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Metabolism Shapes the Tumor Microenvironment

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Abstract

Tumors are strongly influenced by the surrounding normal tissue, which forms a specialized niche termed the tumor microenvironment (TME). The TME is modeled by cancer cells for their own benefit through a complex array of interactions. The identification of new forms of communication within the TME, which are dependent on the tumor's metabolic activity, has expanded our understanding of this heterocellular regulation and has revealed potential therapeutic targets. This review will summarize recent findings on the metabolic regulation of tumor cells by the TME. The concepts to be discussed include the existence of metabolic intratumoral heterogeneity, the contribution of cancer associated fibroblasts (CAFs) to tumor progression, and the regulation of tumor immunology by tumor-secreted metabolites.

Graphical abstract

Introduction

Tumor cells have an extraordinarily elevated requirement for nutrients to sustain their demanding anabolic needs and energy production rates. Thus, extracellular nutrients dictate the rate at which tumor cells proliferate. However, unlike normal cells, cancer cells have greater metabolic plasticity, which allows them to better adapt to lower or changing nutrient conditions [1] in ways that can, in turn, reshape the TME.

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The TME (the non-cancerous components in close proximity to tumor tissue) has recently become the focus of intense research because of its clear role in the establishment and progression of cancer [2]. Thus, understanding the regulatory mechanisms that influence the TME could reveal novel avenues for cancer treatment. In solid tumors, the cancer microenvironment has two main components, cellular and non-cellular, whose proportion and composition vary depending on the tumor location and stage. The cellular components include fibroblasts, mesenchymal stem cells, adipocytes, pericytes, endothelial cells from the mesenchymal lineage, and tumor-infiltrating lymphocytes (TIL) and tumor-resident macrophages (TRM) from the lymphoid and myeloid lineages respectively [3]. Non-cellular components include mainly the extracellular matrix (ECM), which is composed of proteins, glycoproteins, and proteoglycans that act as a scaffold and maintain the tissue architecture [2].

In this review, we will summarize recent key discoveries that add to our understanding of tumor metabolism and how it affects the composition of the two main cellular constituents of the TME: fibroblasts and immune cells.

Tumor Metabolic Heterogeneity

One of the most striking characteristics of cancer cells is their ability to adapt to changing environmental conditions by utilizing a wide range of nutrients [4]. This is usually dictated by the supply of oxygen and nutrients delivered to the TME by the tumor vasculature [5], which is often not uniformly distributed across the tumor bulk. In this regard, a new study from Hensley et al. (2016) found that non-small cell lung cancers (NSCLC) exhibit heterogeneous intratumoral substrate utilization due to variations in tissue perfusion [6]••. By using intraoperative ¹³C-glucose infusion in NSCLC patients [7], the authors found that tumor regions closer to well-vascularized areas, like normal lung tissue, were able to utilize fuels other than glucose to sustain growth, while less perfused regions used glucose as the main carbon source [6]•• (Figure 1A). Importantly, the authors corroborated their model in two different NSCLC oncogenotypes, mutant EGFR and KRAS. Because oncogenes can modulate tumor metabolism [8,9], it still remains to be fully determined if other oncogenic drivers, other tissues of origin, or a more complex oncogenic composition in the tumor bulk [10,11] can also regionally influence substrate utilization. Supporting this concept, Kerr et al. (2016) recently showed that the acquisition of additional mutant KRas^{G12D} alleles was linked to more advanced NSCLC in mice [12]••. Specifically, the authors showed that homozygous mutant KRas^{G12D} cells had increased flux of glucose-derived carbon into the TCA cycle and glutathione biosynthesis, as compared to heterozygous counterparts. Ultimately, the gain in mutant allelic content allowed them to strengthen their glutathione-mediated detoxification [12]•• (Figure 1B). This study provided the first in vivo evidence of how mutagenic load directly affects tumor metabolism and promotes tumor progression. Given that additional mutant KRas^{G12D} are acquired during tumor progression, there is a point in time where homozygous and heterozygous KRas^{G12D}-containing cells with different metabolic activities coexist in the tumor bulk. This suggests that intratumoral mutational differences might, in addition to differential tissue perfusion, also contribute to a tumor's metabolic heterogeneity [6]••.

