Metabolic landscape of the tumor microenvironment at single cell resolution

Zhengtao Xiao, Ziwei Dai & Jason W. Locasale

Department of Pharmacology and Cancer Biology, Duke University School of Medicine, Durham, NC 27710, USA.

Introduction

The tumor milieu consists of numerous cell types each existing in a different environment. Each cell type has unique metabolic demands that enable specific function. In addition, each cancer experiences a distinct nutrient environment, distinct engagement of extracellular signals, and may derive from a different cell of origin thus possibly having distinct mutational patterns. Therefore, at the cellular level, each cell within the tumor is likely to have a different metabolic status. However, nearly all observations of human tumor metabolism in vivo have been conducted using measurements obtained from bulk tumors. Direct observations of cellular metabolism in vivo at single-cell level is not established.

Here, we develop a computational pipeline to study metabolic programs in single cells. In two representative human cancers, melanoma and head and neck, we apply this algorithm to define the intratumor metabolic landscape. We report an overall discordance between analyses of single cells and those of bulk tumors with higher metabolic activity in malignant cells than previously appreciated. Variation in mitochondrial programs is found to be the major contributor to metabolic heterogeneity. Surprisingly, the expression of both glycolytic and mitochondrial programs strongly correlates with hypoxia in all cell types. Immune and stromal cells could also be distinguished by their metabolic features. Taken together this analysis establishes a computational framework for characterizing metabolism using single cell expression data and defines principles of the tumor microenvironment.

Methods

We developed a computational pipeline for analyzing metabolic gene expression profiles at the single-cell level (Fig. 1). We applied missing data imputation and data normalization to gene expression profiles to account for the influence of technical noise. We then characterized the global structure of single-cell metabolic programs using clustering analysis, identified cell type-specific metabolic programs using quantitative metrics we developed, and designed algorithms for quantitation of metabolic heterogeneity of malignant and non-malignant cells. We applied this pipeline to two single-cell RNA-seq datasets for human melanoma and HNSCC, which include an expansive set of gene expression of 4054 cells and 5502 cells respectively.

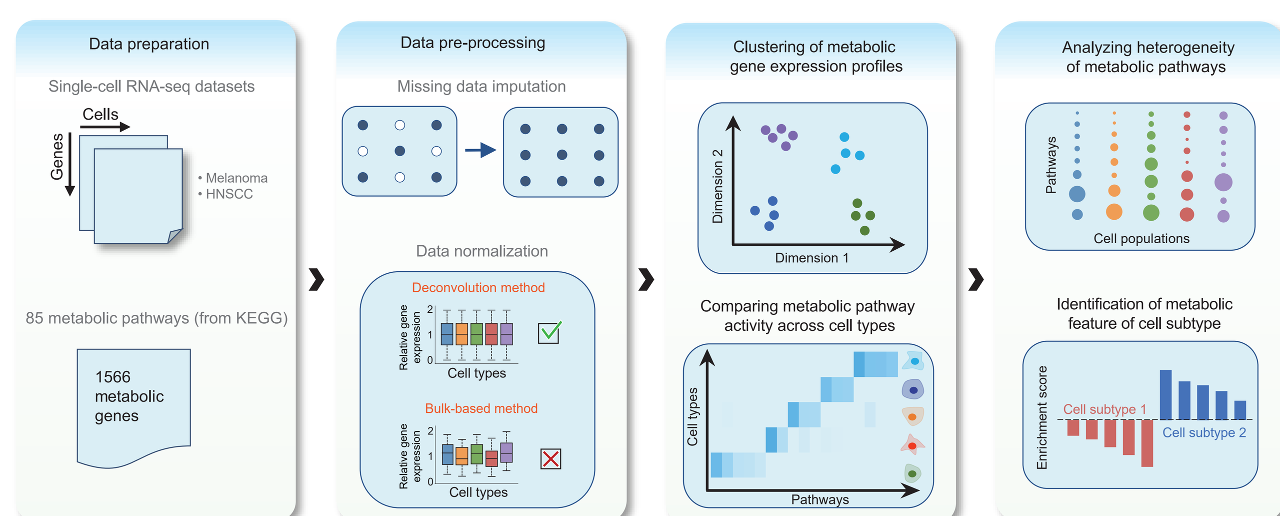

Fig. 1 Schematic representation of the scRNA-seq data analysis pipeline.

