Chapter 5
Immune Cell Metabolism in Tumor Microenvironment

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Abstract Tumor microenvironment (TME) is composed of tumor cells, immune cells, cytokines, extracellular matrix, etc. The immune system and the metabolisms of glucose, lipids, amino acids, and nucleotides are integrated in the tumorigenesis and development. Cancer cells and immune cells show metabolic reprogramming in the TME, which intimately links immune cell functions and edits tumor immunology. Recent findings in immune cell metabolism hold the promising possibilities toward clinical therapeutics for treating cancer. This chapter introduces the updated understandings of metabolic reprogramming of immune cells in the TME and suggests new directions in manipulation of immune responses for cancer diagnosis and therapy.

5.1 Introduction

The tumor microenvironment (TME), first proposed by Lord in 1979, is a complex integrated system for tumor cell growth [1]. This environment is composed of tumor cells, endothelial cells, immune cells, fibroblasts, and extracellular matrix. The immune cells in the TME including macrophages, neutrophils, and lymphocytes play important roles in tumor immune escape, tolerance, and suppression. Traditional theory states that the incidence of cancer is due to failure of immune surveillance [2]. However, this theory was unable to fully explain the interplay between immunity and tumorigenesis. The immunoediting theory, proposed by Schreiber and Dunn,

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elucidated the role of the immune system in cancer development from a new per-spective [3]. Depending on the cellular and environmental context, the immune cells may kill tumor cells but also promote tumor development. This theory divides the carcinogenesis into three stages: immune clearance, immune balance, and immune escape. Immune clearance is similar to the immune surveillance. Immune balance is a stalemate stage that the tumor is not completely removed by the immune system, in which the tumor characteristics are reshaped by the immune system. Immune escape refers to the stage that the tumor escapes immune surveillance after tumor cells are edited by the immune system and progress to clinical tumor stage [4].

During the development of cancer, tumor cells and immune cells interact in a dynamic microenvironment that determines the outcome of tumorigenesis [5]. In the immune clearance phase, the immune system removes tumor cells through antigen-specific and nonspecific mechanisms, wherein lymphocytes are the main effector cells. If the immune surveillance stage functions effectively and the tumor cells are cleared, the immunoediting stage will not ensue. However, the immune system is not always effective in removing every tumor cells, especially the ones with low immunogenicity. These tumor cells often escape temporarily from the immune cell-mediated destruction. The immune balance stage then follows [6]. At this stage, the immune system constantly kills high immunogenic tumor cells, whereas it is "blind" to the tumor cells with low immunogenicity, resulting in the gradual emergence of the tumors with low immunogenic and high malignant pheno-types. The immune system continues to impose pressure to select for the tumor cells with accommodating immunological phenotypes. This Darwin's natural selection-like process is referred to as immune remodeling. The surviving tumor cells with low immunogenicity repeatedly stimulate the immune system and eventually induce the immune tolerance [5–7]. Tumor cells remodeled by the immune system can aberrantly produce inhibitory cytokines including interleukin-10 (IL-10), trans-forming growth factor-β (TGFβ), and vascular endothelial growth factor (VEGF) and induce immunosuppressive cells including regulatory T cells (Tregs) and myeloid-derived suppressor cells (MDSCs), fostering an immunosuppressive TME. In the TME, T cells are tolerant to tumor-associated antigen and functionally suppressive; the professional antigen presentation cells (APCs) are functionally defective, leading to hindered antitumor immune response and the systemic immu-nosuppression [8]. Consequently, establishing the immune-tolerant TME not only reduces the ability of the immune system to reject tumors but also promotes the immune escape of tumors that should have been rejected.

The metabolomics is an emerging direction of immune and cancer research [9, 10]. The metabolism of carbohydrates, lipids, and amino acids is the material basis of all biological processes. For the development of malignancy, the tumor cells must face two challenges: first, obtaining the nutrients needed for the rapid growth; second, evading the surveillance and attack from the host immune system. Tumor cell's unique metabolic program can be used to meet these challenges. Glycolysis is the major metabolic process used by malignant tumors, even when oxygen supply is adequate, which is termed as "Warburg effect" [11]. Glycolysis decreases the pH value of the TME; therefore, tumor cells can inhibit the activities

of antigen-presenting cells (APCs) and cytotoxic T lymphocytes (CTLs) by controlling the acidity of the microenvironment, eventually leading to tumor cell immune escape [12].

Recent studies indicate that tumor-associated immune cells show altered metabolism to affect their differentiation, survival, and function [10]. Since the TME heavily influences carcinogenesis, the metabolic programming and reprogramming of immune cells in the TME and how immune metabolism affects the tumor initiation, development, and metastasis are of great interest and significance [10, 12]. In this chapter, we review the current knowledge on the metabolism of the cell types in the TME, discuss the emerging concept of the metabolic reprogramming in tumor-associated immune cells, and propose the impact of immune metabolism on carcinogenesis and clinical applications.

5.2 Metabolism of Cancer Cells and Cancer Stem Cells in TME

Aberrant proliferation of cancer cells is fueled by altered metabolism (Fig. 5.1). Oncogenic mutations trigger a switch from oxidative phosphorylation (OXPHOS) to glycolysis in tumor cells, and hypoxia further enhances this reprogramming [13, 14]. OXPHOS generates 36 mol ATP from 1 mol glucose, while glycolysis produces only 4 mol ATP [15]. This seeming inefficient metabolic feature relies on abnormal upregulation of the glucose transporters such as GLUT1 [16]. Moreover, mutations in tricarboxylic acid (TCA) cycle enzymes such as succinate dehydrogenase (SDH) or fumarate hydratase (FH) also promote glycolysis and inhibit OXPHOS [14, 17, 18]. Lactic acid is the end product of glycolysis. Lactic acid production can be used as a biomarker of tumor metastasis and overall survival [14, 15]. The secretion of lactic acid by monocarboxylate transporter 4 (MCT4) by cancer cells depends on the intracellular and extracellular concentrations of lactic acid [19, 20]. The extracellular lactate effects include restricting monocyte conversion to dendritic cells (DCs), suppressing cytokine release from DC and CTL, and inhibiting monocyte migration and CTL function. In addition, the release of lactic acid by tumor cells to the extracellular space can block the lactic acid secretion from immune cells to trigger cell death due to excessive intracellular lactic acid [21, 22].

