. The insensitivity of A2P cells to drug treatment suggests that they could exhibit **enhanced plasticity**, both prior to and as a result of treatment. Using RNA velocity analysis on human and mouse tumor sc-RNAseq data, we identified **predicted movement of cell subpopulations** within and between transcriptomically-defined phenotypes. This plasticity, reminiscent of trans-differentiation of normal NE cells to non-NE cell types in the airway epithelium after injury, opens the door for potential perturbations that may induce or prohibit cellular transitions. In conclusion, we propose that A2 cells may play a major role as a **plastic, transit-amplifying** subpopulation that **differentiates into non-neuroendocrine subtypes and repopulates tumors after treatment.**

CLUSTERING SCLC CELL LINES REVEALS 2 ASCL1+ SUBTYPES

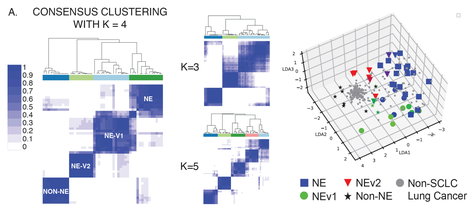


Figure 1 Consensus clustering of 50 CCLE SCLC cell lines reveals 4 transcriptional subtypes of SCLC (left), including 3 neuroendocrine subtypes and one non-neuroendocrine subtype. Both SCLC-A and SCLC-A2p have high expression of ASCL1, but form distinct clusters with differing gene expression profiles.

SCLC SUBTYPES ARE DISTINGUISHED BY GENE EXPRESSION AND DRUG RESPONSE

Analysis of cell lines to 526 drugs and investigational agents shows NEv2 is least sensitive to over half of drugs tested. Specifically NEv2 shows increased resistance to AURK inhibitors.

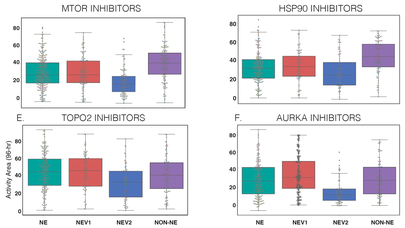


Figure 2 Comparison of drug responses across subtypes for Aurora kinase inhibitors, HSP90 inhibitors, topoisomerase inhibitors and mTOR inhibitors.

SCLC-A AND SCLC-A2p ARE DRIVEN BY ALTERNATE TFS AND CAN FORM DISCRETE ATTRACTORS

Genetic mutations are unable to distinguish between phenotypic subpopulations of SCLC completely; thus, we investigate a transcription factor (TF) network to explain the stability of multiple subtypes. Using TFs from each significant module and the literature, we simulated a network with quasi-Bayesian Boolean rules calibrated to cell line data. This led to 10 attractors that match our four expected phenotypes.

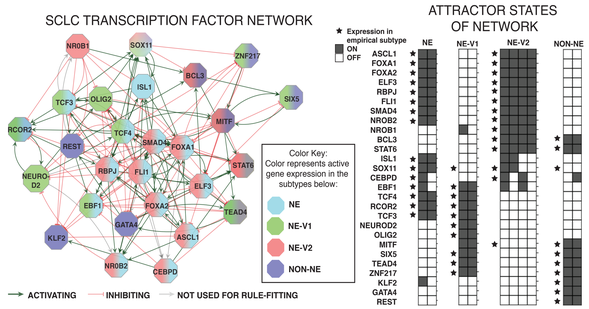


Figure 3 (Left) Gene regulatory network including TFs relevant to SCLC phenotypic heterogeneity. (Right) Simulations of the network are able to recapitulate the expected subtypes. Attractors are defined probabilistically as those states that are more probable than any neighboring state.

IN SILICO NETWORK PERTURBATIONS SUGGEST STRATEGIES FOR REPROGRAMMING SCLC-A2p CELLS

Boolean perturbations (TF held at 0 or 1) allow us to test the outcome of silencing (0) or activating (1) a specific gene. These perturbations suggest experimental strategies for destabilizing a subtype, or "pushing" a cell away from that attractor state. When applied to resistant cell types, such as NEv2 cells, reprogramming may provide a successful strategy for SCLC treatment.

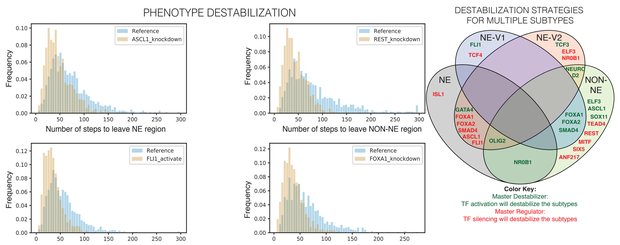


Figure 4 (Left) Examples of destabilization strategies for each subtype. Perturbation allows the simulation to leave a specified state's 4-TF neighborhood faster than a random walk, so a smaller number of steps is required, shifting the distribution of walk lengths to the left. (Right) Destabilization strategies for multiple subtypes. Four-circle Venn diagram shows which perturbations will destabilize (>20% average change in walk length) at least one subtype.

