Tumors are composed of multiple types of interacting cells, including malignant tumors, immune, stromal, and endothelial cells. In malignant melanoma, the cellular diversity poses challenges for cancer therapy. To simultaneously identify the major cellular components associated with melanoma and explore the heterogeneities in the malignant and nonmalignant cell types, Tirosh, Izar, and colleagues used single-cell RNA sequencing (RNA-seq) to better understand the complex tumor cellular ecosystem. A total of 4,645 single cells from 19 patients with melanoma were analyzed, including malignant, immune, stromal, and epithelial cells. To distinguish the different cell types, large-scale copy-number variations were identified, and cells were further grouped by their expression profiles. Nonmalignant cells clustered by cell type independent of tumor origin, whereas malignant cells from each tumor clustered separately, indicating a high degree of intertumor heterogeneity. Malignant cell gene expression analysis also revealed high intratumor heterogeneity in cell cycle genes, indicating low- and high-cycling tumors. Treatment-naïve melanoma cells may harbor subsets of cells that are resistant to targeted therapy. Expression of the transcriptional regulator MITF, a melanoma lineage-survival oncogene, is negatively correlated with expression of the AXL kinase, which is associated with intrinsic resistance to RAF/MEK inhibitors. While bulk tumors could be classified as MITF-high or AXL-high, single-cell analysis revealed the existence of both transcriptional states in every tumor, including treatment-naïve melanoma. Further, RNA-seq of paired BRAF-mutant melanoma biopsies before and after RAF/MEK inhibition indicated that treatment resulted in increased expression of AXL, supporting the initial existence of drug-resistance tumor cell sub-populations. Additionally, enrichment of cancer-associated fibroblasts (CAF) was associated with an AXL-high signature, indicating interactions between melanoma and CAF cells may shape the melanoma cell transcriptome. Taken together, these findings reveal melanoma intratumor and intertumor heterogeneity, identify malignant cell–tumor microenvironment interactions, and detect therapy-resistant tumor subpopulations prior to treatment.

Cells grown as a monolayer take up glucose and glutamine via extracellular matrix–mediated signaling. As cancer cells acquire the capacity for anchorage-independent growth, these growth and survival signals must be replaced through mechanisms that remain unclear. To better understand the metabolic rewiring that occurs during anchorage-independent growth, Jiang and colleagues showed that H460 lung cancer cells grown as anchorage-independent spheroids displayed less pyruvate dehydrogenase activity compared to cells grown in a monolayer, as evidenced by reduced conversion of glucose-derived carbon into citrate, lower oxygen consumption, and increased inhibitory phosphorylation of pyruvate dehydrogenase kinase. During hypoxia, reductive carboxylation supports metabolic independence. To better understand the metabolic requirements during anchorage-independent growth, Jiang and colleagues showed that H460 lung cancer cells grown as anchorage-independent spheroids displayed less pyruvate dehydrogenase activity compared to cells grown in a monolayer, as evidenced by reduced conversion of glucose-derived carbon into citrate, lower oxygen consumption, and increased inhibitory phosphorylation of pyruvate dehydrogenase kinase. During hypoxia, reductive carboxylation supports redox homeostasis during anchorage-independent growth. Nature 2016;532:255–8.

CTP inhibited the mitochondrial uptake of labeled citrate. Moreover, detachment of cells from a monolayer promoted the production of reactive oxygen species (ROS), particularly within the mitochondria, and was exacerbated upon deletion of IDH1 or CTP, or inhibition of the pentose phosphate pathway (PPP), a known regulator of ROS during matrix detachment. Keeping mitochondrial ROS levels low enough to sustain cell growth required IDH1-dependent reductive carboxylation in the cytosol, followed by transfer of citrate and/or isocitrate into the mitochondria via CTP, and oxidation of isocitrate by IDH2. This cycle transfers NADPH from the PPP into the mitochondria. Deletion of IDH1 or IDH2 specifically reduced the proliferation of detached cells in a manner that was dependent on mitochondrial ROS. Together, these results suggest that anchorage-independent growth promotes mitochondrial oxidative stress that must be counterbalanced by IDH1-driven changes in reductive carboxylation and citrate metabolism.
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IDH1-Driven Reductive Carboxylation Supports Anchorage Independence

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