Cancer cells also obtain energy from high levels of glutamine to support the proliferation [23]. Glutaminolysis is the main metabolic pathway regulated in mitochondria, through which glutamate is catabolized into alpha-ketoglutarate (α-KG) and glutamate. α-KG is then converted to pyruvate via TCA cycle and then to lactate [24]. Although combined activation of c-Myc and HIF-1 can induce lactate dehydrogenase A (LDHA) and pyruvate dehydrogenase kinase 1 (PDK1) that contribute to glycolysis [25, 26], mTOR-SIRT4-glutamate dehydrogenase (GDH) axis and c-Myc orchestrate glutaminolysis. Moreover, c-Myc can induce glutamine transporters (SLC5A1) and glutaminase 1 (GLS1) [27, 28]. Glutaminolysis enables
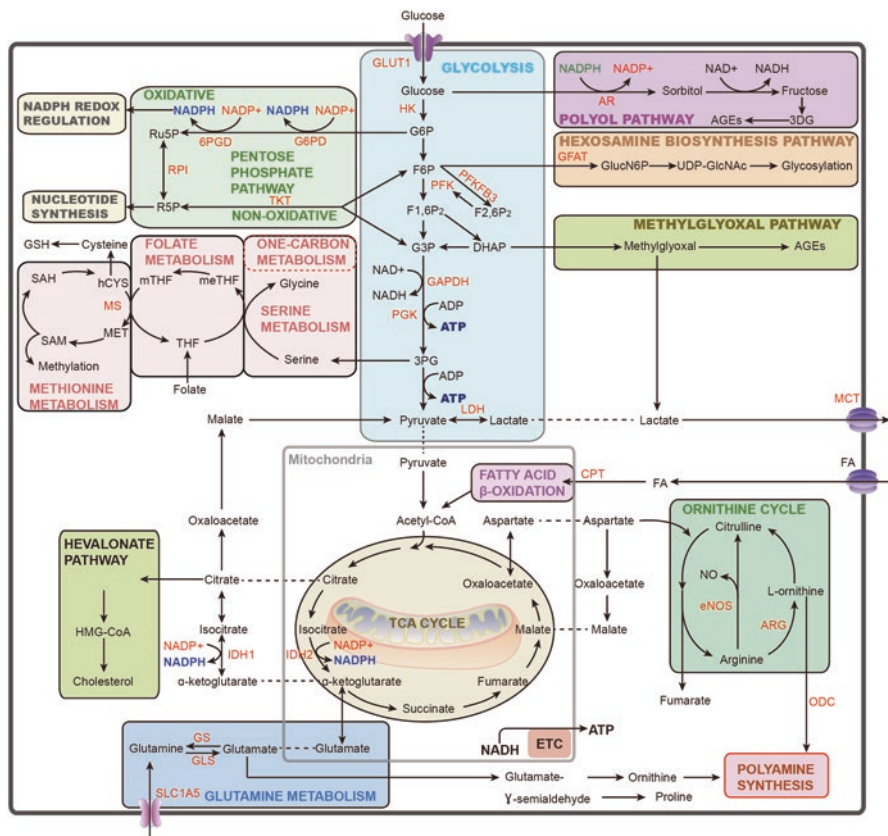
Fig. 5.1 Overview of cancer cell metabolism. This diagram depicts the cellular metabolic pathways in cancer cells. *Abbreviations*: *3DG* 3-deoxyglucosone, *3PG* 3-phosphoglycerate, *6PGD* 6-phosphogluconate dehydrogenase, *AGE* advanced glycation end product, *AR* aldose reductase, *ARG* arginase, *ATP* adenosine triphosphate, *CPT* carnitine palmitoyltransferase, *DHAP* dihydroxyacetone phosphate, *eNOS* endothelial nitric oxide synthase, *ETC* electron transport chain, *F6P* fructose 6-phosphate, *F1,6P2* fructose 1,6-bisphosphate, *F2,6P2* fructose 2,6 bisphosphate, *FA* fatty acid, *G6P* glucose 6-phosphate, *G6PD* glucose 6-phosphate dehydrogenase, *GAPDH* glyceraldehyde 3-phosphate dehydrogenase, *GFAT* glutamine-6-phosphate amidotransferase, *GlucN6P* glucosamine-6-phosphate, *GLS* glutaminase, *GLUT* glucose transporter, *GS* glutamine synthetase, *GSH* glutathione, *hCYS* homocysteine, *HMG-CoA* hydroxymethylglutaryl coenzyme A, *IDH* isocitrate dehydrogenase, *LDH* lactate dehydrogenase, *MCT* monocarboxylate transporter, *ME* malic enzyme, *MET* methionine, *meTHF* 5.10-methylene-tetrahydrofolate, *mTHF* 5-methyltetrahydrofolate, *MS* methionine synthetase, *NAD* nicotinamide adenine dinucleotide, *NADPH* nicotinamide adenine dinucleotide phosphate, *NO* nitric oxide, *ODC* ornithine decarboxylase, *PFK1* phosphofructokinase-1, *PFKFB3* 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase-3, *PGK* phosphoglycerate kinase, *ROS* reactive oxygen species, *RPI* ribose-5-phosphate isomerase, *SAH* S-adenosylhomocysteine, *SAM* S-adenosylmethionine, *TCA cycle* tricarboxylic acid cycle, *THF* tetrahydrofolate, *TKT* transketolase, *UDP-GlcNAc* uridine diphosphate N-acetylglucosamine

cancer cells to reduce NADP+ to NADPH which is an electron donor for reductive steps in lipid synthesis and nucleotide metabolism and the maintenance of reduced glutathione (GSH); thus, glutaminolysis is essential for cancer cells to regulate redox state [29].

In the presence of extracellular nutrients and enough oxygen, cancer cells synthesize fatty acids. However, under metabolic stress, cancer cells scavenge extracellular lipids to maintain viability and growth. Mechanistically, hypoxia, oncogenic RAS, and mTORC1 stimulate cancer cells to uptake lysophospholipids and desaturated fatty acids [30–32]. Also, fatty acid oxidation is enhanced in cancer cells to enable survival and proliferation [33]. Cancer cells express high levels of monoacylglycerolipase (MAGL), an enzyme that hydrolyze endocannabinoid 2-arachidonoylglycerol (2-AG) and convert monoacylglycerols to free fatty acid and glycerol that are essential for supplying energy for cancer cells [34].

Cancer stem cells (CSCs) are a small group of tumor cells with stem cell characteristics that initiate and maintain tumor growth and correlate with tumor metastasis. CSCs possess unlimited self-renewal, capacity to propagate tumors through asymmetric cell division, and therapeutic resistance [35, 36]. Tumor cells compete with CSCs for the space and energy generating macromolecules in the TME to limit CSC division and to drive tumor dormancy. The death of tumor cells therefore will relieve the CSCs from such competition and lead to the self-renewal and proliferation of CSCs [37]. Accumulating evidence indicates that the metabolic reprogramming is essential for CSCs to maintain stemness [38, 39]. Activated mitochondrial metabolism by genotoxic stress or hypoxia can lead to increased ROS and prostaglandin E2 that awaken dominant G0-phased CSCs to proliferative state [40]. The monocarboxylate transporter 1 (MCT1) is highly expressed in cancer stem-like cells to promote lactate uptake for the self-renewal and invasion [19, 20]. A recent study showed that CSCs express CD36, a scavenger receptor, which uptake oxidative low-density lipoproteins (ox-LDL) to maintain stemness [41]. Redox stress is a hallmark of cancer tissues that mediates robust metabolism in adjacent MCT1-positive proliferating CSCs which utilize lactate derived from glycolytic cancer cells to fuel mitochondrial metabolism. According to the distinct metabolic changes, cancer cells and CSCs dynamically regulate tumor progression.

5.3 Metabolism of Immune Cells in TME

The immune cells infiltrated in TME include cell subsets belong to both innate and adaptive immune systems. The current findings suggest that metabolic reprogramming is a common feature of both cancer cells and immune cells in the TME. The adjustment of the metabolic program regulates the differentiation and functions of tumor-associated immune cells and thus the progression of tumors [9, 10, 12]. Understanding how the immune metabolism changes and how the altered metabolism regulates immune cells in TME is of great interest and vital for developing effective therapies to treat cancer.

5.3.1   Macrophages

Macrophages have both anti- and pro-tumor functions by regulating tumor growth, angiogenesis, invasion, and metastasis [42, 43]. Based on the phenotypes, tumor-associated macrophages were divided arguably into two types, M1 and M2. M1 cells can be induced by interferon (IFN) γ and lipopolysaccharide (LPS), secrete pro-inflammatory cytokines including TNFα and IL-12, and express high levels of MHC molecules and nitric oxide synthase (NOS). M1 cells play crucial roles in pathogen clearance and tumor antigen presentation. M2 cells can be induced by IL-4 and IL-10, express moderate levels of MHC molecules and IL-12, but produce abundant anti-inflammatory cytokine such as IL-10, mannose receptor, and arginases to promote immunosuppression, tumor cell extravasations, and metastasis [44]. Most tumor-promoting tumor-associating macrophages (TAMs) are M2 type, while both M1 and M2 cells do coexist in the TME. The function of TAMs appears plastic as they have been found to display an inflammatory phenotype in the early phase of tumor initiation but exhibit immunosuppressive characteristics during tumor progression and metastasis [42]. These observations are consistent with the immunoediting theory that TME is a dynamically changing system.