Results

• Landscape of metabolic gene expression at single-cell level

Malignant cells exhibit high metabolic plasticity which leads to both patient specific (Fig 2 a-b) and genotype specific (Fig 2 c-d) metabolic reprogramming. In contrast, non-malignant cells in the tumor microenvironment (TME) exhibit no distinguishable metabolism differences between patients (Fig2. e-f).

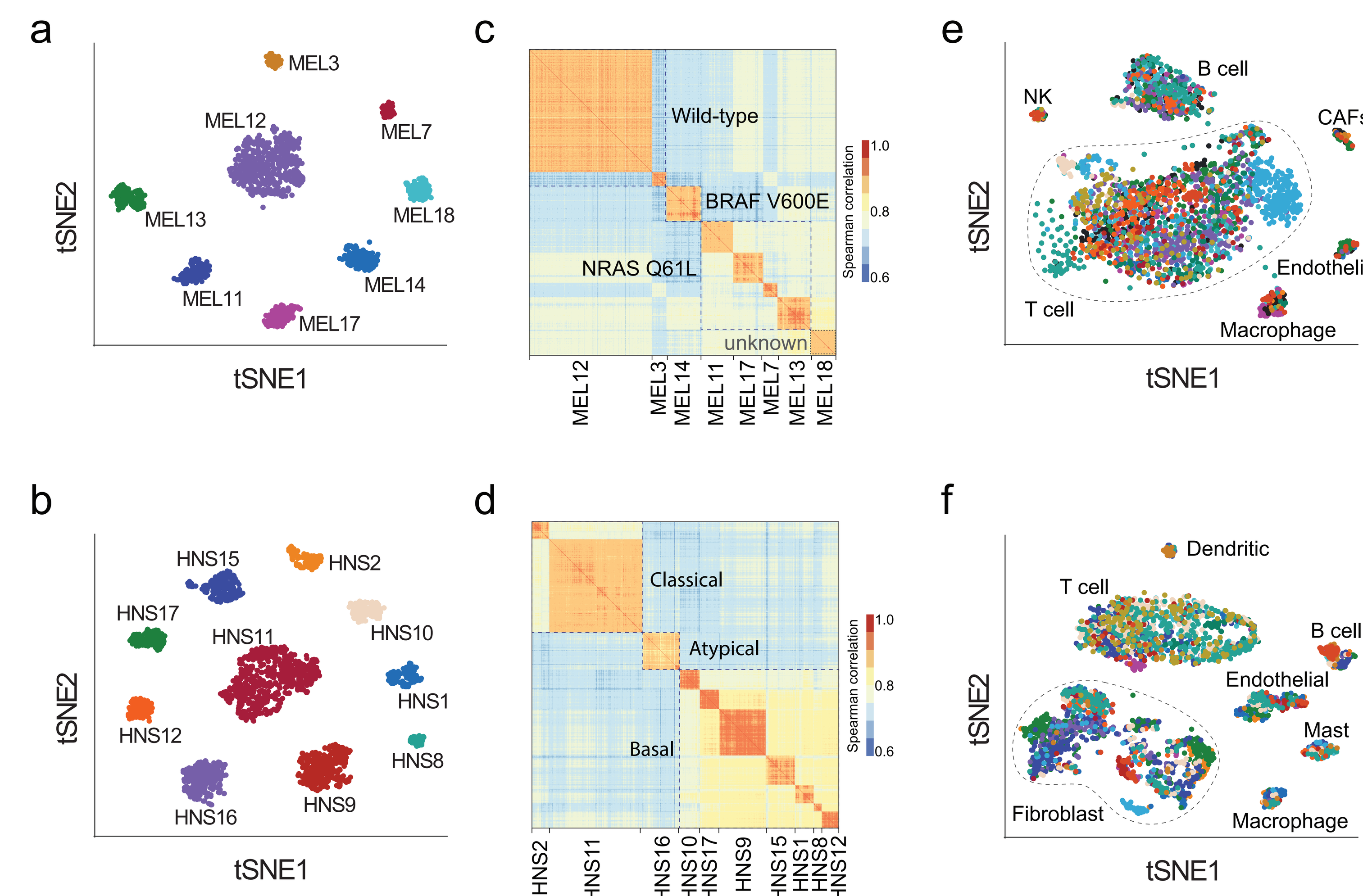

Fig. 2 Landscape of metabolic gene expression at single-cell level. (a) t-SNE plot of metabolic gene expression profiles of malignant cells from the melanoma dataset. The color of each dot indicates the tumor which the cell comes from. (b) Same as in (a) but for the head and neck squamous cell carcinoma (HNSCC) dataset. (c) Clustered correlation matrix showing Spearman's rank correlation coefficients of metabolic gene expression profiles between malignant cells in the melanoma dataset. (d) Same as in (c) but for the HNSCC dataset. (e) t-SNE plot of metabolic gene expression profiles of non-malignant cells from the melanoma dataset. The color of each dot indicates the tumor which the cell comes from. (f) Same as in h but for the HNSCC dataset.

