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A New Perspective on the Heterogeneity of Cancer Glycolysis

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Abstract

Tumors are dynamic metabolic systems which highly augmented metabolic fluxes and nutrient needs to support cellular proliferation and physiological function. For many years, a central hallmark of tumor metabolism has emphasized a uniformly elevated aerobic glycolysis as a critical feature of tumorigenecity. This led to extensive efforts of targeting glycolysis in human cancers. However, clinical attempts to target glycolysis and glucose metabolism have proven to be challenging. Recent advancements revealing a high degree of metabolic heterogeneity and plasticity embedded among various human cancers may paint a new picture of metabolic targeting for cancer therapies with a renewed interest in glucose metabolism. In this review, we will discuss diverse oncogenic and molecular alterations that drive distinct and heterogeneous glucose metabolism in cancers. We will also discuss a new perspective on how aberrantly altered glycolysis in response to oncogenic signaling is further influenced and remodeled by dynamic metabolic interaction with surrounding tumor-associated stromal cells.

Key Words: Glycolysis, Heterogeneity, Tumor microenvironment, Stroma, Metabolism, Cancer

INTRODUCTION

Advances in our understanding of cancer biology have revolutionized the conception of anticancer drug development and patient treatment, with the divulgence of the genetic and molecular alterations driving the growth of specific cancers leading to the development of clinically effective molecularly targeted therapies (Neal and Sledge, 2014). At the heart of these achievements is the realization of the extensive genetic and molecular diversity of tumors found beyond the conventional classifications of tissue of origin and histology. Despite this diversity, many cancers display a similar set of characteristics necessary for their growth and proliferation, often described as the "hallmarks of cancer" (Hanahan and Weinberg, 2011). Among such well-recognized hallmarks as unlimited replicative capacity and the ability to invade and metastasize to other tissues, tumors cells have been known to have a unique metabolic preference for converting glucose into lactate under aerobic conditions resulting in a far less efficient method of ATP production, a process known as aerobic glycolysis or the Warburg effect (Vander Heiden *et al*., 2009; Koppenol *et al*., 2011; DeNicola and Cantley, 2015; DeBerardinis and Chandel, 2016). This phenomenon was uncovered by the pioneering work of Otto Warburg in the early twentieth century (Warburg, 1925, 1956). This cancer-cell specific metabolic preference has been validated in many tumor types and is the clinical foundation for the detection and diagnosis of cancer via ^{18}F-Fluorodeoxyglucose (^{18}F-FDG) positron emission tomography (PET) (Kayani and Groves, 2006; Farwell *et al*., 2014). As research on cancer metabolism has greatly expanded, the importance of other nutrients such as glutamine and acetate, and of processes of nutrient acquisition such as macropinocytosis, has revealed extensive metabolic heterogeneity in tumors comparable to their genetic and molecular diversity (Reitzer *et al*., 1979; DeBerardinis *et al*., 2007; Commisso *et al*., 2013; Comerford *et al*., 2014; Mashimo *et al*., 2014). The revelation of these atypical metabolic processes has greatly enhanced our understanding of how cancer cells acquire the necessary nutrients to maintain their growth and proliferation and of the genetic aberrations that facilitate these pathological processes. Yet a thorough understanding of glycolytic metabolism in cancer has eluded us, stagnated by the