Distinct polarization leads to different metabolic modes of macrophages (Fig. 5.2). M1 macrophages undergo glycolysis, while M2 macrophages exhibit increased oxygen consumption rate and show increased OXPHOS and decreased lactate release [45, 46]. TME is heterogeneous and dynamic during carcinogenesis. The glucose metabolism of TAMs varies in distinct phases. Indeed, at the early inflammatory stage of cancer initiation, TAMs favor glycolysis; in contrast, TAMs show OXPHOS at the later stage of tumor progression [47, 48]. This shift is mediated by cytokines and lactic acid. The lactate released from tumor cells promotes hypoxia and induces TAMs by enhancing the expression of arginase 1 (ARG1) to catalyze the metabolism of arginine to ornithine and polymines to promote collagen synthesis and tumor growth [49]. The activation of PI3K-Akt pathway may also contribute to the glucose uptake and glycolysis since it upregulates the expression of glucose transporters (e.g., GLUT1) and key enzymes (e.g., hexokinase and phosphofructokinase-1); promotes acetyl-CoA synthesis to link the metabolism of fatty acids, glucose, cholesterol, and amino acids; and facilitates the diversion of citrate from TCA cycle to acetyl-CoA by phosphorylating and activating ATP citrate lyase [46, 50, 51]. The switch to glycolysis in TAMs is controlled by Akt-mTOR-HIF-1 axis, resulting in the abundant TCA cycle intermediates and succinate accumulation [52, 53]. Factors in TME such as HMGB1 and DAMPs can stimulate Toll-like receptors (TLRs) that activate PI3K-Akt in myeloid cells like TAMs resulting in glycolysis and enhanced inflammation in TME [54]. PI3K-Akt activation in myeloid cells is also involved in resistance to anti-angiogenic therapy [55]. The activation of mTOR, a downstream molecule of PI3K-Akt, counterintuitively promotes M2 cells [52]. Of interest, this activation feedback negatively regulates PI3K [56]. The activation of c-Myc skews macrophage to M2 polarization and promotes tumor-promoting function of TAMs by increasing CCL18, TGFβ, VEGF, and MMPs [57]. However, whether and how c-Myc controls the metabolic reprogramming of TAMs remains unclear.
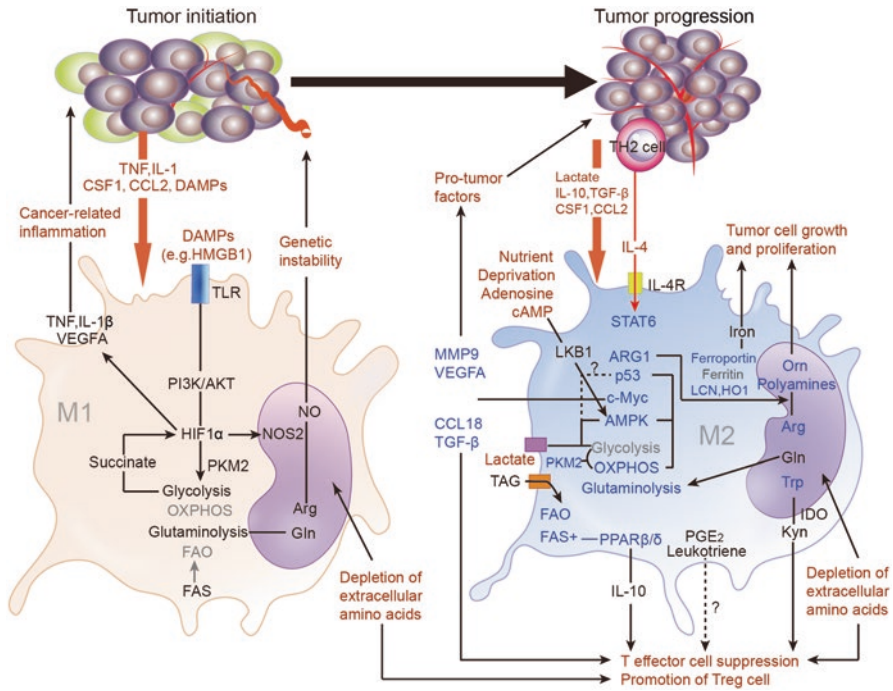
Fig. 5.2 Metabolic reprogramming of TAMs. An overview of the key metabolisms in M1 and M2 macrophages and their roles in tumor initiation and progression. During tumor initiation, macrophages are in M1 phenotype and metabolize through a glycolytic shift, HIF-1 activation, and impaired OXPHOS to mediate the expression of NO, ROI, IL-1β, and TNF, to support genetic instability and cancer-related inflammation that leads to tumorigenesis. HIF-1 also enhances angiogenic molecule VEGF-A. In TAMs (M2 macrophages) in the tumor progression stage, AMPK is activated via nutrient deprivation, Th2-derived IL-4, lactate accumulation, and activated PKM2 suppresses glycolysis while upregulating OXPHOS. This induces immunosuppressive macrophages that promote tumor growth. Amino acid, iron, and fat metabolism that contribute to this process are also shown

Moreover, inflammatory TAMs also express PKM2 which in its inactive dimeric form binds to HIF-1 to promote glycolysis in M1 macrophages, while in its active tetrameric form switches macrophages into M2 phenotype [58]. TAMs enhance cancer-related inflammation via HIF-1 which transcribes several key enzymes in glucose metabolism, such as GLUT1, HK2, and PGK1 [59, 60]. HIF-1 can also induce ROS and RNI production which contributes to the genetic mutation and transformation [61]. Moreover, hypoxic TME-increased glycolysis leads to lactic acid accumulation in the TME and skews TAMs to an M2 phenotype with high expression of ARG1, VEGF-A, Tie-2, and IL-10. These immunosuppressive and pro-angiogenic factors promote TAM-related tumorigenesis [49, 62, 63]. The distinct modes of arginine metabolism in TAMs lead to different functions, with M1 macrophages producing NO showing antitumor effect, while polyamine-producing

M2 macrophages induce cancer cell proliferation, remodeling, and growth [64–66]. TAMs that express ARG1 also contribute to T cell immunosuppression via inducing T cell apoptosis [67].

The lipid metabolism in TME macrophages is altered in response to a variety of stimuli. LPS and IFN-γ, the M1 macrophage inducers, suppress fatty acid intake and oxidation, while M2 macrophages are prone to increase fatty acid oxidation (FAO) [68]. The uptake of lipids, especially triacylglycerol (TAG), is also critical for FAO and M2 activation [69]. The underlying mechanism involves peroxisome proliferator-activated receptors (PPARs), liver X receptors (LXRs), and signal transducer and activator of transcription (STAT) [68, 70–72]. PPARs and LXRs are nuclear receptors activated by lipids, such as free fatty acids, eicosanoids, and cholesterol metabolites. PPARγ mediates M2 macrophage polarization to promote tumor progression and metastasis. PPARδ activation in macrophages is triggered by the clearance of apoptotic cells. The fatty acid synthase and PPAR activation are induced in TAMs to contribute to tumor growth. The phagocytosis of apoptotic tumor cells containing oxysterols activates LXRs in macrophages, leading to an M2 immunosuppressive phenotype.

Arachidonic acid metabolism also mediates the switch of macrophage phenotypes. For example, M1 stimulation leads to increased prostaglandin E2, a cyclooxygenase (COX)-derived eicosanoid, while IL-4 induces the upregulation of 15-lipoxygenase (15-LOX) in macrophages [73, 74]. The alveolar macrophages express high levels of COX-1 and 5-LOX, but TAMs express high levels of COX-2 [72]. Anti-inflammatory factors including IL-10, IL-4, and TGFβ induce AMPK activation which drive TAMs to an immunosuppressive M2 phenotype and induce OXPHOS [75]. These findings indicate distinct metabolic modes in TAMs mediate both anti- and pro-tumor responses.

Glutamine metabolism is another important pathway for the differentiation and functions of TAMs, and macrophages express high levels of glutaminase. Glutamine is required for macrophage phagocytosis and antigen presentation. Also, key enzymes in glutamine metabolism, such as AKG, GPT2, GLUL, and GATM, are enhanced in M2 macrophages [49, 76]. However, the mechanisms by which glutamine metabolism regulates TAMs remain unknown. In addition, M2 macrophages can generate indoleamine-2, 3-dioxygenase (IDO), an enzyme degrading tryptophan [77]. Since IDO upregulates regulatory T cells via tryptophan catabolite, TAMs promotes Treg cell generation to inhibit T cell function and establish an immune-tolerant microenvironment [78].

Polarized macrophages show altered iron metabolism. M1 macrophages express high level of H-ferritin, a protein for iron storage, but M2 macrophages express increased ferroportin, the iron exporter. Thus, M1 macrophages favor iron sequestration and inhibit tumor growth, while M2 macrophages exhibit enhanced iron release which promotes tumor progression [79, 80]. Hemeoxygenase-1 (HO-1), an iron-releasing enzyme metabolizing heme to carbon monoxide (CO), biliverdin, and ferrous iron, is inhibited in M2 but not in M1 macrophages [81]. Importantly, iron is also involved in regulating HIF-1 stability by activating prolyl hydroxylases (PHDs) in TAMs [82, 83]. The evidence suggests the iron metabolism is also crucial for TAM-mediated regulation of carcinogenesis.

