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Immuno-Oncology Crosstalk and Metabolism

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Immune Cell Metabolism and Function

1

Ajay Dixit and Mahendra Singh

Abstract

Immune cells are highly dynamic by nature and rely on metabolism to adapt to different conditions. Many recent studies have shown the importance of immune cells and their metabolism in the pathogenesis of many diseases. Cellular metabolism acts as a guiding force to regulate immune cell activation, differentiation, and cellular behavior, thus regulating the extent of the immune response. Here in this chapter, we have discussed different metabolic signatures and pathways that control the activation status of immune cells and how the change in the metabolic status affects the immune response, disease pathobiology, and homeostasis especially in cancer.

Keywords

Metabolism · Immune-metabolite · Glycolysis · Lipids · Amino acids · Redox

1.1 Introduction

Immune cell metabolism refers to the study of changes in intracellular metabolic pathways that take place in immune cells during the process of immune activation that results in the alteration of their function. Recent studies of metabolic pathways in immune cells particularly in the last several years have clearly demonstrated a complex interplay between immunity and metabolic reprogramming, which presents

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an extra layer of complexity for us to understand the role of the immune system in health and disease. Most relevant metabolic pathways to the immune cells are glycolysis, tricarboxylic acid (TCA) cycle, pentose phosphate pathway, fatty acid synthesis, fatty acid oxidation, and amino acid. Immune cells have characteristic metabolic pathways which are specific their lineage and phenotype. For example, fatty acid synthesis and glycolysis are key features of lipopolysaccharide (LPS)-activated macrophages, while interleukin-4 (IL-4)-activated macrophages primarily depend on the use of oxidative phosphorylation and fatty acid oxidation to generate energy. Similarly, T cells have their own characteristic metabolic pathways: for example, memory T cells are characterized by oxidative metabolism, where as effector T cells are highly glycolytic in nature.

Metabolism of these immune cells is so important that enzymes such as glyceraldehyde 3-phosphate dehydrogenase (GAPDH), pyruvate kinase isoenzyme M2 (PKM2), and enolase as well metabolites like succinate and citrate have important role in promoting specific event in immune cell activation process. Recent advances in the field of immunotherapies especially in the area of immune oncology have provided a body of evidence suggesting that small molecules that can target metabolic pathways and have potential to alter the phenotypes of immune cells, are now being studied and developed as possible therapeutic intervention strategies.

Interestingly, Early discoveries of cellular metabolic pathways were historically conducted on cells of lymphoid origin such as lymphocytes. Usually when lymphocytes prepare themselves for an immune response they undergo a high degree of activation and proliferation. However, the characterization of energy metabolism of other cell types such as myeloid cells and natural killer (NK) cells has also received an increasing amount of attention. A large number of studies have demonstrated that myeloid cells undergo unique metabolic reprogramming after stimulation of the Toll-like receptor 4 (TLR4) using LPS [1, 2].

Myeloid cells undergo a switch from oxidative phosphorylation to glycolysis despite the abundance of oxygen. It is important to note that activated lymphocytes also exhibit similar metabolic signature. These observations prompted researchers to hypothesize that activated myeloid cells exhibit a general characteristic feature such as “Warburg effect” (aerobic glycolysis) during the induction of innate immunity [3, 4].

Myeloid cells are one of the major stromal populations in the tumor microenvironment (TME) and comprised of macrophages, myeloid-derived suppressive cells (MDSCs), and dendritic cells (DC) [5–7]. Myeloid cells support the development and maintenance of immunosuppressive microenvironment in various cancers for which these cells have to undergo necessary metabolic adaptations and [8, 9] and due to the fact that myeloid cells play an important role in the activation of both adaptive and innate immune responses, we will first discuss the metabolic diversity underlying the myeloid mediated immune responses while specifically emphasizing how change metabolism alters myeloid cells-generated immune response.

1.2 Macrophages

Macrophages are found in almost every tissue type and are crucial for maintaining immunity and homeostasis [10]. As the name suggests they are the *big eaters*; they detect, engulf, and destroy pathogens. Additionally, they act as specialized antigen-presenting cells where they present the digested antigens on MHC II to activated adaptive immunity. Interstingly, Macrophages are naturally highly plastic in nature and can change their phonotype based on the cues in the tissue microenvironment and can originate in tissues either from yolk sac during gestation period ex: tissue resident macrophages [11] or bone marrow ex: monocytes derived macrophages, which accumulates in tissues during inflammation via CCL-2/CCR-2 pathways [12]. Of note, Tissue-resident macrophages can maintained by self-replication and are longed lived whereas monocyte-derived macrophages are terminally differentiated and short lived [13, 14].

Phenotypically they are traditionally classified into two major types: M1 and M2. M1 macrophages known also as classically activated and are activated by IFN-γg and/or LPS, known for their proinflammatory nature while M2 macrophages also known as alternatively activated and are obtained by activating by interleukin (IL)-4 and IL-10 and have immunosuppressive and wound-healing functions [15, 16]. However, many recent studies have challenged this over -simplistic dichotomous classification and suggests that macrophages display rather broad spectrum of phenotype representing various activation stages which may not fit in this dichotomous M1 and M2 phenotypic classification [17, 18]. Future studies are required to understand how these diverse macrophages effects disease progression and outcome.

While the role of macrophages in adaptive and innate immunity has been known for over a century and macrophage biology is an extensive area of research in many pathological conditions such as atherosclerosis [19], diabetes [20], and pancreatitis [21], their role in cancer biology has been recognized more recently as much as that macrophages are considered to be the seventh hallmark of the cancer [22]. This chapter mainly focuses on macrophage metabolism in tumors. Macrophages are considered as the largest myeloid cell population that are known to infiltrate many types of solid tumors [6] which is why they are also commonly referred as tumor-associated macrophages or TAMs. Almost all stages of tumorigenesis like initiation, progression, immunosuppression, metastasis, as well as resistance to therapies are known to involved TAMs [23–28]; hence, it is an active area of research in immune oncology [29]. TME is rich in metabolite which are generated as result of myriad of biochemical pathways operating in cancer and stromal cells. TAMs compete with other cell types especially cancer cells for available metabolites and thus are forced to reprogram their metabolism in order to survive and maintain their phenotype. Although many biochemical pathways and metabolites posses the ability to modulate immunity but for simplicity sake we have focused on major pathways/metabolite as consequences of carbohydrates, amino acid and lipid metabolism.

1.2.1 Carbohydrate Metabolism

Glycolysis is one of the most simple metabolic pathways where glucose is converted into pyruvate and generates two molecules of ATP in the process. Besides ATP, glycolysis also produces several intermediate metabolites required for ribose, amino acids, and fatty acids metabolism supporting cells' basic needs. The pyruvate generated at the end of glycolysis usually enters mitochondria and participates in oxidative phosphorylation to produce more ATP molecules.

Macrophage plasticity is supported by metabolic shift between glycolysis and oxidative phosphorylation [30]. Proinflammatory M1 macrophages show high dependency on glycolysis [31] whereas M2 cells with anti-inflammatory functions are more dependent on oxidative phosphorylation (OXPHOS) [32] suggesting that a shift between different glucose metabolic pathways is necessary to support a particular phenotype. Most tumors are hypoxic in nature in which Hif-1a, a transcription factor central to hypoxic response in all types of cells, plays an important regulatory role. Hif-1a regulates many key enzymes involved in glucose metabolism like pyruvate dehydrogenase kinase 1 (Pdk1), glucose transporter 1 (Glut1), phosphoglycerate kinase 1 (Pgk1), glucokinase (Gck), lactate dehydrogenase, and pyruvate kinase isozymes M2 (Pkm2) [33]. The upregulation of enzyme Glut1 plays a crucial role in supporting the glycolytic activity of M1 macrophages [34]. Besides, there are more such enzymes which play important roles in all this, for example lactate dehydrogenase which converts pyruvate to lactate and takes it away from mitochondria, and pyruvate dehydrogenase kinase which is known for inactivating pyruvate dehydrogenase and limiting pyruvate entry into the Krebs cycle, thereby reducing OXPHOS. Further, the conversion of pyruvate into lactate is very important for maintaining NAD+ levels and its flux through the glycolytic pathway.

Another important pathway is pentose phosphate pathway (PPP) which is involved in maintaining M1 phenotype. PPP is important for maintaining NADPH pool inside the cell. The oxidative phase of PPP converts $NADP^+$ to NADPH., Enzyme NADPH oxidase utilizes NADPH to generate reactive oxygen species (ROS). ROS regulates several functions in macrophages including but not limited to phagocytosis, bacterial killing, and polarization [35]. Mitochondrial ROS also help in the secretion of cytokines like TNF-α, IL-6, and IL-1β. In a recent study led by Bulua et al., it has been demonstrated that defects in mitochondrial ROS result in reduction in inflammatory cytokine production after LPS stimulation suggesting a role for ROS in maintaining inflammatory phenotype. Unsurprisingly, M2 macrophages have lower capacity to generate ROS [36].

The deletion of gene encoding 6-phosphogluconate dehydrogenase (PGD), which converts 6-phosphogluconate into ribulose 5-P, is known to reduce proinflammatory response in macrophages in a medical condition called hypercholesterolemia indicating the important role of PPP [37]. In a study by Haschemi et al. [38], it has been shown that carbohydrate kinase-like protein, CARKL, catalyzes the conversion of sedoheptulose into sedoheptulose-7-phosphate which is an orphan reaction in the PPP. This is how CARKL helps the refocusing of cellular metabolism to a high redox state upon physiological or artificial downregulation. These investigators also

reported that CARKL-dependent metabolic reprogramming is required for both M1- and M2-like macrophage polarization. Overexpression of CARKL in macrophages results in defective M1 polarization as well as dampened inflammatory response [38]. CARKL promotes the non-oxidative steps in the pentose phosphate pathway, which can in turn lead to increased ribose-5P production, required for nucleotide and UDP-GlcNAC synthesis. UDP-GlcNAC is essential for the process of N-glycosylation of the key M2-specific proteins such as CD206, which is abundantly expressed on the surface of M2 macrophages. M2 macrophages express higher levels of the glycolytic enzyme 6-phosphofructo-2-kinase B1 (PFKFB1), which breaks down fructose-2,6-bisphosphate [39], an activator of glycolysis to fructose-6-phosphate, resulting in reduced glycolytic rate.

1.2.2 Amino Acid Metabolism

Both M1 and M2 macrophages utilize arginine metabolism differently [40]. M1 macrophages metabolize L-arginine into nitric oxide (NO) and L-citrulline. The NO generated in this process has tumoricidal properties. On the other hand, M2 macrophages upregulate ARG1, which catalyzes the conversion of L-arg to L-ornithine, and polyamine synthesis [41]. Polyamines are known to support tumor growth [42].

ARG1 is a urea cycle enzyme that has long been known as a marker of alternatively activated macrophages. ARG1 converts L-arginine into ornithine and urea. Even though the function of macrophage-derived ARG1 is not fully well understood, recently a large number of reports have emerged which indicate that in hypoxic conditions lactate can mount the expression of the ARG1 gene [43, 44]. A genetically engineered mouse model in which ARG1 has been knocked out in their macrophages develops significantly smaller tumors than their wild-type counterparts, suggesting that tumor progression can be influenced by macrophage-derived ARG1 [45]. Interestingly, in the same study it was shown that TAMs have increased expression of all enzymes from the urea nitrogen cycle, as compared to the tumor cells.

In another study, TAMs either isolated from glioblastomas or co-cultured with cell lines derived from glioblastoma were reported to have increased expression of genes pertaining to glutamate transport and its metabolism. This is of particular importance because this shows that glioblastoma tumor microenvironment contains large amounts of glutamate [46]. Furthermore, tryptophan metabolism, L-arginine-derived metabolites, and cysteine/cysteine play important roles by mediating the immunosuppressive activity of MDSCs. Therefore, production of high levels of NO may also help mediate the immunosuppression of MDSCs [1].

1.2.3 Lipid Metabolism

Lipids play critical roles during macrophage polarization [47] and metabolic reprogramming in macrophages is linked to their activation. M1 macrophages can kill pathogens by sustaining inflammatory responses, mediated by their reliance on aerobic glycolysis and fatty acid biosynthesis. While glycolysis is a way of producing ATP at a faster pace in the M1 macrophages, but fatty acids act as precursors for the synthesis of inflammatory mediators in the M1 macrophages. On the contrary, anti-inflammatory M2 macrophages mediate the resolution of inflammation and tissue repair, switching their metabolism to fatty acid oxidation and oxidative phosphorylation. However, discoveries in recent years have challenged this classical view and suggest towards a rather complex metabolic network during macrophage activation. It has been shown that lipid metabolism plays a critical role in the activation of both M1 and M2 macrophages. A body of work demonstrates that inflammasome activation in M1 macrophages essentially occurs owing to the fatty acid oxidation while glycolysis plays a crucial role in fueling fatty acid oxidation in M2 macrophages [48].

However, metabolism of macrophages during activation is way too more complex than it has been thought; therefore, in order to unravel the metabolic signature of macrophages more studies are needed. It is important to note that most studies in this regard have been conducted in mouse models while many differences exist between human and murine macrophages in terms of gene expression signatures and corresponding metabolic pathways activated during polarization. This makes the extrapolation of these research findings difficult from mouse models to human subjects, especially in order to determine as to whether or not the reprogramming of macrophage polarization by metabolic interventions would be helpful in the treatment of human diseases [1].

Environmental cues are also crucial in order to determine the course of cell metabolism in macrophages. This is particularly important for those tissue macrophages which encounter a specific set of environmental signals. Furthermore, the presence of chronic inflammation may alter the tissue microenvironment, not only in promoting the influx of macrophages but also in affecting the metabolic signature of resident macrophages. Therefore, the tissue microenvironment plays an important role in determining the chronicity or severity of various diseases characterized by inflammation. For example, recent observations made in tumor-associated macrophages (TAM) have shown that the metabolic routes used by TAMs are greatly influenced by tumor cell-derived compounds such as lactate, which instigates proinflammatory reprogramming and prompting tumor angiogenesis [49]. Recent studies indicate that in addition to TAMs, tissue microenvironment-dependent metabolic rewiring of immune cells accumulating in the vessel wall or in the joints promotes inflammation and disease progression in certain diseases such as atherosclerosis and rheumatoid arthritis [50, 51]. There is a great body of evidence that suggests that targeting lipid metabolism in macrophages might improve the outcome of metabolic diseases as well as it could be a key to therapeutic strategies in tumor tissues [52–54].

1.2.4 Others

In a recent study, an age-related increase in the production of lipid messenger prostaglandin E2(PGE2) was observed in the mouse model of Alzheimer's disease. PGE2 binds to receptor protein EP2 on the cells, which in turn results in suppression of oxidative phosphorylation and glycolysis pushing macrophages into an energy-deficient state limiting the beneficial functions of macrophages and increase in inflammation. Inhibition of EP2 restores the function of macrophages and protects the aging brain [55].

1.3 Myeloid-Derived Suppressor Cells (MDSCs)

Myeloid-derived suppressor cells are a group of immature myeloid cells which are highly heterogenous in nature that possess potent immune-suppressive properties. MDSCs are comprised of two major subsets: polymorphonuclear (PMN)-MDSCs and monocytic (M)-MDSCs. Phenotypically, murine PMN-MDSCs are characterized as CD11b+Ly6ClowLy6G+, while M-MDSCs are CD11b +Ly6ChiLy6G−. Human PMN-MDSCs are instead defined as HLA-DRlow/− CD14−CD11b+CD15+, while M-MDSCs as CD11b+CD14+HLA−DRlow/− CD15− [56, 57]. However, it is difficult to discriminate proinflammatory cells such as neutrophils and inflammatory monocytes from immunosuppressive PMN- and M-MDSCs as all these cells share cellular origin and phenotypic markers.

Suppression of antitumor immunity by MDSCs is well established [58, 59]. In the patients with cancers, MDSCs' occurrence is regulated by a network of transcriptional regulators which promote immature and immunosuppressive activation of myeloid cells. STAT3 was the first transcription factor that was characterized for its capability to drive MDSC expansion and accumulation [60]. NF-κB and JAK/STAT signaling is known to upregulate immunosuppressive molecules such as inducible nitric oxide synthase (iNOS) (in M-MDSCs), arginase 1 (ARG1) (in PMN-MDSCs), and reactive oxygen species (ROS) [61]. Several cytokines and chemokines (e.g., G-CSF, CCL2, GM-CSF, CXCL1) induce the mobilization of MDSCs from bone marrow to the peripheral lymphoid organs and to the TME [62], where they promote tumor immune evasion. Further, MDSCs are known to express higher level of PD-L1 which inhibits T-cell activity [63, 64]. The role of AMPK in regulating MDSC immunosuppressive functions has also been reported. It has been shown that increased level of phosphorylation and activation of adenosine monophosphate-activated protein kinase leading to reduction in NO production also reduce IL-6 levels and inhibit MDSC migration suggesting that AMPK can be a potential drug target to reduce immunosuppressive behavior of MDSCs in tumors [65].

1.3.1 Carbohydrate Metabolism

MDSCs have been demonstrated to exhibit the Warburg effect while they are going through their maturation with high glucose and glutamine uptake rates as well as reduction in their oxygen consumption rate (OCR). Approximately 95% of the total ATP generated in the MDSCs is obtained through a glycolysis-dependent mechanism [66]. The metabolic reprogramming of cancer cells such as the use of aerobic glycolysis (the Warburg effect) affects the tumor microenvironment and infiltrating immune cells through changes in glucose metabolism. M-MDSCs are known to get differentiated into M1- or M2-like TAMs and to TNF-α and inducible nitric oxide synthase (iNOS)-producing dendritic cells (DCs) in the tumor microenvironment, while monocytes also convert into monocytic-MDSCs (M-MDSCs) [67]. While going through maturation and activation, tumor-derived MDSCs exhibit an increase in central carbon metabolism, including glycolysis, PPP, and TCA cycle. Granulocytic-MDSCs (G-MDSCs) have also been demonstrated to utilize both glycolysis and OXPHOS in mouse models of various types of cancers [68].

Tumor-derived MDSCs show upregulation in the glycolysis, and its metabolite produced during this process known as phosphoenolpyruvate could protect MDSCs from apoptosis and contribute to their survival [66]. Owing to the high uptake rates of glucose in both tumor cells and MDSCs, immune cells do not have any metabolic plasticity in order to acclimatize to the condition of low oxygen tension and limited glucose availability, which could result in immune cell dysfunction and death, indirectly facilitating tumor escape and progression.

In addition to glycolysis, another metabolic activity in these cells which is known as glutaminolysis plays an important role in ensuring an adequate supply of intermediates and energy during tumor progression. A recently conducted study demonstrates that glutaminolysis supports the maturation and immunosuppressive function of MDSCs through iNOS activity in vitro [69]. Thus, a growing body of studies now point to the high metabolic plasticity of immune cells, which can change their differentiation and function according to the context required.

1.3.2 Amino Acid Metabolism

In TME, MDSCs in the presence of IFN-γ show higher uptake of L-Arg by inducing the cationic amino acid transporter 2 (CAT2), iNOS, and ARG1. Further, depletion of L-Arg by G-MDSCs blocks CD3zeta expression in T cells leading to the inhibition of antigen-specific T-cell proliferation. Ablation of CAT2 impairs L-Arg uptake and reduces immunosuppressive and pro-tumoral activities of MDSCs [70]. MDSCs are known to help sequester L-cysteine, thereby causing its deprivation in the tumor microenvironment. L-cysteine deprivation also decreases the expression of CD3zeta and inhibits T-cell proliferation. Thus, MDSCs can effectively block the activation of T cells by sequestering cysteine, as T cells lack the cystathionase required to convert methionine to cysteine [71]. Metabolites derived from L-Arg metabolism

such as tryptophan and cysteine play an important role in regulating the immunosuppressive activity of MDSCs [72].

1.3.3 Lipid Metabolism

A subset of MDSCs that infiltrate tumors undergo both metabolic and functional reprogramming to become highly immunosuppressive cells so as they could support tumor growth. MDSCs express lectin-like oxidized low-density lipoprotein receptor-1 (LOX-1) that in turn enables these cells to specifically associate with endoplasmic reticulum (ER) stress and lipid metabolism, which possess potent immunosuppressive activity promoting T-cell-suppressive functions [72, 73]. Further, high PPAR-γ activity restrains ROS production in G-MDSCs [74], thereby helping in the processes of impairing cancer cell proliferation and metastasis.