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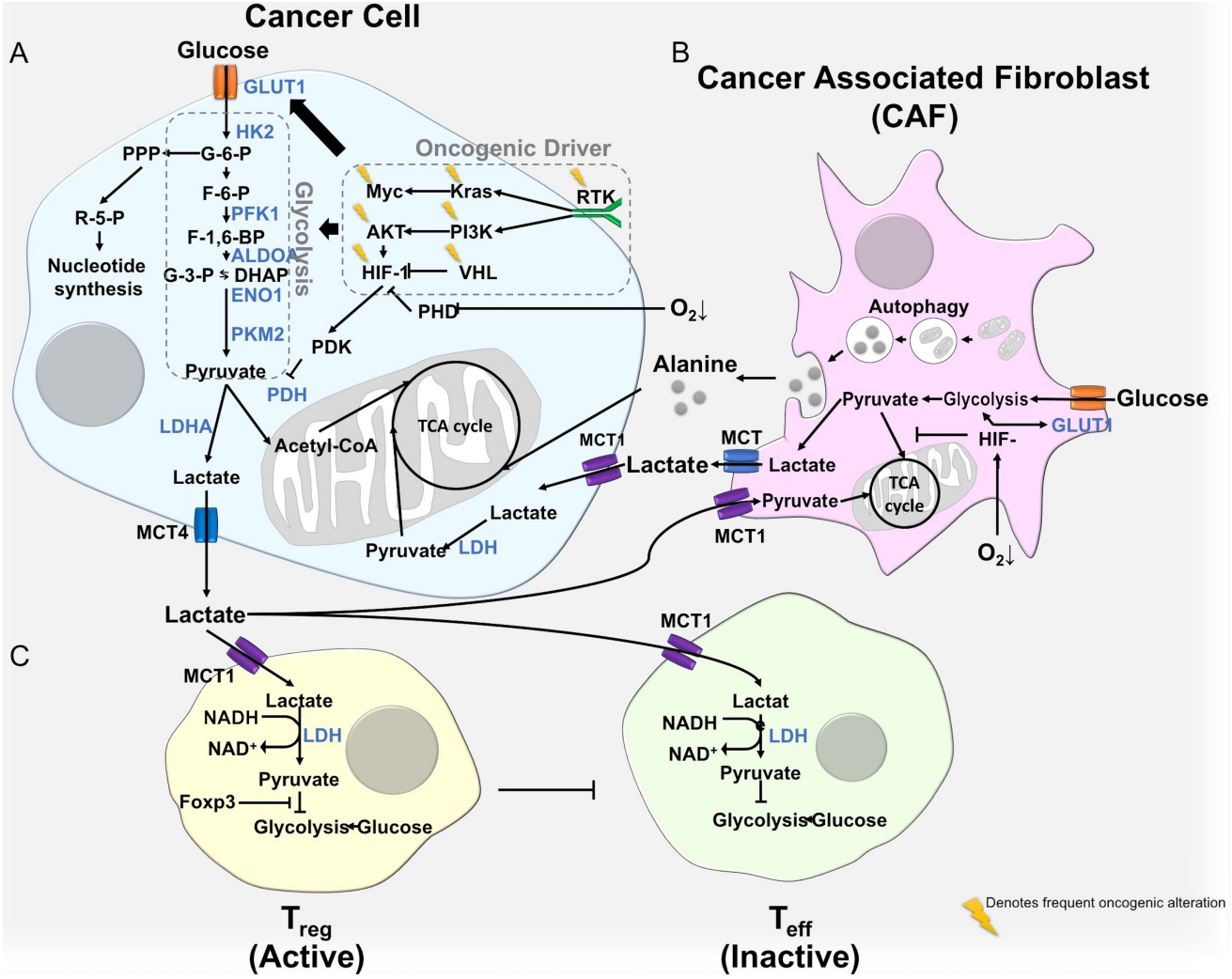

Fig. 1. Intrinsic and extrinsic variables affect the tumor metabolic signature. (A) Genetic oncogenic alterations strongly drive cancer cell glycolytic metabolism. (B, C) Secreted metabolites can be used as an alternative fuels source by various tumoral subpopulations, such as cancer associated fibroblasts (CAFs) (B) and immune cells (C), which form symbiotic or competitive relationships within the diverse tumor microenvironment (TME).

prevailing assumption that aerobic glycolysis or the Warburg effect is a relatively uniform distortion of normal cellular metabolism in cancer, and a poor understanding of how tumor glycolysis shapes and interacts with the tumor microenvironment to promote the growth and proliferation of tumor cells. In this review, we will explore the genetic and molecular alterations driving these distinctive glycolytic signatures in various types of cancer. We will further discuss new developments that illustrate how aerobic glycolysis is as potentially diverse and heterogeneous as other more newly described metabolic processes and the consequences of these metabolic interactions with the tumor microenvironment of specific tissues (Fig. 1).

THE GENETIC FOUNDATION OF AEROBIC GLYCOLYSIS

Shortly after the discovery of aerobic glycolysis in cancer cells, Herbert Crabtree provided the first evidence of glycolytic heterogeneity in cancer by demonstrating that glycolysis was not uniformly elevated in tumors, even within tumors of the same type (Crabtree, 1929). Further studies revealed that aerobic glycolysis was not restricted to tumor cells but was found in other various neoplastic and normal tissues (Murphy and Hawkins, 1925; Crabtree, 1928). Crabtree attributed this glycolytic heterogeneity to various genetic and environmental influences, but it was not until after the discovery of oncogenes that the genetic foundation of cancer metabolism was uncovered. The normal growth and proliferation of cells is strictly regulated by external growth signals and the availability of nutrients, so it is not surprising that malignant cells have acquired oncogenic mutations that exquisitely integrate mitogenic signaling pathways with metabolic pathways to provide continuous access to nutrients (Ward and Thompson, 2012). The pathways described below are a few of the manifold dysregulated signaling pathways whose independent, coopera-

tive, and overlapping elements coalesce to form the glycolytic heterogeneity first described by Herbert Crabtree in the early twentieth century.