5.3.2   Neutrophils

Neutrophils, also known as polymorphonuclear cells (PMNs), account for 50–60 % of the peripheral blood leukocytes, with a potent phagocytic function. Neutrophil to lymphocyte ratio is a risk predictive index for tumor recurrence [84]. In the TME, neutrophils also have two biological phenotypes: antitumor N1 exerts tumor cyto- toxicity to reject tumor by enhancing antitumor immune memory; pro-tumor N2 plays the opposing roles, i.e., enhancing tumor growth, invasion, and metastasis, promoting tumor angiogenesis, mediating immunosuppression, and producing enzymes to damage normal tissue cells to facilitate tumor growth, invasion, and metastasis. Such dichotomy of neutrophils is similar to that of macrophages [85, 86]. Tumor-derived TGFβ can switch N1 neutrophils to N2 which blunts CD8+ T cell responses to promote tumor growth [87]. Similar to macrophages, tumor- associated neutrophils (TANs) can produce several factors such as ARG1, ROS, MMPs, IL-6, and IL-1β to promote cancer progression, angiogenesis, and metasta- sis [85, 86].

The metabolic reprogramming, aerobic glycolysis and pentose phosphate path- way (PPP), controls the functions of neutrophils [88, 89]. Very few mitochondria are in neutrophils; OXPHOS and ATP production are ineffective in these cells. Neutrophils rely on PPP to produce NADPH that is essential for maintaining redox balance and cell survival [90]. The chemotaxis, calcium mobilization, and oxidative burst are driven by glycolysis since G6P deficiency blunts these functions of neutro- phils [91]. Glycolysis and PPP are also involved in the formation of neutrophil extracellular traps (NETs), a mixture of DNA, histones, and antimicrobial peptides that traps and kills bacteria [90, 92]. NETs segregate circulating tumor cells and accumulate in the vasculature to promote the inflammatory adherence, contributing cancer-induced organ failure and metastasis [93]. Moreover, neutrophil-derived leu- kotrienes contribute to the colonization of distant tissues via selectively expanding the sub-pool of cancer cells to retain high tumorigenic potential. Knocking down of 5-lipoxygenase (5-LOX), the key enzyme for leukotriene synthesis, blunts the pro- metastatic activity of neutrophils [94]. Together, these observations suggest that metabolic change regulates the TAN functions and tumor development. Whether and how the lipid and amino acid metabolism are reprogrammed and how these metabolic changes regulate neutrophil functions await further investigation.

5.3.3   Basophils and Eosinophils

Basophils, a cell population derived from myeloid cells, respond to IgE-dependent and IgE-independent stimuli and crosstalk with other immune cells such as lympho- cytes, macrophages, and DCs [95]. These cells are involved in Th2 responses through producing cytokines including IL-4, IL-13, and IL-25 and also contribute to immunoglobulin synthesis, tumor angiogenesis, and hematopoiesis by secreting IL-6, GM-CSF, and VEGF and arguably present antigens to T cells. Hence,

basophils regulate both innate and adaptive immunity. However, the metabolic reprogramming in tumor-associated basophils remains unknown.

Eosinophils are granulocytic leukocytes derived from hematopoietic progenitors. They interact with both innate and adaptive immune cells. IL-3, IL-5, and GM-CSF are crucial for eosinophil development, while CCL11, CCL24, and CCL26 contribute to eosinophil chemotaxis [96, 97]. Eosinophils express MHC-II and costimulatory molecules such as CD40 and CD80/86 to promote T cell activation and proliferation [98]. Eosinophils also produce IDO and TGFβ to mediate Treg and Th2 polarization [99]. Interestingly, the infiltration of eosinophils in TME associates with improved prognosis in various types of solid tumors [96] but with poor outcome in Hodgkin lymphoma [100]. The antitumor activity of eosinophils is through their degranulation in the tumor. Consistently, eosinophils with CCL11 deficiency exhibit impaired antitumor potential [101]. Also, the necrosis and chemokines in cancer tissues induce the differentiation and migration of eosinophils [102]. Systemic IL-2 and IL-25 therapy promotes eosinophil degranulation [103, 104]. The recruitment of eosinophils in TME is mainly mediated by high-mobility group box 1 (HMGB1), a factor in damage-associated molecular patterns, which elicits eosinophil degranulation by binding to the receptor for advanced glycation end products (RAGE) [105]. Some receptors normally expressed in NK cells such as NKG2D and 2B4 are also expressed in eosinophils to mediate tumor cytotoxicity [106, 107]. These observations suggest that reprogramming the metabolism in eosinophils may trigger the degranulation and improve antitumor immunity.

5.3.4   Mast Cells

Mast cells are derived from bone marrow hematopoietic stem cell-differentiated precursor cells. These precursor cells enter the cavity or mucosa from blood and then mature. As an important class of innate immune cells, mast cells play a key role in allergic diseases such as asthma. After activation, mast cells not only produce and release a variety of cytokines and chemokines, including histamine, serotonin, interleukins, leukotrienes, prostaglandins, and proteases to promote inflammation, but regulate the functional activity of DC, T cells, CD4+ CD25+ regulatory T cells, B cells, and other immune cells in TME [108]. Similar to that in TAMs, PI3K-Akt activation in mast cells correlates with enhanced glycolysis [109]. The metabolic reprogramming of the tumor-associated mast cells is yet unknown.

5.3.5   CAFs

Fibroblasts are a large proportion of cells in the TME. These cells synthesize collagen, laminin, fibronectin, and other matrix components to establish a structural framework in the matrix. Fibroblasts can be activated during wound healing,

inflammation, and stress. The TME-activated fibroblasts called cancer-associated fibroblasts (CAF) or myofibroblasts specifically express α-smooth muscle actin (αSMA) [110]. Apart from the local fibroblasts, CAFs can be derived from vascular smooth muscle cells, pericytes, marrow-derived mesenchymal cells, and through epithelial-mesenchymal transformation (EMT) [111]. CAF can reshape the extracellular matrix by secreting matrix-degrading enzymes, particularly metalloproteinases and derivatives to contribute to tumor drug resistance [112]. CAFs promote tumor growth, angiogenesis, and metastasis by secreting several factors, including insulin-like growth factor, hepatocyte growth factor, basic fibroblast growth factor, Wnt ligands and MMPs, as well as cytokines and chemokines such as CCL7, CXCL12, and VEGF-A [110, 111].

It has been observed that CAFs engage in aerobic glycolysis. Mechanistically, IDH3α reduced by TGFβ or platelet-derived growth factor (PDGF) breaks the equilibrium between α-KG and fumarate/succinate that are allosteric regulators of PHDs, thereby in turn increasing HIF-1 transactivation and enhancing glycolysis [113]. CAFs also display increased glutamine metabolism and decreased OXPHOS [114, 115]. They provide lactic acid, amino acids, and ketone bodies to cancer cells, while cancer cells produce ROS to activate HIF-1 in CAFs to maintain the glycolysis [110, 111]. The metabolic change of CAFs impacts the secretion of cytokines and chemokines. For example, α-KG and fumarate/succinate are allosteric regulators of lysine-specific demethylase, PHDs, and methylcytosine demethylase that are epigenetic regulators [116]. Also, glycolysis and glutamine metabolism in CAFs are regulated by p62-mTORC1-c-Myc pathway that promotes ROS and IL-6 production and enhance tumor progression [114, 117].

Distinct from cancer cells, although CAFs favor glycolysis, the proliferation is much slower when compared with normal fibroblasts, suggesting that the biosynthesis of CAFs is not dependent on glycolysis [118]. However, cancer cells uptake the CAF-secreted lactate for tumor anabolic metabolism, growth, and metastasis [117]. GLUT4 is overexpressed on CAFs to release lactic acid, while GLUT1 is upregulated in cancer cells to import glucose and metabolites. The lactate released from CAFs further acidifies the TME to facilitate tumor progression and drug resistance [119]. Hence, CAFs not only secrete growth factors but also fuel cancer cells by providing lactate and other glucose metabolites. The cooperation of metabolites shuttling between CAFs and cancer cells aggregates the TME that facilitate tumor development.

5.3.6 *NK and NKT Cells*

Natural killer cells (NK cells) are a subtype of lymphocytes in innate immune system. They express Ly49, NCR, and CD16 and play antitumor action by secreting IFN-γ and cytotoxic molecules such as perforin and granzyme or by antibody-dependent cell-mediated cytotoxicity (ADCC) and T cell activation [120, 121]. Under resting state and short-term activation, they favor OXPHOS, while upon

prolonged activation by high-dose IL-15, NK cells switch to glycolysis [122]. In the TME, both IL-15 and hypoxia can lead to enhanced glycolysis in NK cells [123]. However, the mechanism by which TME affects the metabolic reprogramming and how the altered metabolism modulates the activity of NK cells remain to be elucidated.