• Cell type-specific metabolic reprogramming

Compared to bulk samples, single cells provide high resolution in characterizing metabolic heterogeneity. While bulk RNA-seq measures the average expression levels over a mixture of different cell types thus masking the difference between cell types in the same sample. Our results show that pathway activities are more variable between different types of single cells than between bulk tumors and normal tissues. The malignant cells undergo a global up-regulation of metabolic pathway activity which can only be detected at the single-cell level.

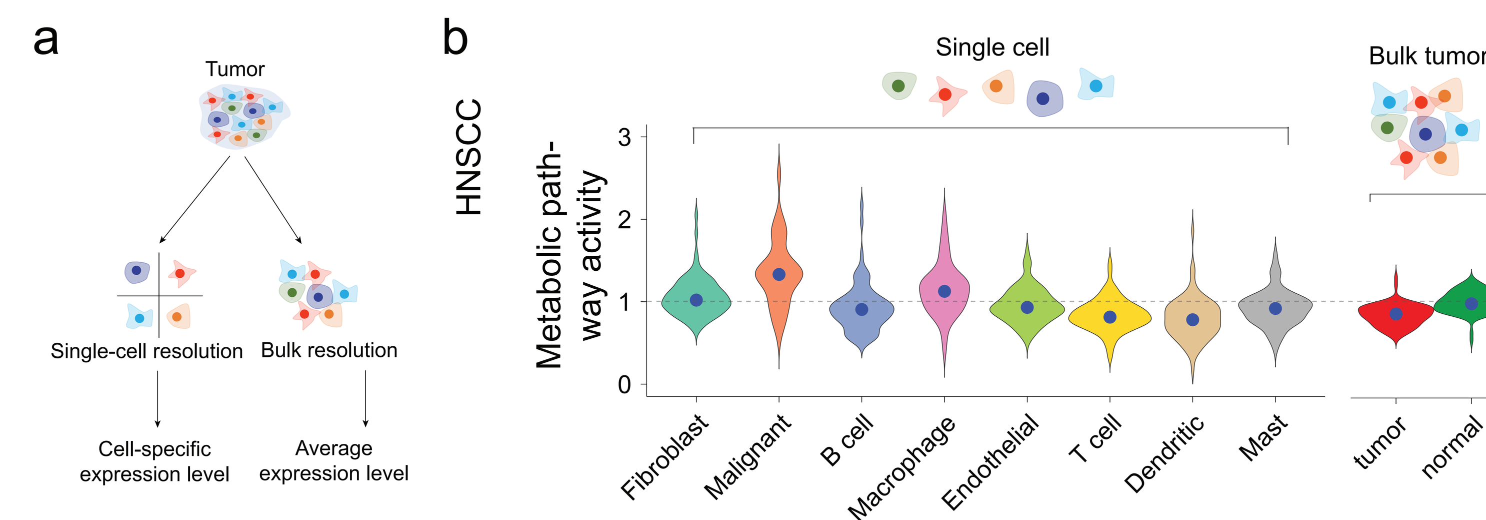

Fig. 3 Cell type-specific metabolic reprogramming. (a) Difference between bulk and single-cell RNA-seq in characterizing gene expression profiles in tumors. (b) Distributions of pathway activities in different cell types from the HNSCC scRNA-seq dataset (left) and in bulk tumors and normal samples from TCGA (right).