1.4 Dendritic Cells

Dendritic cells (DCs) are a subtype of antigen-presenting cells which undertake a job on capturing antigens coming from tumors or pathogens and presenting these antigens to the T cells so as to invoke immune response. Cellular processes such as the development, polarization, and maturation of DCs are controlled by the metabolism of DCs as metabolic pathways provide energy support for these cellular processes as well as for the functions of DCs [75]. However, the immune functions of DCs in tumor microenvironment (TME) are generally inhibited. Abnormal metabolism of tumor cells results in metabolic changes in TME, such as hyperglycolysis, lactate and lipid accumulation, acidification, and tryptophan deprivation, leading to the limited function of DCs and occurrence of tumor immune escape [76]. Metabolic regulation of DCs in combination with immunotherapy can strengthen the ability of antigen presentation and T-cell activation of DCs, improve the existing antitumor therapy, and overcome the defects underlying DC-related therapies in the current stage, which has great potential in oncology-focused therapies.

Glucose is vital for migration of DCs to C-C motif chemokine ligand 21 (CCL21). Blocking glycolysis results in the destruction of the optimal migration of DCs to the draining lymph nodes. Activated form of DCs depends on glycolysis and PPP to maintain their energy production and membrane integrity; they also provide elements for the generation of an inflammatory mediator, and sustain their ability to migrate [77]. Inhibition of glycolytic pathway impairs various functions of DCs including antigen presentation, T-cell stimulation, and cytokine production [78]. Surface of DCs does have MHC II proteins; upregulation in the expression of MHC II on the surface of DCs requires molecule redistribution of endocytic compartments via lysosome tubulation [79], which also needs energy support.

Fatty acid metabolism has been shown to be critically involved in the development, maturation, and function of the DC [80]. Because of its integration with

mitochondrial function, the fatty acid synthesis (FAS) affects the derivation of DCs, which can not only block monocyte-derived DC formation from human PBMCs but also prevent the generation of DCs in primary and secondary lymphoid organs. FAS also helps decrease the expression of MHC II leading to the increased CD40 expression on the DC surface. Further, oxidized lipoproteins can accumulate in the tumor-resident dendritic cells via scavenger receptor-mediated internalization where they form lipid droplets [81, 82]. Unfortunately, no effective way has been developed as of now to modify metabolic pathways in the DCs. However, there is a great potential for new therapies in this direction. Future studies so as to understand metabolic pathways in the DCs may provide new insights into the more effective treatment of tumors.

1.5 T Cells

The ability of T cells especially CD8 T cytotoxic T cell to detect and eliminate neoplastic cells in the body has been the main focus of cancer immunology research in the past few years. Metabolic studies in T cell have shown that the function of T cells is critically dependent on their ability to metabolically adopt in the tumor microenvironment. Differentiation of naïve T cells into various forms of T helper is metabolically demanding and the inability of T cell to adopt could hamper this process, thus effecting physiological and pathological Th1 and Th2 immune responses [83].

Many recent studies have pointed out that naïve T lymphocyte activation process requires a dramatic change in their metabolism [84–87]. Therefore, investigating the signaling pathways and clues that regulate these metabolic changes and their functional consequences in T-cell development and activation has been a focus of T-cell response research during the recent years.

1.5.1 Carbohydrate Metabolism

T-cell activation alters cellular metabolism characterized by an increased glucose uptake and glycolysis [88], which has also been reported in cancer cells. However, in cancer cells, this altered metabolic program is primarily driven by cellular dysregulation due to the alteration in genes due to many mutations, whereas in T cells this well-regulated physiological response is essential for proper functionality. As in naïve T cells the requirement for macromolecule is minimal; hence, the cellular metabolism is geared towards the production of energy for basic needs, which is mostly by oxidative phosphorylation. However, upon activation, the naïve T cells increase their glucose uptake and aerobic glycolysis [89] to meet the increase in energy demands.

The increased uptake of glucose upon activation/antigen encounter is regulated by PI3K (phosphoinositide 3-kinase)–Akt and dependent on mTOR- and MYC-regulated pathways [90, 91]. Naïve T cells largely depend on oxidative

catabolic metabolism and undergo a dramatic shift from catabolic to anabolic metabolism upon activation. During this process T cells that fail to undergo this metabolic reprogramming do not gain effector functions suggesting the importance of the metabolic shift. Tumor cells learn to utilize glucose more efficiently in hypoxic conditions. Once activated the effector T cells use the glycolytic pathway to support their rapid growth and production of various effector molecules. Besides T-cell, tumor cells also rely on glucose for growth and survival. Thus, tumor cells and activated T cells compete for the available pool of glucose in TME. However, as observed in most cases, tumor cells outcompete immune cells for glucose by over-expressed higher levels of glucose transporter GLUT1. Indeed, higher expression of GLUT1 in cancer cells correlates with lower CD8 T-cell infiltration in many cancers such as PDA [92], renal cell carcinoma [91], SCC [93], and ovarian cancer [94]. Thus, inadequate levels of glucose and other nutrients hamper T-cell proliferation [95, 96], cytokine production [97, 98], and TCR signaling [99]. Therefore, the ability of tumor cells to take up glucose more efficiently results in their increased proliferation which ultimately suppresses antitumor immunity. Apart from ATP production, the intermediates generated during glucose catabolism via aerobic glycolysis enter in many pathways for the synthesis of proteins, nucleic acids, and lipids. Thus, glucose metabolism is an essential key regulatory pathway in T-cell activation and function.

1.5.2 Amino Acid Metabolism

Besides glucose, tumor and immune cell also compete for amino acids and acetate which are utilized in various other pathways. Glutamine metabolism regulates T-cell responses.

Effector T cells increase the glutamine uptake upon activation and can differentiate into Treg if glutamine availability is limited. Effect of glutamine metabolism differs between T-cell subsets which is metabolized by the enzyme glutaminase (GLS) to glutamate. Glutamine activates CD4 Th17 and pharmacological inhibition or genetic deletion of GLS has been shown to reduce inflammation in various inflammatory diseases such as airway inflammation [100], inflammatory bowel disease [101], and psoriasis [102]. Activated T cells show decreased proliferation and cytokine secretion when exposed to lower glutamine concentrations [103], therefore suggesting that decreased glutamine levels can hinder immune responses in situ.

Amino acid L-arginine (Arg) is also known to play a critical role in regulating antitumor T-cell immunity. Arginine is metabolized by two key enzymes, namely nitric oxide synthase (NOS) and arginase (ARG1). Arginine metabolism affects T-cell differentiation [104] suggesting that arginine levels within the TME can promote T-cell dysfunction. Therefore, manipulating NOS and ARG activity in tumors is lucrative to enhance the efficacy of T-cell-based therapies. Local NO produced by intra-tumoral DCs augments adoptively transferred CD8 cytotoxic T cell [105] mediated tumor killing suggesting that NO can be pro- or antitumor

depending on the context. Higher arginine levels in tumors correlate with higher amount of suppressive TAMs [28] in TME. Arginine promotes T-cell effector function during the T-cell activation. Arginine also promotes their survival as well as differentiation into the memory T cell [104] suggesting that lower arginine levels can promote T-cell dysfunction with TME. Thus, manipulation of NOS and ARG1 activity within tumor sites could enhance the efficacy of T-cell-based therapies.

Tryptophan (Trp), an essential amino acid, also has important immune-physiological functions. Trp can be metabolized into different end products by the host or intestinal microbiota in the gastrointestinal tract. For instance, the intestinal microbiota can catabolize Trp to indoles and its many derivatives, which also play an important role in the regulation of intestinal immune tolerance. Trp is commonly catabolized by kynurenine pathway (KP) to produce kynurenine (Kyn) and other metabolites like 3-hydroxyanthranilic acid (KA), anthranilic acid (AA), 3-hydroxykynurenine (3-HK), xanthurenic acid (XA), and QA [106]. These KP metabolites can play a role in immune regulation. Trp-derived metabolite 3,3-′-diindolylmethane (DIM) has been shown to bind to aryl hydrocarbon receptor (AhR), a known regulator of T-cell response. AhR binding causes induction of FoxP3 expression in T cells which causes enhanced $FoxP3^+$ Treg generation [107]. Tregs have immunosuppressive properties and in turn regulate the function of various other immune cells specifically cytotoxic T cells to induce immune tolerance. Tregs play an important role in many inflammatory diseases as well as in many cancers. Higher Treg correlates with poor prognosis in many cancers. Hyperactivation of KP is reported in many cancers [108]. Higher expression of indoleamine 2,3-dioxygenase (IDO), as the key enzyme in Trp metabolism, correlated with poor prognosis in hematologic malignancies, breast cancer, lung cancer, glioma, melanoma, prostate cancer, and pancreatic cancer.

1.5.3 Lipid Metabolism

Lipids act as structural molecules and are required for the cell membrane formation, many recent studies have suggested its role beyond just structural molecule. The fatty acid metabolism in T-cell activation and differentiation is now well accepted in the field of T-cell biology. Fatty acid metabolism involves fatty acid synthesis (FAS) and fatty acid oxidation (FAO). FAS produces key lipids important for cell membrane formation, which supports cell proliferation, while fatty acid oxidation (FAO) generates ATP and many metabolic intermediates required for important physiological functions.

As observed in other cells, T cells can use fatty acids as an energy source by β-oxidation. Preferential FAO has been shown to regulate differentiation, fates and functionality of CD8+ memory T (Tmem) cells, and induction of CD4+ regulatory T (Treg). Activated T cells rely on FAS [109] where PI3K/Akt pathway activates sterol regulatory element-binding protein (SERBP)-1 which leads to upregulation of ATP citrate lyase (ACLY) and fatty acid synthase (FASN) [110], whereas naïve T cells and memory T cells rely on FAO to maintain basic functions such as membrane

functional integrity [111]. In some cases, FAO may also prevent the activation of Teff cell by upregulating programmed cell death protein 1 (PD-1) expression as well as by upregulating carnitine palmitoyltransferase 1A, one of the rate-limiting enzymes in FAO, leading to inhibition of IFN-γ secretion [111]. On the other hand, FAO also favors more Treg cell formation via MAPK activation [112]. Immunosuppression is one of the hallmarks of most solid tumors and Tregs are an important component of it. Treg promotes SERBP-1-dependent lipid metabolism in the tumor microenvironment which hampers $CD8^+$ T cell's ability to produce IFN-γ and kill tumor cells, thus supporting the generation of immunosuppression of tumor-associated macrophages. Besides SERBP1, peroxisome proliferator-activated receptor (PPARs) is also known to regulate lipid metabolism and promote immunosuppression in solid tumors. Increased PPAR-γ activity inhibits lipolysis, limits OXPHOS in T cells, and promotes differentiation of Tregs [113].

Besides fatty acids, cholesterol metabolism in immune cells in the tumor microenvironment can also play an important role in their functionality. Some recent studies show an increase in cholesterol content in activated CD8 T cells [114]. Activation of TCR is accompanied by increased activity of enzymes involved in cholesterol biosynthesis. ACAT-1 and ACAT-2 genes encode cholesterol esterification enzyme that converts free cholesterol to cholesteryl esters for the storage. ACAT-1 is expressed in $CD8^+$ T cells and plays an important role in the early stage of T-cell activation. ACAT-1 deletion leads to decreased cholesterol esterification but increased cholesterol biosynthesis of cholesterol results in increased membrane cholesterol in $CD8^+$ T cells [115] and the increase in membrane cholesterol enhances TCR clustering and efficient immunological synapse formation.

Intracellular cholesterol and its derivatives can inhibit Tc9 cell differentiation. SUMOylation of liver X receptor (LXR) decreases binding of P65 to the IL-9 promoter, thus reducing the expression of IL-9 [116]. Further, cholesterol in tumor-infiltrating lymphocytes (TILs) can upregulate the expression of endoplasmic reticulum stress receptor XBP1 [117], which may cause higher expression of the immune checkpoint causing weaker T-cell activity and decreased antitumor response.

1.6 Natural Killer (NK) Cells

Natural killer (NK) cells are the major subset of the innate immune system that are of the lymphoid lineage. NK cells are characterized by their cytolytic functions against tumor cells in TME or virally infected cells. NK cells constitute ~5–20% of the total population of peripheral blood lymphocytes and share many common surface markers with T cells. NK cell functionalities, as seen in other cell types, are regulated by their metabolism. Conversely, NK cell metabolism and its antitumor responses are impaired in TME due to metabolic competition with cancer cells. It has also been demonstrated that the immunosuppressive effect of the tumor microenvironment (TME) alone limits the antitumor potential of NK cells. In the TME, various tumor and tumor-associated cells produce and secrete factors like IL-6, IL-10, transforming

growth factor-β (TGF-β), prostaglandin E2 (PGE2), and indoleamine 2,3-dioxygenase (IDO) that may directly or indirectly dampen NK cell activation [118] by down receptors NKp30, NKp44, or NKG2D [119] and tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) [120]. Furthermore, HLA-E expressed on the surfaces of cancer cells can activate inhibitory receptors (CD94/NKG2A) on NK cells [121] which tilt the balance between activation/inhibition signals in NK cells.

TME strongly affects NK cell metabolism and regulates full effector functions. As seen in other cell types, reduced availabilities of glucose substantially impact NK antitumor activity. Cong et al. reported that glucose deprivation dampens NK cell antitumor activity. In their study decrease in glycolytic rates attenuated cytotoxicity and cytokine production which was due to enhanced fructose-1,6-bisphosphatase (FBP1), an enzyme that inhibits glycolysis [122]. The increase in glycolysis is regulated by sterol regulatory element-binding proteins (SREBP) which promote citrate–malate shuttle. Blocking the activation of SREBP protein or citrate–malate shuttle inhibited the interferon-γ production and NK cell cytotoxicity [123] suggesting a crucial function of SREBP regulating glucose metabolism and thus for NK cell effector function.

Besides glucose, amino acid metabolism also plays a role in NK functionality. Reducing arginine in the media has been shown to impair the proliferation and IFNγ in NK cells [124, 125]. Conversely, mTOR signaling, which is important for regulating glycolysis, is inhibited in leucine-depleted media [126] suggesting the important role of amino acid metabolism in NK biology. Experts in the field believe that it is crucial to explore the NK cell metabolism to determine the way it keep its antitumor activity intact in the metabolically restrictive TME. The more we delineate the finer details of immunometabolism of NK cells, the better we can understand the effector functions of the NK cells. Further studies are needed to determine the ways through which TME shapes NK cell metabolism, which could be targeted to improve NK cell-based immunotherapies.

1.7 Conclusion

Cancer immunotherapy is an encouraging and fast-growing therapeutic modality for human cancers which has increasingly sought attention of both biomedical researchers and patients. Despite the recent success of immune checkpoint inhibitors and CAR T-cell therapy more specifically in hematologic malignancies, the application of immunotherapy in solid tumors is still facing several obstacles resulting from the heterogeneous expression of antigens as well as the induction of immunosuppressive tumor microenvironment. Tumor cells do exhibit specific metabolic requirements in order to survive and proliferate so as to progress into bigger tumors. Within a microenvironment where immune cells share resources with tumor cells, survival of immune cells completely depends on competing metabolic pathways with tumor cells for their development and effector function. This competing shared microenvironment results in acidification, hypoxia, and nutrient depletion that in

turn can alter the antitumor immune response and even could promote resistance to immunotherapies such as adoptive cell transfer and immune checkpoint blockade. Therefore, newer strategies to overcome the inhibitory effect of the TME on proinflammatory immune cells are currently being explored in the field. Once developed these strategies are going to prove as key for the success of immunotherapies especially in solid tumors.

While the study of immunometabolism undoubtedly is becoming a significant topic for cancer researchers, we must realize that during immunotherapy, targeting the metabolism of immune cells is still a challenging task. The major reason behind this task being so difficult lies in the fact that both cancer cells and immune cells with proinflammatory functions utilize quite similar metabolic pathways. Therefore, targeting a given pathway, for example, a metabolic pathway, underlying glucose metabolism to reverse the Warburg phenotype by use of glycolysis inhibitors in cancer cells may also concurrently disrupt the antitumor functions of the immune cells present in the same TME. For better success of immunotherapy in future, researchers will have to study subtle differences between the immune cells and the cancer cells particularly differences in their metabolic profiles in the TME so that immune cell-targeted metabolic modulation strategies could be developed.

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Cancer Metabolism and Aggressive Tumor Behavior

2

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Abstract

Aggressive tumor behavior poses a serious threat to the success of cancer therapy. Altered cancer metabolism is a hallmark feature of tumor initiation, progression, and metastases. During these processes, the tumor cells suffer bioenergetic and nutrient demand, which is met by metabolic reprogramming or preferential nutrient usage facilitated by the acquisition of driver oncogenic mutations and inactivation of tumor-suppressor genes. The metabolic heterogeneity and plasticity of tumor cells provide cellular fitness and survival advantage in the harsh tumor microenvironment (TME), resulting in aggressive tumor growth and resistance to chemotherapies. Besides, other cell types, including stroma, immune cells, and extracellular matrix in the TME, undergo metabolic switching that

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influences disease progression. Because aberrant glucose metabolism is central to tumor cell metabolic reprogram, various clinical trials targeting glucose uptake and its metabolites in combination with other molecular targets have been focused on reducing tumor progression by inhibiting the metabolic interplay. Here, we describe in detail how the metabolic plasticity of cancer cells and TME results in tumor progression and aggressiveness. In addition, we highlight the current approaches being explored for therapeutic intervention. This overview will help in understanding the intricated metabolic networks and open new avenues of cancer treatment.

Keywords

Glucose metabolism · Heterogeneity · Cancer stem cells · Immune cells · Hypoxia · Tumor progression · Lactic acid · Chromatin

2.1 Introduction

Metabolic alterations are a characteristic hallmark feature of tumor cells, facilitating tumor cell proliferation, invasion, immune evasion, and metastases [1]. These aggressive features impose a serious therapeutic hurdle in cancer treatment [1] and are responsible for almost 90% of cancer-related mortality and morbidity [2]. Typically, cancer metastasis involves three main steps—invasion, intravasation, and extravasation. The initial step of metastasis involves detachment of tumor cells from the primary site and invasion of the local milieu directly via blood vessels (intravasation) or lymphatic system. The invasion or dissemination of tumor cells from the primary site to surrounding tissue/stroma occurs either as single cells or clusters [3, 4]. However, only a small subset of disseminated tumor cells survive the shear stress and protective immune cells attached to the endothelial linings of blood vessels and extravasate to facilitate successful metastasis [5].

The tumor mass also harbors a small population of "stemlike" cells known as cancer stem cells (CSCs) that influence various aspects of tumor biology. CSC was first identified in acute myeloid leukemia in 1994 and its potential role in tumor aggressiveness, therapy, relapse, and metastasis of hematological and solid tumor cells was subsequently recognized [6, 7]. These CSCs (0.05–1%) are characterized by the expression of distinct surface markers based on the origin of tumors [8]. Like pluripotent stem cells, CSCs show several salient features such as surviving for longer periods, quiescence, resistance to apoptosis, and ability to undergo self-renewal and differentiation [6, 7]. Such self-renewal property allows CSCs to initiate uncontrolled proliferation with diverse molecular, cellular, and metabolically active phenotypes, subsequently resulting in the significant increase in heterogeneity of primary and metastatic tumors [7, 9]. The acquisition of heterogeneous tumor phenotypes increases the survival advantage during treatment with chemotherapy causing therapy resistance and relapse in various cancer types [9, 10]. To fulfill their energy and biosynthetic demand, tumor cells and CSC increase their nutrient uptake

(glucose and glutamine) from the environment [1, 11]. The marked increase in the glucose consumption by tumor cells compared to normal cells in the presence of oxygen (O_2) was first discovered by Otto Warburg (1926) and is known as the Warburg effect [12]. The Warburg effect is well established in a variety of tumors [1] and has been exploited for tumor diagnosis and staging by positron-emission tomography (PET) using radiolabeled glucose analog ^{18}F-fluorodeoxyglucose (^{18}F-FDG) [13].