Reprogrammed CAFs and Stellate Cells Sustain Tumor Growth

CAFs make up a key stromal component that plays a fundamental role in tumor initiation, growth, invasion, and dissemination [13]. While normal fibroblasts (NF) undergo a reversible process of activation upon acute injury, known as the wound healing response [14], CAFs are chronically activated by environmental cues, mostly derived from the tumor's activity. Activated CAFs (also termed myofibroblasts) differ phenotypically from activated NFs in several ways including lower contractility, increased survival potential, increased proliferation, and augmented ECM remodeling ability [14].

Metabolic reprogramming is an emerging hallmark of CAF activation. Although more commonly attributed to tumor cells [15], a growing body of evidence shows that CAFs also undergo radical changes in their metabolism during activation. For instance, CAFs use aerobic glycolysis to sustain their augmented proliferation activity instead of relying on oxidative phosphorylation (OXPHOS) [16]. Their metabolism is also characterized by an increase in autophagy [17–20]• as a mechanism to mobilize internal sources of nutrients to provide the TCA with metabolic intermediates. A new study from Sousa et al. 2016 has provided a novel link between a myofibroblast-like cell, pancreatic stellate cells (PSCs), and tumor behavior. They showed that activated PSCs, in the context of pancreatic ductal adenocarcinoma (PDAC), secrete autophagy-derived alanine to sustain tumor metabolism [21]••. They also found that PSC-supplied free alanine could supplant glucose-derived carbon in TCA-cycle metabolites while diverting glucose utilization towards the serine and glycine one-carbon (SGOC) pathway, which functions in the de-novo synthesis of nucleotides [22] (Figure 2). This newly described mechanism could contribute to the resistance to nutrient stress observed in PDAC tumors by relieving the dependence on glucose and other nutrients. Similarly, Yang et al. 2016 recently reported that, under glutamine scarcity such as that observed in core regions of ovarian tumors, tumor-engaged CAFs harnessed carbon from diverse sources to produce glutamine for tumor cells [23]. Importantly, branched-chain amino acids (BCAA) and aspartate were the major substrates contributing to the nitrogen supply for glutamine synthesis in ovarian CAFs. Ultimately, the authors showed that combining simultaneous tumor and stromal targeting of glutamine usage pathways could efficiently prevent tumor growth in a mouse model of ovarian cancer [23]•• (Figure 2). Both studies, Sousa et al. 2016 and Yang et al. 2016, reported new tumor-feeding strategies of activated PSC and CAFs, respectively, which add to our understanding of TME crosstalk and offer potentially actionable targets. However, it is worth noting that PSC and ovarian CAFs rely on different mechanisms to mobilize amino acids (Figure 2). The specific signaling cascades that dictate which mechanism is used by CAFs and PSC to meet the nutritional demands of the tumor are not known. Additionally, since other tumor-feeding strategies have been discovered, such as exosome-mediated delivery of amino acids and TCA intermediates [24], it is yet to be determined whether there are tumor-derived cues that engage CAFs to deliver a specific nutrient or input back to the tumor. In this regard, recent work by Tape et al 2016 reported that a particular oncogene in tumor epithelial cells was able to induce CAF-dependent signaling. Specifically, they showed that KRas^{G12D} was able to affect additional signaling pathways in tumor cells by non-cell autonomously engaging local heterotypic fibroblasts through SMO/Gli activity to signal back to tumor cells

through an IGF1R/AXL-Akt-dependent axis. In this way, distinct metabolic, proliferative, anti-apoptotic, and anchorage-independent growth phenotypes in tumor cells would only be activated when tumor cells are in the presence of CAFs [25] (Figure 2). Taken together, these results suggest that there are, indeed, specific signaling mechanisms in the tumor that can hijack stromal components to promote tumor growth. However, it is yet to be determined whether other oncogenes and tumor suppressors can exert similar reciprocity with fibroblasts or other components of the TME.