NKT cells are a heterogeneous group of T cells that share properties of both NK cells and T cells. They recognize non-polymorphic CD1d (an APC molecule) that binds self and foreign lipids and glycolipids [124]. Upon activation, these cells produce abundant IL-2, IL-4, IFN-γ, GM-CSF, and IL-21 so that they play antitumor functions in TME [125]. However, the metabolic reprogramming in tumor-associated NKT cells is yet unknown.

5.3.7   Endothelial Cells

In the TME, vasculatures deliver nutrients and oxygen to the tumor, which is the basis of tumor survival and development. Tumor cells release pro-angiogenic signals to drive the metabolic reprogramming of endothelial cells (ECs) [126]. Inhibition of VEGF signaling is a clinically approved strategy, although the benefits are limited since tumors acquire drug resistance within months after treatment [127].

It has been found that the structure and function of vessels in TME and in normal tissues are significantly different. They are dilated, tortuous, and hyperpermeable. The ECs are poorly connected and lack a regular pattern. The basement membrane has nonuniform thickness and composition [128]. These cause deprived oxygen and nutrients. Hypoxia switches cancer cell metabolism away from OXPHOS to glycolysis, from glucose to glutamine as the major substrate for fatty acid synthesis (FAS). Tumor-associated ECs resemble cancer cells to undergo a shifting from quiescence to rapid growth during vessel sprouting. These ECs are highly plastic [126, 129]. They require a baseline glycolysis flux to function as an endothelium and maintain vascular barrier homeostasis. ECs preserve high concentration of oxygen in the blood. Also, ECs protect themselves from oxidative stress using glycolysis. They can also move from normoxic to hypoxic areas [130]. Glycolysis can produce ATP faster than OXPHOS. Hence, similar to cancer cells, glycolysis contributes to vascular sprouting and the survival and proliferation of ECs (Table 5.1) [131, 132]. Lactate dehydrogenase B, GLUT1, and glycolytic enzyme 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase-3 (PFKFB3) are increased in tumor-associated ECs. PFKFB3 promotes the synthesis of fructose-2,6-bisphosphate(F2,6P2) and the activation of 6-phosphofructo-1-kinase (PFK1) which converts fructose-6-phosphate (F6P) to fructose-1,6-bisphosphate (F1,6P2) [133]. Indeed, interfering with glycolysis by inhibiting PFKFB3 (by 3-(3-pyridinyl)-1-(4-pyridinyl)-2-propen-1-one (3PO)) or other key enzymes blunts angiogenesis-associated tumor growth [134]. However, the systemic complete and permanent inhibition of glycolysis may also induce undesired effects. Fortunately, Partial and transient reduction of glycolysis

Table 5.1 Cancer cell versus endothelial cell metabolism

	Cancer Cell	Endothelial Cell
Glycolysis	Warburg effect Upregulated through oncogenes and tumor hypoxia Switch PKM1 to PKM2	Compartmentalized Activated by growth factors
OXPHOS	Reduced TCA activity	Few mitochondria, low respiration Upregulated in low glucose and stress
Glycogen Metabolism	Glycogen metabolized under hypoxic stress	Glycogen synthesis Stores depleted in low glucose, not in hypoxia
FAO	Source of NADPH Response to oxidative stress	Possibly used for energy Might promote angiogenesis Energy source in low glucose
PPP	Redox homeostasis Regulate cell death via ROS	Redox homeostasis Might promote angiogenesis G6PD regulates ROS signaling High PPP for proliferation
AA Metabolism	Glutamine as nitrogen source for polyamine synthesis and anaplerosis Energy and biosynthesis	Glutamine as alternative energy source under stress GLS blockade reduces proliferation Glutamine inhibits NO production

renders ECs more quiescent without overt detrimental side effects [134]. Glycolytic metabolites such as lactate can be uptaken by ECs through MCT1. Instead of being metabolized, lactate induces HIF-1 activation and competes with α-KG to bind to prolyl hydroxylase 2 (PHD2), thereby enhancing the expression of angiogenesis-related genes [135, 136]. Therefore, the partial and transient reduction of glycolysis may be sufficient to inhibit pathological angiogenesis in the TME. These results indicate that targeting glycolysis in ECs inhibits angiogenesis, but the viability should be concerned.

The decreased supply of glucose in ECs can also be compensated by glycogenolytic production of glucose-1-phosphate (G1P), which can be converted into glucose-6-phosphate (G6P). The glycogenolysis-derived G6P only minimally contributes to energy production in normal or low glucose conditions. G6P catabolism might be important in the oxidative PPP (oxPPP) process to generate energy [137, 138]. PPP also produce ribose-5-phosphate (Rb-5-P) which can feedback into glycolysis through the biogenesis of F6P [139, 140]. Moreover, NADPH produced by PPP protects ECs against ROS, enhances NO synthesis, and contributes to ATP production [141]. Therefore, PPP may promote angiogenesis and regulates redox homeostasis.

ECs have a high level of glutaminolysis to support ATP synthesis and fuel cell proliferation in the conditions of decreased glucose supply (Table 5.1) [142]. ECs can uptake glutamine from extracellular milieu and also produce glutamine from glutamate. Inhibition of glutaminolysis induces EC senescence [143]. Glutamine metabolism also promotes ornithine synthesis, a precursor of mitogenic polyamines [144]. In addition, glutamine metabolite glucosamine can inhibit oxPPP and NO

production by reducing NADPH [145]. How glutaminolysis mediate angiogenesis has not been described.

Most ECs contain mitochondria that compose less than 5 % of the cell volume. The mitochondria-derived ROS activates HIFs by inhibiting PHDs, which in turn enhances glycolytic metabolism and angiogenesis [146]. Glycolysis-derived ATP also in part contributes to maintaining EC mitochondrial network. However, the role of mitochondria in tumorigenesis is yet unknown. FAO is induced in ECs upon glucose deprivation, in which process AMPK is activated [147]. Whether and how FAO influences angiogenesis is also unclear.

5.3.8   Dendritic Cells

DCs are divided into immature (imDCs), semi-mature (smDCs), and mature (mDCs). imDCs show low expression of MHC class I molecules, lack of B7 costimulatory molecules, etc. These cells induce immune tolerance since they cannot effectively activate T cells [148]. Recent studies showed that semi-mature and mDCs can also induce immune tolerance. smDCs have a unique feature which can be induced by granulocyte-macrophage colony-stimulating factor (GM-CSF), IL-4, and TNF with bone marrow cells in vitro. smDCs obtain a significantly different molecular phenotype, as compared with mDCs and imDCs, showing high expression of MHC class I molecules and moderate expression of costimulatory molecules, but they do not secrete inflammatory cytokines such as IL-1, IL-6, and IL-12 [149, 150]. These cells produce inflammation-inhibiting factors including IL-10 and can enhance the activation of CD4$^+$CD25$^+$ Tregs [151].

As a major population of professional antigen-presenting cells, DCs are activated and mature by sensing pathogen-associated or damage-related stimuli, displaying upregulation of MHC molecules and costimulatory molecules (e.g., CD80/86, CD40), cytokines (e.g., IL-12), and chemokine receptors (e.g., CCR7). Then DCs migrate to lymphoid organs to present antigens and activate T cells. However, tumor-associated DCs are immunosuppressive [152–154]. Moreover, increased imDC numbers and decreased mDC and DC numbers with impaired functions were observed in cancer microenvironment [149, 152].

The differentiation and function of DCs also rely on the metabolic reprogramming. Under resting state, DCs favor OXPHOS, while they switch to glycolysis after activation [155]. Also, glycolysis is essential for upregulated costimulatory molecules (CD80/86, CD40) and cytokines (IL-12) and DC survival [50]. Indeed, the activated DCs show increased NO production, enhanced PI3K-Akt activation, and impaired OXPHOS [50, 155].