Altered cancer cell metabolism is associated with various stages of tumorigenesis. As altered metabolism enhances the cellular fitness of tumor cells by increasing the nutrient uptake, it is essential to understand how these nutrients are utilized, and what metabolic changes occur as a result of preferential nutrient uptake in the tumor microenvironment (TME) in order to promote the tumor progression [1].

This chapter describes in detail the role of altered glucose metabolism in tumor progression and metastasis, metabolic heterogeneity of CSCs, and its association with chemoresistance. In addition, we summarize how the metabolic plasticity of tumor cells influences the TME, leading to disease aggressiveness or therapeutic resistance. We also highlight the potential therapeutic approaches being used to target cancer metabolism.

2.2 Altered Glucose Metabolism in Tumor Cells

Human somatic cells cultured in petri dish undergo limited cell division and become senescent to die due to the "Hayflick limit" named after the first observation by Leonard Hayflick in 1961 [14]. However, tumor cells overcome this "limit" to facilitate limitless cell division, by accumulating oncogenic mutation, inactivating tumor-suppressor genes, and sustaining telomerase activity. This process is driven by the metabolic rewiring of tumor cells to improve their cellular fitness and selective survival advantage [1]. Typically, in normal cells, the influx of glucose is driven by extracellular signals rather than bioenergetic demand. For instance, mammary epithelial cells cultured in detached condition from extracellular matrix have suppressed glucose uptake despite high glucose present in the medium, resulting in decreased mitochondrial function and ATP production [1]. However, constitutive activation of AKT alone can stimulate glycolysis to restore the mitochondrial function and maintain ATP levels despite growth factor deprivation. In normal cells, glucose diffuses into the mitochondria, where it enters the tricarboxylic acid cycle (TCA) to oxidize glucose to carbon dioxide and generate NADH and $FADH_2$ molecules with a little amount of lactate generation via oxidative phosphorylation (OXPHOS) pathway. NADH and $FADH_2$ then enter the electron transport chain to generate net two ATP molecules per glucose consumed.

In 1926, Otto Warburg observed that cancer cells preferentially utilize glycolysis even in the presence of O_2 to support their energy requirement (Warburg effect) [12]. The aerobic glycolysis generates building blocks for macromolecules (proteins, lipids, and nucleotides) required to maintain enhanced growth and proliferation of cancer cells [1]. However, aerobic glycolysis is highly inefficient as it generates only

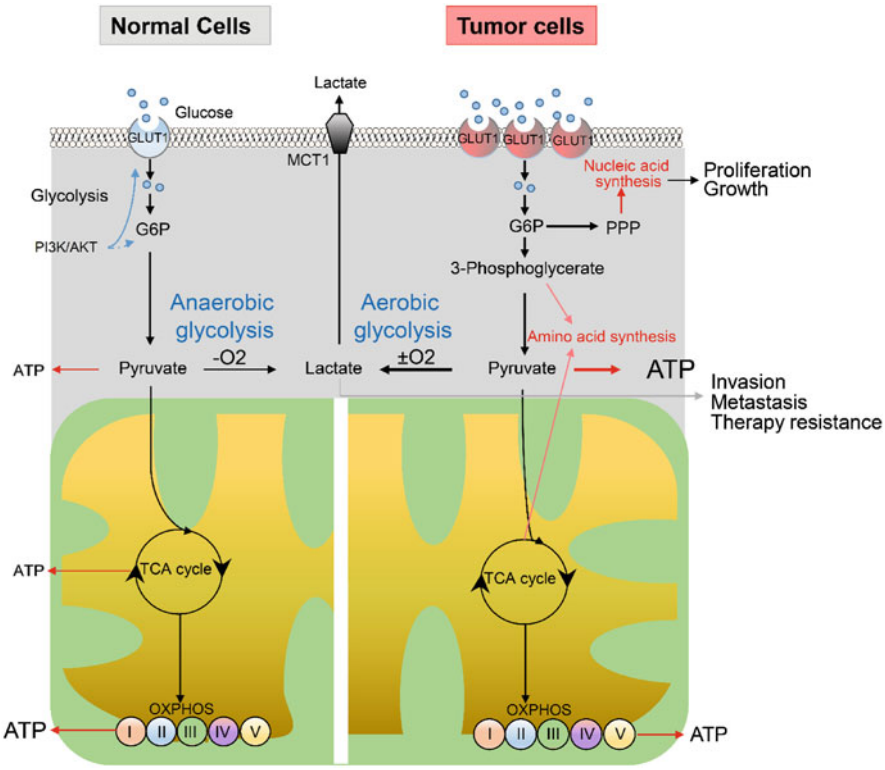

Fig. 2.1 Metabolic reprogramming in tumor and normal cells

two ATP molecules per molecule of glucose metabolized compared to 36 ATP molecules generated via OXPHOS. This low energy production is compensated by PI3K/AKT signaling, a key master regulator of glucose uptake. During PI3K/AKT signaling, AKT drives the transcription of the glucose transporter GLUT1 and its translocation to the cell surface.

AKT also induces the hexokinase (HK) activity to phosphorylate glucose and prevents effluxing of glucose back to the extracellular space. In addition, AKT also activates the phosphofructokinase and thus promotes the irreversible function of glycolysis. Increased GLUT1 and HK activity increases the glucose uptake by 100-fold in tumor cells, leading to the generation of more ATP molecules during aerobic glycolysis than OXPHOS [12]. However, during aerobic glycolysis, the tumor cells generate high amounts of lactate as a by-product (Fig. 2.1). Inhibiting this pathway by inhibitors targeting PI3K or receptor tyrosine kinases can result in the blockade of glucose uptake by the tumor cells [15, 16]. Moreover, aberrant activation of the PI3K/AKT pathway is shown to induce growth factor-independent tumor progression [1].

Apart from PI3K/AKT signaling, oncogenic proteins such as Ras are known to increase the transcription of *GLUT1* [17, 18]. In pancreatic cancer, *Kras* mutation is

an early oncogenic insult that initiates pancreatic intraepithelial neoplasia development and later progresses to pancreatic ductal adenocarcinoma (PDAC) with additional genetic mutations, including Trp53. Increased glycolysis is a key feature of *Kras*-driven tumorigenesis [17, 19]. Abrogation of *Kras* signaling in the PDAC murine model has been shown to result in tumor regression along with severe reduction of *Glut1* transcription and rate-limiting glycolytic enzymes [20]. Apart from elevated glycolysis, *Kras* also fuels the glycolytic intermediates to pentose phosphate and hexosamine biosynthesis [20]. At the molecular level, *Kras*-driven glycolysis is mediated by the activation of MAP kinase, which increases the *cMyc*-dependent transcription of glycolytic enzymes. During cellular stress, such as starvation, mutant *Kras* cooperates with other antioxidant enzymes such as paraoxonase 2 (*Pon2*) to increase glycolysis in PDAC [21]. In lung cancer, mutant *Kras* is responsible for metabolic heterogeneity and metabolizes the glucose differently based on the degree of lesion (low to high grade) in $Kras^{G12D}$;$Trp53^{-/-}$ lung tumors [22]. In addition, lung cancer patients and NSCLC cell lines (49%) also gain homozygous mutation for *Kras* (G12D) [23, 24], which influences the glycolytic switch, maintenance of redox balance, channeling of glucose metabolites to the TCA cycle, and biosynthesis of glutathione [22, 25]. Increased glutathione in the homozygous mutant *Kras* in NSCLC protects the cells from reactive oxygen species (ROS)-mediated abnormalities, thereby increasing the selective growth of these cells during lung tumor progression [26].

2.3 Cancer Stem Cells Exhibit Heterogeneous Metabolic Characteristics

Stem cells are undifferentiated cells with a unique capacity for self-renewal and multiple differentiation in multicellular organisms [27, 28]. As somatic cells have limited cell division, replenishing of the damaged cells is achieved by stem cells and self-renewing its progenitors for maintaining the tissue homeostasis. At physiological condition, stem cells reside in the hypoxic microenvironment, which enables them to maintain their undifferentiated state, proliferate, and commit to cell fate [29]. Due to spatial residence, stem cells rely heavily on anaerobic glycolysis to support their energy requirement [30]. The reliance of stem cells on glycolysis is due to fewer or immature mitochondria, which protects the genome from ROS generated by OXPHOS and limits oxidation of proteins and lipids [31]. A key driver for glucose metabolism in a low-O2 environment is the activation of transcription factor hypoxia-inducible factor 1 α (HIF1α). During anaerobic glycolysis, HIF1α heterodimerizes with HIF1β to promote the transcription of glycolytic genes [32]. The hypoxic condition stabilizes the HIF1α protein by preventing hydroxylation and facilitates the expression of pyruvate dehydrogenase kinase (PDK2 and 4) to prevent pyruvate from entering into the TCA cycle, thus blocking mitochondrial respiration. However, depletion of HIF1α in stem cells results in the reversal of this phenotype, thereby allowing the cells to undergo mitochondrial respiration rather than glycolysis. The transition from glycolysis to mitochondrial respiration is

responsible for the exhaustion of hematopoietic stem cells, and thus suggests the pivotal role of HIF1α in maintaining the hematopoietic stem cell function [33].

Like stem cells, CSCs have the ability to self-renew and maintain an undifferentiated state, remain quiescent, and activate DNA repair machinery. CSCs are associated with tumor initiation, relapse, therapy resistance, and metastatic dissemination [7, 10]. Several studies have identified and characterized CSCs in various malignancies for use as biomarkers or targeted therapies [34]. The stemness features are tightly regulated by several transcription factors (TF) such as OCT4, SOX2, KLF4, and Nanog. Shinya Yamanaka, in 2006, first demonstrated that four TFs (Oct4, cMyc, Sox2, and Klf4) could induce pluripotency in the mouse embryonic fibroblast suggesting the importance of TFs in stemness [35]. Like cancer cells, CSCs also undergo metabolic adaptation to the cellular environment, such as hypoxia versus normoxia and proliferative versus quiescence. Such changes in the cellular environment cause a shift in the metabolic states that gives rise to cellular heterogeneity in CSCs [11, 36]. The existence of heterogeneity in tumor cells and CSCs represents a major therapeutic hurdle in several cancers.

Though CSCs are metabolically very active, controversy regarding their energy metabolism (glycolytic or mitochondrial respiration) is still under scrutiny. In general, glycolytic activity is mainly responsible for maintaining the stemness traits of stem cells, embryonic stem cells, and induced pluripotent stem cells. For example, increased glycolysis in non-small cell lung cancer (NSCLC) leads to the elevation of ABCG2 transporter in the side population [37] via activation of the AKT pathway. Constitutive expression of active AKT also increases the glycolytic rate and aerobic glycolysis independently of the growth factor [37, 38]. Apart from glycolysis, CSCs also utilize OXPHOS for alternative energy generation in response to their physiological needs, suggesting its metabolic flexibility. Recent findings have shown that liver CSCs are highly OXHPOS dependent compared to the non-stem cells, which was evident from increased mitochondrial DNA copy number, mitochondrial content, and ROS. In addition, as a result of the treatment with 2-deoxy-D-glucose (2-DG), the high OXPHOS liver CSCs promote the expression of stemness surface markers CD133 and CD44 [39]. Overall, we now understand that CSCs can undergo metabolic reprogramming (glycolysis or OXPHOS) to support their stemness.

2.4 Metabolic Plasticity Drives Cancer Cell Metastasis

As tumor cells are highly active metabolically, there is a dramatic change in the TME with increased hypoxia, nutrient shortage, and lactic acid buildup. Most of the metabolic pathways are interconnected and flexible, allowing the tumor cells to reprogram their metabolic activity for glucose catabolism and maintain the redox balance during changing microenvironment. The metabolic plasticity ensures the survival of the tumor cells by increasing their cellular fitness during nutrient starvation. For example, in the case of chronic glucose starvation in serous ovarian cancer cells, tumor cells undergo metabolic reprogramming to generate cell types that are highly heterogenic. Such generation of heterogenic cell types is driven by the

ZEB1-dependent transcription of NNMT (nicotinamide N-methyltransferase), which is highly expressed in the metastatic and recurrent tumors compared to matched primary carcinoma. In addition, ZEB1-dependent expression of NNMT also confers resistance to glucose dependence and increases the migration of ovarian cancer cells suggesting metabolic adaptation during glucose restriction [40].

Tumor cells increase their metastatic potential by metabolic reprogramming by shifting from glycolysis to OXPHOS [41]. The metabolic shift to OXHPOS is coordinated by transcriptional coactivator PGC-1α (peroxisome proliferator-activated receptor-gamma coactivator-1α), a key regulator involved in mitochondrial biogenesis and metabolism [42]. Recent studies using the systems biology approach by utilizing AMPK and HIF1 signatures in The Cancer Genome Atlas indicated the presence of a hybrid phenotype that enables the cells to consume various types of nutrients [41, 43]. It also provides cellular advantages such as efficient energy production through multiple metabolism pathways, synthesizes biomass for rapid cell proliferation, and maintains ROS at a moderate level to favor ROS-mediated signaling [44]. Such phenotype was evident in circulating tumor cells isolated from highly metastatic mouse basal type breast cancer cell line (4T1) [45]. The hybrid phenotype is characterized by high levels of HIF1/pAMPK (AMP-activated kinase), which favors both glycolysis and OXPHOS. In contrast, another phenotype with high HIF1/low pAMPK expression and low HIF1/high pAMPK expression in triple-negative breast cancer exclusively favored glycolysis and OXPHOS, respectively [41]. Such metabolic plasticity creates a major clinical hurdle, considering that the current clinical strategies targeting metabolism have been largely ineffective. Thus, simultaneous targeting of both the pathways (glycolysis and OXPHOS) may be critical to eliminate these metabolically highly flexible tumor cells [41, 46].

2.5 Lactic Acid Secretion, Utilization, and Tumor Progression

As a result of increased metabolic rate in tumor cells, there is a significant accumulation of lactic acid and H^+ in the cytosol. Almost 85% of the incoming glucose is converted to lactic acid, which needs to be eliminated from the tumor cells to prevent acidosis and support higher rates of glycolysis. This elimination of lactic acid and H + from the cytosol to the microenvironment is assisted by the increased expression of monocarboxylate transporter isoforms (MCT1 and 4) and Na-driven proton release, respectively [47, 48]. Overexpression of MCT1 and 4 has been associated with poor prognosis and high mortality in several cancers [47]. The dependence on MCTs to expel lactate is based on the fact that lactic acid is a weak acid, which prevents them from diffusion across the membrane. However, studies have shown that the dissociation of lactate to H+ generation is not the primary cause for acidosis. Rather the coupling of ATP hydrolysis and glycolysis is the major source of H+ production which contributes to acidification (low pH) [49].

Heterogeneous distribution of glucose in the intratumoral area, apart from activating HIF1α, also activates the oncogene cMYC to upregulate LDHA (lactate dehydrogenase A), leading to the generation of NAD+ which in turn activates

glycolysis, thus maintaining the vicious cycle [50, 51]. Besides HIF1α and cMYC, lactate also regulates the transcription of *RAS*, *PI3KCA*, *E2F1*, tumor-suppressor genes (*BRCA1* and *BRCA2*), and genes that mediate cell cycle and cell proliferation [52]. On the contrary, cMYC and tumor suppressor P53 also activate the transcription of *MCT1* to favor lactate uptake [53, 54]. HIF1α activates the transcription of *MCT4* to expel lactate from the cells [55]. Under physiological conditions, lactate concentration in the blood and normal tissues ranges between 1.5 and 3 mmol/L [56]. The levels can rise up to 40 mmol/L concentrations in tumors [57]. When lactate is not eliminated from the cells, it can lead to lactic acid acidosis, which is common in most highly mitotic tumors. Tumor-associated acidosis was first documented in acute leukemia patients in 1963 [58]. In general, lactic acid acidosis in cancer patients results from a failure in lactate clearance from the liver due to deficiencies in thiamine and/or riboflavin. Thiamine functions as a cofactor that facilitates the conversion of pyruvate to acetyl-CoA by pyruvate dehydrogenase. Due to thiamine deficiency, this conversion from pyruvate to acetyl-CoA prevents the entry of the latter into the TCA cycle [59]. Thus, balancing lactic acid production and expulsion by cancer cells is essential to prevent intracellular acidification and apoptosis.

Though lactate was previously considered as a "metabolic waste" product of glycolysis, recent studies have demonstrated the role of lactate levels in driving tumor progression, immune escape, angiogenesis, cell migration, and drug resistance [51, 56]. TME is composed of stromal cells, endothelial cells, and immune cells. Immune cells primarily surveil the body to eliminate any pathogen, including tumor cells. However, tumor cells release anti-inflammatory cytokines and recruit immunosuppressive cell types in the TME to inhibit the immune response [60]. Lactate accumulation also dampens the antitumor activity of NK cells and NKT cells by inducing apoptosis [61, 62]. In several tumors, tumor-associated macrophages undergo polarization in response to lactate-induced transcription of vascular endothelial growth factor (VEGF) and arginase 1 [63]. Furthermore, lactate also assists the tumor cells in evading immune response by expressing its receptor G protein-coupled receptor 81 (GPCR81). In lung cancer cells, the activation of GPCR81 receptor results in the upregulation of programmed death-ligand 1 (PD-L1) in the membrane, which blocks the antitumor immune response. On the contrary, blocking the LDHA enzyme which converts pyruvate to lactate in the tumor cells increases the efficiency of programmed cell death 1 (PD1) therapy [64].

Higher lactate in the TME is associated with an increased metastasis in various cancers [48] and correlates with poor clinical outcome [56]. The mechanisms by which lactate promotes metastasis are multifactorial: (1) modifies several cell adhesion molecules, such as integrins, which assist in cell binding to the extracellular matrix, making them more migratory [65], and (2) induces the expression of proteases (MMP9, cathepsin B, and hyaluronidase) to degrade the surrounding tissues, thereby allowing tumor cells to metastasize [66, 67]. Apart from metastasis promotion, lactate buildup is also associated with the induction of therapy resistance. In NSCLC, prolonged treatment with tyrosine kinase inhibitors (EGFR and MET) results in a metabolic shift towards increased glycolysis and lactate production. This

lactate, in turn, promotes the secretion of hepatocyte growth factor by cancer-associated fibroblast (CAF) in an NFkB-dependent manner to activate MET signaling to induce therapy resistance [68]. Thus, targeting lactate metabolism or uptake has proven to be an important strategy for cancer therapy.

2.6 Glucose Metabolism, Chromatin Structure, and Chemoresistance

Changes in the global chromatin structure are associated with gene expression, DNA repair, and tumor progression [69]. Typically opening and closing of chromatin structure is facilitated by the acetylation of histones (H3, H4, H2A, and H2B in nucleosome core) catalyzed by the balanced action of histone acetyl transferase (HAT) and histone deacetylase (HDACs). During harsh metabolic reactions, tumor cells meet the increasing demand for energy and precursors for biosynthesis by initiating the distinct transcription of metabolic genes via chromatin remodeling [70]. The metabolites generated during the metabolic reaction are taken up by the cells actively or passively through the plasma membrane or nuclear membrane to modify the chromatin structure or processed by the metabolic enzymes to function as a substrate or cofactor for the chromatin-remodeling enzymes. Acetyl-CoA is one such metabolic by-product that functions as a substrate for HAT activity. The canonical histone acetylation involves addition of acetyl group at lysine residue which is derived from the metabolite acetyl-CoA. Acetyl-CoA generated during glucose metabolism is funneled through mitochondrial metabolism via a citrate intermediate, which is exported and lysed in the cytosol by ATP-citrate lyase to generate acetyl-CoA. Therefore, nutrient availability is vital in regulating the chromatin structure and gene expression during metabolic reprogramming.

The study by Liu et al. (2015) has shown that inhibiting glycolysis with 2-DG or silencing two rate-limiting enzymes, hexokinase-1 (HK1) and pyruvate kinase (PKM), results in condensing of the chromatin structure and reduced tumor cell proliferation [71]. Besides, increased glycolysis results in higher accumulation of cellular acetyl-CoA, a substrate for acetyltransferases, which increases the histone acetylation, thereby enabling the cells to undergo efficient DNA repair and induce chemoresistance [71].