Ras/Raf pathway

Ras, named after the transforming rat sarcoma virus, was one of the first retroviral and human oncogenes discovered (Harvey, 1964; Kirsten and Mayer, 1967). Members of the Ras family are found to be mutated in nearly 30% of all human cancers, making them the most frequently mutated genes involved in tumorigenesis with Kras as the most prevalent (Fernández-Medarde and Santos, 2011). The discovery of the Ras oncogene and its involvement in cellular signaling was followed closely by observations that the activation of Ras directly influenced glucose metabolism (Boerner *et al*., 1985; Flier *et al*., 1987). Further delineation of the Ras signaling pathway revealed that cells harboring $Kras^{G12D}$ mutations constitutively activated mitogen activated protein kinase (MAPK) and phosphatidylinositol-3-phosphate (PI3K) signaling, which drove uncontrolled cell growth and proliferation (Malumbres and Barbacid, 2003). Kras activation of MAPK upregulates expression of the transcription factor Myc, another potent oncogene that regulates cell growth, proliferation, apoptosis, and differentiation (Sears *et al*., 1999). Myc can act as a primary oncogene through translocation or amplification events, such as in Burkitt's lymphoma, however under constitutive Ras signaling Myc acts as a downstream effector whose activation is dependent on the Raf-MEK-MAPK signaling cascade, or through inhibition of glycogen synthase kinase-3 (GSK-3) by the PI3K pathway which prevents Myc degradation (Ying *et al*., 2012). Among its numerous target genes, Myc directly regulates several glycolytic enzymes such as glucose transporter 1 (GLUT1), hexokinase 2 (HK2), phosphofructokinase (PFKM), and enolase 1 (ENO1), as well lactate dehydrogenase A (LDHA), which is necessary for the regeneration of NAD+ from NADH during aerobic glycolysis (Shim *et al*., 1997; Osthus *et al*., 2000; Semenza, 2010). Alternatively, Ras can influence glycolytic metabolism through the PI3K-mTOR pathway (discussed below), or by upregulating glucose flux through other glycolytic-intermediate utilizing pathways such as the hexosamine biosynthesis pathway (HBP), or the pentose phosphate pathway (PPP) (DeNicola *et al*., 2011; Ying *et al*., 2012).

PI3K/mTOR pathway

The PI3K signaling pathway is one of the most frequently dysregulated pathways in cancer, with a reported 38% of solid tumors exhibiting aberrant PI3K pathway alterations (Millis *et al*., 2016). PI3K is normally activated by ligand-bound receptor tyrosine kinases (RTK), where it phosphorylates the second messenger phosphatidylinositol-3,4,5-triphosphate (PIP_3) to recruit downstream effectors Akt and mammalian target of rapamycin (mTOR) (Whitman *et al*., 1988; Heitman *et al*., 1991). Accordingly, the PI3K signaling pathway regulates cell growth, proliferation, differentiation, metabolism, and cell motility (Fruman *et al*., 2017). Cell motility is regulated in part by reorganization of actin filaments by PI3K activated Rac. In this process, the glycolytic enzyme aldolase A is mobilized from filamentous-actin as actin fibers are disassembled and reassembled, allowing the cell to rapidly increase glycolytic flux in response to cell movement in a coordinated manner (Hu *et al*., 2016). Downstream of PI3K, Akt is a potent modulator of aerobic glycolysis, increasing the synthesis and membrane localization of glucose transporters and activating regulatory glycolytic enzymes such as HK2 and 6-phosphofructo-2-kinase/fructose-2,6-bisphosphate 3 (PFKFB3) (Clarke *et al*., 1994; Okada *et al*., 1994; Deprez *et al*., 1997; Barthel *et al*., 1999; Rathmell *et al*., 2003; Miyamoto *et al*., 2008). Akt further activates mTOR complex 1 (mTORC1), which functions as a nutrient sensor and initiator of protein and ribosome synthesis (Kim *et al*., 2002). Upon stimulation by growth factor or oncogenic signaling, mTOR increases the synthesis of transcription factors Myc and hypoxia inducible factor-1α (HIF-1α) via internal ribosome entry site (IRES)-dependent translation (Laughner *et al*., 2001; Galmozzi *et al*., 2004; Silvera *et al*., 2010). Like Myc, HIF-1α activates a transcriptional profile that shifts cellular metabolism towards glycolysis and away from oxidative phosphorylation by induction of numerous glycolytic genes such as GLUT1, HK2, PFKM, aldolase A (ALDA), phosphoglycerate kinase 1 (PGK1), LDHA, and pyruvate dehydrogenase kinase 1 (PDK1) (Semenza, 2010).