Since tumors clearly benefit from engaging CAFs, the TME offers a potential target that could be exploited therapeutically [26]. Moreover, fibroblasts are genetically stable and more easily accessible by the vasculature than tumor core regions. However, finding actionable targets that allow simultaneous targeting of tumor epithelia and TME components remains a considerable challenge. One of the reasons for this is that some potential target proteins have opposite cell-dependent functions. That is, they behave as tumor promoters in one cellular compartment and tumor suppressors in another. For instance, metabolic reprogramming of prostate CAFs is mediated by the downregulation of the signaling adaptor *Sqstm1* (p62), which impairs metabolic detoxification and increases IL-6 secretion through inhibition of the mTORC1/c-Myc axis, which, in turn, promotes inflammation and tumorigenesis [20]•. Conversely, in the prostate tumor epithelium, p62-dependent mTORC1 activation in response to amino acids promotes tumor proliferation [27] (Figure 3A). A similar cell-dependent role for p62 is also found in liver tumors. While p62 accumulation in hepatocytes, due to partial autophagy inhibition, inflammation, and oxidative stress, leads to non-mutational activation of mTORC1 that can initiate hepatocellular carcinoma (HCC) development [28]••, hepatic stellate cell (HSC)-specific KO of p62 potentiates liver fibrosis, inflammation, and HCC progression [29]••. Importantly, full-body KO of p62, in the context of a commonly used HCC inducer cocktail, diethylnitrosamine (DEN), and high-fat diet administration, enhanced HCC development [29]••, which suggests that the tumor-suppressor role of p62 in the stromal fibroblast compartment [20,23,29] outweighs its tumor-promoting effects in the tumor epithelium [27–31]. Mechanistic studies revealed that p62 loss in the HSC compartment reduced the anti-fibrotic and anti-inflammatory effects of vitamin D receptor (VDR) agonists, thus promoting HSC activation [29]•• (Figure 3B). These results are of immediate clinical relevance as there are several ongoing clinical trials testing vitamin D analogs as a way to block stellate cell activation [32]• (clinicaltrials.gov, NCT02603757). Given that p62 degradation is concomitant to HSC activation, highly inflamed and fibrotic tumors with reduced levels of p62 will probably not respond to the tumor-suppressor effects of vitamin D analogs [29]••. Thus, p62 levels in the stroma could be used clinically to determine potential responders. Taken together, these results illustrate how inhibition of an oncogenic target in tumor cells could potentially backfire by increasing stromal reactivity and inflammation.

Tumor-Derived Metabolic Intermediates Influence Immune Cell Function

While it has long been known that cancer cells can present cancer-specific peptide-MHC complexes to T cells [33], it was not known why naturally occurring immune responses failed to stop tumor growth [34]. Currently, evasion from immune surveillance is considered a hallmark of cancer that allows tumor cells to grow despite acquiring potential neo-antigens

[35]. The mechanisms by which tumors dodge the immune system include display of immunomodulatory proteins on the tumor cell surface, secretion of soluble cytokines, and, as recently reported, changes in localized concentrations of tumor-derived metabolites [36].