Another chromatin-associated protein, MORC2, a member of the Microrchidia family CW-type zinc finger (MORC) family of proteins, is upregulated in several cancers [72]. It also regulates transcription by modifying the chromatin structure [73, 74]. During tumorigenesis, MORC2-mediated transcription is catalyzed by the interaction with histone HDAC1, HDAC4, and EZH2 [75]. Likewise, during glucose metabolism, cMYC directly targets the expression of *HK2*, *PFKM*, *ENO1*, *GLUT1*, and *LDHA* [76], while MORC2 is known to regulate *LDHA* by cooperating with cMYC to promote the migration of breast cancer cells [75, 77, 78]. As numerous metabolic pathways converge onto cMYC regulation, attempts to block or restore altered pathways driven by cMYC can lead to novel strategies in cancer treatment.

2.7 Cross Talk Between Tumor Microenvironment and Metabolism in Disease Progression

As discussed earlier, metabolic plasticity allows tumor cells to adapt themselves to changing TME [1]. Aberrant tumor vasculature in the TME causes heterogeneous perfusion (O_2 and nutrients) across the tumor vessels, which promotes a hypoxic environment [79]. The competitive metabolic milieu in the TME also results in the variable nutrient utilization among tumor cells, immune cells, and stromal population [80, 81]. Besides, tumor cells adapt to their metabolic needs in the hypoxic conditions of TME through HIF1, which activates enzymes of glycolytic flux. Overall, the intratumoral metabolic heterogeneity by the nonuniform distribution of nutrients is influenced by various factors, including the composition of TME, disease stage, and mutation load [82]. Here, we will discuss in detail how stroma, extracellular matrix (ECM), and immune cell metabolism are reprogrammed by tumor cells and influence the disease progression.

***Stroma*:** The contribution of stroma for tumor growth and progression is well established in different cancers, but how alterations in stromal composition support tumor growth are still unclear. The metabolic interplay between cancer cells and TME is a well-recognized hallmark of tumors. The accumulation of different metabolic intermediates and their by-products in the TME activates stromal cells through paracrine signaling and alters their phenotype [83]. Stroma modulation by growing tumor is synonymous to the regeneration of damaged tissue and involves (a) monocyte recruitment and activation to pro-inflammatory M1 phenotype for clearance of necrotic tissue and subsequent transition to M2 phenotype; (b) fibroblast recruitment, their differentiation to myofibroblasts, and secretion of ECM for surrounding cells survival; and (c) immunosuppressive milieu characterized by T_{regs}, M2 macrophages, and myeloid-derived suppressor cells [84]. This stromal regeneration by tumors is driven by alteration of metabolic consumption in the TME, which includes autophagy in stromal fibroblasts by glucose depletion and AMPK activation and secretion of nonessential amino acids, which leads to enhanced tumor growth [85, 86]. CAFs are the main component of tumor stroma and engage in tumor progression by promoting tumor cells to undergo EMT and enhancing the stem cell traits and metastatic dissemination [87, 88]. Accumulating evidence shows that CAFs undergo metabolic reprogramming during their activation, including utilization of aerobic glycolysis and increased autophagy for mobilization of the nutrients into the TCA cycle [89, 90]. Also, CAF-derived exosome is seen to mediate metabolic reprogramming [91]. While in PDAC, the oncogenic mutation is observed to regulate signaling in both the tumor cells and adjacent stromal cells. By cell-specific proteome labeling and multivariate phosphoproteomics, it is observed that tumor cell KRAS (KRASG12D) interacts with fibroblast to initiate reciprocal signaling in tumor cells. This reciprocal signaling results in distinct tumor cell phosphoproteome, which regulates tumor cell proliferation and apoptosis and increases mitochondrial capacity [92]. Tumor cells also interact with the CAFs and reprogram their cellular metabolism to adapt to the nutrient deprivation in the harsh TME. One such classic

example is the reciprocal interplay between prostate cancer cells and CAFs which results in EMT and metabolic shift in the tumor cells. As prostate cancer cells come in contact with CAFs, it reprograms the metabolism of cancer cells towards aerobic metabolism, thereby decreasing the dependence on glucose and shift towards aerobic metabolism. This process is driven by reducing GLUT1 expression and increasing the lactate load by MCT1. Therefore, prostate cancer cells by inducing symbiosis with CAFs utilize their by-products, favoring them to grow in a low-glucose environment [88]. While MCT1 can induce lactate uptake or secretion in cancer cells, MCT4 promotes lactate efflux in CAFs through HIF1α induction under hypoxic conditions and results in tumor promotion [93]. In fact, triple-negative breast cancer patients with high stromal MCT4 expression show poor prognosis [94]. In addition, stroma-associated pancreatic stellate cells also secrete nonessential amino acids, decreasing the tumor cell dependence on glucose and serum-derived nutrients [85]. Likewise, CAFs in ovarian tumors utilize carbon to produce glutamine for cancer cells. This shows the existence of novel cross talk between tumor cells and CAFs in metabolic regulation of tumor cells [95]. Thus, targeting the glutamine pathway in both tumor and stroma resulted in a significant decrease in tumor growth [96]. However, the mechanistic link between CAFs and tumor nutrient demand is not clear. A detailed understanding of these pathways would help in dissecting the actionable targets, including targeting both tumor and TME simultaneously. This approach of simultaneous targeting is limited by the cell-dependent function of different actionable target proteins. These targets are present in both tumor epithelium and TME but possess opposite functions. For example, prostate tumor epithelium-mediated downregulation of p62 in stromal fibroblasts resulted in impaired metabolism through reduced mTOR activity and cMYC expression and release of ROS and IL-6, which in turn enhanced epithelial invasion and tumorigenesis [97]. Therefore, inhibiting their activity in tumor cells could be compensated by increased stromal reactivation.

***ECM*:** Extracellular matrix (ECM) consists of an intricate network of secreted proteins that provide biochemical and mechanical support to different tissues and organs. Tumor cells interact with ECM via transmembrane integrin receptors to control cell migration, proliferation, and metabolism. Tumor relieves anchorage dependence and gets disengaged from the ECM for metastases and dissemination. However, ECM detachment results in impaired glucose uptake, reduced cellular ATP levels, and increased ROS production. Tumor cells endure this stressful environment by altering their nutrient utilization from glycolysis to glutamine-derived TCA metabolism mediated by AMPK-regulated NRF2 expression [98]. Glutamate production through AMPK-mediated glutamine metabolism helps to reduce oxidative stress following anchorage independence. ECM composition and organization are influenced by the presence of CAFs in TME [99]. Higher collagen content has been correlated with altered metabolism in breast cancer due to reduced oxygen and glucose consumption and increased glutamine consumption by tumor cells [100]. In head and neck squamous cell carcinoma (HNSCC), cancer cell-derived glutamate promotes ECM remodeling by maintaining the redox state in CAFs, and aspartate from CAFs sustains cancer cell proliferation [101]. These opposite results

might be due to different tumor types and altered TME composition. ECM undergoes continuous remodeling by expressing a variety of matrix-degrading enzymes, resulting in altered nutrient uptake by the tumor cells. For instance, hyaluronan degradation in ECM enhances transporter GLUT1 mobilization to the plasma membrane and promotes glucose uptake and increased migration of cancer cells [102]. In a nutshell, the studies mentioned above fill a gap in understanding the varying metabolic requirements of cells in anchorage-dependent and -independent conditions. A better understanding of the underlying mechanisms of ECM remodeling and metabolic rewiring in tumors could encourage the development of novel therapeutic interventions.

***Immune cells*:** The hallmarks of TME, including hypoxia, low pH, lactate accretion, waste accumulation, and very high demand for nutrients, create a competitive niche for different cells present in the TME [81, 103]. Multiple studies have demonstrated that this nutrient-competitive milieu favors tumor progression and dampens effector T-cell functions but not necessarily their proliferation [104–106]. Metabolic heterogeneity in the TME plays a key role in the differential intratumoral immune cell recruitment. Metabolic reprogramming by cell-intrinsic and -extrinsic nutrient availability in the TME results in the differential activity of immune cells [107, 108]. Also, tumor cells, by employing the Warburg pathway, limit the nutrient supply to immune cells, thereby inducing the immunosuppression [103]. Increased glycolysis is a hallmark of metabolic alterations of activated immune cells, including macrophages, NK cells, dendritic cells, B cells, and effector T cells [109]. Multiple studies have described T-cell activation by complex metabolic regulation [110, 111]. Earlier studies have shown the association between the differentiation state of T cells (naïve, effector, or memory) and their metabolic activity [112]. Naïve T cells have basal glucose requirements and depend mainly on fatty acid oxidation and glutaminolysis for their nutrient supply, while activated T cells undergo metabolic switching towards glucose metabolism. For T-cell activation, CD28 costimulation promotes glucose uptake via the PI3K-AKT pathway, and TCR activation induces glutaminolysis through ERK/MAPK pathway [113]. Additionally, enhanced mTOR activity results in the activation of CD8+ T cell and stabilization of HIF1α required for CD4+ T-cell proliferation and activation. Effector T-cell subsets, including TH17, TH1, TH2, and activated $CD8^+$ T cells, have been shown to possess high glycolytic activity as seen by increased mTOR activation. Thus, metabolic reprogramming in activated T cells through PI3K-AKT, mTOR, AMPK, and HIF1α signaling pathways gives rise to similar metabolic profiles of both cancer cells and activated T cells [114–116]. This has been one of the major challenges posed by therapeutic interventions directed towards cancer cells.

Glycolysis is important in immune cell programming from TH_{17} to T_{reg} type [117, 118]. The different metabolic requirement of various immune cells is dictated by their functional activity. This is consistent with the idea that CD28 signaling for T-cell activation is dependent on increased glucose uptake while M2 macrophages and T_{regs} can survive in low-glucose conditions as they utilize fatty acid oxidation for nutrient requirement [119, 120]. In fact, switching of T_{reg} metabolic pathway to

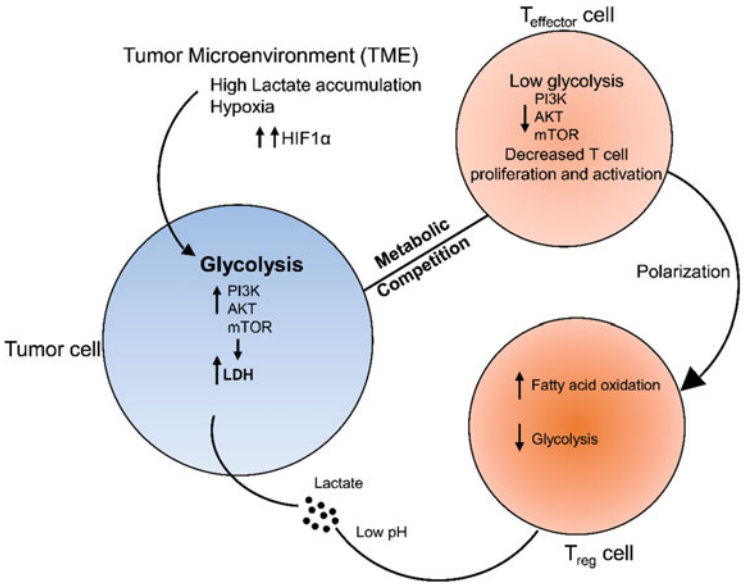

Fig. 2.2 Effect of high lactate accumulation and hypoxia in tumor microenvironment (TME) on T-cell effector function and reprogramming to immunosuppressive Treg cells

fatty acid oxidation may be due to suppression of mTOR by AMPK [81, 121]. Also hypoxia in TME induces high adenosine concentrations by tumor cells; it exerts an immunosuppressive effect through the binding of adenosine receptors in various immune cells [122]. Likewise, lactate accumulation by excessive glycolytic activity in the TME engenders metabolic reprogramming of both tumor and immune cells and angiogenesis through increased VEGF secretion [56, 88]. In one study, excessive lactate accumulation resulted in reduced T-cell effector function and polarization towards T_{reg} phenotype [123, 124] (Fig. 2.2). In addition, reduced activation of infiltrated immune cells (T cell, B cell, and NK cell) and poor monocyte differentiation by excessive lactate concentrations in the TME endow tumor cells with the ability to proliferate at higher levels. While T cells rely solely on glycolysis for their nutrient requirement, hypoxia-induced mitochondrial function loss has also been linked to T-cell exhaustion through MYC-regulated pathway [125]. Nevertheless, there remains a gap in metabolic heterogeneity and its association with immune cell type due to limitations in traditional technologies that help determine the metabolic profile. Recent advancements in flow cytometry and mass spectrometry-based analysis have encouraged researchers to develop innovative approaches of profiling patient samples at a single-cell level. CyTOF-based multiplexing in flow cytometry has allowed single-cell metabolic profiling of human $CD8^+$ T cells in colorectal carcinoma patients [126]. This study suggested that the metabolic heterogeneity in the peripheral and tumor-infiltrating $CD8^+$ T-cell subsets causes differences in their functional attributes. Therefore, delineating the effect of metabolic reprogramming

on tumor immune cell function and distribution will allow intervention with pharmacological inhibitors to remodel the immune response.

2.8 Therapeutic Targeting of Glucose Metabolism

Developing therapeutic strategies targeting the Warburg pathway in tumors has been a long-standing approach to eliminate or delay tumor progression. Several drugs targeting enzymes and intermediates of glycolytic pathways have been evaluated in clinical trials [127] with little success. It is now becoming clear that cancer cells exhibit hybrid metabolism (glycolysis and OXPHOS) under stress conditions induced by the oncogenic activation of Ras, MYC, and c-SRC or ROS generation [128, 129]. Such metabolic plasticity orchestrates the tumor cell proliferation and metastasis by maintaining ROS levels and efficient energy production [45]. In fact, there exist reports indicating the synergistic effect of a combination of glycolytic inhibitor 2-DG decreasing the glucose uptake and metformin inhibiting OXPHOS activity on the growth and metastatic potential of tumor cells [130]. Regardless of the impressive data with 2-DG in several preclinical studies, clinical data are not very satisfactory [131, 132]. A recent clinical trial in PC patients with 2-DG was stopped due to slow accrual. Likewise, clinical trials of other cancers with 2-DG were not satisfactory and unambiguous. Clinical trials combining 2-DG with other chemotherapeutic agents including cisplatin, docetaxel, or radiation are currently ongoing [127]. Data obtained from initial trials are quite encouraging and might open new avenues for cancer treatment. Several other anti-glycolytic agents target different enzymes and intermediates of the glycolytic pathway, including glucose uptake and phosphorylation, fructose phosphorylation, glucotriose metabolism, pyruvate formation, oxidation, lactate dehydrogenase, and tumor acidosis [127]. One of the most effective anti-glycolytic agents, 3-bromopyruvate (3-BrPA), a pyruvate analog, acts by targeting GAPDH and inhibiting both tumor glycolysis and mitochondrial OXPHOS. As a result, cancer cells undergo energy deficiency through ATP diminution and apoptosis and eventually die, leading to decreased tumor growth. In addition, studies have shown the anticancer effect of 3-BrPA through suppressing tumor invasion, angiogenesis, and metastasis. 3-BrPA has shown antitumor potential not only as a single agent but also acting synergistically in combination with cytotoxic agents and ABC transporters to restore drug sensitivity [133, 134]. As 3-BrPA is stable in the acidic TME, it has the potential for efficient tumor cell killing with reduced off-target toxicity. However, nonspecific alkylation by 3-BrPA can induce toxicity in the normal immune and stem cells. Therefore, several attempts are being made for local-regional delivery of 3-BrPA through catheters, microencapsulation, or intra-arterial routes to minimize the toxicity [135, 136]. Likewise, synergistic inhibition of glycolysis and OXPHOS by a combination of metformin with bromodomain and extra-terminal motif (BET) inhibitor, JQ-1, has been tested in pancreatic cancer [137]. These combinatorial targeting strategies could provide ways to overcome therapy resistance and achieve durable responses. The metabolic plasticity of cancer cells in the harsh TME is mediated by a cross talk between

gene regulation and metabolic pathways [1]. A recent study devised a theoretical framework to couple gene signatures and metabolic interplay in the hybrid metabolism phenotype. This study indicated a direct correlation between AMPK and OXPHOS, and HIF1 and glycolysis, highlighting the significance of targeting abnormal metabolism in cancer by modulating both genes and metabolic pathways [41]. The multifaceted interactions between different signaling pathways regulate metabolic reprogramming in cancer cells, allowing them to proliferate and sustain therapeutic resistance. The inhibition of key metabolic regulators, including KRAS, MYC, P53, HIF1α, and PI3K/AKT/mTOR pathways, could be an effective approach towards tumor killing. For instance, targeting KRAS in PDAC patients showed promising results in preclinical studies; however, it had no positive influence on patient survival [17]. Similarly, preclinical studies targeting EGFR and CDK4/6 by afatinib and palbociclib have shown great promise in reducing tumor progression by reducing metabolic reprogramming in HNSCC [138]. Several ongoing preclinical and clinical studies targeting HIF1α, MYC, and P3K/mTOR pathways in various cancers are under progress. Nevertheless, the metabolic plasticity of cancer cells poses a serious therapeutic challenge in targeting a specific pathway as they can overcome the inhibitory effect by activating the alternative metabolic pathways. In addition, other cells of TME, including stroma, fibroblasts, and immune cells, also influence the metabolic milieu of tumor cells and help them survive in a stressful environment. Therefore, current approaches focus on combining anti-glycolytic agents that target different metabolic pathways or their combination with other chemotherapeutic agents to overcome the therapeutic resistance. Overall, the knowledge acquired from these studies will help develop an understanding on future therapeutic perspectives based on metabolic reprogramming.

2.9 Concluding Remarks

Metabolic reprogramming is employed by tumor cells/CSCs to survive and grow in the harsh TME to generate energy and precursors for the biosynthetic process and maintain their redox balance. This reprogramming is achieved by acquiring mutations in the oncogene and tumor-suppressor genes which activates the downstream signaling pathways associated with tumor progression, metastases, and therapy resistance. Apart from metabolic switching from glycolysis to OXPHOS, tumor cells also acquire a hybrid phenotype and utilize both metabolic pathways. While most studies are limited to investigating altered metabolism in tumor cells, a broader understanding of metabolic cooperativity between the tumor cells and stromal compartments may help delineate intricated metabolic pathways and exploit them for novel anticancer therapies.

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Immuno-onco-metabolism and Therapeutic Resistance

3

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Abstract

Cancer is a complex succession of disease progressively associated with uncontrolled growth and capability of cells eluding the body's natural mechanism of defense and cell death. Primary metabolic processes are critical for mammalian cells in terms of producing energy, sustaining redox potential, and precursors for macromolecule production. Therefore, disrupted metabolism of immune cells as a result of their differentiation and stimulation has given rise to a new concept of immuno-metabolism. Several preclinical immunotherapy models and cancer patient analyses have lately resulted in a number of cancer immunological understandings. Increasing evidence suggests that metabolic machinery in the tumor microenvironment (TME) plays a role in the development of immune checkpoint inhibitor (ICI resistance). Due to the current successes of cancer immunotherapy in the treatments of a variety of cancers, researchers are focusing their attention on elucidating changes in cancer and immune cell metabolic patterns throughout their interactions in the context of cancer development and immunotherapy. Potential benefits for individuals with aggressive tumors have resulted from advancements in our understanding of tumor immune biology and the establishment of cancer immunotherapies. Current studies have shown that both competition for vital nutrients and deprivation of certain amino acids can cause T-cell malfunction; therefore, the metabolic landscape of the tumor microenvironment is of special interest. Within the TME, nutrients and oxygen are scarce, forcing immune cells to undergo metabolic reprogramming to adapt to harsh conditions. Cancer-induced metabolic deregulation in immune cells can

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attenuate their anticancer properties, but can also increase their immunosuppressive properties. Therefore, targeting metabolic pathways of immune cells in the TME may strengthen the efficacy of ICIs and prevent ICI resistance. In this, chapter we discuss the interactions of immune cells and metabolic reprogramming of immune cells in the tumor microenvironment, as well as developing treatment options to increase immune cell metabolic efficiency and boost the antitumor immune response.

Keywords

Tumor microenvironment · Immune cells · Cancer immunotherapy · Immunotherapy resistance · T cells · Glycolysis · Oxidative phosphorylation · Hypoxia · Metabolic reprogramming · Immune checkpoint inhibitors · Cytotoxic T-lymphocyte antigen-4 · Programmed death receptor-1

Highlights

1. A brief history and introduction of immunotherapy.
2. Introduction to various immune cells present in tumor microenvironment.
3. Understanding the metabolic reprogramming of immune cells.
4. Discuss the immune checkpoint inhibitors and resistance to immunotherapy.
5. Strategies to overcome resistance to immunotherapy.