VHL/HIF-1 pathway

As part of the cellular oxygen sensing network, hypoxia inducible factors (HIF) respond to decreases in oxygen availability in the cellular environment, with HIF-1α and HIF-1β as the most prevalent members of the HIF family of transcription factors involved in tumorigenesis (Semenza, 2012). Under normal oxygen tension, the oxygen sensitive HIF-1α subunit is hydroxylated by prolyl hydroxylases (PHD) using molecular oxygen and α-ketoglutarate as cofactors (Ivan *et al*., 2001; Jaakkola *et al*., 2001). Hydroxylated HIF-1α is then recognized by von Hippel Lindau (VHL) and polyubiquitinated (Maxwell *et al*., 1999). This process continuously marks HIF-1α for proteasomal degradation, however the absence of molecular oxygen under low oxygen tension allows HIF-1α to escape hydroxylation and accumulate in the cytosol where it heterodimerizes with HIF-1β and translocates into the nucleus to initiate transcription of hypoxia response genes (Wang *et al*., 1995). The HIF-1 mediated hypoxic response encompasses transcriptional upregulation of angiogenesis, erythropoiesis, cell survival, and glucose metabolism, with the downregulation of oxidative phosphorylation via expression of pyruvate dehydrogenase kinase 1 (PDK1) (Semenza, 2012). PDK1 phosphorylates pyruvate dehydrogenase (PDH), inhibiting the conversion of pyruvate to acetyl-CoA and entry into the citric acid (TCA) cycle. This metabolic switch serves two critical functions under hypoxia: amelioration of the cytotoxic ROS burst accompanying electron transport in oxygen depleted environments, and the diversion of pyruvate towards lactate fermentation and regeneration of cytosolic NAD+ for the continued production of ATP through glycolysis (Denko and Giaccia, 2001; Kim *et al*., 2006; Papandreou *et al*., 2006). HIF-1 is often found highly expressed in solid tumors due to aberrant vascularization and poor perfusion which leads hypoxic stabilization of HIF-1α (Semenza, 2012). However, oncogenic stabilization of HIF-1 can occur through a mutation or loss of VHL, which occurs with up to 90% frequency in clear cell renal cell carcinoma (ccRCC) (Gossage *et al*., 2015), or through mTOR-mediated transcriptional stabilization after activation of the PI3K pathway (Laughner *et al*., 2001; Silvera *et al*., 2010). As a consequence of its position at the convergent end of multiple signaling pathways, HIF-1 is uniquely equipped to integrate intrinsic signals with external environmental cues to

regulate cellular glycolytic metabolism.

Accumulated research has revealed the complexity and variety of oncogenic stimuli and the relationship they have with driving cellular metabolism. Given that many mechanisms of oncogenic control impart unique metabolic rewiring and that many tumors harbor multiple oncogenic alterations, human tumors exhibit a large array of metabolic differences. As a result, the heterogeneous nature of tumor metabolism among different tumor types, harboring distinct oncogenic alterations, has become a sizeable topic of interest to further understand cancer pathophysiology.