Tumor-derived metabolites accumulate in the TME as a result of an accelerated and imbalanced metabolism. For example, hypoxic environments lead to high concentrations of extracellular tumor-produced adenosine, which exerts immunosuppressive actions by binding to adenosine receptors present in various immune cell types [37]. In addition, lactate, a by-product of glycolysis, is also frequently found at high concentrations in solid tumors as a result of hypoxic environments and extreme glycolytic rates [38,39]. Importantly, build-up of extracellular lactate has both cell-intrinsic effects on metabolism and non-tumor cell autonomous effects that drive tumorigenesis [40], including metabolic reprogramming [41], tumor inflammation [42], and angiogenesis [43,44]. The tumor-promoting actions of lactate include blocking the differentiation and activation of monocytes and T cells [45,46]. Recently, Colegio et al. 2014 demonstrated how lactate promotes vascular endothelial growth factor secretion and M2 macrophage polarization by an HIF1 α -dependent mechanism [47]••. In turn, secretion of Arginase1 by M2-polarized macrophages signaled back to tumor cells and promoted growth [47] (Figure 4). In another study, Brand et al. 2016 described a link between secreted lactate and the immunosuppressive effect observed on tumor-infiltrated T and NK cells [48]••. In a proof-of-principle experiment, the authors reported that LDHA-low tumor cells had impaired growth compared to control cells when implanted into WT mice, while removing T and B cell populations (Rag2 $^{-/-}$) or T, B, and NK cell populations (Rag2 $^{-/-}$ γ C $^{-/-}$) from recipient mice had a permissive effect on tumor growth of LDHA-low cells. Because control cells grew equally well in mice regardless of their immunocompetent status, they concluded that neither NK nor T cells could block tumor growth unless the tumor cells were deficient in lactate production. Mechanistically, the authors found that lactate directly impaired nuclear factor of activated T- cells 1 (NFAT) activity, which resulted in reduced IFN- γ production (Figure 3). On the other hand, LDHA activity in the immune compartment has been recently linked to the maintenance of high levels of Acetyl-CoA to support histone acetylation and transcription of inflammatory cytokines such as IFN- γ [49]. Taken together, these results offer a new perspective on the functions of lactate in shaping the composition of the TME and offer new rationale for therapeutic strategies that target its extracellular secretion [39].

Environmental cues [50], nutrient availability [51], and oxygen concentration [52] all act in concert to produce proper T cell effector responses. Because nutrients are typically scarce in the TME, any available nutrients can have a substantial impact on activation and differentiation of T cells, which rely mainly on augmented glucose, fatty acid, and amino acid consumption to sustain increased activity and proliferation [53,54]. In this regard, Geiger et al. 2016 reported that T cell activation and memory formation is dependent on intracellular arginine [55]••. They described how increased intracellular arginine concentrations could promote a shift from glycolysis to oxidative phosphorylation during T cell memory formation, thus endowing cells with a central memory-like phenotype that included increased survival and anti-tumor capacity [55] (Figure 4). This study offers a novel mechanism by which extracellular nutrients can directly influence T cell activation and differentiation to control tumor evolution. Although arginine provided through the diet

was able to affect T cell function [55], it is not known whether tumor metabolism could likewise dictate extracellular arginine levels to affect T cell function. Additionally, new studies are focusing on how nutrient availability influences epigenetic regulation and tumor behavior that can ultimately lead to remodeling of TME composition [56–60]••. For example, the intratumoral shortage of glutamine has been shown to contribute to tumor metabolic heterogeneity and therapeutic responses by limiting epigenetic enzyme cofactors such as α -ketoglutarate, whose intracellular decrease leads to global H3K27 hypermethylation [61]•• (Figure 4). This regulatory mechanism could explain other reported examples of epigenetic regulation, including the Polycomb repressor complex 2 (PRC2)- and DNA methyltransferase 1 (DNMT1)-dependent silencing of inflammatory cytokines CXCL9 and CXCL10 by the tumor, with a direct impact on T cell helper 1 trafficking to the TME [56]•.

Tumor metabolic heterogeneity [6] could also play a role in differential intratumoral immune recruitment. Of note, the nature, density, functional orientation, and location of adaptive immune cells within the tumor (the “Immunoscore”) has proven to be highly predictive of patient outcome [62–64]• and has allowed for the discovery of mechanisms controlling anti-tumor T cell responses [65]•. The composition and degree of immune infiltration in different areas of a given tumor is now known to be a key aspect of tumor biology. Therefore, future studies should explore how different metabolic activities within one tumor influence immune cell distribution in the tumor and how pharmacologic intervention could switch the TME to a more permissive ecosystem that allows anti-tumor immune responses.