In the hypoxic TME, HIF-1 drives the transcription of mTOR and glycolysis-associated genes, thereby promoting DC glycolysis, maturation, and activation [156]. However, hypoxia also inhibits the recruitment of monocyte-derived DCs from peripheral blood [157]. Hypoxia upregulates adenosine receptor (A2b), and adenosine can bind this receptor to blunt DC differentiation and switch them to a

Th2-promoting profile, i.e., express IL-10, TGFβ, and COX-2 that lead to abnormal differentiation of myeloid cells thereby causing Treg activation and DC defection [158]. Mechanistically, adenosine can induce AMP-activated protein kinase (AMPK) that promotes OXPHOS and inhibits glycolysis [50]. The lactic acid also contributes to inhibit DCs by inhibiting glycolysis [159]. These impaired DCs also secrete VEGF-A to promote angiogenesis and tumor growth [160].

PI3K-Akt activation also contributes to enhanced expression of GLUT1, the glucose transporter key for glycolysis, and disabled-2 adaptor (DAB2) on DCs which suppresses T cell response against tumors [161]. The mTOR inhibition by rapamycin in BMDCs promotes their antitumor activity, while mTOR inhibition in human monocyte-derived and plasmacytoid DCs blunts the immunostimulatory actions and T cell response [155].

Similar to macrophages, tumor-associated DCs (TADCs) can express enhanced ARG1, IDO, and iNOS that deplete arginine and tryptophan in the TME so that suppression of CD8$^+$ T cell function and survival is mediated [162]. Moreover, the retinoic acid metabolism in TADCs can promote Treg-mediated immunosuppression [163]. These amino acid metabolism pathways suggest potential targets for cancer immunotherapy. Recently, it was found that vaccines can trigger GCN2, a nutrient sensor, in DCs that in turn activate the antigen-presenting function of DCs and CTL response [164].

TADCs are also regulated by lipid metabolism. During DC activation, the FAS can promote the antigen presentation by increasing ER and Golgi expansion [165, 166]. The scavenging receptors such as CD36 and MSR1 upregulated in TADCs contribute to the uptake of lipids [167]. The lipid accumulation subsequently attenuates DC function and T cell activation. Hence, it has been proposed that the switch of glucose metabolism from glycolysis to OXPHOS in the TADCs may turn on the FAS and lipid uptake, therefore impairing DCs in TME and inducing an immunotolerogenic condition [155, 165, 168]. Furthermore, the limited nutrients in TME can induce ER stress that will induce excessive lipid accumulation via TCA cycle in TADCs and further inhibit CTL priming [169].

Abovementioned finding suggests that the altered metabolic programming in TADCs impairs their activation and antitumor function. Rectifying the abnormal metabolism may restore DCs' ability to reject tumor in the TME.

5.3.9 Myeloid-Derived Suppressor Cells (MDSCs)

MDSCs, originally discovered in the 1970s, are a group of myeloid immature cells in tumor-bearing mice [170, 171]. The morphology of these cells is similar to the granulocyte-monocyte progenitor cells that are yet to differentiate into macrophages, DCs, or granulocytes. These cells are CD11b$^+$Gr1$^+$ and can be subcategorized into monocytic (Ly6C^{high}Ly6G$^-$) and granulocytic (Ly6C^{low}Ly6G$^+$) based on the expression of Ly6C and Ly6G. In humans, MDSCs also express hematopoietic stem cell surface markers CD34 and immature marker CD31, hardly expressing

MHC class I molecules and maturation markers [172, 173]. MDSCs can secrete MMP9 and undergo endothelialization to induce angiogenesis and promote tumor survival and invasion [174]. With the development and progression of tumor, large amounts of cytokines, such as VEGF, IL-10, and TGFβ, can be released in the TME. These cytokines not only recruit MDSCs to tumor but also promote their differentiation into immunosuppressive cells [175]. On the other hand, MDSCs are immunosuppressive through multiple means: MDSCs can suppress the maturation and antigen presentation function of DCs directly or indirectly [176]. By direct interaction, MDSCs suppress IFN-γ production by CD8 T cells [177]. MDSCs secrete TGFβ, arginine enzymes, and reactive oxygen and nitrogen species to inhibit T cell activation and proliferation [177] and to promote Treg cell generation [178].

Glycolysis, glutaminolysis, and TCA cycle metabolism and arginine metabolism are upregulated during MDSC maturation. Fatty acid uptake and FAO mediate the immunosuppressive function of MDSCs in TME [179]. Amino acid metabolism and oxidative stress also mediate MDSC immunosuppressive functions. By expressing IDO, MDSCs metabolize tryptophan to kynurenine, induce Treg expansion, and inhibit T cell functions [180]. L-arginine and L-cysteine can be depleted by MDSCs, leading to the downregulation of CD3ζ and the inhibition of T cell activation [181, 182]. MDSCs express high levels of ARG and iNOS. ROS such as peroxynitrites are produced under the conditions of limited L-arginine availability. ROS induces T cell apoptosis by nitrotyrosylating and preventing tyrosine phosphorylation of key signaling proteins for T cell activation [183]. Peroxynitrites also nitrate TCR, IL-2R, and CD8 molecules, leading to T cell signaling disruption [184, 185]. Recent studies indicated that a lipid mediator derived from arachidonic acid via cyclooxygenase 2 (COX-2), i.e., prostaglandin E_2 (PGE_2), can be secreted by tumor cells and MDSCs [186, 187]. PGE_2 enhances MDSC development by inducing ARG, iNOS, and IDO and promotes MDSC recruitment to the TME by inducing CXCL12 [188]. Taken together, the metabolic reprogramming of MDSCs provided a potential target for regulating the immunosuppressive network in the TME.

5.3.10   T Cells

The infiltration and activation of T cells in TME control tumor progression. CD8+ T cells are the major effector cells in tumor immunity. Upon activation by APCs, CD8+ T cells migrate to the tumor tissue to kill target cells through perforin (to damage cell membranes), granzymes (to enter target cells and degrade DNA), and FasL. In addition, CD8 T cells secrete cytokines such as IFN-γ and TNFα to promote antitumor immune response [189]. CD4+ T cells differentiate into distinct subtypes to promote or repress tumorigenesis. CD4+ Th1 T cells produce IFN-γ to promote tumor immune rejection. Th1 cell infiltration in TME is associated with good clinical prognosis. In contrast, Th2 cells and Tregs temper tumor rejection and facilitate tumor immune escape. Th17 cells may both promote and inhibit tumor progression in a tumor-type and stage-dependent manner [190].

Table 5.2 T cell metabolism

	Naïve CD8	EffectorCD8	Memory CD8	Exhausted CD8	Th1	Th2	Treg	Th17
Glycolysis		+++			+++	+++		++
OXPHOS	+++		+	++			+++	
PPP		++			++			++
FAS		++						++
FAO	++		++	++			++	
Glutaminolysis	+	++		+	++	++		++
Tryptophan metabolism		++			++	++		++

For activated T cells to proliferate and release cytotoxic factors and cytokines, they switch the metabolism to aerobic glycolysis to increase the uptake of glucose and glutamine [191, 192]. However, the activation of PD-1 can inhibit the uptake and utilization of these nutrients and promote FAO, thereby inducing T cell anergy, exhaustion, and autophagy [193–195]. Since H^+ secretion increases when tumor cells release lactic acid, TME becomes acidic, leading to reduced T cell function [196].

In the TME, tumor cells compete for nutrients to hinder antitumor functions of T cells and lead to the metabolic reprogramming of T cells (Table 5.2). The efficiency in tumor cell uptake of glucose is ten times of that in activated T cells. Because glucose is the sole source of the energy required for effector T cells [192]. The lack of glucose severely affects IFN-γ production and the cytolytic activity of CTLs. Moreover, the accumulation of metabolic wastes in TME, such as lactate and kynurenine, can also inhibit T cell function [197]. Acidification and hypoxia of TME also impairs the proliferation and function of CTL [192, 196]. De novo FAS is critical for the development of effector T cells, while the generation and survival of memory T cells need FAO and OXPHOS [198].