HETEROGENEITY OF GLYCOLYSIS AND METABOLIC DEMANDS IN CANCER

The metabolic demands of transformed cancer cells are substantially different than those of normal quiescent cells. Highly proliferative cancer cells exhibit significantly increased metabolite catabolic and anabolic fluxes, which support a cell-autonomous reprograming of cellular metabolism to fuel proliferative capacity. During proliferation, cellular bioenergetic and anabolic demands reach a maximum, requiring increased uptake of nutrients to support physiology (Warburg, 1956; Vander Heiden *et al*., 2009; Hanahan and Weinberg, 2011). Augmented glycolysis fuels many metabolic pathways which can bolster proliferative capacity in cancer cells. Increased glucose flux, mediated by elevated expression of glucose transporters (GLUTs), fuels metabolic fluxes such as the pentose phosphate pathway (PPP), acting as the major metabolic contributor to NADPH pools, for cellular redox homeostasis, ribose-5-phosphate, a key building block in the biosynthesis of nucleoid acids, and erythrose-4-phosphate, needed for the synthesis of aromatic amino acids. *De-novo* serine and glycine biosynthesis from 3-phosphoglycerate is likewise dependent on glycolytic flux and acts as the metabolic bridge between catabolic glucose metabolism and the one-carbon folate cycle, which is important for nucleotide metabolism, glutathione biosynthesis, and cellular methylation reactions (Vander Heiden *et al*., 2009; Ducker and Rabinowitz, 2017; Gentric *et al*., 2017; Vander Heiden and DeBerardinis, 2017). These cellular metabolic needs are essentially liked cancer cell proliferation and physiology. As a result, augmented nutrient intake and metabolism fundamentally supplies the requisite energy and building blocks to support tumorigenecity. However, a clear understanding of the various fuels used in tumors is not yet fully established. Developing a more complete model of how tumor cells fulfill heterogeneous metabolic demands will critically contribute to our understanding of cancer pathobiology and possible targets for therapeutic intervention.

Recent work has begun to shed light on a more complete and diverse picture of tumor metabolism, beyond the canon of a single cancer-specific metabolic program (DeBerardinis, 2014). The turning point which has lead the to the recent advances in the scientific understanding of tumor metabolism occurred when researchers began to compare different tumors to one another instead of comparing tumors to cancer associated normal tissues (Gentric *et al*., 2017). It is now evident that many cancer-specific metabolic phenotypes exist. Both inter- and intra-tumor metabolic heterogeneity can be observed within and between tumors. The former was well characterized by Hensley *et al*. (2016), in human lung carcinomas by infusing universally labeled, heavy glucose (U-C^{13} glucose) into the blood of lung carcinoma patients. The U-C^{13} glucose was allowed to perfuse through the patient and tumor samples were collected for metabolomic and transcriptomic analysis. Interestingly, the balance between nutrient uptake was significantly altered in regions of high perfusion as compared to regions of lower perfusion. High perfusion regions of the tumor seem to have more access to various carbon sources to fuel metabolism, whereas regions of lower perfusion have a restricted nutrient pool and rely more on glucose metabolism (Hensley *et al*., 2016). These observations suggest the existence of a high degree of spatial metabolic heterogeneity within tumors and highlight a significant challenge in the development of metabolically targeted therapeutic strategies, originating from the apparent plasticity of the cancer cell metabolome. This work also underscores an important consideration for research targeting cancer metabolism, the apparent wide diversity of carbon sources which can be utilized within the tumor to support tumorigenesis. Recently, Faubert *et al*. (2017) and Hui *et al*. (2017) have shown that human lung tumors can utilize lactate as a metabolic fuel. Using innovative approaches of *in vivo* isotopic labeling of lactate, these studies were able to show that lactate contributes more carbon to the TCA cycle than that glucose. It has, as well, been shown in acute myeloid leukemia that glycolysis can be alternately supported by fructose uptake rather than glucose through the increased expression of the GLUT5 transporter (Chen *et al*., 2016). Together, these findings bare whiteness to the complexity and plasticity of tumor metabolism beyond aerobic glycolysis.

With recent mounting evidence for multiple levels of metabolic heterogeneity existing between tumors, intense research has been focused on identifying metabolic signatures unique to specific cancer types. To this end, significant efforts being made in the comprehensive molecular profiling of cancers have identified metabolic subtypes enriched in different patient subpopulations. Early observations of cancer subtype specific metabolic preferences were made by microarray profiling of a cohort of diffuse large B-cell lymphoma, which identified a cluster of patients with significantly elevated expression of genes involved in mitochondrial oxidative phosphorylation (Monti *et al*., 2005). This observation revealed the possible existence of metabolically distinct subtypes among patients with essentially identical clinical diagnoses. More current progress in the field of cancer metabolism has revealed differentially preferred metabolic needs among different cancers, highlighting possible avenues for the development of targeted strategies for the treatment, diagnosis, and prognosis of a wide spectrum of cancers displaying unique metabolic phenotypes. Goodwin *et al*. (2017) have recently characterized the existence of extensively differing glucose metabolic capacity among the major histological phenotypes of non-small cell lung cancer (NSCLC), lung adenocarcinoma (ADC) and lung squamous cell carcinoma (SqCC). This study revealed a distinct upregulation of the GLUT1 glucose transporter in lung SqCC, fueling highly augmented glycolytic capacity and lactate production as compared to lung ADC. The observation of the uniquely elevated capability of lung SqCC to uptake and metabolize glucose underscores a key metabolic signature, distinct to lung squamous tumors, and is a metabolic characteristic which can be effectively targeted for therapeutic development (Goodwin *et al*., 2017). It is becoming clear that there

