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Discovering Multiple Pathways to Drug Resistance in Melanoma Cells with Single-Cell Proteomics and Single-Cell Metabolomics

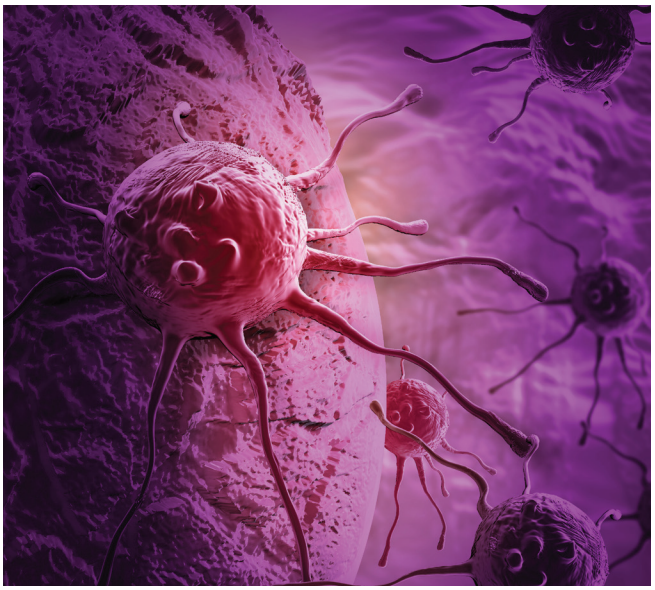
Cancer cells evolve constantly, regularly adopting new states in response to acute and chronic stimuli. This poses a great challenge when it comes to treatment, as cells that are initially responsive to pharmacological agents can quickly adopt drug-resistant states through both genetic and non-genetic mechanisms. In a recent *Nature Communications* article, a team from the California Institute of Technology outlined a multi-omics approach for capturing the diverse range of trajectories that individual cells can take *en route* to drug resistance.

Characterizing cellular transition to drug resistance with functional phenotyping

Cells develop drug resistance through changes in signaling, metabolism, transcription, and gene expression. Previous studies elucidated key mechanistic insights in this area, but those studies lacked cellular-level resolution or did not characterize how cells transited from drug sensitivity to drug resistance. Hypothesizing that multiple potential trajectories existed for cells to adopt drug-resistant states, James Heath's team at the California Institute of Technology investigated changing cellular states in $BRAF^{V600E}$ mutant melanoma cancer cells using predictive single-cell functional proteomic and metabolic assays.

Using unique single-cell functional proteomics and metabolomics to track cellular states

$BRAF^{V600E}$ cells are highly plastic, transitioning rapidly from a naïve state to a drug-tolerant one upon BRAF inhibition. Lead author Yapeng Su and his co-authors examined how this process develops by isolating $BRAF^{V600E}$ cells after 0, 1, 3, and 5 days of treatment with the BRAF inhibitor vemurafenib. They then characterized those isolated cells using integrated single-cell proteomics and metabolomics.



Cellular heterogeneity became evident as expression of several important markers, such as the metabolic regulators HIF1α and p-AMPKα and the proliferation marker Ki67, varied from cell to cell prior to treatment (day 0). At the same time, the researchers noted similarities: most cells presented high glucose uptake and maintained uniform expression of the metabolic enzymes lactate dehydrogenase (LDH) and PKM2. BRAF inhibition quickly resulted in the suppression of Ki67, signaling phosphoproteins, and most metabolic regulators, as well as reduced glucose intake (day 1). However, a small sub-population remained Ki67-high, indicating a slower drug response in those cells.

Cellular state changes became prominent around day 3, as most probed analytes exhibited a sharp but transitory increase in variance. Indeed, all of the metabolic regulators

except p-LKB, all of the resistant state markers and regulators except Slug, all of the metabolic enzymes, and all of the signaling phosphoproteins displayed this phenomenon, suggesting the possibility of one cellular state change having occurred by day 3. By day 5, most cells were senescent. Moreover, the expression of Slug, which is an epithelial-mesenchymal transition-related transcription factor, increased in variance and abundance, indicating that some cells trended toward a mesenchymal phenotype. Finally, the team observed increased levels of single cell proteomic markers associated with drug resistance such as AXL, N-cadherin, NGFR, and TNFR.

Predicting multiple pathways of resistance with functional phenotyping

For Su et al., these results indicated that cells presented initial drug responses on day 1, drug-induced cell-state changes by day 3, and emerging drug tolerance by day 5. Single-cell proteomics and metabolomics analysis revealed two subpopulations, MITF-low and MITF-high. Each appeared to take a different path to becoming drug tolerant. These two distinct pathways explain why researchers observe such varied responses when treating melanoma patients.

Su et al. found that expression of the transcription factor MITF determined cellular trajectory upon BRAF inhibition. MITF-low cells expressed less Ki67 than MITF-high counterparts, and accordingly proliferated more slowly. MITF-low cells and MITF-high counterparts also presented different signaling protein expression profiles, which is important for cancer treatments that target known mediators of oncogenic pathways. MITF-low cells were more susceptible to NFκB inhibition, while MITF-high cells showed more vulnerability to antagonism of PKM2, a glycolysis pathway enzyme.

Having inferred the existence of two independent paths to drug resistance using single-cell proteomics and metabolomics, Su et al. predicted that, relative to monotherapy targeting BRAF or a single pathway, a combination therapy targeting multiple pathways would overcome drug resistance and yield the best response. Indeed, while monotherapy or dual-inhibition approaches slowed tumor growth somewhat, the combination therapy predicted using single-cell proteomics and metabolomics—a BRAF, NFκB, and PKM2 triple-inhibition approach—resulted in a superior response compared to any of the other drug combinations.

Characterizing functional diversity can lead to more effective therapeutic combinations

Using single-cell functional proteomics and metabolomics, Su et al. characterized the cellular heterogeneity within a cell population at a static timepoint and quantitively connected multiple timepoints to characterize dynamic heterogeneity on an individual cell-level. With this information, they showed that cancer cell responses to a common stimulus may entail multiple divergent functional pathways, while still resulting in the same genomic phenotype. Understanding these functional adaptations provides a potentially powerful methodology for predicting effective therapeutic combinations.

Reference

Y. Su et al., "Multi-omic single-cell snapshots reveal multiple independent trajectories to drug tolerance in a melanoma cell line," *Nat Commun*, 11:2345, 2020.