Naive T cells get energy from OXPHOS, fatty acid oxidation (FAO), and low levels of glutaminolysis, whereas these cells need much more nutrient for activation [191, 192, 198]. Therefore, activated T cells show enhanced glycolysis, PPP, and glutaminolysis and decreased FAO (Table 5.2). Glycolysis is required for the functions of effector T cells, while PPP and glutamine metabolism are involved in biosynthesis. Upon activation, signaling pathways involving PI3K-Akt, mTOR, HIF-1, and c-Myc are triggered in CD8+, Th1, Th2, and Th17 cells to promote the expression of key factors in nutrient metabolism, such as GLUT1, PDK1, and HK2 [191–193, 198]. This in turn leads to further enhanced glycolysis and glutaminolysis. In addition, the activated T cells exhibit a noncanonical Myc-dependent transcriptome coupling glycolysis and glutaminolysis to polyamine biosynthesis to maintain T cell proliferation [198, 199]. The mTOR activation regulates the balance between effector and memory T cells by modulating T-bet, a key transcription factor for Th1 cell differentiation [200]. The energy sensor AMPK, activated by increased ratio of AMP and ATP, nutrient deprivation, and anti-inflammatory cytokines (e.g., IL-4,

IL-10, and TGFβ), suppresses IFN-γ and granzyme B production and induces OXPHOS and glutamine-dependent mitochondrial metabolism in T cells to suppress T cell-mediated antitumor response [201, 202]. Tumor-derived oxysterol induces LXR activation to inhibit neutrophil recruitment and DC migration, switches on M2 polarization, and suppresses T cell response to aid tumor immune tolerance [70].

The metabolism of amino acids, such as arginine, tryptophan, glutamine, and cysteine, is important for TIL functions (Table 5.2). For example, deficiency of arginine impairs protein synthesis in TILs, leading to reduced TIL activation [203, 204]. L-arginine metabolism is dependent on the activities of NOS and ARG. NOS converts arginine to NO and citrulline, and ARG hydrolyzes arginine into urea and ornithine. Administration of ARG and NOS-specific inhibitors can activate TILs [205]. Mechanistically, NO can react with ROS to produce reactive nitrogen species (RNS) such as peroxynitrite which induce lymphocyte anergy and apoptosis by nitration of tyrosine residues or the mitochondrial permeability transition pore (voltage-dependent anion channel) [184, 206]. Moreover, RNS modifies chemokines such as CCL2, by nitration or nitrosylation, to inhibit T cell infiltration into the tumor. Indeed, drugs targeting nitration in TME induce T cell infiltration [207]. Effector CD8+ T cells can also impair the CAF-mediated chemoresistance by inhibiting cysteine and glutathione metabolism in fibroblasts [208], suggesting a novel intersection for combined chemotherapy and immunotherapy in cancer treatment.

As mentioned earlier, tryptophan deprivation in TME contributes to tumor progression. IDO, the rate-limiting enzyme in tryptophan metabolism, inhibits the proliferation of effector T cells by depleting tryptophan in the TME [209, 210]. IDO is mainly expressed in mesenchymal cells, such as ECs, macrophages, and DCs. Tumor cells also express IDO upon IFN stimulation [211, 212]. By metabolizing tryptophan, IDO leads to the release of kynurenine. The reduction of tryptophan and the increase of kynurenine synergistically inhibit the activation and proliferation of antitumor T cells [209]. Therefore, IDO upregulation in cancer patients correlates with impaired T cell accumulation, proliferation, and function and poor prognosis. Agents inhibiting IDO, such as INCB024360 and 1MT, can promote the antitumor T cell function [209, 213].

A recent study revealed a new mechanism that cholesterol metabolism regulates the antitumor responses of CD8+ T cells [214]. Cholesterol is abundant in the plasma membrane, which is key for the TCR clustering and immunological synapse formation. The deficiency of ACAT1, a key cholesterol esterification enzyme, led to potentiated effector function and proliferation of CD8+ T cells. However, ACAT1 knockout in CD8+ T cells could not affect the glycolysis, OXPHOS, and FAO levels. Inhibition of PD-1 did not alter the expressions of ACAT1 and other cholesterol esterification genes. Combination of anti-PD-1 antibody and ACAT1 inhibition synergistically blunted the tumor development.

Tregs are generally divided into natural regulatory T cells (nTregs, CD4+CD25+) and induced regulatory T cells (iTregs, CD4+CD25−), both of them express Foxp3. They express IL-10, TGFβ, and IL-2 receptor α-chain (CD25), but do not produce IL-2. Tregs suppress immune response and T cell activation through cell-cell inter-

action and cytokines [215, 216]. Tregs are present in various tumors and play an immunosuppressive role. AhR-enhanced IDO1 and kynurenine mediate Treg generation [217], while mTOR activation inhibits Treg development [218]. Thus the elimination of Tregs in the TME, to some extent and excluding in colorectal cancer, inhibits tumor growth. The function of regulatory T cells (Tregs) is not affected by lactic acid and acidic environment. Of interest, Tregs favor OXPHOS, FAO, and activated AMPK for the nutrient metabolism (Table 5.2) [218–220]. Hence they survive well in the nutrient-deprived TME. Moreover, the HIF-1 upregulation in the hypoxic TME promotes Treg expansion [221]. Interestingly, HIF-1 shows an important metabolic checkpoint for the differentiation of Tregs or Th17 cells [222].

Th17 cells are a newly discovered class of T helper cell subsets. Naive CD4$^+$ T cells preferentially differentiate to Th17 under the stimulation of TGFβ and IL-6. In addition, IL-23 is a key factor for the maintenance and expansion of Th17 cells [223]. Recent studies found that the presence of Th17 in the TME antagonizes the IFN-γ-producing Th1 cells to favor tumor growth [224]. Th17 cells also rely on glycolysis and FAS for differentiation and activation [225] (Table 5.2).

5.3.11   B Cells

By producing antibodies and immune complexes, B cells can regulate the functions of myeloid cells to promote tumor growth. It has been reported that c-Myc, but not HIF-1, mediates LPS and antigen-stimulated activation of B cells and triggers the glycolysis and mitochondrial metabolic activity. Tumor cells express BAFF which can also induce the glycolysis and antibody production of B cells by a GLUT1-dependent manner [226]. Therefore, metabolic reprogramming of B cells will be also of interest to be investigated to better understand the immunoediting in tumor progression.

5.4   Clinical Diagnostic and Therapeutic Applications

5.4.1   PET/CT and PET/MRI

Cancer cells favor glycolysis to metabolize glucose, regardless of oxygen tension, which is termed as Warburg effect. Anaerobic glycolysis (fermentation) is more rapid but less efficient than OXPHOS to generate ATP. This process produces lactate and contributes to immunosuppression. At present, Warburg effect has offered an opportunity to diagnose and monitor therapy response in many clinical cancers. Position emission tomography-computed tomography/magnetic resonance imaging (PET/CT or PET/MRI) is a clinical imaging technique combining PET and CT/MRI [227–230]. PET imaging shows the spatial distribution of metabolic activity

(especially the Warburg effect), while CT/MRI precisely aligns the anatomic imaging. PET with distinct radiotracers can evaluate altered metabolisms of glucose, fatty acids, amino acids, and other cancer markers. Hence, molecular imaging with PET is very precise for detection and directing therapy and has been applied widely in several cancer types.

5.4.2   PD-L1, PD-1, CTLA-4, and IDO

Recent breakthrough in cancer immunotherapy based on the clinical application of monoclonal antibodies targeting T cell immune checkpoints, including PD-1 and CTLA-4, clearly demonstrates the significance of effector T cell activation in antitumor response [231]. PD-L1:PD-1 and CTLA-4 signaling dampens antitumor responses. The expression of PD-L1, a key immune checkpoint, can be induced by hypoxia in tumor-associated DCs. PD-L1 is a target gene of HIF-1 and NF-κB, two central transcriptional factors in hypoxic responses. PD-L1/PD-1 pathway inhibits glycolysis and promotes FAO and lipolysis to mediate T cell metabolic reprogramming [232–234]. Of interest, PD-1 is also expressed in cancer cells such as melanoma cells. Activation of melanoma-PD-1 promotes tumor progression by mTOR pathway [235]. Preclinical data suggested that inhibition of PD-1 and prostaglandin E synthases synergistically promotes tumor eradication [236]. Therefore, it has been proposed to use PD-1 as a radiolabeled PET imaging tracer to efficiently distinguish PD-L1-positive and PD-L1-negative tumors.

CTLA-4 is a target gene of Foxp3 and has a major role in enhancing Treg activity and suppressing T helper cells [237]. IDO activity can be induced by CTLA-4 in plasmacytoid DCs via reversing CD80 signaling [238]. The increase of IDO, PD-L1, and CTLA-4 in the peripheral blood of cancer patients correlates with advanced disease and poor outcome, independent of the stages of cancer [239, 240]. Therefore, combination treatments targeting several of these markers to modulate metabolisms in immune cells may have a synergistic effect.