is not one single, uniform metabolic program which cancer cells employ to fuel tumorigenecity. With the advent of global, molecular characterization and metabolomic profiling, significant progress is being made which is painting a very diverse picture of the cancer metabolic profiles and how cancers use nutrients to fuel their tumorigenecity.

It has been the goal of many scientific endeavors to understand the cancer-specific heterogeneous metabolic requirements in order to facilitate the development of novel therapeutic, diagnostic, and prognostic avenues in the treatment and management of cancer (Tennant *et al*., 2010; Hamanaka and Chandel, 2012; Vander Heiden, 2013; Hay, 2016). However, for many years, theories attempting to model cancer bioenergetics have, until recently, provided limited translational insights into the metabolic processes required for tumorigenecity, leaving Otto Warburg's seminal discovery of the cancer cell's glycolytic preference as the defining characteristic of tumor metabolism. With current progress toward a more complete understanding of tumor metabolic requirements and adaptation, we may soon be able to effectively ascertain heterogeneous metabolic pathways which exhibit candidacy for the development of precision medicine as well as targeted therapy.

ROLE OF TUMOR MICROENVIRONMENT AND GLYCOLYSIS

Malignant cells do not exist in isolation from non-cancerous, "normal" cells. They coexist with the normal cells of the tumor stroma in a highly complex tissue structure called the tumor microenvironment (TME) (Ansell and Vonderheide, 2013; Junttila and de Sauvage, 2013). Due to extensive metabolic demands of the various highly proliferative members of the TME, nutrient availability can be scarce. The frequent lack of sufficient vascularization within the TME results in large regions of both transient and permanent hypoxia, nutrient depravation, and cellular waste accumulation (Chang *et al*., 2015). The harsh conditions of the TME lead to a state of metabolic competition between various cellular subpopulations for cellular function and survival. In order to maintain the diversity of cell types within the TME, coexisting stromal and tumor cells can aggressively consume shared metabolic fuels or form a symbiotic relationship in which the metabolic byproducts of one population become a preferred nutrient source for another (Hanahan and Weinberg, 2011; Lyssiotis and Kimmelman, 2017). Here, we will discuss the metabolic demands of major cellular subpopulations within the tumor stroma and how these populations remain metabolically competitive in the nutrient limited TME.

Cancer-associated fibroblasts

One major cellular subpopulation within the tumor stroma consist of cancer-associated fibroblasts (CAFs), which are known to exhibit both tumor limiting and tumor promoting functions in a highly context dependent manner. Tissue resident fibroblasts, upon activation with cytokines and growth factors like transforming growth factor beta (TGF-B), differentiate into activated CAFs with augmented metabolism which supports an altered physiology (Bhowmick *et al*., 2004; Kalluri, 2016; Lyssiotis and Kimmelman, 2017). CAF metabolism shares some common features with cancer cell metabolism, exhibiting highly elevated glycolytic metabolism and lactate secretion (Zhang *et al*., 2015). However, whereas in cancer cells, elevated glycolysis can be stimulated by abhorrent oncogenic signaling, the CAF elevated glycolytic capacity seems to be associated with stabilization of HIF-1A and activation of hypoxia related signaling (Kim *et al*., 2012). With multiple highly glycolytic cellular subpopulations within the TME, such as CAFs and highly proliferative tumor cells, the essential glycolytic fuel, glucose, becomes limited, especially in tumor regions with decreased vascular nutrient supply. The high glycolytic capacity of CAFs fuels significant amounts of lactate secretion into the TME, which can ostensibly be used by other cellular populations as a metabolic fuel (Pavlides *et al*., 2009; Bonuccelli *et al*., 2010; Fiaschi *et al*., 2012; Kalluri, 2016; Faubert *et al*., 2017). However, this is only one example of a symbiotic metabolic relationship between TME cellular populations. Recent work has highlighted the seemingly high diversity in nature of metabolic needs within different tumor cellular subpopulations, with most work focused on the metabolic needs of the cancer cells. With the observation of a high degree of metabolic heterogeneity and differing tumor cell glycolytic capacity between different tumors, it is also conceivable that the tumor cell metabolism can essentially support stromal populations. Indeed, it has been observed that tumor cell nutrient secretion can support CAF metabolic needs (Koukourakis *et al*., 2006; Rattigan *et al*., 2012).