Results

• Intratumoral metabolic heterogeneity

We developed a workflow (Fig. 4a) to quantitate the intratumor metabolic heterogeneity. We found that the mitochondrial OXPHOS is the most important contributor to intratumoral metabolic heterogeneity for both malignant (Fig. 4b) and non-malignant cells (Fig. 4c).

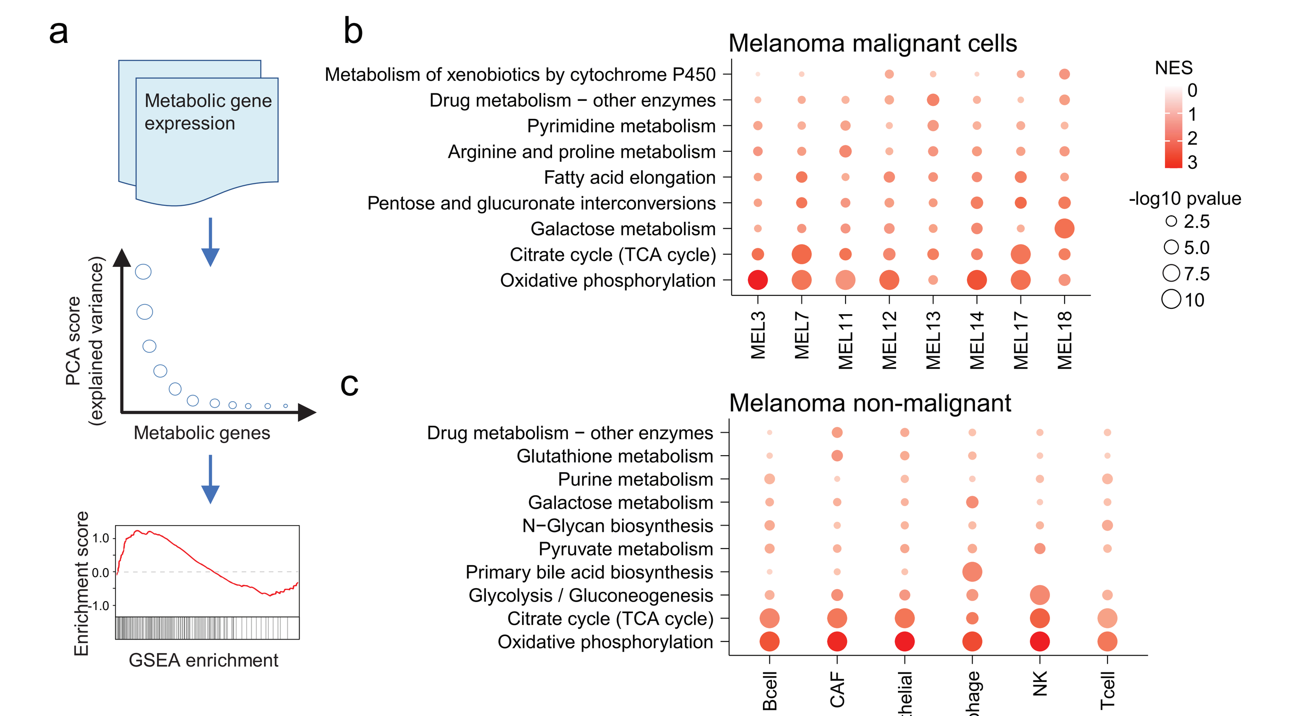

Fig. 4 Intratumoral metabolic heterogeneity. (a) Workflow for quantitating metabolic heterogeneity. (b) Metabolic pathways enriched in genes with highest contribution to the metabolic heterogeneities among malignant cells from different tumors in the melanoma dataset. (c) same as in (b) but for different types of non-malignant cells.