3.1 Introduction

Since late 1800s, the importance of cancer immunology and the utilization of the immune system as a means to destroy cancer cells have been marked [1–3]. The pioneer of cancer immunotherapy, William B. Coley, MD, documented cases wherein cancer disappeared after a person acquired erysipelas, a bacterial illness, in 1893. Dr. Coley was mistaken in thinking that the bacteria were eliminating the tumors; nevertheless, scientists now believe that the infections prompted a powerful immune response that eradicated the cancers. Following these findings, Dr. Coley created "Coley's toxins," a combination of dead *Serratia marcescens* and *Streptococcus pyogenes* that he delivered to individuals with several forms of incurable carcinomas. Coley's toxins were efficient in healing certain tumors, especially sarcomas, and were utilized as a treatment for cancer for years [4]. In 1899, Parke-Davis and Company started manufacturing Coley's toxins and has been continuing to do this for several years. The toxins were utilized in a number of European and American hospitals, notably the Mayo Clinic, but the outcomes were inconsistent [5]. Due to limitations in therapeutic effectiveness, keenness for Coley's toxins waned, and radiation became the favored treatment for cancer with much more consistent effects [6]. Even Memorial Hospital (now Memorial Sloan Kettering Cancer Center in New York City), with which Dr. Coley was affiliated, set a policy in 1915 saying that people with cancer should be treated with radiation rather than Coley's toxins [5]. Chemotherapy was established during the World War II which

further lowered attention in Coley's toxins and other cancer immunotherapies. In 1965, the American Cancer Society classified toxins developed by Coley as an "unverifiable" therapeutic (a classification that has since been rescinded) [1]. Helen Coley Nauts, Dr. Coley's daughter, researched many of her father's case records after his death in 1936 and felt persuaded that her father's research was noteworthy [7]. Despite her efforts to reawaken attention on his work, specialists, particularly cancer specialists at Memorial Sloan Kettering, remained unconvinced. This nonprofit organization has become a significant source of support for cancer immunotherapy research awarding more than $29 million in scientific grants in 2015 [7, 8]. In 1957, E. Donnall Thomas took another significant step forward in cancer immunotherapy by investigating stem cell transplantation. Dr. Thomas initially treated individuals with advanced leukemia with bone marrow from normal people and had some success. The treatment, though (currently called as allogeneic hematopoietic stem cell transplantation [allo-HSCT]), was extremely hazardous. Nonetheless, allo-HSCT has been utilized as a therapeutic agent for hematologic malignancies since its inception. Steven A. Rosenberg achieved the next major breakthrough in cancer immunotherapy in the late 1980s, when he delivered cytokines and activated immune cells to melanoma patients [8]. Dr. Rosenberg has documented recoveries in a number of individuals with advanced melanoma, and he continued to explore the field of cancer immunotherapy, focusing on melanoma and renal cell carcinoma (RCC) research [5]. Throughout the twentieth century, immunotherapy cancer treatments were being developed [9]. Even when President Richard Nixon proclaimed a war on cancer in 1971, little was known about cancer genesis, oncogenes, and environmental factors, and even less about cancer-relevant immune system functions. Despite this, most researchers were aware that the immune system was essential in cancer diagnosis and eradication, and they believed that immunodeficiency was the cause of cancer growth [10, 11]. Over the last few decades, scientists have made significant progress in comprehending how cancer is diagnosed, removed, and avoided by the immune system [12]. Developments in molecular and tumor biology, notably in the last 15 years, have radically altered cancer treatment approaches. Recently the use of immune checkpoint inhibitors (ICI) in treating cancer has revolutionized the approach to eradicate cancer cells by reactivating immune responses. Previously, malignancies were only identified and treated based on histomorphological characteristics and the organs of genesis. It is also becoming clear that the widespread use of cytotoxic chemotherapeutic medications has achieved a clinical limit, and that therapies should rather be targeted to specific molecular changes. Table 3.1 shows a timeline that highlights few of the key milestones in cancer immunotherapy. Two key breakthroughs in cancer studies and treatments are currently addressing the demand for targeted therapies. Some of these developments are based on substantial advances in cancer immunotherapy, which have enabled novel therapies to boost the body's antitumor immune capabilities [8, 13]. Since this strategy is so successful, scientists named "cancer immunotherapy" the "Breakthrough of the Year" in 2013. Experimental studies with immune checkpoint blockade (ICB) treatments or chimeric antigen receptor (CAR) T-cell therapy have demonstrated to have been possibly

Table 3.1 Showing a timeline that highlights few of the key milestones/events in cancer immunotherapy

Historical dates	Events/key milestones in cancer immunotherapy
• 1893	Dr. William B. Coley invented Coley's toxins
• 1899	Parke-Davis and Company manufactured Coley's toxins
• 1915	Memorial Hospital (Memorial Sloan Kettering) banned Coley's toxins and preferred radiation treatments
• 1943	Use of chemotherapy began for cancer treatment
• 1953	Helen Coley Nauts (Dr. Coley's daughter) founded the cancer Research Institute in New York City
• 1957	Dr. E. Donnall Thomas successfully treated patients with advanced leukemia with allo-HSCT
• 1971	President Richard Nixon declared a war on cancer
• 1986	Recombinant INF-α was approved by the FDA for hairy cell leukemia
• 1988	Dr. Steven A. Rosenberg published work regarding curative treatment of melanoma patients with active immune cells and cytokines
• 1992	rhIL-2 approved by the FDA for RCC
• 1996	First mAB approved by the FDA for selective B-cell malignancies
• 2010	First DC-based cancer vaccine approved by the FDA for prostate cancer
• 2011	First CTLA-4 ICB FDA approved for melanoma
• 2014	First PD-1 ICB FDA approved for metastatic melanoma

lifesaving [6]. Tumors have vanished and terminal cancer has gone into dormancy for years in some individuals who have received treatments with cancer immunotherapies [12].

Pharmaceutical companies, governments, and charities have all expressed interests in and invested in the emerging subject of cancer immunotherapy, which is backed by good clinical data [12]. As a result, ClinicalTrials.gov had almost 1700 clinical investigations linked to cancer immunotherapy as of May 2017. The detection and targeting of treatable genetic changes in oncogene-driven malignancies are the target of a second revolution currently underway. It enables genotype-directed treatments to be customized to particular subsets of individuals with unique genetic aberrations across various tumor types. Based on theory, therapies that target tumor-specific molecular aberrations should be more successful and less harmful [8]. The term "immunometabolism" was coined as a consequence of a recent success in immune cell metabolic reprogramming. In light of the recent achievement of cancer immunotherapy in curing a variety of cancers, researchers are focusing their efforts on elucidating changes in cancer and immune cell metabolic patterns during their interactions in the context of cancer development and immunotherapy. Although immune system metabolic regulation is not clear-cut, there is rising attention in the developing role of "immunometabolism" as a key modulator of immune cell fate and functioning [14–19]. It is well understood that immune cell modifications in critical metabolic processes are caused not just by nutrition or oxygen levels, but even by immunological signals [20]. It is evident that, in addition to energy generation and

biosynthesis, immune cell phenotype and functioning are governed by a variety of metabolic processes. Cancerous cells undergo a complicated and dynamic metabolic reprogramming in response to the bioenergetic and biosynthetic demands for growth and adjustment to the "stressful" tumor microenvironment (TME). Hypoxia, pH, and the "Warburg effect," or cancer cells' preferential utilization of glycolysis for ATP production, are all important factors in determining the metabolic TEM [21–25].

Cancer cells influence the immune compartments through their metabolic activities which directly or indirectly hampers immune cell activation, fitness, and effector function by aggressively competing for critical nutrients (e.g., glucose, amino acids, lipids, and glutamine) or generating metabolic by-products [19, 26–30]. As a consequence, such faulty immune cells not just fail to kill cancer cells, but also transform into cells that support tumor, allowing cancer to spread and invade more easily. The basic influence of metabolic reprogramming on immunological cells within the TME or in cancer immunotherapy, on the other hand, is relatively small. The elimination of invading pathogens as well as maintaining tissue homeostasis are the primary functions of the immune system of the host. This complex and strongly engaging immune system, which includes lymphoid organs and different kinds of immune cells, as well as immune-modifying factors such as chemokines, cytokines, and surface molecules, can be activated to support a well-controlled immune response to eradicate pathogens or altered cells while trying to avoid tremendous tissue damage. Dysfunction of this system, on the other hand, can lead to deadly infections, autoimmunity, cancer, and a variety of other diseases [31, 32]. There are two major arms of the immune system, the innate and acquired, each having specialized cells associated with it. Macrophages or monocytes, NK cells, and neutrophils are examples of innate immune cells that can give rapid immunity as a first line of defense. T and B lymphocytes are adaptive immune cells that can be stimulated to produce long-lasting antigen-specific immune responses [33]. In recent years, a complex interaction between immune cell formation (or functioning) and metabolic reprogramming has evolved. An immense organization of immunometabolism studies is trying to focus on clarifying metabolic alterations linked with phenotypic differentiation and stimulation of major subsets of immune cells, such as T cells and macrophages, and, more notably, the prognosis of metabolic reprogramming of that kind of immune cells. This complete consequence has increased fundamental comprehension of the various metabolic modifications in determining the immune cell variety and functional adaptability. The tumor microenvironment (TME) is populated by immune cells, endothelial cells, stromal cells, and cancer-associated fibroblasts [34]. Cancer cells tend to evade immune surveillance and killing through a variety of methods (as shown in Fig. 3.1). Novel techniques such as mass cytometry and single-cell RNA sequencing have recently been developed and applied in tumor immunology to assist to define the immune cell landscape within the TME at the single-cell level, making it simpler to examine and recognize new subsets of tumor-associated immune cells [35, 36]. The importance of such tumor-associated immune cells in TME, as well as their applicability in cancer immunotherapy, will be described in the sections to follow. The cellular tumor microenvironment, in combination with physical settings, promotes invasion

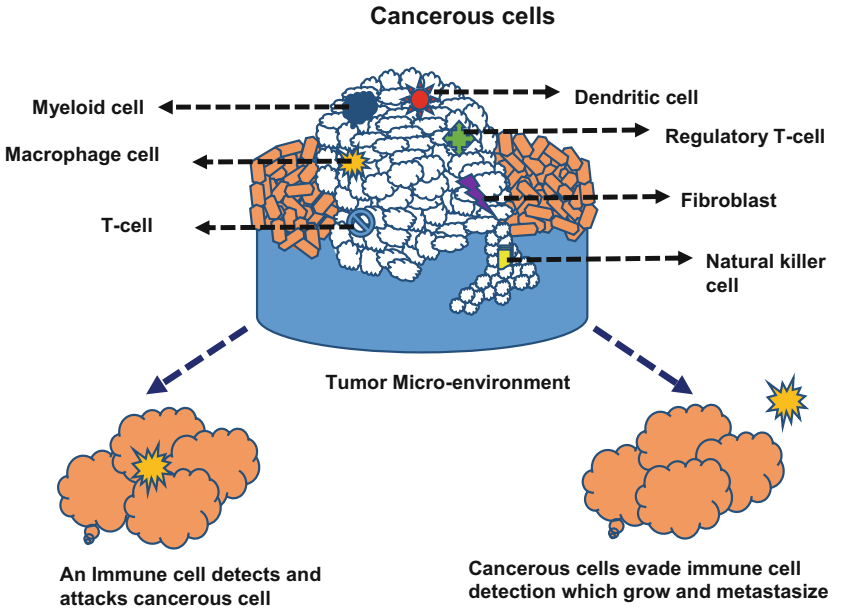

Fig. 3.1 The tumor microenvironment consists of heterogeneous populations of cells

through a variety of mechanisms, the most important of which is the role of immune cells. Tumor cells' biological contact with stromal immune cells results in a variety of effects, varying from tumor inhibition to tumor activation. Metastasis and invasion are a series of events that necessitate remodeling of the whole extracellular matrix (ECM), such as the disintegration of the basement membrane, neovascularization, and loss of cell cohesion, that acts as a mode of transport to a new location where colonization finishes the process. For such a long period, it was assumed that immune cells were responsible for clearing tumor cells from the body. While this is true for immune cells like NK cells and cytotoxic T cells, the role of mast cells, TAMs, and several other immune cells in the promotion of tumor cannot be ignored [37–39]. Tumor cells have immunoediting capabilities in two ways: they can evade immune recognition due to dysregulated APC pathways, or they can change the phenotype of immune cells. Figure 3.2 illustrates how immune cells play a part in tumor development, invasion, and metastases, as well as how it compares to the usual inflammatory response. Tumor cells that have their antigen expression downregulated are able to evade the immune system's clearance mechanisms. The phenotypic alteration of immune cells is caused by a cytokine environment created by the interplay of stromal cells and tumor cells which begins to favor metastasis tumor development. CD4+ T helper cells in the Th1 phenotype are recognized to enhance the tumor-clearing activity of CD8+ cells, whereas similar CD4+ T helper cells in the Th2 phenotype are believed to delay the activity of CD8+ cells. Likewise, the N1 and N1 phenotypes of neutrophils and macrophages accordingly are

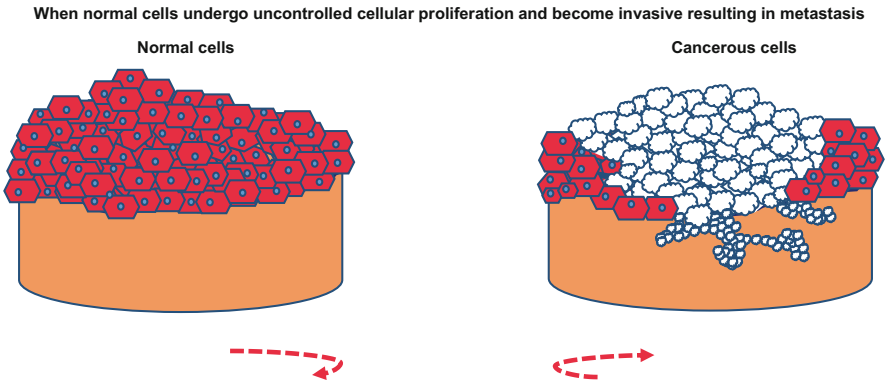

Fig. 3.2 Role of immune cells in tumor progression, invasion, and metastasis

renowned for their tumor-suppressing properties, but transition to the N2 and M2 phenotypes promotes tumor growth [40]. Simultaneously, CAFs have been found to release a variety of growth factors as well as certain matrix-degrading enzymes.

Such modifications cause ECM remodeling, which allows metastatic tumor cells to easily enter the neovasculature, infiltrate, and establish themselves [41, 42].

Immune cells in tumor microenvironment (TME): Tumor-associated immunological cells can be split into two categories: tumor-antagonizing immune cells and tumor-promoting immune cells. Both cell types play an important part in tumor growth at various stages.

Tumor-antagonizing immune cells: T cells (particularly CD8+ cytotoxic T cells and effector CD4+ T cells), DCs, NK cells, neutrophils, and macrophages are among the tumor-antagonizing immune cells.

(a) **Effector T cells:** T cells are adaptive immune cells that play a role in host protection, homeostasis, immunological tolerance, and memory. T-cell receptors (TCRs) are activated to identify antigens from infiltrating infections, cancerous cells, as well as the environment. T cells are divided into two groups, i.e., CD8+ and CD4+ T cells, depending on lineage markers and capabilities. After recognizing antigenic peptides in association with class I MHC molecules, CD8+ T cells can be stimulated and differentiated into CTLs. CD4+ T cells, on the other hand, can be stimulated by antigen in the presence of class II MHC molecules found on professional APCs, such as DCs, B cells, and macrophages. Based on the cytokine environment of the microenvironment, CD4+ T naïve cells develop into functional helper T cells after stimulation [43–47]. CTLs may exert direct lethal effects on target cells (for example, infected or altered cells), while CD4+ T cells primarily assist CTL functioning. Even though most of the effector T cells die after elimination of cancer cell or infection, a diverse pool of memory T cells stay alive and persist in the body where they can be mobilized to mount a much more quick and powerful immune response when confronted with secondary infection from a morphologically same pathogen [48–

50]. Increasing evidence suggests that when T cells are activated, they undergo metabolic reprogramming which results in the imprinting of their different destinies and roles. While naïve T cells generate energy primarily through OXPHOS of numerous nutrients such as amino acids and glucose, differentiating effector T cells transition to glycolysis after encounter of antigens and stimulation to support their quick proliferation and function, such as cytokine production [51–53]. The metabolic reprogramming of stimulated T lymphocytes is governed by several signaling mechanisms. Binding CD3 and CD28 on T cells activates signaling cascades including Akt and PI3K in addition to starting signals which are intracellular that promote cell proliferation [54–57]. The role of Akt in cell survival and cytokine synthesis, which are both essential for active T cells, is clearly documented [58–61]. Such signaling cascades increase glycolysis by stimulating glucose transporters (e.g., GLUT1), transporters of amino acids, and glycolysis rate-limiting catalysts (e.g., HK2) [60, 62]. It is important to note that most of the metabolic and signaling changes in stimulated T cells are similar to those seen in cancer cells. T-cell activation is properly controlled by immune checkpoint inhibitors or co-inhibitory signals in addition to co-stimulated signals to avert abnormal immunological responses, such as those that promote autoimmunity. During T-cell activation, the immunological checkpoint molecule CTLA-4 is activated, which has a considerably stronger affinity for the co-stimulatory B7 molecules on APCs. Competing for B7 molecule binding inhibits co-stimulation signaling via CD28 and prevents activation of T cells [63]. Another immune checkpoint molecule that restricts T-cell response is PD-1 [64] that can lead to exhaustion of effector T cells in chronic diseases and cancer immune escape [65–67]. The activation of PD-1 signaling in human CD4+ T lymphocytes has lately been found to limit their capacity to absorb and use glucose, glutamine, and amino acids. Rather, these T cells have a higher rate of fatty acid oxidation (FAO) that is linked to an increase in the important enzyme CPT1A [68]. CTLA-4, like PD-1 blockage, decreases glucose absorption in T cells after co-stimulation [69, 70]. CTLA-4, on the other hand, suppresses glycolysis by downregulating GLUT1 without affecting CPT1A levels or FAO rate implying that CTLA-4 plays a function in regulating the metabolic activity of non-stimulated cells. Further research found that both CTLA-4 and PD-1 signaling inhibits CD28-mediated Akt and PI3K activation [62, 70, 71], implying that Akt is a universal pathway employed by immunological checkpoint molecules to reduce the activation of T cells. T cells that have been activated also enhance the absorption of fatty acids (FAs) and boost lipid synthesis [19]. An enzyme ACC1 converts acetyl-CoA to malonyl-CoA, which is necessary for the production of long-chain FAs. ACC1 deletion in T cells prevents antigen-specific CD8+ T cells from expanding because of increasing cell death. Exogenous FAs can help ACC1-deficient CD8+ T-cell survival, indicating that FA production plays an important role in CD8+ T-cell expansion and engagement [72]. De novo FA production via ACC1 is also required for differentiation of Th17 inflammatory cells. Suppression of ACC1 reduces cell differentiation of Th17 by boosting the

anti-inflammatory Foxp3+ regulatory T-cell polarization [73], emphasizing the role of FA source specificity in controlling T-cell lineage development. Unlike active effector T cells, which favor glycolysis, Tregs or CD8+ memory T cells rely heavily on OXPHOS and FAO for energy metabolism. During Treg development in an asthmatic model, substantially active AMP-activated kinase protein in Tregs reduces GLUT1 expression and favors significant lipid oxidation rates [74]. CD8+ memory T cells that survive after pathogen elimination [75] have a metabolic profile with increased mitochondrial FAO as shown in Fig. 3.3. CD8+ memory T cells, unlike effector CD8+ T cells, employ extracellular glucose to promote OXPHOS and FAO, showing that lipid synthesis is required to supply substrates to FAO [76]. Memory T cells need IL-7 and IL-15 for longevity and self-renewal, according to several sources of research [77–79]. To maintain cellular longevity throughout infection with L. monocytogenes, CD8+ memory T lymphocytes have a significant mitochondrial spare respiratory capacity (SRC). CPT1A expression was observed to be boosted by IL-15 [80]. TAG production has been proven to be a critical component of human and mouse CD8+ memory T-cell survival when they are supported by IL-7 [81]. The mammalian glycerol channel aquaporin 9 (AQP9) can be induced by IL-7 in CD8+ memory T cells. Glycerol transfer into memory CD8+ T cells for triacylglycerol (TAG) production and storage is hampered when AQP9 is absent [81–83]. These findings add to our knowledge of how cytokines regulate mitochondrial metabolism to define certain T-cell activities. Another important factor in T-cell formation and function is amino acid metabolism, which serves as a source of fuel and also a precursor for the synthesis of macromolecules. L-arginine, which is also a precursor of immunomodulatory metabolites including polyamines and NO, is required for protein synthesis [16]. Increased L-arginine levels perceived by transcription factors PSIP1, TSN, and BAZ1B in activated T cells induce a metabolic transition from glycolysis to OXPHOS, enabling the production of central memory T cells with improved survival capability [84], highlighting the importance of L-arginine in reprogramming metabolism and T-cell functionality. Furthermore, glutamine, which is the most abundant amino acid in blood, is essential for energy synthesis in activated T cells to promote rapid proliferation [85]. TCR activation can cause amino acid transporter activation. Th1 and Th17 cell differentiation is mediated by ASCT2-induced glutamine and leucine uptake [86]. Glutamine seems to be important for CD8+ CTL fitness and CD8+ memory T-cell growth [87]. These numerous investigations, taken together, reveal the intricacy of metabolic alterations that might affect immune cell stimulation and differentiation. CTLs have long been considered to be the most important lymphocyte subsets for killing cancer cells that exhibit class I MHC molecules [88]. CD8+ T cells can be converted into cytotoxic effector CD8+ T cells when antigens from DCs are presented to them [89]. Through the expression of CXCR3, stimulated CTLs can move into the inflammatory environment under the direction of chemokines (such as CXCL9 and CXCL10) released by DCs [90]. The association between DC ligands (CD70 and CD80-