Beyond a shared capacity for glycolytic metabolism, tumor stromal fibroblast populations have been shown to symbiotically contribute to TME through the generation of multiple extracellular nutrient pools (Ghesquière *et al*., 2014; Lyssiotis and Kimmelman, 2017). CAFs have been shown to contribute multiple amino acids to the TME, which can be used as carbon sources to fuel energy producing metabolic fluxes in nutrient limited conditions. Sousa *et al.* (2016) showed that pancreatic stellate cells, a myofibroblast-like cellular population, contribute to alanine secretion through autophagic metabolism. Secreted alanine helps to support proliferation in low-nutrient enviroments, contributes a carbon source for tumor cell TCA cycle anaplerosis, supports lipid biosynthesis, and shunts glucose to de novo serine biosynthesis through glycolytic 3-phosphoglycerate flux (Sousa *et al*., 2016). Recently, Linares *et al.* (2017) have shown that p62-deficient tumor stromal CAFs rewire their metabolism under glutamine deprived conditions to maintain TCA cycle anaplerosis through glucose-derived mitochondrial pyruvate flux. A key feature of this metabolic adaptation is the generation of aspartate from oxaloacetate through transamination. They show that accumulated aspartate is then converted to asparagine through the action of asparaginase and subsequently secreted into the TME, contributing to extracellular nutrient pools (Linares *et al*., 2017). Together, these observations and recent advances highlight the heterogeneous and complex nature of the CAF-tumor cell metabolic relationship.

Cancer-associated immune cells

Diverse members of the innate and adaptive immune system, such as neutrophils, macrophages, and various populations of T-cells, take up residence and exert functional roles within the TME (Gajewski *et al*., 2013). A significant amount of research is currently focused on delineating the tumor-associated immune populations and the roles they play within the TME. Immunological profiling and immunogenomics is es-

sentially contributing to the development of potent, immunotherapeutic options for cancer patients by elucidating ways to bolster and educate the host immune system against tumor cells antigens. Following these recent developments, the field of cancer immunometabolism has developed in order to better understand the metabolic demands of cancer-associated immune cells. Similar to other members of the TME, cancer-associated immune cells must adapt their metabolism to maintain metabolically viable within the nutrient limited TME (Biswas, 2015). The T cell, for example, is greatly attuned to adapting its metabolic profile to overcome environmental stressors. When activated, the T cell greatly upregulates glycolysis and lacated production, even in the presence of sufficient oxygen (Buck *et al*., 2015, 2016). Interestingly, recent evidence shows that T-cells are distinctly affected by the TME metabolic signature, exhibiting a potent sensitivity to lactate. Angelin *et al*. (2017) have shown that T-cells can switch to lactate uptake in a glucose starved TME. However, the reversal of the LDH reaction to generate pyruvate drains the cellular NAD+ pools, effectively inhibiting GAPDH activity and glycolytic flux. This is particularly detrimental to cytotoxic and T-effector cells as these cellular populations strongly rely on high glycolytic flux for activation, differentiation, and physiological function. Interestingly, the immunologically inhibitory T-regulatory cells exhibit resistance to lactate inhibition through Foxp3 downregulation of c-MYC and subsequent decrease in glycolytic dependence (Angelin *et al*., 2017). Theoretically, it is possible to envision a TME metabolic signature which supported tumor progression through lactate inhibition of cytotoxic and T-effector cell populations, leaving resistant T-regulatory cells to maintain immunosuppressive rule over TME associated immune cells. Elevated glucose metabolism within the stromal compartment is a feature shared by multiple cell types who use augmented glucose flux in diverse ways. For example, Semba *et al*. (2016) show that the HIF-1A/PDK1 promotion of cellular glucose flux to lactate is critically important for macrophage migration during inflammation and tumor immunosurveillance. Tumor-associated macrophages have, as well, been implicated in depleting the tumor microenvironment of the amino acids, tryptophan, and arginine, resulting in the secretion of immunosuppressive catabolites and amino acid deficiency, which metabolically stress various TME cellular populations (Munn *et al*., 1999; Geiger *et al*., 2016). With a robust amount of research now being focused on a better understanding of tumor-associated immunometabolism, one can hypothesize as to the critical metabolic alterations occurring in the tumor immunometabolome, which affect tumor progression. The diverse interactions between tumor-associated immune cells and other cellular populations within the TME highlights the existence of a novel route to explore for immunometabolic modulation in the development of more effective immunotherapeutic options.