SUBTYPES ARE REPRESENTED ACROSS HUMAN AND MOUSE TUMORS

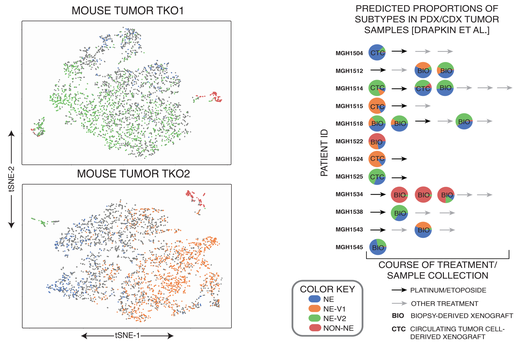
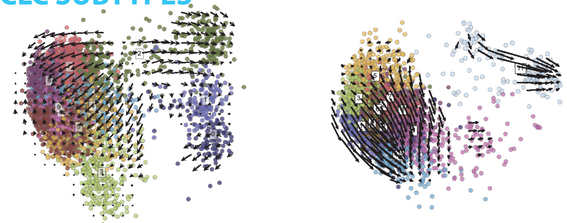


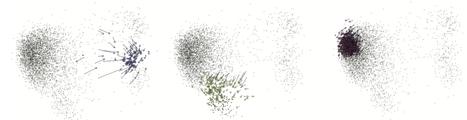
Figure 5 Using a nearest neighbor analysis, scRNA-seq data from two independent mouse tumors shows correlations with human cell line subtypes. CIBERSORT (Newman, 2015) was used to detect proportions of subtypes in human tumors before and after treatment (Drapkin, 2018).

RNA VELOCITY QUANTIFIES PLASTICITY OF SCLC SUBTYPES



Steady states within tumor correspond to cell-line subtypes

Cluster 1: NON-NE Cluster 3: NE Cluster 8: NEv2



Single-cell RNAseq shows heterogeneous subtypes with high plasticity and possible transitions between subtypes

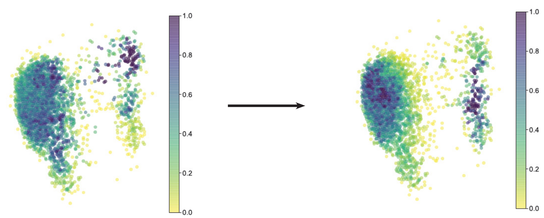


Figure 6 (Top) RNA velocity [La Manno, 2018] gradient field for scRNA-seq of two triple knockout (TP53, RB1, P130) mouse SCLC tumors. (Middle) Clusters show steady states correlate with subtypes derived from bulk human cell line RNA-seq. (Bottom) Distribution of starting point and ending point probabilities for a random walk through the landscape (random diffusion + velocity drift).

A2P CELLS SHOW ENHANCED PLASTICITY AND TRANSITION TO NON-NE SUBTYPES

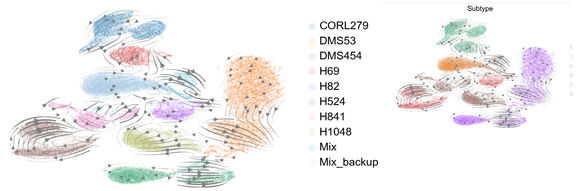


Figure 7 : Stochastic RNA velocity shows plasticity and subgroups of cell lines from each of the four subtypes. Green: NE, Red: NEv1, Purple: NEv2, Brown: Non-NE, Orange: Mixed cells.

A2P CELLS MAY REPRESENT A PLASTIC, TRANSIT-AMPLIFYING SUBPOPULATION OF SCLC TUMORS

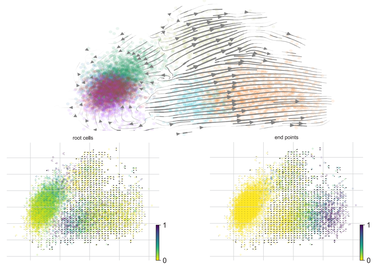


Figure 8 : Transitions within a single cell line suggest plasticity of subtypes that may apply in tumors as well. NEv2 cells move towards a more canonical neuroendocrine gene signature, suggesting NEv2 may be able to give rise to NE cells.

CONCLUSIONS AND FUTURE DIRECTIONS

- Consensus clustering identified four phenotypic clusters defined by WGCNA gene modules, with differential drug sensitivity and gene expression patterns.
- Stable states in a quasi-Boolean GRN recapitulate empirical results, and perturbations of these states suggest strategies for reprogramming. siRNA screens to text these predictions and their effects on drug response are underway.
- RNA velocity analysis suggests relatively plastic phenotypic states, and master TF expression dynamics show movement within the phenotypic landscape.
- Pseudo-temporal single cell data along an RNA velocity trajectory will be used to constrain the GRN model and define movement through the SCLC phenotypic landscape. States with low RNA velocity (sinks) should be represented as attractors of this network.

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