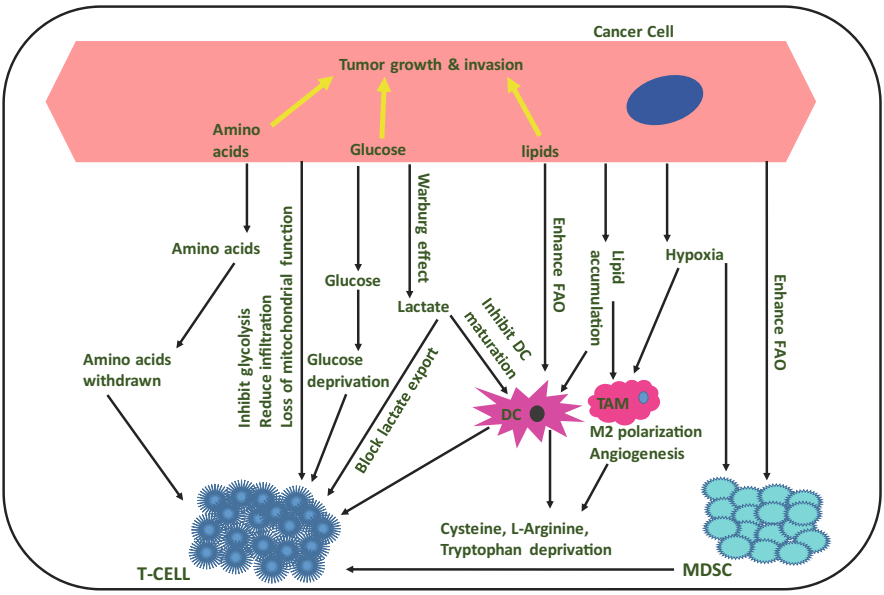

Fig. 3.3 Cancer cells metabolically change tumor microenvironment via enhanced FAO, lipid, glucose, and amino acid accumulation which leads to an amplified immune suppression and tumor growth and invasion

CD86) and CD8+ T-cell receptors (CD27 and CD28) was thought to be one of the most important processes in priming CD8+ T cells. The supporting signals from CD4+ T cells, in addition to the stimulus signals from DCs, were revealed to be critical for CTL priming [91–93]. On the one hand, CD4+ T cells can aid CD8+ T-cell activation by interacting with CD40 and CD40L, as well as stimulate CD8+ T-cell proliferation by producing IL-2 [94–97]. CD4+ T cells, on the other hand, can aid DC activation and licensing of CD8+ T cells by cross-presentation of tumor antigens to CD8+ T cells or by promoting the release of cytokines as well as other stimulatory substances [98]. Furthermore, CD4+ T cells can aid in the maturation of CD8+ T cells into memory CTLs [99]. CTLs could destroy the target 113 cells through granular exocytosis and Fas ligand (FasL)-mediated apoptosis in the "stimulation" state; CTLs might also release IFN-γ and TNF to promote cancer cell cytotoxicity [100]. CTL activation and regulation involve two signals: the first from the TCR, and the second from additional receptors known as immunological checkpoints [101]. There are two types of immunological checkpoints: inhibitory checkpoints, e.g., CTLA-4, PD-1, TIM-3, LAG-3, TIGIT, and CD96 [102–107], and stimulatory checkpoints, e.g., ICOS, OX-40, 4-1BB, CD27, CD40L, GITR, and HVEM [108–112]. We previously understand that in tumors, cells block CTL activation by expressing ligands such as PD-L1 that bind to inhibitory checkpoints, which is thought to be a key way for cancer cells to avoid being detected by the

immune system. Furthermore, in the presence of prolonged antigen and inflammation inside the TME, CTLs would get exhausted, contributing to T-cell malfunction and tumor growth [113].

(b) **NK cells:** Innate immune lymphocytes (NK cells) are a type of immune cell that acts as a first line of defense towards infections and cancerous cells. These cells have a role in host defense through cytotoxicity and cytokine release (e.g., IFN-γ), as well as by influencing the actions of other immune cells (i.e., T cells, DCs) [114–118]. In their resting state, NK cells primarily utilize mitochondrial OXPHOS. When cytokines (e.g., IL-2) activate NK cells, substantial metabolic reprogramming occurs, as seen by increased glycolysis rates. Glycolysis inhibition is enough to limit IFN-γ and granzyme B release by activated NK cells [119]. For cytokine-mediated metabolic reprogramming of NK cells, the transcription factor Srebp is required. Srebp is thought to facilitate glucose metabolization to cytosolic citrate through the citrate-malate shuttle during increased glycolysis and OXPHOS in NK cells. Srebp inhibition reduces NK cell activity and cytotoxicity against cancer cells [120]. As a result, metabolic reprogramming to glycolysis is required for NK cell effector activity. Even when IL-12 plus IL-18 stimulation of NK cells is independent of glycolysis or mitochondrial OXPHOS, activation of IFN-γ release by activating NK receptors demands glucose-driven OXPHOS [121] implying that metabolism can behave as a second signal for IFN-γ release resulting from receptor activation of NK cells.

NK cells are also a subpopulation of tumor-antagonizing immunological cells that play a role in tumor immunosurveillance. In humans, NK cells are CD3CD56+ cells that make up 5–15% of circulating lymphocytes [122]. TME's NK cells provide the same function as CD8+ T cells [122]. Following the supervision of chemokines released by DCs, NK cells can be drawn to inflammatory or cancerous tissues. The tumor-killing response is mediated mostly by NK cells producing perforin and granzymes, which cause target cells to undergo apoptosis [123]. NK cells also release pro-inflammatory chemokines and cytokines (such as IFN-, IL-6, TNF, CCL5, and GM-CSF) to aid in antitumor action [122]. In addition, the NK cell may influence T-cell antitumor response by promoting antigen cross-presentation to CTLs [123]. Despite the fact that NK cells play an important role in tumor immunosurveillance, numerous studies show that the lethal function of tumor-infiltrating NK cells is constantly reduced [122]. Tamara Krneta and coworkers, for instance, found that NK cells inside the TME of breast tumors were immature, as demonstrated by their lowered expression of DX5, CD27 low CD11b low phenotype, enhanced expression of NKG2A, and reduced levels of NKp46, perforin, and granzyme B; however, therapy with IL-12 and anti-TGF-151 improved the maturity of this subset of tumor-infiltrating NK cells. The defective subset of liver-infiltrating NK cells with CD11b-CD27 has even been linked to the advancement of hepatocellular carcinoma, according to Qiong-Fang Zhang and coworkers. Because inhibitory immune checkpoint signaling (e.g., CTLA-4, PD-1, KIR2DL-1/2/3, Tim-3, CD96, NKG2A, TIGIT) inhibits NK cell

function, inhibiting these inhibitory mechanisms may reinstate NK cell antitumor capability [122]. Blocking the checkpoint receptor TIGIT avoids NK cell depletion and generates robust antitumor immunity, according to a new study by Qing Zhang et al.

(c) **Dendritic cells (DCs):** DCs are specialized APCs that play a key role in both innate and adaptive immunity. Through PRRs, these cells can detect a vast array of inflammatory signals coming from microbiological pathogens or altered cells and induce beneficial immune responses [14, 124]. DCs at various stages of maturation or activation have been shown to be sustained by several types of cellular metabolism to meet their bioenergetic and biosynthetic requirements. Human monocytes develop into DCs after being exposed to GM-CSF and IL-4, a mechanism that needs lipid metabolism and mitochondrial synthesis [125, 126]. Human DC formation from peripheral blood mononuclear cell progenitors is significantly reduced when FA production is blocked [127]. DCs, on the other hand, absorb more glucose and generate more lactate when toll-like receptor (TLR) ligands are activated [128–130]. The suppression of OXPHOS by nitric oxide (NO) generated during DC activation is required for this metabolic transition towards glycolysis. Glucose restriction significantly reduces DC activity and longevity after TLR stimulation [128, 129]. DCs, in addition to glucose, express specialized isoforms of glycogen-metabolizing enzymes to aid maturation and effector function, especially during the early stages of activation or during glucose-restricted situations [128]. TLR activation causes a significant glycolytic flow, which is required for de novo FA production. The enlargement of the Golgi and ER for the production of co-stimulatory substances and the release of inflammatory cytokines can then be supported by FA synthesis [131]. Tolerogenic DCs, in comparison with activated or mature DCs, have high mitochondrial oxidative activity and a significantly higher FAO rate. As a consequence, inhibiting FAO can help tolerogenic DCs to stimulate T cells more effectively [132]. Nevertheless, in tumor-associated DCs which are recognized to be operationally defective or tolerogenic, the molecular and metabolic shift from glycolysis to OXPHOS has yet to be characterized. As previously stated, DCs primarily serve as specialized APCs. They can display antigens and offer T-cell activation co-stimulatory signals. NK cells and B cells can also engage with DCs [133, 134]. Recruitment and immune activation of immune effector cells increase when matured, active DCs infiltrate tumors. Tumor cells, on the other hand, have the ability to suppress the activities of DCs. Michielsen et al., for instance, discovered that tumor-prepared media made from cultivating human colorectal tumors explant tissues included elevated levels of CCL2, CXCL1, CXCL5, and VEGF, and that pretreatment of DCs in vitro using this media inhibited maturation [135]. Tumors can immobilize DCs by inducing PD-1 expression, according to Krempski and coworkers. As a result, DC-based vaccinations primed with patient-specific neoantigens, either alone or in conjunction with inhibitory checkpoint inhibition to reinstate DC antigen-presenting activity, should be considered as possible methods for cancer immunotherapy.

(d) **Macrophages:** Macrophages are multipurpose innate immune cells that play a role in a variety of physiological and pathological processes such as host protection, tissue homeostasis, tumor development or elimination, as well as T-cell immunity control. The discovery of two primary phenotypes of macrophage activation or polarization has been made based on preliminary investigations employing in vitro stimuli. Inflammatory stimulation, such as IFN-γ plus lipopolysaccharide (LPS), can cause classic macrophage activation (i.e., M1 phenotype), which is described by the inflammatory cytokine production (e.g., TNF-α, IL-12) and reactive nitrogen and oxygen intermediates (RNIs and ROIs), and also improved antimicrobial functions. In comparison, anti-inflammatory signals (e.g., IL-4, IL-13, glucocorticoids) frequently promote M2 polarization or alternative stimulation of macrophages which is linked with a functional switch towards tissue repair or immunosuppression [136–138]. M1/M2 polarization of macrophages is associated with different metabolic processes. Unlike M1 macrophages, which prefer to consume glucose [15], differentially activated macrophages primarily rely on FAO and mitochondrial synthesis [139]. LPS activation of macrophages causes an increase in succinate levels, which is a TCA cycle intermediate, as well as overexpression of glycolytic genes and decreased expression of mitochondrial biosynthesis genes. Glycolysis suppression in macrophages by 2-deoxyglucose reduces IL-1β generation caused by LPS [140]. In macrophages, signal transducer and activator of transcription 6 (STAT6) and PPAR-coactivator-1 (PGC-1), that connect inflammatory and lipid homeostasis cellular processes, induce metabolic reprogramming towards FAO and mitochondrial synthesis in response to IL-4 [141, 142]. M1 macrophages upregulate the GLUT1 [143], whereas M2 macrophages stimulate lipoprotein lipase and CD36, which control triacylglycerol intake and transportation [144, 145]. Exogenous triacylglycerol uptake and lipolysis create FAs for FAO and stimulate the gene expression that identifies M2 macrophages [145]. However, more research into FAO's role in M2 macrophages is needed. Although CPT2-deficient macrophages displayed a deficiency in FAO, they were appropriately polarized to an M2 phenotype in vitro and in vivo, according to a recent study. Furthermore, etomoxir-mediated suppression of FAO in human macrophages had no impact on IL-4-induced M2 polarization [146]. Furthermore, cholesterol metabolism was found to regulate macrophage activity. In macrophages, activating type I interferon (IFN) transmission causes a metabolic transition as seen by a reduction in cholesterol production and an elevation in cholesterol import. The production of IFN-inducible genes is induced in macrophages when this metabolic transition is deliberately induced, which improves the antiviral immune responses [147]. Restricting flow via the cholesterol biosynthetic pathway activates a type I IFN response that is STING (stimulator of interferon genes) dependent, which not only identifies a metabolic inflammatory circuit that included lipid biosynthesis and innate immunity, but also does provide scientific proof for metabolic reprogramming to modify host immunity. The functional regulation of macrophages has been linked to amino acid metabolism. In macrophages,

IFN-γ and LPS activation can increase the activity of inducible nitric oxide synthase (iNOS or NOS2), which enhances the transformation of L-arginine to NO. IL-4, on the other hand, causes macrophages to produce arginase 1, which mediates the transformation of L-arginine to L-ornithine [148]. The uniqueness of arginine metabolism has been linked to a variety of macrophage activities, such as the removal of microbial infections and cancer cells, tissue remodeling, and, in some situations, tumor growth support [149, 150]. In macrophage M2 polarization, glutamine degradation and UDP-GlcNAc-linked modules are necessary, according to a recent study. M2 polarization and generation of the chemokine CCL22 can be suppressed by glutamine deficiency or suppression of N-glycosylation [151]. The NLRP3 inflammasome (NOD-like receptor family pyrin domain containing 3) is a well-studied intracellular PR multi-protein complex that controls the innate immune responses and the generation of pro-inflammatory cytokines like IL-1β and IL-18 [152–154]. Lipid biosynthesis is increased by mitochondrial uncoupling protein-2 (UCP2) to control NLRP3 inflammasome activation in macrophages, as evidenced by lower IL-1β and IL-18 production in LPS-challenged UCP2-deprived mice. Reduced expression of FASN led to impaired lipid synthesis due to the lack of UCP2 (Moon, Lee, et al., 2015). Surprisingly, FAO increased NLRP3 inflammasome activation in macrophages via NOX4-dependent overexpression of carnitine palmitoyltransferase 1A (CPT1A), a rate-limiting enzyme that regulates mitochondrial FAO [155]. Furthermore, saturated FA palmitate can activate the NLRP3 inflammasome in macrophages, whereas unsaturated FA oleate cannot [156]. Suppression of glycolysis in macrophages reduces both caspase-1 activity and IL-1β maturation in reaction to LPS and ATP, indicating that activation of a glycolytic phenotype in macrophages is connected to NLRP3 inflammasome activation [155]. The phenotypic polarization and operational stimulation of macrophages are both dependent on metabolic reprogramming. Macrophages, which are immune cells produced from circulatory monocytes, are an essential kind of immune cell in TME. They are often classified as pro-inflammatory (M1 polarized) or anti-inflammatory (M2 polarized). Conventionally activated macrophages (M1) create pro-inflammatory cytokines and ROS or RNS reactive that are important for host defense and tumor cell death, and are hence regarded as "good" macrophages [157]. Narayanan et al. discovered that tumor-infiltrating M1-polarized macrophages led to the tendency of increased survival in MSI-H individuals in a recently released study indicating the tumor-antagonizing activity of M1-polarized macrophages [158]. M2-polarized macrophages, on the other hand, not just release anti-inflammatory cytokines and decrease immunosurveillance against tumor cells, but also enhance angiogenesis and matrix remodeling, hence promoting tumor development and metastasis [157]. Tumor-associated M2-polarized macrophages are often thought to be the primary source of myeloid-derived suppressive cells (MDSCs) [159], which will be explored more in the portion regarding tumor-promoting immune cells.

(e) **Neutrophils:** Neutrophils are also another type of immune cells observed in invading many different forms of malignancies. Neutrophils' classical roles involve infection defense via a variety of processes (primarily phagocytosis, antimicrobial agent release, and creation of neutrophil extracellular traps) and triggering an inflammatory response by the secretion of several chemokines and cytokines [160–162]. In theory, neutrophils may be effective antitumor effector cells because the different microbicidal and cytotoxic chemicals found in neutrophil granules can kill malignant cells, and chemokines and cytokines released by neutrophils can also attract other antitumor cells [163]. In fact, in artificial environments in which neutrophils have been powerfully stimulated, it has been proposed that neutrophils may lyse tumor cells through antibody-dependent cell-mediated cytotoxicity (i.e., when antitumor antibody is present) [164]. Increasing data suggests, however, that tumor-associated neutrophils (TANs) may aid in tumor growth. TANs are classified into N1 and N2 phenotypes, which are identical to the anticancer (M1) and pro-tumor (M2) phenotypes of macrophages. The morphology of N2-polarized neutrophils is identical to that of granulocytic or polymorphonuclear myeloid-derived suppressive cells (PMN-MDSCs) [165], which will be addressed in the section on tumor-promoting immune cells.

Tumor-promoting immune cells: The majority of tumor-promoting immune cells are regulatory T cells (Tregs) and myeloid-derived suppressor cells (MDSCs).

(a) **Regulatory T cells (Tregs):** Treg cells, as members of the T-cell family, play an important role in the regulation of immunological homeostasis and peripheral tolerance [166]. Tregs express Foxp3 as a dependable marker in addition to the CD4+ marker on the surface of cells, which is important for Treg lineage determination and modulation of inhibitory signals [167]. Foxp3+ Tregs' regulating role on effector T cells renders them a double-edged weapon in the body. Tregs, on the one hand, can repress an overactive immune response, like autoimmune disease. On the other side, the suppressive role of Tregs within the TME may hinder CTLs from responding effectively to cancer cells. Indeed, recent single-cell sequencing demonstrated that Tregs in malignancies are highly heterogeneous and expanded clonally, and Treg function is tightly linked to patient prognosis [168].