Cancer-associated endothelia cells

Blood vessels supply tissues and tumors with essential nutrients, oxygen, and are responsible for removing cellular waste products. Blood vasculature is lined by cells called endothelial cells, which mediate may functions of new vessel formation. Recent work has shed light on newly emerging concepts, suggesting that the endothelial cell angiogenic potential is critically linked to its cellular metabolism. Given the endothelial cell's advantaged position at the interface between nutrient rich blood and perfused tissue, it is logical to hypothesize that endothelial cells, having no restriction to oxygen or nutrient pools, use mitochondrial respiration to generate the vast majority of cellular bioenergetic needs. However, multiple groups have shown that endothelial cells from large and small vessels exhibit a dependence on glycolytic metabolism (Dobrina and Rossi, 1983; Culic *et al*., 1997; Cantelmo *et al*., 2016). The reasons for this glycolytic dependence have not yet been fully established. However, given that one of the basic functions of vasculature is the supply of oxygen to perfused tissues, endothelial aerobic glycolysis may act to sustain blood oxygen levels to insure diffusion into vascularized tissues (Eelen *et al*., 2015; Vandekeere *et al*., 2015; Treps *et al*., 2016). Given tumor cells' need for metabolic fuels, oxygen, and waste removal, tumor angiogenesis is critically important for cellular viability within the TME (Treps *et al*., 2016). Tightly associated tumor endothelial cells form the lining of tumor vasculature and fundamentally contribute to tumorigenecity by maintaining regional tumor blood supply and mediating new vascular growth into tumor regions starved of blood access. Recently, Cantelmo *et al.* (2016) have shown that tumor vascular normalization can be induced by inhibition of endothelial cell glycolysis. By inhibiting the glycolysis stimulating enzyme, PFKFB3, Cantelmo *et al.* (2016) show that endothelial cell endocytosis of VE-cadherin is impaired, leading to vascular normalization, decreased metastasis, and increased chemotherapeutic efficacy. Targeting cancer associated angiogenesis has now become a well-recognized, viable option for the development of new tumor targeting therapies (De Bock *et al*., 2013). By targeting endothelial glycolytic metabolism, future therapies may be able to not only decrease tumor metastasis by normalizing vascular permeability, but also increase tumor perfusion, allowing more efficient delivery of cancer cell-targeting therapeutic agents.

With such diversity in cellular populations and metabolic needs within the TME, it is prudent to ascertain which metabolic relationships are impactful on tumor development and may harbor specific targets for further therapeutic-oriented study. It is clear that stromal cells have both distinct and competing metabolic relationships with cancer cells. However, questions still arise about the nature of the metabolic relationship between tumor and stroma. For example, how does a symbiotic interaction between the tumor and stroma evolve over the course of tumorigenesis? Does the metabolic phenotype of the tumor essentially direct that of the stroma or visa versa? If so, how can modern translational science interact with and manipulate this intricate metabolic relationship for the benefit of cancer patients? Currently ongoing work is attempting to answer these essential questions.

CONCLUDING REMARKS

Given that for many years, the field of tumor metabolism has researched concept of uniformly elevated glycolysis as a unifying metabolic feature of tumor cells, early clinical efforts attempting to target glycolysis and glucose metabolism among a wide selection of human tumors were expected to generate clinically meaningful responses. Unfortunately, most clinical trials testing the efficacy of glycolytic targeting have exhibited dismal results (Vander Heiden, 2013; Vander Heiden and DeBerardinis, 2017). However, emerging research has

uncovered highly diverse and complex metabolic phenotypes among human tumors, with different tumor types displaying varying capacities and dependencies for glycolytic metabolism and glucose utilization (Goodwin *et al*., 2017). With these observations becoming more functionally understood, keen research in now focused on translating glycolytic heterogeneity into the clinic with the goal of identifying patient populations who will benefit the most from inhibiting glucose metabolism. The potential for developing new routes of therapeutic, diagnostic, and prognostic options for patients through targeting tumor glycolysis in heavily glycolytic tumor subtypes will need to be rigorously tested in pre-clinical and clinical settings. The horizon seems bright for a resurgence of glycolytic targeting in human tumors.

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