Review

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Famine versus feast: understanding the metabolism of tumors *in vivo*

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To fuel unregulated proliferation, cancer cells alter metabolism to support macromolecule biosynthesis. Cell culture studies have revealed how different oncogenic mutations and nutrients impact metabolism. Glucose and glutamine are the primary fuels used *in vitro*; however, recent studies have suggested that utilization of other amino acids as well as lipids and protein can also be important to cancer cells. Early investigations of tumor metabolism are translating these findings to the biology of whole tumors and suggest that additional complexity exists beyond nutrient availability alone *in vivo*. Whole-body metabolism and tumor heterogeneity also influence the metabolism of tumor cells, and successful targeting of metabolism for cancer therapy will require an understanding of tumor metabolism *in vivo*.

Studies to understand cancer metabolism

Investigation into the mechanisms governing metabolic adaptations in cancer cells has undergone a dramatic expansion in recent years. Studies using *in vitro* culture systems have led to important insights regarding nutrient utilization and the regulation of metabolic pathways by describing how cancer cells exploit existing metabolic programs to fuel proliferation and survival. Examining tumor metabolism *in vivo* introduces new complexities, but taking this step is crucial to gain a deeper understanding of how whole-animal physiology impacts nutrient availability, as well as to appreciate the role of tumor heterogeneity and interactions between different cell types in tissues. Gaining this insight will be crucial for developing new therapies that exploit metabolic pathways and improve patient therapies. In this review we first discuss the current understanding of cancer cell metabolism gained primarily from cell culture studies, and then focus on emerging insights arising from experiments using patients and mouse models with the intent of highlighting the strengths and limitations of each experimental context and calling attention to key unanswered questions.

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Defining proliferative metabolism using cell culture

Cancer is defined by unconstrained proliferation of transformed cells. Establishing cell lines in culture selects for the fastest-growing malignant clones from the tumor with concomitant loss of non-dividing and slowly proliferating cancer cells, as well as any other cell types that were part of the original tumor tissue (Figure 1A). To illustrate, one clone with a slight 5% proliferation advantage will almost completely eliminate a second clone in fewer than 65 generations (Figure 1B). Thus, by its nature, cell culture selects for a relatively homogeneous population of cancer cells, generating clean systems with which to investigate the contributions of specific oncogenic mutations to metabolic programs and the underlying metabolic requirements of cell proliferation.

The common oncogenic drivers *Ras* and *Myc* both promote cell-autonomous metabolic changes associated with malignant transformation, namely the diversion of metabolic substrates into anabolic (see Glossary) pathways. Oncogenic Ras increases glucose and glutamine consumption [1,2], while Myc enhances glutamine metabolism through a transcriptional program that increases the expression of genes involved in glutamine uptake and catabolism [3,4]. Myc also ties increased glutaminolysis to changes in glucose metabolism [5], and can directly control expression of genes involved in aerobic glycolysis

Glossary

Anabolic: biochemical reactions requiring the input of energy for the synthesis of new macromolecules in cells.
ATP: adenosine triphosphate. The primary energy currency of the cell.
Catabolic: biochemical reactions that provide energy for use by cells via the oxidation of various nutrients.
Flux: rate of metabolite flow per unit time through a metabolic pathway.
Glutaminolysis: oxidative metabolism of glutamine by the tricarboxylic acid (TCA) cycle.
Macromolecules: large polymers that compose much of the structure of a cell including DNA, proteins, and lipids
Macronutrient: general term describing nutrients required in large amounts such as carbohydrates, proteins, and fats.
NADPH: reduced NADP. A cofactor for intracellular redox reactions that is used primarily as a source of electrons for reduction reactions. It is important for many anabolic reactions and for coping with reactive oxygen species.
Quiescent: non-proliferating.
Tracer: a version of a metabolite in which one or more of the atoms has been replaced with a different isotope version of that atom such that the difference can be tracked using mass spectrometry (MS) or nuclear magnetic resonance (NMR) spectroscopy. If the isotope used is radioactive, radioactivity detection methods can also be used.