(b) **Myeloid-derived suppressor cells (MDSCs):** MDSC, which was first proposed roughly 10 years ago, is another important kind of tumor-promoting immune cells found in TME [169]. Granulocytic or polymorphonuclear MDSCs (PMN-MDSCs) and monocytic MDSCs (M-MDSCs) are two types of MDSCs. PMN-MDSCs resemble N2-polarized neutrophils morphologically, whereas M-MDSCs resemble M2-polarized macrophages [170]. The interaction of MDSCs and cancer cells is thought to be crucial for tumor growth. MDSCs would be activated and proliferate in TMEs in reaction to cytokines and chemokines from the inflamed tumor environment [171]. MDSCs, in turn, may stimulate angiogenesis by producing MMP9, VEGF, and prokineticin

2, as well as induce cancer cell migration to endothelial cells and enhance metastasis [167]. A recent study revealed that tumor-associated M2-polarized macrophages, also known as MDSC, can control aerobic glycolysis in BC cells in an extracellular vesicle-reliant manner. MDSCs may also limit T-cell activity by producing arginase (ARG), iNOS, and immunosuppressive cytokines such as TGF-β and IL-10. A recent series of preclinical investigations found that blocking MDSC transport improved the effectiveness of T cell-based immunotherapy [169]. Myeloid cells are generated from bone marrow and could be mononuclear (MN) or polymorphonuclear (PMN) in nature, with MN consisting of macrophages, monocytes, and dendritic cells and PMN consisting of basophils, neutrophils, eosinophils, and mast cells [172]. Pro-inflammatory cytokines stimulate myeloid progenitor cells to multiply and develop into MDSCs in chronic inflammation [173]. As a result, MDSC accumulation is typically observed when a pathological situation prevails, and they are mainly missing in a healthy body. MDSCs weaken the immune system and aid in cancer growth in a variety of ways. These cells can either help to recruit Treg cells to the TME or develop into TAMs [174, 175]. MDSCs can also help fibroblasts to differentiate into CAFs. CCL-5 and CSF-1 attract monocytic-MDSCs (M-MDSCs) to the TME, whereas chemokine (C-X-C motif) ligand 12 (CXCL-12), CXCL-1, CXCL5, CXCL-6, and CXCL-8 enhance PMN-MDSC infiltration [176]. Surprisingly, the cytokine CCL-2 can attract both PMN-MDSCs and M-MDSCs. In comparison to PMN-MDSCs, M-MDSCs are found in greater quantities in TME. Hypoxia has been found in solid tumors to drive the differentiation of MMDSCs into TAMs in order to maintain immune repression [177]. Researches have also shown a higher risk of cancer-related death in individuals with a higher number of circulating MDSCs [178, 179].

As previously stated, nutritional requirements of fast-dividing cells are met by increased glucose absorption, which is the cell's principal source of carbon. In the cancer region, there is a change to aerobic glucose metabolism, which eventually results in acidosis. Generally, we may say that the emergence of one circumstance results in the emergence of another [180, 181]. Following the role of this mechanism in tumor progression, several antimetabolites and energy inhibitors have been recommended for successful treatment. These drugs can be used alone or in conjunction with other chemotherapy drugs. These antimetabolites are generally harmful due to their impact on normal cell metabolism. Marracheet et al. used a nanoparticle-based technique to target the anti-glycolytic drug. Aside from this reliance on increased glucose utilization, lipid is another factor that plays a role in cancer growth. The function of lipid in cell-to-cell interactions in TME was well described by Djefaflia et al. [182]. In this aspect, nanoparticle-based techniques are not yet fully established, but they can be tested in the future to stop tumor progression.

3.2 Modifications in the Tumor Microenvironment (TME)

Through a series of multistep genetic aberrations, a normal cell becomes a cancerous cell. The major causes in genomic abnormalities are germline mutation and somatic mutation. Numerous scientists have recently re-examined the carcinogenic process, with a special emphasis on epigenetic modification and TME changes. TME components such as stromal fibroblasts, myofibroblasts, myoepithelial cells, macrophages, neutrophils, endothelial cells, leukocytes, and ECM may inhibit or slow tumor growth in tumor cells and stromal cells through modulating gene expression. An all-encompassing examination of molecular abnormalities in the TME can help to identify the pathways that enable the development of cancers and can be exploited to generate better anticancer drugs [39, 183]. Many investigations have found general and specific cell epigenetic alterations linked to TME in the heterogeneous cell populations. Some of the possibly best epigenetic changes responsible for carcinogenesis and tumor progression include hypo- or hypermethylation of DNA, makeover of miRNAs, histone modifications, and chromatin remodeling. The majority of these changes are tied to a certain tumor type's gene expression profile. DNA methyl transferases of various types are accountable for DNA methylation on cytosine residues and maintenance of methylation sequences. The cancer epigenome is commonly believed to be characterized by DNA hypomethylation as well as gene-regulated hypermethylation, each of which has distinct effects on gene expression. DNA hypomethylation is known to activate R-Ras, melanoma-associated antigen (MAGE), cyclin D2, and loss of imprinting (LOI) genes, which favor carcinogenesis pathways. Gene expression pattern, on the other hand, has an impact on DNA methylation, as suppression of tumor-suppressor genes like RB1 results in DNA hypermethylation and histone hypo-acetylation. Histone acetylation and deacetylation are two processes that cause aberrant gene expression linked to cancer when they are modulated. TME has been reported to have changes in ATP-dependent chromatin-remodeling factors and miRNAs [24]. Researchers have done a number of experiments to look for epigenetic changes in various cancer types. Figure 3.4 depicts some of the common epigenetic changes involved in carcinogenesis and progression. The function of epigenetics on cells in TME with Swarm rat chondrosarcoma (SRC) carcinogenesis, which affects epigenetic and gene expression profiles, was examined by Hamm et al. Researchers discovered that the epigenetic and gene expression profiles in SRC differed from those in normal tissue, and that the profiles varied dramatically depending on the transplanted site. When compared to normal rats, rats with SRC tumors displayed DNA hypomethylation. Furthermore, various ECM components, metalloproteases, cathepsins, thymosin-β4, C-fos, AP-1, VEGF, TGFβ2, and connective tissue growth factor (CTGF) were expressed differently in SRCs than in normal rat cartilage. These studies demonstrated the importance of an epigenetic pattern in SRC carcinogenesis [184]. Drug resistance, which inhibits apoptotic processes of drug candidates and cancer cell death, can be addressed by understanding epigenetic modifications connected to TME. Studies conducted in vitro demonstrated that CAFs are involved in the phosphorylation of ER-α at serine-118 in cancer cells, which makes them

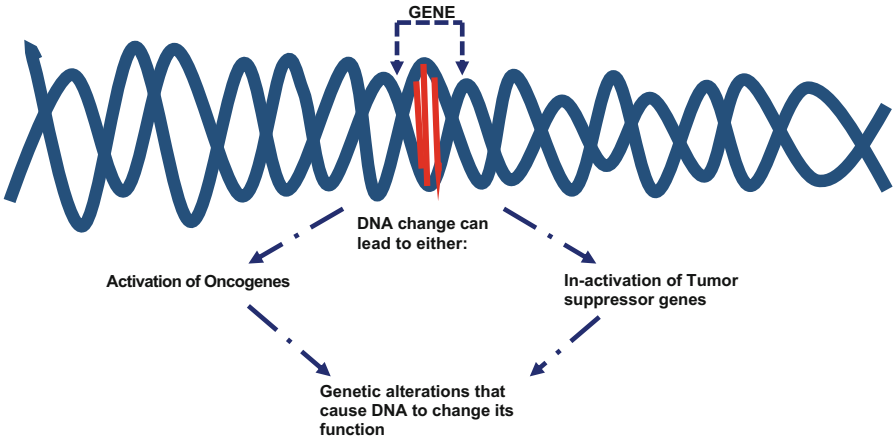

Fig. 3.4 Genetic modifications involved in tumorigenesis and progression

refractory to tamoxifen therapy [185]. With a good understanding of epigenetic modifications in the TME of individual cancers, inefficient treatment options such as gene therapy can be eliminated.

Metabolic alterations in immune cells during cancer progression: During carcinogenesis and progression, metabolic reprogramming is a common feature of cancer [24, 34]. For promoting cell growth and multiplication, cancer cells prefer to utilize glucose as a source of energy (Warburg effect) [21, 186]. Growth factor signaling in the TME stimulates PI3K-Akt, which increases the expression of a variety of glycolytic genes (including GLUT1, PFKFB3, HK2, and LDHA) as well as PDK, which inhibits the TCA cycle [187]. As a result of these molecular changes, the TME is deprived of glucose and amino acids, causing tumor-infiltrating immune cells to become dysfunctional. In addition to promoting cancer cell development, metabolic reprogramming produces waste products like arginine, lactate, phosphoenolpyruvate, and tryptophan by-products; this can also contribute to an immune-suppressing milieu and influence the fate and function of immune cells.

Immune evasion through metabolic competition: Despite their intrinsic antitumoral ability NK cells often fail to prevent tumor growth because of functional abnormalities in the TME [188–191]. Upregulation of fructose-1,6-bisphosphatase (FBP1) in NK cells limits glycolysis, reducing their ability to kill tumors in a Kras-driven lung cancer transgenic model [192]. Cancer cells also require a lot of glutamine besides glucose [193, 194]. Activation of the TF c-Myc during NK cell activation is inhibited by glutamine removal or systemic blockage of L-amino acid transport. The deletion of c-Myc limits NK cell growth and tumor cytotoxicity [195], implying that amino acids and glutamine are critical for NK cell activity. Growing data suggests that glucose deprivation mediated by the tumor Warburg effect reduces tumor-reactive T lymphocytes' effector activity. By boosting glycolysis through the production of HK2, cancer cells can evade CD4+ T cell-mediated immune surveillance. Because of metabolic competition, CD4+ T cells with inadequate glycolytic

metabolite phosphoenolpyruvate (PEP) experience an upsurge in SERCA-mediated Ca2+ absorption and poor T-cell activation [196]. Competitive glucose intake by tumors limits glycolytic ability, mTOR activity, and IFN-γ production inside T cells in a mouse sarcoma model, thus impairing T cells' tumor-protective potential [29]. Furthermore, in human melanoma and lung cancer specimens that are poorly invaded by T cells, a significant increase in glycolysis-related genes is frequently observed, showing that tumor glycolysis likely acts as a barrier to T-cell infiltration [197]. Increased glycolysis in cancer cells leads to an increase in lactic acid (LA) synthesis in the TME that can alter T-cell metabolism and functioning by preventing LA export from T cells. This is consistent with a favorable relationship among lactate levels in serum and tumor load in cancer patients [30]. As a result, it is possible that tumor-induced glucose constraints influence T-cell response, at least partially, and that glucose intake may act as an additional mechanism underpinning tumor immune evasion. T cells infiltrating murine and human cancers have been found to lose mitochondrial mass and functionality, according to a new research. This reduced mitochondrial synthesis in tumor-specific T cells was caused by faulty Akt-PPAR-γ coactivator 1 (PGC1α) signaling [198], implying that regulation of oxidative metabolism in tumor-infiltrating T cells repeals their effector activity by restricting metabolic demands.

3.3 Metabolic Reprogramming and Dysfunction of Tumor-Associated Antigen-Presenting Cells (APCs)

Lactic acid (LA) buildup is known to occur in cancer cells and stromal cells (e.g., cancer-associated fibroblasts or CAFs) during active glycolysis [199]. Tumor-derived lactic acid (LA) is a crucial component in tumor escape from immune surveillance, as evidenced by its ability to block IL-12 production by DCs and induce a phenotype identical to tumor-associated DC during DC development [200]. Increased fatty acid (FA) production in DCs in reaction to TLR stimulation leads to increased lipid accumulation [131, 201]. Increased lipid content in tumor-associated DCs, on the other hand, has been linked to TME DC dysfunction. Prostaglandin E2 (PGE2) is a prostanoid lipid that has been shown to improve cancer cell survival, proliferation, migration, immunosuppression, and angiogenesis. The active enzymes for PGE2 generation, cyclooxygenase (COX)-1 and -2, are substantially increased in breast, colorectal, lung, stomach, and pancreatic malignancies [202, 203]. Melanoma cells that are responsible for the production of PGE2 have been shown to prevent CD103+ DC activation and accumulation, as well as decrease the expression of antitumor type I immunity molecules like T-bet, IFN-γ, and IL-12 [204]. In addition, DCs from tumor-bearing mice or individuals with cancer collect more lipids than DCs from healthy ones. Increased level of scavenger receptor A (CD204), which is a negative regulator of DC immunogenicity, is thought to be a major source of lipid buildup in tumor-associated DCs [203, 205–207]. Lipid-laden DCs do indeed have a diminished ability to digest antigen and trigger allogenic T cells. The application of an acetyl-CoA carboxylase inhibitor or anti-SRA

antibodies to lower fat content in DCs can significantly improve DC vaccination's antitumor efficacy [208]. Oxidized neutral lipids which are tumor derived, like cholesterol esters, FAs, and triglycerides, but not non-oxidized lipids, reduced peptide class I MHC complex cell surface expression and inhibited DC tumor antigen cross-presentation [209]. The X-box-binding protein 1 (XBP1) is a key TF that mediates the ER response to stress, and has been shown to aid in carcinogenesis by boosting cancer cell survivability and spread [210–212]. The XBP1's constitutive activation in ovarian tumor-associated DCs can also stimulate a triglyceride biosynthetic pathway, leading to abnormal lipid buildup that dampens antitumor immunity [213], implying that targeting the XBP1-induced ER stress response could offer a novel strategy for metabolically enhancing the immunogenicity of DCs. While it is widely known that abnormal β-catenin signaling plays a significant role in cancer formation and metastasis [214, 215], its role in immune cell dysfunction is only beginning to be understood. Cancer cells can boost β-catenin signaling in DCs, preventing T lymphocytes from cross-priming [216]. Tumor-infiltrating DCs use β-catenin signaling to metabolize vitamin A and generate retinoic acid, which aids in the regulatory T-cell response and immunological tolerance. In DCs, deleting β-catenin or inhibiting the β-catenin pathway significantly reduces regulatory T-cell response and inhibits mice melanoma progression [217]. Melanoma produces an immunological privileged milieu via a paracrine Wnt5a-β-catenin-peroxisome proliferator-activated receptor-γ (PPAR-γ) pathway, which upregulates CPT1A expression for enhanced FAO in DCs, according to a recent research. This metabolic switch towards FAO enhances the activation of indoleamine 2,3-dioxygenase-1 (IDO1) while lowering the synthesis of immunostimulatory cytokines (e.g., IL-12), resulting in a tolerogenic DC phenotype and Treg expansion [218]. Depending on these findings it is proposed that modifying lipid accumulation in DCs can increase antitumor immunity and clinical impacts of DC-based cancer vaccines.

3.4 Metabolic Reprogramming in Suppressive Immune Cells

Despite the fact that the metabolic circumstances in the TME reduce tumor-specific effector T-cell antitumor action, Tregs can continue to inhibit the immune system because of their metabolic dependence on FAO [74]. The generation of amino acids and lactate by hypermetabolic cancer cells, as well as the expression of hypoxia-inducible factor-1α (HIF-1α) in the tumor, can aid in the growth of Tregs, which limit the stimulation and cytolytic activity of effector T cells within TME [26, 219]. Cancer cells' interactions with tumor-associated macrophages (TAMs) or monocytes via metabolic processes or signals may also play a significant role in tumorigenesis. Tumor-produced lactic acid has the ability to alter TAM function, hence amplifying tumor-promoting inflammatory response in the TME. Mouse and human monocytes/macrophages triggered by TLR ligands exhibit increased transcription of IL-23p19 in the presence of tumor-derived lactic acid [220]. Through activation of the matrix metalloprotease MMP-9, proliferation of inflammatory Th17

cells, and prevention of CTL tumor invasion, IL-23 enhances cancer-supporting inflammatory response [221, 222]. The glycolytic flow and lactate release are triggered by LPS activation of human monocytes. Because adding lactic acid to limit lactate export or preventing glycolysis with 2-deoxyglucose greatly suppresses monocyte/macrophage activation-associated TNF generation, the abundance of tumor-derived lactic acid can also affect monocyte activation [223]. The fact that lactic acid from cancerous cells promotes HIF-1-dependent vascular endothelial growth factor and arginase 1, which increase tumorigenesis in syngeneic mouse cancer models, adds to the notion that lactic acid plays a role in TAM dysfunction [224]. TAMs' lipid profile appears to be significantly altered, according to growing evidence. In an orthotopic lung cancer model, alterations in numerous genes involved in lipid signaling were found, indicating a reprogramming of lipid metabolism in macrophages. TAMs had higher levels of COX-2 in comparison to healthy controls, which were linked to greater tumor angiogenesis [225, 226]. In renal cell carcinoma-associated macrophages, high expression of 15-lipoxygenase 2 (15-LOX2) and its lipid product 15(S)-hydroxyeicosatetraenoic acid was also linked with the levels of the immunosuppressive cytokine IL-10 and chemokine CCL2, both of which aid in cancer-supporting inflammation and immune escape [227]. To accomplish their tumor-promoting activity, cancer cells also control FA metabolism in TAMs. To promote tumor cell invasion, Lewis lung carcinoma cells aggressively generate macrophage-colony-stimulating factor (M-CSF), which induces the synthesis of macrophage-intrinsic fatty acid synthase (FASN) and IL-10 [228]. FASN operates upstream of the nuclear receptor PPARβ/δ, a critical regulator of tumor angiogenesis, according to mechanistic investigations [229, 230]. Fatty acid-binding proteins (FABPs) are lipid chaperones that bind hydrophobic ligands (such as eicosanoids, saturated or unsaturated long-chain fatty acids, and other lipids) reversibly and control their biological activity [231, 232]. Epidermal FABP (E-FABP) is strongly increased in M1-like macrophages in the stroma of mice mammary tumors, while macrophages with low E-FABP expression are more M2 oriented, as demonstrated by differential IFN-β production. The anticancer activity of macrophages is much enhanced when E-FABP is activated in an antagonistic manner [233, 234]. As a result, TAMs' pro-tumor phenotype can be determined by their intracellular metabolic lipid patterns. There may be a relationship between amino acid or nutritional deficiency and tumor-associated myeloid cell dysfunction. Cells can obtain cysteine, which is needed for cell proliferation and protein synthesis, by importing extracellular disulfide-bonded cystine through the Xc- membrane cystine transporter and reducing it to cysteine [235, 236], or using cystathionase to convert intracellular methionine to cysteine [237, 238]. Myeloid-derived suppressor cells (MDSCs) are a diverse population of immature cells that are phenotypically defined as CD11b+Gr1+ cells in tumor-bearing mice and CD11b+CD14 CD33+ cells in people with cancer [172]. MDSC expansion is a key factor in cancer's ability to evade antitumor immunity. Cysteine export is restricted in MDSCs due to the lack of the alanine-serine-cysteine (ASC) transporter. MDSCs, on the other hand, can import extracellular cysteine via the Xc- transporter. As a consequence, MDSCs sequester cysteine

in the TME, restricting cysteine availability to T cells [239]. Because T cells lack cystathionase and the Xc- transporter, they rely on cysteine from other cells for activation and proliferation [240]; deficiency of cysteine in the TME impairs T-cell activation [239]. Likewise, MDSCs limit T-cell activation by depleting arginine via overexpression of arginase I, which reduces CD3ζ expression on T cells [241]. Arginase I is abundantly expressed in mature myeloid cells invading mouse lung carcinoma and human NSCLC, in addition to MDSCs. These myeloid cells, rather than tumor cells or infiltrating lymphocytes, are thought to be the predominant source of intra-tumor arginase I and are capable of depleting extracellular L-arginine through cationic amino acid transporter 2B. Tumor-associated mature myeloid cells, like MDSCs, deplete L-arginine, which inhibits antigen-specific T-cell activation by downregulating CD3ζ expression [242]. Upregulation of IDO by tumor-infiltrating myeloid cells, like DCs, MDSCs, or TAMs, catalyzes tryptophan metabolism in the kynurenine pathway, which can limit T-cell activation by depleting tryptophan and expanding Tregs [243–245]. As a result, tumor-associated myeloid cell deprivation of amino acids and the resulting TCR signaling impairment or T-cell activation are the primary methods through which cancerous cells avoid immune detection and/or attack. Some recent investigations have also found metabolic pathways to be involved in the suppressive actions of MDSCs. Tumor-infiltrating MDSCs from mice and humans have a metabolic profile similar to activated FAO [246]. Increased FAO and FA absorption are linked to higher levels of arginase I and also an increase in MDSCs' ability to suppress T cells. In response to a higher level of FAO, MDSCs release cytokines that could support MDSC expansion (e.g., G-CSF, GM-CSF, IL-6, IL-β1, and IL-10), which is inhibited by FAO suppression [246]. G-CSF and GM-CSF, which are produced by cancer cells, can signal in a paracrine way through STAT3 and STAT5 to promote the expression of lipid transporters and lipid absorption in MDSCs [247]. Intracellular lipid buildup boosts oxidative metabolism and promotes MDSC immunosuppressive activity that can be reverted by inhibiting STAT3/5 signaling or removing the FA translocase CD36. Lipid transport proteins are dramatically increased in both tumor-infiltrating and peripheral blood MDSCs in humans [247]. Polymorphonuclear MDSCs (PMN-MDSCs) from people with cancer express lectin-type oxidized LDL receptor 1 (LOX-1) but not those from healthy persons. PMN-MDSCs that express LOX-1 have a gene profile that is linked to immunological suppression [248]. As a result, tumor-derived lipids produce significant metabolic alterations in MDSCs that can affect their capacity to inhibit antitumor T-cell effector function.