• Metabolic features of non-malignant cell subtypes

The subpopulations of non-malignant cells reprogram their metabolism to establish their roles in interacting with other cell types and modulating the tumor microenvironment.

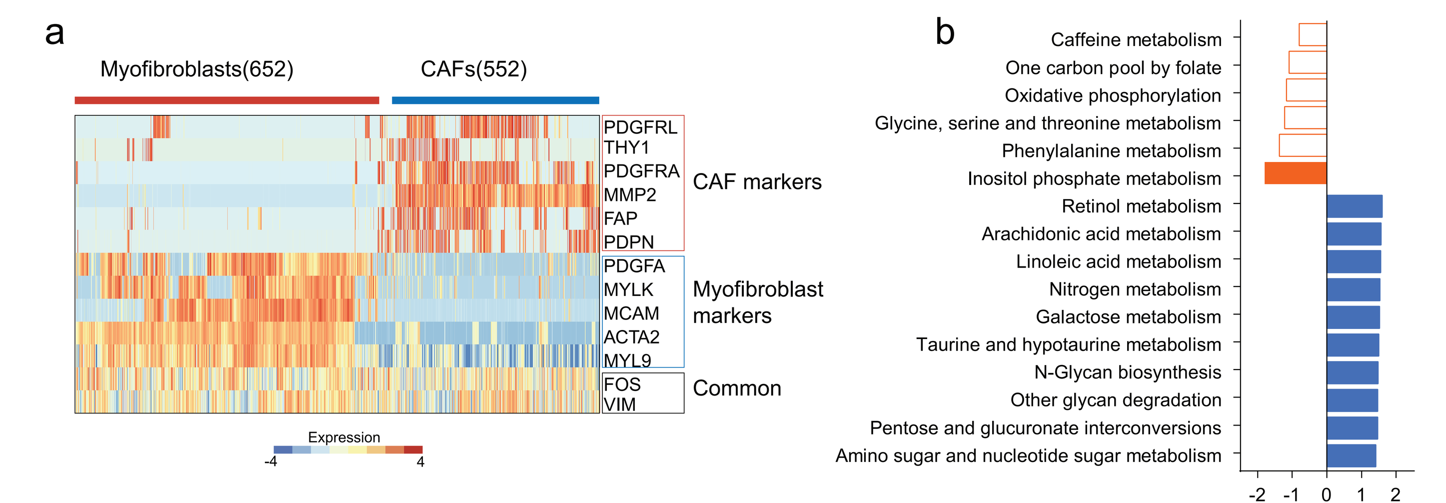

Fig. 4 Metabolic features of non-malignant cell subtypes. (a) Gene markers and their expression levels used for classifying fibroblast cells in the HNSCC dataset into CAFs and myofibroblasts. (b) Top 10 metabolic pathways enriched in CAFs or myofibroblasts in the HNSCC dataset. Significantly enriched pathways with GSEA p-value < 0.05 are highlighted in red (higher in myofibroblasts) or blue (higher in CAFs)

Conclusion

In this study, we analyze metabolic gene expression profiles of more than 9000 single cells from two representative human tumor types including melanoma and squamous cell carcinoma of the head and neck (HNSCC). We find that activities of metabolic pathways in malignant cells are in general more active and plastic than those in non-malignant cells, and the metabolic features of single cancer cells are poorly captured by measurements done with bulk tumors. Variation in mitochondrial activity is the major contributor to the metabolic heterogeneities among both malignant cells and non-malignant cells, and, strikingly, the activities of glycolysis and oxidative phosphorylation both correlate with hypoxia at the single-cell level. We also identify metabolic features of different immune and stromal cell subtypes and find patterns distinct from behaviors of these cells in ex vivo culture conditions. These findings begin to unravel principles of how malignant transformation affects the metabolic phenotypes of tumor and non-tumor cells within the tumor microenvironment.