5.4.3   CAR-T

Cancer immunotherapy based on the adoptive transfer of autologous T cells has shown promising efficacies. Chimeric antigen receptor (CAR)-T cells have been used to exert potent antitumor effect [241, 242]. CARs consist of cytoplasmic domain of the Fc receptor γ chain or CD3ζ modules and that of costimulatory cytoplasmic domains such as CD28, 4-1BB, and ICOS [243]. Little is known about the metabolic reprogramming of CAR-T cells. As described in the above section, naïve and memory T cells rely on fatty acid oxidation, while activated effector T cells shift to glycolysis and enhanced OXPHOS. A recent report showed that CD28 or 4-1BB CD3ζ CAR-T cells exhibited increased survival and proliferation, promoting

central memory T cells. CD28-CD3ζ CAR-T cells favored aerobic glycolysis, while 4-1BB-CD3ζ CAR-T cells preferred FAO [243]. The choice of CAR impacted the T cell metabolic reprogramming and differentiation, suggesting that modulating the metabolism in T cells may also be important to enhance the antitumor effects of CAR-T cells.

5.4.4 Drug Repositioning

Drug repositioning, also known as drug repurposing, re-profiling, re-tasking, or therapeutic switching, is the application of known drugs and compounds to new indications. It is an emerging and important application in drug development for cancer therapy [244]. The computational approaches can enhance the efficiency and success rates, particularly in terms of high-throughput shotgun repurposing. For example, proton pump inhibitor (PPI) is an acid-activated drug that inhibits H/K--ATPase to treat gastric cancer. PPI can synergistically modulate the acidic TME with chemotherapy and improve chemoresistance [245, 246]. Terfenadine is a histamine receptor H1 antagonist, which can prevent VEGF secretion from mast cells in hypoxic microenvironment and induce ROS-mediated apoptosis and autophagy of melanoma cells [247]. Simvastatin specifically inhibits HMG-CoA reductase so that it restrains p53 mutation from activating mevalonate pathway for cholesterol synthesis in breast and ovarian cancer cells [248]. System xc⁻ cystine/glutamate antiporter, a heterodimer composed of the 4F2 heavy chain (SLC3A2) and the light chain xCT, is a membrane amino acid transporter that mediates the exchange of extracellular cystine and intracellular glutamate [249]. Sulfasalazine is a specific inhibitor of xCT cystine transporter. It blocks the reduced GSH synthesis, leading to oxidative stress in cancer cells, resulting in the suppression of NSCLC and gastric tumor progression and breast cancer metastasis [249–251]. Metformin is an oral drug used to treat type 2 diabetes mellitus by suppressing glucose production by the liver [252]. Recently, metformin was reported to inhibit tumor progression and ameliorate the prognosis [253–255]. By inhibiting ATP-binding cassette subfamily G member 1 (ABCG2) and ectonucleotide pyrophosphatase/phosphodiesterase family member 1 (ENPP1), metformin suppresses cancer cell chemoresistance to drugs [256]. These drugs are being applied in clinical trials and show promising results.

5.4.5 Metabolism-Based Antiangiogenic Therapy

The current paradigm for anti-angiogenic therapy is to block VEGF and VEGFRs [257]. However, the tumor cells can rely on other signaling pathways for proangiogenesis. The hypoxic conditions caused by treatment often lead to the outgrowth of resistant tumor clones [258]. The EC metabolism requirement potentially provides novel anti-angiogenesis therapeutic opportunities. Silencing PFKFB3 by

3PO is capable to reduce EC glycolysis and vessel sprouting, without switching to aerobic respiration [134]. More importantly, the effect of 3PO on glycolysis is reversible, as normal sprouting was recovered after administration for 6 h in vivo The moderate reduction in glycolysis by 3PO is sufficient for increasing the fraction of quiescent ECs and reducing EC proliferation and migration [132, 133]. Combination of 3PO and VEGFR tyrosine kinase inhibitor SU5416 significantly impaired angiogenesis, as compared with the optimal doses of any one of these inhibitors alone [134]. In addition, 3PO is a chemotherapeutic agent to block tumor proliferation [259]. Together these findings indicate that targeting tumor-associated EC metabolism is a potential therapeutic strategy.

5.5 Concluding Remarks

The metabolism of cancer cells and immune cells in TME is instrumental for tumor initiation, progression, and metastasis. The deprivation of nutrients from the environment suppresses antitumor immune cells, such as CD8$^+$ T cells, M1 macrophages, and N1 neutrophils, promoting the differentiation and activation of pro-tumor immune cells, including MDSCs, M2 macrophages, and Tregs [260, 261]. The extent to which metabolism pathways represent true vulnerabilities for tumor development remains unclear. Targeting glycolysis, glutaminolysis, and FAO has provided clinical benefits; the strategies integrating redox homeostasis and PPP may also generate new opportunities [9, 10, 119].

The most serious challenge in reshaping the immune profiles in TME is to understand the metabolic heterogeneity which is extremely complex depending not only on tumor and immune cell types but also on tumor stages and etiology (Fig. 5.3). Activation of signaling pathways including PI3K-Akt, mTOR, HIF-1, c-Myc, etc. in tumor-associated immune cells regulates their metabolism for survival, differentiation, and pro- or antitumor functions [10]. For example, HIF-1 activation in cancer cells and immune cells may upregulate glycolytic metabolism and enhance cancer-related inflammation during the initiation. In the tumor progression stage, HIF-1 elevation in TAMs, TADCs, MDSCs, and Tregs contributes to immunosuppression and angiogenesis by PD-L1 expression, lactate release, and adenosine-adenosine receptor interaction that facilitate tumor growth [32, 46, 58, 60, 136, 221, 232, 233]. Other factors and molecules, such as noncoding RNAs, complements, and coagulation-related factors, also regulate immune cell survival, differentiation, and functions in TME [262–264]. However, whether and how these molecules regulate metabolic reprogramming in tumor-associated immune cells will be of interest to be investigated.

The metabolite exchange adds an essential dimension of heterogeneity in the TME to contribute to tumor growth, metastasis and clinical resistance [12, 260, 261, 265]. Nonetheless, we posit that targeting the immune cell metabolism in TME in addition to the traditional cancer therapies will lead to more precise and efficient diagnosis and treatment of these fatal diseases.
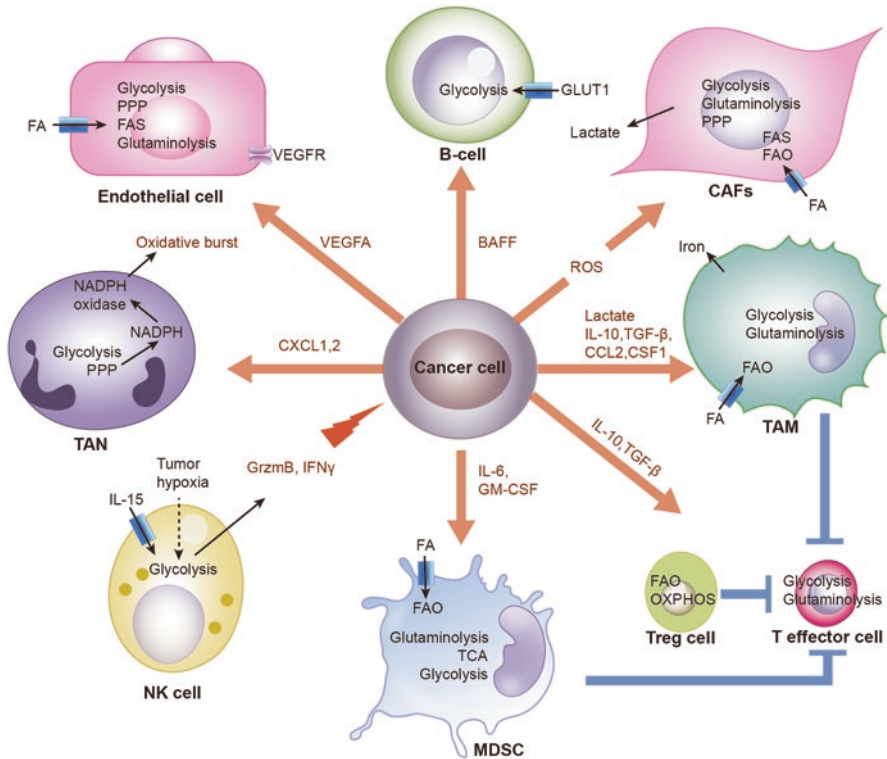
Fig. 5.3 Metabolic crosstalk between cancer cells and TME-immune cells

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