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COMMENTARY

Metabolism in the tumor microenvironment: insights from single-cell analysis

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ABSTRACT
The metabolism of both cancer and immune cells in the tumor microenvironment (TME) is poorly understood since most studies have focused on analysis in bulk samples and *ex vivo* cell culture models. Our recent analyses of single-cell RNA sequencing data suggest that the metabolic features of single cells within TME differ greatly from those of the bulk measurements. Here, we discuss some key findings about metabolism in cancer and immune cells and discuss possible relevance to immunotherapy.

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Cancer metabolism; immunometabolism; tumor microenvironment; single-cell RNA sequencing

Tumors are complex ecosystems where malignant cells coexist with diverse groups of nonmalignant cells (*e.g.* immune, stromal, endothelial cells) that together constitute the TME. These cells alter their metabolic gene expression to control the rates of metabolic reactions and vary the concentrations of metabolites to support their function in the context of their environment. However, traditional approaches profile the metabolic gene expression in tumors from bulk cell populations which preclude our ability to detect the metabolic program in individual cells and the heterogeneity among cells. Such information can be obtained by the emerging single-cell RNA sequencing (scRNA-seq) technologies designed for characterizing the transcriptome of single cells, but this rich resource of data has yet to generate new insights that help advance our understanding of cancer cell metabolism. To fill this gap, we recently developed a computational framework for analyzing metabolic gene expression profiles based on scRNA-seq data, and applied this workflow to scRNA-seq datasets from two human cancers, melanoma, and head and neck.[1] Our study defines the metabolic landscape in TME from three aspects: (1) the general metabolic programs for both malignant and nonmalignant cells; (2) the major contributor of metabolic heterogeneity for different cell populations; (3) the metabolic features of different immune cell subtypes. In this commentary, we will briefly introduce our key findings that challenge the current understanding of cancer metabolism and discuss their relevance to cancer therapy.

The general metabolic program in tumors has been widely investigated by bulk level transcriptome analyses. For example, by comparing the expression patterns of metabolic genes between tumor tissues and adjacent normal tissues, Hu *et al.*[2] and Gaude *et al.*[3] suggest that although some metabolic pathways (*e.g.* nucleotide biosynthesis and glycolysis) are significantly altered in most tumors, many aspects of metabolism are similar to that in the corresponding normal tissues. However, these studies implicitly assume that the cell populations are homogeneous, thereby masking the variability across individual cells. Besides, the use of *ex vivo* models (*e.g.* cultured cell lines)[4] allows better accuracy of measurements but does not reflect the genuine environment in the TME. A systematic and thorough study of cell metabolism in the TME requires methods that can both characterize cell composition in the tumor and profile metabolic gene expression in each cell. The emerging scRNA-seq technology has been applied to capture the diversity of cell types in the TME of various cancer types. Further comparison of expression levels of metabolic genes across different cell types can help identify overall metabolic features of malignant and nonmalignant cells. We demonstrated that most metabolic pathways are upregulated in malignant cells and display higher variation compared to nonmalignant cells, which differ markedly from conclusions drawn based on bulk measurements. The global up-regulation of metabolic pathway activities in malignant cells may help guarantee their high demands of nutrients and energy for proliferation purposes. Single malignant cells also exhibit high metabolic plasticity that allows them to adapt to the patient-specific and genotype-specific environment. In addition to malignant cells, nonmalignant cells in the TME shape their metabolism and appear to influence malignant cell metabolism.[5] Different functional populations of nonmalignant cells were found to each use a specific metabolic program without showing distinguishable difference between different patients. Therefore, it appears that nonmalignant cells establish a consistent metabolic program to exert their function in the TME. Further studies are needed to clarify the relationship between metabolism and cell type-specific function of nonmalignant cells in the TME.

Besides genetic and cell type-specific factors that have a profound impact on cellular metabolism, other factors such as nutrient and oxygen availability and cell–cell interactions also influence metabolic programs adopted by each single cell, contributing to intra-tumoral metabolic heterogeneity. Metabolic heterogeneity is also known to influence responses to anti-tumor therapeutics and predict clinical outcomes. Understanding how