Future Perspectives

The naturally occurring reprogramming of the TME is likely to benefit a tumor’s growth. Therefore, it is a priority to evaluate newly identified actionable targets that can be used to disengage the cellular components of the TME and allow a permissive anti-tumor state. To this end, new studies on stroma reciprocity in oncogene-driven tumors have revealed the importance of using heterocellular systems for drug screening to account for additional non-tumor-dependent effects. Additionally, a growing interest in the metabolite-dependent epigenetic reprogramming of cellular components of the TME is likely to yield interesting insights into the “field cancerization” effect [66,67] that is currently poorly understood. Finally, the newly reported tumor metabolic heterogeneity in NSCLC will likely spur further research on how it might affect irregular distribution of TME cellular components, such as local CAF activation or immune infiltration.

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Highlights

- Differential vasculature perfusion and oncogenic load promote tumor metabolic heterogeneity
- Activated cancer associated fibroblasts and pancreatic stellate cells supply nutrients to foster tumor growth
- Opposing cell-dependent functions in the tumor microenvironment promoted by different signaling routes
- Tumor metabolic activity halts efficient immune responses

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Figure 1. Tumor Metabolic Heterogeneity

(A) Differential tumor vasculature perfusion promotes different carbon source usage in NSCLC. Low perfused areas use mainly glucose while highly perfused areas use other sources. (B) Oncogenic $KRas^{G12D}$ mutation load dictates a metabolic switch to an increased TCA-supported metabolism and augmented glutathione biosynthesis that increases the ROS-detoxifying ability.

Figure 2.

Activated CAFs and PSCs maintain tumor growth by supplying free amino acids. Activated CAFs and PSCs mobilize free aminoacids by increasing glutamine biosynthesis and autophagy, respectively. Free aminoacids are fed to the tumor to sustain its metabolic demands of Non-Essential Amino Acids (NEAA), lipids and nucleotides. Also, oncogenic KRas^{G12D} is able to extent cell-autonomous signaling by hijacking CAFs metabolism to obtain reciprocal signaling and support tumor growth, survival and metabolism.

Figure 3. Opposed and cell-dependent role of p62 in tumor epithelia vs stromal CAFs and HSCs
(A) Amino acids (AAs) can activate mTORC1 in a p62-dependent manner in Prostate Cancer (PCa) epithelial cells, which promotes anabolism and cell growth and blocks autophagy. Autophagy inhibition leads to accumulation of p62 and further mTORC1 activation. In the CAF compartment, p62 is downregulated, which negatively affects mTORC1 and c-Myc's ability to sustain adequate metabolic detoxification, which leads to elevation of ROS and paracrine and autocrine signaling by IL6 and TGFβ. **(B)** In the liver, accumulation of p62 leads to increased NRF2 and NFκB transcriptional activation that in turns mediates redox resistance and supports HCC development. Also, p62 promotes mTORC1 and c-Myc activity which sustains proliferation and metabolism conducive to HCC. In the stromal compartment, HSC activation leads to lower p62 levels, which prevent adequate VDR-RXR heterodimer formation and anti-fibrotic effects of Vitamin D.

Figure 4. Tumor metabolic activity exerts immunosuppressive functions

Low oxygen, nutritional competition and altered tumor metabolism induce an immunosuppressed TME. Accumulation of LDHA-derived lactate inhibits NK and T cell function, while polarizing macrophages into an M2 state that secretes Arginase 1 to the tumor. Also, low levels of arginine prevent memory-like T cell formation while low levels of glutamine induce an epigenetic rearrangement in tumor cells that lead to immune evasion.