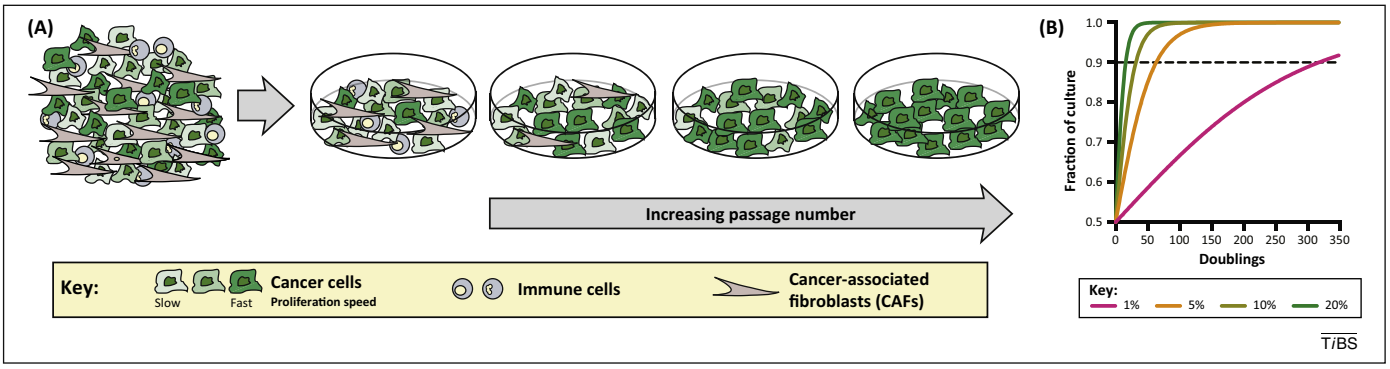

Figure 1. Establishing tumor-derived cell lines in culture selects for the fastest-proliferating clones in the population, and non-dividing and less-proliferative cells are lost upon serial passaging. This inevitable consequence of cell culture is illustrated graphically in **(A)**, using the example of cell line generation from a tumor. **(B)** A model demonstrating how many cell doublings are required for a clone to take over the culture population if that clone has the proliferation advantage indicated (key; proliferation advantage indicated as % faster than control doubling-time). The model assumes competition between two distinct clones plated at equal density with one clone having a fixed advantage that is invariant over time. We further define one clone representing greater than 90% of the cultured population as having taken over the culture. This threshold is reached after 317 doublings with a 1% proliferation advantage, 64 with 5%, 32 with 10%, and only 16 with 20%. Additional details of the model are included in the supplementary material online.

[6]. Mutations in other key cancer genes also influence metabolism. Loss of p53 promotes glucose uptake and metabolism [7,8], and can impact how glucose is used by cells [9]. In all cases, genetic alterations associated with cancer are accompanied by metabolic alterations that favor anabolism, enabling the acquisition and utilization of nutrients to satisfy increased ATP demands and produce the nucleotides, lipids, and proteins needed for rapid cell division [10].

The metabolic differences between proliferating and non-proliferating cells have received less scrutiny. Studies utilizing mammalian primary fibroblasts and lymphocytes where culture conditions are manipulated to promote quiescent or proliferative states have demonstrated that, in contrast to proliferating cells, quiescent cells favor catabolic metabolism [5,11] (Figure 2). Maintaining homeostasis requires nutrient breakdown to generate ATP, as well as NADPH production to cope with redox stress [11,12]. Quiescent cells also strive to balance fatty acid and protein degradation with synthesis [11], a finding consistent with the absence of an increase in cell mass in these non-proliferating cells. Nevertheless, these cultured cells still rely on glucose and glutamine, whereas many differentiated mammalian tissues use other nutrients [13]. For example, the heart can consume fatty acids, glucose, ketones, or amino acids to support the large amount of ATP required for electrical activity and continuous mechanical contraction [14], while the brain relies almost exclusively on glucose metabolism, only switching to ketones when glucose is not available [15]. Thus, caution is needed when generalizing studies of specialized quiescent cell systems in culture to diverse cell types in intact tissues in an organism.

Nevertheless, the different metabolic phenotypes of proliferating and non-proliferating cells in culture illustrate that these states have different metabolic requirements. At a first approximation, proliferating cells favor biomass production while non-proliferating cells favor biomass maintenance. Relevant to understanding tumor metabolism, not all cancer cells actively proliferate in many solid tumors [16], and the mechanics of serially passaging cancer cell lines selects against quiescent or more slowly proliferating cancer cells (Figure 1B), limiting the study of these tumor cell populations to date. It is important to recognize that studies of cancer cell metabolism in culture fail to capture the metabolic phenotype of less-proliferative tumor cells.

Glucose and glutamine: primary substrates of proliferative metabolism *in vitro*

Glucose

In addition to taking up more glucose, proliferating cancer cells in culture metabolize glucose differently from non-proliferating cells, converting most of the pyruvate derived from glucose to lactate rather than oxidizing it via the tricarboxylic acid (TCA) cycle. For most normal cells, increased conversion of glucose to lactate is favored in oxygen-limited conditions, whereas cancer cells exhibit this phenotype even when oxygen is abundant, an observation first described by Otto Warburg [17]. Termed aerobic glycolysis or the Warburg effect, this metabolic phenotype is a well-described feature of cancer cells that has been extensively studied [10].

Increased glucose flux through glycolysis is thought to promote shunting of metabolites into branch pathways for the synthesis of macromolecules [10]. For instance, metabolism of glucose-6-phosphate via the oxidative arm of the pentose phosphate pathway (PPP) produces NADPH and ribose-5-phosphate, two critical components for new cell generation. NADPH is crucial for managing redox stress and for reductive biosynthetic reactions, while ribose-5-phosphate is a required precursor for *de novo* nucleotide synthesis [18]. In addition, some cancer cells depend on flux of downstream glycolytic intermediates into the non-oxidative arm of the PPP for ribose-5-phosphate production (Figure 2) [19,20]. Glucose metabolism also contributes to the generation of nucleotide bases, and slowing this production can limit proliferation in some situations [21].

Diverting fructose-6-phosphate, another product of glycolysis, into the hexosamine biosynthetic pathway (HBP) provides the necessary substrates for glycosylation of proteins and lipids, an abundant modification that has been implicated in several aspects of tumor progression