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cancer cell metabolism is influenced by these factors requires that we identify metabolic pathways most responsive to variation in these factors. At the level of individual cells, the heterogeneity of each metabolic pathway can be quantified with higher precision. We showed that the group of genes defined in KEGG (Kyoto Encyclopedia of Genes and Genomes) as oxidative phosphorylation (OXPHOS) exhibits the most heterogeneous behavior of all metabolic pathways for both malignant and nonmalignant cells. Notably, OXPHOS was previously reported to be heterogeneous across bulk tumor samples of both the same tumor type and different tumor types.[2] Thus, these findings indicate that mitochondrial programs are influenced not only by the environmental or genetic factors across different tumors but also by the physiological conditions in the same tumor. One possible reason is that mitochondrial activity is sensitive to changes in oxygen availability and the nutritional supply. The low oxygen concentration in some regions may induce expression of transcription factor HIF-1α which promotes the expression of glycolytic genes and a number of other genes. Therefore, it was interesting to investigate the relations among OXPHOS, hypoxia, and glycolysis. Surprisingly, our results suggested that the activity of OXPHOS is correlated with both glycolysis pathway expression and the expression of genes that respond to hypoxia, which is not observed in analyses from cultured cancer cell lines. This finding contrasts with the dominant view that hypoxia-inducible HIF1-α stabilization shifts OXPHOS to glycolysis. Nevertheless, OXPHOS is reported to serve as a sensor of oxygen availability,[6] perhaps is actively regulated by the hypoxia response genes. OXPHOS and glycolysis expression may also increase simultaneously through increased substrate delivery and enzyme expression in tumor cells.[7] It is worth noting that contrary to the conventional wisdom that OXPHOS is downregulated in all cancers, our analyses suggest that the activities of mitochondrial energy pathways (OXPHOS and TCA cycle) are higher in malignant cells than nonmalignant cells. This perspective is also supported by increasing evidence that some cancers depend on mitochondrial metabolism.[6] The reason for these seemingly perplexing findings on mitochondrial metabolism could be our lack of knowledge on various aspects of mitochondria biology. Overall, these analyses suggest mitochondria is a major driver of the tumor metabolic heterogeneity and plays an important role in the TME.

Nonmalignant cells acquire adequate nutrients to engage the metabolism that supports their function in the TME and determines their fate. For example, tumor-infiltrating T cells are one of the intensively investigated nonmalignant cell types due to their anti-tumor functions. The differentiation of T cells is profoundly influenced by the activity of metabolic pathways.[8] However, challenges still largely remain in characterizing the *in vivo* metabolic dependencies of different T cell states and the role of metabolic pathways in each T cell state. To address these challenges, it is necessary to identify the differentiation states of T cells in the TME and study their metabolic programs. scRNA-seq analyses combined with prior knowledge of marker genes enable us to identify different subclasses of nonmalignant cells and parse the metabolic features in each subclass. We demonstrated that the metabolic program for T cells is not static but regulated according to differentiation status. $CD4^+$ T cells, compared to $CD8^+$ T cells, are found to have higher OXPHOS and glycolysis activities. Regulatory T cells (Tregs) compared to helper T cells (Ths) exhibit higher glycolysis and OXPHOS activities, which contradicts previous studies from mice showing that Ths tend to be more glycolytic compared to Tregs. These findings suggest that T cells reprogram their metabolism to form different metabolic features in different states but that their metabolic status may not be consistent with what is found in *ex vivo* models. Further studies are needed to identify the metabolic program utilized by each subset of T cells and its relevance to their function.

Finally, the findings from this work have some potential implications that could be considered to guide the current tumor therapeutics which target tumor metabolism. Building a conceptual framework to better understand metabolic reprogramming in different cell types could help to design effective therapies that precisely target malignant cells but not nonmalignant cells. The computational framework we developed could also be applied in studying other tumor types and physiological processes to understand the metabolic alterations in different tumors and under different conditions. Metabolic heterogeneity allows cells to acquire resistance to antitumor drugs by different mechanisms which is a major difficulty faced by almost all cancer treatments. It is thus important to understand precisely how metabolic pathways are regulated in different cells *in vivo*. The findings that OXPHOS is the most heterogenous metabolic pathway at the levels of different cells, cell types, tumors, and patients suggest that the responses to therapies targeting OXPHOS might also be highly heterogeneous among patients and cancer types. Further clinical trials should consider determining which cancer types or patients may benefit from treatments targeting OXPHOS. Cancer immunotherapy is an increasingly successful strategy for treating cancers. Our study could be helpful for understanding the metabolic features of differentiation states of immune cells, particularly T cells, but further studies are needed to devise appropriate strategies to modulate the metabolism of highly functional T cells for promoting their antitumor activity *in vivo*.

In conclusion, our study using scRNA-seq data uncovered distinct metabolic features for both cancer and immune cells compared to the bulk measurements. These findings encourage further attention to cancer metabolism at single-cell resolution. There are some potential caveats in our work which can be overcome by the development of new techniques. One limitation is that metabolic features are inferred from gene expression but not directly metabolic flux measurements. The development of single-cell metabolomics technologies will lead to the ultimate goal of understanding the metabolism at the single-cell level. Another obvious challenge of our study is the lack of spatial information of cells in the TME, which could be very useful for studying metabolism in different regions. It's worth noting that the advent of approaches for profiling the gene expression using single-cell sequencing in 3D allows us to measure the regional metabolic heterogeneity in human cancer.[9] Efforts to precisely characterize the metabolism in individual cells would make it possible to tackle the metabolic complexity of cancer.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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