Resistance to therapy: Cancer cells are hard to treat specifically with traditional chemotherapeutic medicines because they grow from the body's normal cells [34]. These drugs generally work by interfering with metabolic reprogramming [249], stopping the cell cycle at various stages [250], triggering apoptosis and reducing cancer cell growth [251], and other cellular mechanisms. The action of powerful anticancer drugs on noncancerous cells in the body, such as the skin, liver, spleen, and several other organs, has resulted in an increase in toxicities. The resistance of distinct kinds of chemoprevention drugs is caused by cellular TME, and also the physiological factors that surround it. As mentioned in the previous

section, extracellular matrix (ECM) in TME has a higher stiffness and thickness. TME's features create physical barriers to drug entrance, which leads to resistance [252]. Cancerous cells that show chemoresistance in vivo have been found to be reactive to the similar chemotherapeutic drug when examined in vitro. TME has been found to be the cause of this occurrence in studies undertaken in these areas. The chemotherapeutic resistance can be acquired or *de novo*. Acquired resistance is the result of a lengthy process that begins with the acquisition of genetic level modifications that are eventually responsible for resistance.

De novo resistance, on the other hand, is an environment-mediated drug resistance (EMDR) that develops via a series of signaling pathways launched by TME's cellular components. Soluble factor-mediated drug resistance (SFMDR) and cell adhesion-mediated drug resistance (CAMDR) are two types of EMDR (CAMDR). SFMDR is triggered by chemokines, cytokines, and other growth factors, which are mostly released by cancer-associated fibroblasts (CAFs) and render tumors resistant to chemotherapeutic drugs as well as radiotherapy. CAMDR, on the other hand, is triggered by tumor cell integrin attachment to ECM cells, resulting in mutations and drug resistance [253]. Hwang et al. investigated the role of human pancreatic stellate cells in the progression of pancreatic cancer and discovered that they have a major impact on pancreatic cancer cell proliferation, infiltration, migration, and metastasis in vivo. When these stellate cells coexist with malignant pancreatic cells, resistance to treatments like cisplatin increases [254]. In ovarian cancer cells, Sharmen et al. revealed increased collagen VI in the ECM as the primary factor implicated in cisplatin resistance. The scientists observed that ECM cells with distinct transcription profiles were accountable for cisplatin resistance [39, 249]. Through the formation of oxygen free radicals, physiological circumstances such as hypoxia alter the DNA-damaging activity of drugs. Elastin, collagen, hyaluronan, polysaccharides, proteoglycans, related enzymes, and growth factors are all constituents of the ECM that control cell proliferation. When these elements are combined, they are accountable for a wide range of biological processes as well as cell behavior regulation [255]. The scaffolding and supportive mechanisms of ECM are determined by physical features like density, porosity, stiffness, rigidity, and orientation in maintaining the integrity of the related tissue. TME alters the biomechanical and physical properties of ECM in such a manner that it promotes metastasis and carcinogenesis. Increased stromal rigidity and density are caused by upregulated cross-linking of collagen and its components, which is started by lysyl oxidase. This increased collagen cross-linking offers superior mechanical support for tumor growth while also preventing chemotherapeutic drugs from entering the tumor, resulting in treatment resistance. CAFs and immune cells release proteolytic family enzymes (cathepsins, urokinase, and matrix metalloproteinases) that break down ECM and produce bioactive fragments that promote cell migration, invasion, and angiogenesis [23]. Figure 3.5 illustrates how ECM affects tumor growth and metastasis.

CD8 T cells invading tumors, in particular, depend largely on aerobic glycolysis for their proliferation and effector activity and must compete with cancerous cells for nutrients [256]. CD8 T cells are confronted with an immunosuppressive

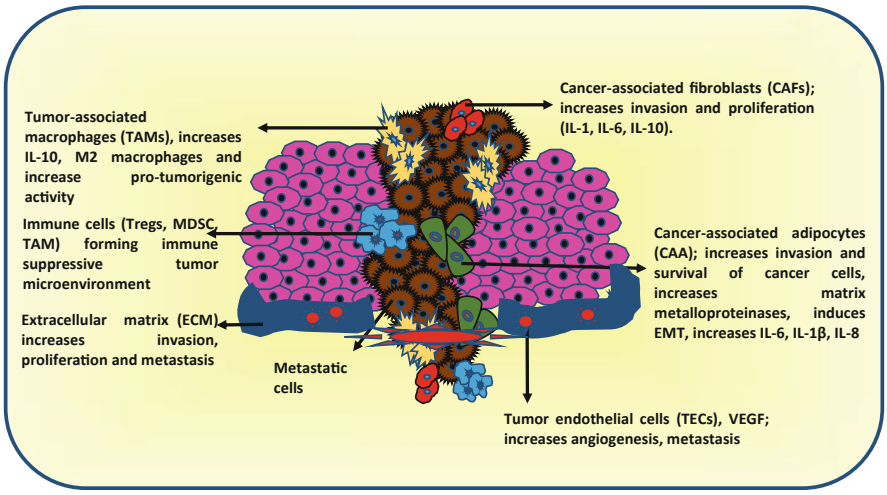

Fig. 3.5 ECM and its involvement in tumor progression and metastasis

environment created locally by cancerous cells in order to weaken immune effectors and elude destruction, besides metabolic hurdles inside the tumor microenvironment. Cancer cells induce the migration of stromal cells, such as myeloid-derived cells, cancer-associated fibroblasts, and Tregs, that are arranged to express immunomodulatory factors such as IL-10, TGF-β, and IL-355 [257]. Simultaneously, cancer cells release a variety of surface ligands which are able to activate co-inhibitory checkpoint receptors located on T cells, such as PD-1 [258]. Negative co-stimulatory signals are transferred when PD-1 is activated by its cognate ligands, PD-L1 or PD-L2, to diminish T-cell activation, mainly via dephosphorylation of CD28 coreceptor complex signaling components and to a lesser extent via desensitization of proximal TCR signaling molecules [70, 259, 260]. Tumor cells are likely to generate a local condition of immune privilege by co-opting such signals, which are mainly used to maintain immune tolerance. Because of our growing understanding of how such co-inhibitory receptor interactions impact immune regulation, therapeutic antibodies to inhibit such suppressive axes have been developed. Ipilimumab was the first of them to be licensed by the FDA and it demonstrated exceptional effectiveness and long-term relapses in patients with advanced carcinoma [261]. Antibodies that block PD-1/PD-L1 signaling were licensed shortly after for a variety of indications, including NSCLC, head and neck squamous cell carcinoma, as well as any solid cancer with a mismatch repair defect [262]. PD-1 blockade is currently licensed for more than 20 indications, rendering it the most extensively used PD-1 combination immunotherapy. Furthermore, ipilimumab in conjunction with nivolumab therapy for advanced melanoma individuals has been approved, giving better therapeutic benefit than either monotherapy [263]. However, the number of patients with all cancers still does not experience long-term therapeutic benefit from the FDA-approved immunotherapeutic treatments currently

available [264, 265]. The discipline of immunotherapy has shifted its focus to discovering therapeutic strategies that can help patients with "immune-cold" cancers that are resistant to existing treatments. However, progress in this endeavor will need a better comprehension of the mechanisms through which the tumor microenvironment of such resistant malignancies acts to inhibit the antitumor T-cell response locally, and the development of therapies to diminish these suppressive modifications. Hanahan and Weinberg posited two evolving hallmarks of cancer in 2011, immune system avoidance and deregulated metabolism [266], and also how T cells need high amounts of energy to sustain and maintain effector function, but pose a harsh intra-tumoral landscape with restricted nutrients and a buildup of toxic metabolic by-products. Tumor cells use this process to manipulate the chemical composition of the extracellular microenvironment in order to form an extra barrier that prevents the antitumor immune response from activating. Reducing these obstacles to antitumor immunity is critical, and a better knowledge of the interface of tumor cell and T-cell metabolism will lay the foundation for future therapeutic approaches that enhance the effectiveness of immune-based therapies.

Resistance to immunotherapy: Immunotherapy is an emerging and a potential method to treat different cancer types [267]. It was William B. Coley in 1890s who first anteceded the role of immune system in regulating tumor development, since immune system has a divergent role in the suppression as well as progression of tumors at different stages of development [268]. The overall reaction of immune system to tumor inception occurs in accordance with unique interconnected immune cells significantly identifying and destroying cancerous cells [269, 270]. Due to the dynamic and emerging immune responses, resistance to immune-based therapies is associated with a number of factors including genetic alterations, tumor microenvironment which supports the enhancement of barrier to drug entry, and a consequent development of resistance to immunotherapeutic agents. In spite of the novel enduring reactions with immunotherapies [271, 272], still much of the responders show resistance to treatments. Such resistance mechanisms are categorized into primary and secondary resistance. Primary resistance (or adaptive resistance) describes a condition in which there is no response to the first cancer immunotherapy [273–275]. Figure 3.6 shows the example of immune checkpoint inhibitors which block those immune cells that help in tumor promotion, like CTLA-4, PD-1, and PD-L1 inhibitors, where CTLA-4 and PD-1 receptors are present on T cells and PD-L1 on tumor cells. Secondary resistance (or acquired resistance) on the other hand responds primarily to the cancer immunotherapy and then relapse after a certain period of time promoting tumor progression [276–278]. Resistance mechanism for both primary (adaptive) and secondary (acquired) depends upon two major factors: intrinsic tumor cell factors and extrinsic tumor cell factors or tumor microenvironment factors [270]. The main reason for primary resistance against the immunotherapy involves lack of tumor antigens, lack of antigen-presenting cells, deletion of transporter associated with antigen-processing (TAP) protein complex, deletion of beta-2-microglobulin (B2M), and mutations in human leukocyte antigen (HLA) complex favoring immune escape. The principal metabolic [271] pathways connected to the mechanism of primary resistance recognized include the following:

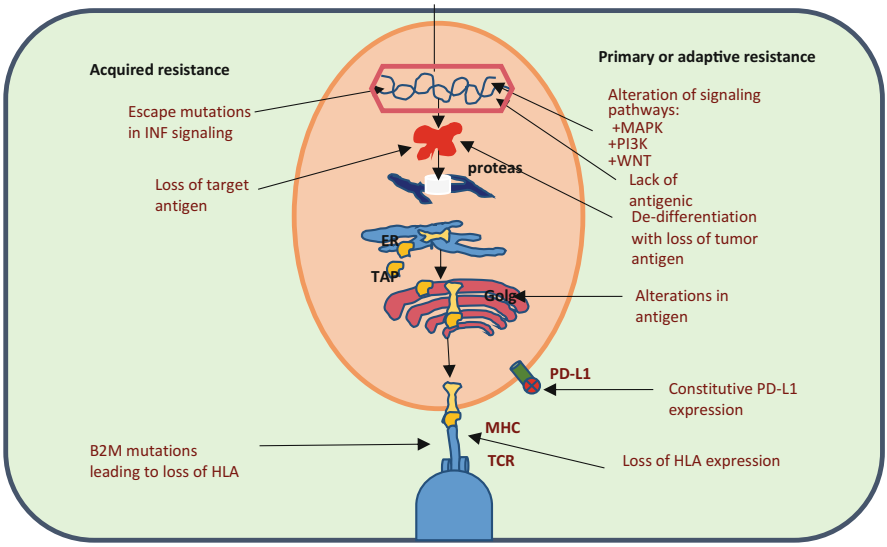

Fig. 3.6 Primary and secondary immune resistance

- **RTK/RAS pathwa**y: The oncogenic signaling pathway mitogen-activated protein kinase (MAPK) activation directs the T-cell recruitment and function inhibition via the production of several factors like VEGF and IL-8, which is a chemokine-promoting angiogenesis [272].
- **PI3K/AKT/mTOR pathway:** With the loss of PTEN gene, PI3K/AKT pathway gets activated as noted in several types of cancers, which result in the development of resistance against immunotherapies. For example, melanomas were reported to show 30% activation of such signaling. Moreover, with the loss of PTEN, there is significant reduction in the gene expression levels of INF-γ, granzyme-B, and $CD8^+$ T cells [273].
- **Wnt pathway:** The constitutive potential of Wnt signaling pathway results due to the stability of β-catenin, thereby inducing T-cell suspension/ejection. Presence of β-catenin results in the reduction of dendritic cells (DC) as shown in some mouse models. Tumors which lack β-catenin show an effective response against immunotherapy while tumors with β-catenin show resistance to such therapies [274].
- **PD-L1 expression and molecular control:** One of the most central mechanisms to immunotherapeutic resistance is the constitutive expression of cell surface ligand PD-L1 on the tumor cells effectively inhibiting the antitumor T-cell responses. Apart from this deletion of PTEN or PI3K/AKT signaling pathway alterations lead to the expression of PD-L1 tumor cell surface ligand continuously [276]. EGFR, MYC signaling pathway CDK5 disturbances, as well as increase in the PD-L1 transcription all lead to the PD-l1 expression and such an elevated expression results in the inhibition of antitumor T-cell activation [278].
- **INF-γ pathway:** It is an emerging vital metabolic signaling pathway in resistance mechanisms against immunotherapeutic agents. This pathway shows both

positive and destructive consequences performing the antitumor responses. Under normal expression of INF-γ, it induces antitumor immune responses via increased antigen presentation, increased MHC protein molecule expression, engaging other immune cells, and drawing direct pro-apoptotic and antiproliferative influence on tumor cells. When INF-γ is continuously expressed, it leads to the inhibition of antitumor immune responses via immune editing of tumor cells which ultimately results in immune escape. Mutations in the INF-γ receptor chains JAK1 or JAK2 and STATs (signal transducer and activator of transcription) conclude in the immune escape by tumor cells resulting in the loss of antitumor effects of interferon gamma. For example, anti-CTLA-4 therapy results in the development of primary resistance due to the high frequency of mutations in INF-γ receptors preventing signaling in response to interferon therapy promoting immune escape from T cells. Moreover, this pathway also plays an important role in the inducible PD-L1 expression [279].

- **Epigenetic modifications:** Epigenetic control on the DNA in tumor cells directs modifications in the expression of immune-associated genes, which play a very critical role in primary resistance to immunotherapeutic agents. In preclinical mouse melanoma models, histone deacetylase inhibitors preceded with the increased MHC and tumor-associated antigen expression and a decreased competition for endogenous lymphocytes with an advantage for adoptive cell transfer therapy (ACT), thereby enhancing the antitumor T-cell response. Similarly, in lymphoma mouse model, an increase in CD80 expression was observed by hypomethylating agents with a consequent increase in $CD8^+$ T cells. Use of demethylating agents empowers immune-associated genes to re-express with a potential therapeutic impact [280, 281].

Tumor microenvironment mechanisms (TME): This includes all other components within the TME apart from tumor cells consisting of regulatory T cells (Tregs), myeloid-derived suppressor cells (MDSCs), M2-macrophages, fibroblasts, and a number of other stromal cells which are involved in the inhibition of antitumor immune responses.

(a) **Regulatory T cells (Tregs)** are one of the most critical immune cells having a role in maintaining tolerance, identified by the expression of FOXP3 transcription factor, and are known to suppress effecter T-cell responses (Teff) via release of inhibitory cytokines like IL-10, IL-35, and TGF-β and even direct cell contact. A number of human tumor cells have shown that Treg cell infiltration and their depletion from TME can reform antitumor immunity. Certain preclinical mouse models have shown that immunotherapy (anti-CTLA-4) results in an increased Teff-to-Treg cell ratio [282–284].

(b) **Myeloid-derived suppressor cells (MDSCs):** MDSCs appear to be one of the major regulators of immune responses in cancer cells. These have been characterized by the expression of CD11b and Gr-1 marker in mouse models. Human MDSCs express $CD11b^+$ and $CD33^+$ markers that are involved in advancing angiogenesis, invasion, and metastasis of tumor cells. Moreover,

occurrence of MDSCs is linked with the reduced survival of breast cancer and colorectal cancer patients. MDSCs in TME have been identified to decrease the efficacy of immunotherapy. Consequently, destroying MDSCs can enhance the antitumor immunotherapeutic responses [171, 285–287].

(c) **Tumor-associated macrophages (TAMs):** TAMs are categorized into two major types: M1 and M2 macrophages represent one more class of immune cells (leukocytes) influencing antitumor immunotherapeutic responses. M1 macrophages are tumor suppressors promoting antitumor immunity while M2 macrophages are tumor promoters suppressing the antitumor immunity [288]. Preclinical studies on mouse models of lung adenocarcinoma as well as colon cancer, cutaneous T-cell lymphoma, breast cancer, and melanoma have shown that decrease in TAMs due to downregulation of M2 macrophages resulted in the reduction of tumor growth with the inactivation of chemokine and cytokine signaling (CCR2, CCL2). Role of macrophages in resistance mechanisms suggests direct suppression of T-cell response via PD-L1 in hepatocellular carcinoma and ovarian carcinoma (B7-H4). To overcome this potential resistance mechanism, certain immunotherapeutic agents in combination to show improved tumor regression are under clinical trials [42, 136, 289, 290]. In addition to the above mechanisms in promoting resistance against immune checkpoint inhibitors, co-stimulation of certain immune responses directs the activation of inhibitory signals. For example, INF-γ overexpression leads to the stimulation of effector T cells (Teff) as well as PD-L1, indoleamine-2,3 dioxygenase (IDO) [290] which is a tryptophan-metabolizing enzyme promoting tolerance and suppression of Teff cell function, and carcinoembryonic antigen cell adhesion molecule-1 (CEACAM-1), all silencing the antitumor immunity response [291, 292]. In addition, T-cell activation leads to the increased expression of inhibitory CTLA-4 receptor via TCR and CD28 co-stimulatory signaling. As per the preclinical mice model study [293, 294], relapse of tumor cells was observed after anti-PD-1 therapy due to the increased T-cell immunoglobulin mucin-3 (TIM-3, an immune checkpoint receptor) expression on T cells of lung adenocarcinoma [295, 296]. Likewise relapse in lung cancer patients was found due to increased TIM-3 expression on T cells after PD-1 therapy. An immunosuppressive cytokine transforming growth factor-β (TGF-β), stimulated by Tregs, has a role in angiogenesis and suppression of antitumor activity of immune cells is linked with worse prognosis in a number of different cancer types. A combination of TGF-β inhibitor and anti-CTLA-4 showed synergy in a melanoma model leading to antitumor responses. Likewise, adenosine is showing an inhibitory role in T-cell proliferation and cytotoxic function by A2A receptor on T cells and metastasis on tumor cells by A2B receptors. One more inhibitor is CD73 enzyme dephosphorylating adenosine monophosphate (AMP) releasing adenosine promoting tumor progression. Besides there are certain chemokines and their receptors which help in supplying MDSCs and Treg cells in TME, for example CCR1, CCR4 (expressed by Tregs), and CXCL12 (promotes immunosuppression) [175, 297].

Secondary resistance (acquired resistance): This mechanism of resistance represents patients with initial tumor response and after a certain period of time the tumor progression relapses. The main reason for such resistance mechanism involves loss of T-cell recognition, loss of antigen presentation, loss of T-cell function, and advancement of immune escape via mutations in different cancer types [270]. Deletion or loss of B2M as already discussed in primary resistance is required for *human leukocyte antigen* (HLA) class I folding and transport to cell surface. Its deficiency due to mutation can reduce $CD8^+$ T-cell recognition. Similarly, loss of function) In case of metastatic colorectal carcinoma, in which immune response to TIL ACT immunotherapy was shown for 9 months and later it relapsed due to mutation altering antigen-presenting machinery and interferon signaling. Furthermore, immune checkpoint inhibitors that have shown increased expression in TME are LAG-3, TIGIT, VISTA, etc. There are several ongoing clinical trials identifying many more [298, 299].

Strategies to overcome resistance to immunotherapy: The concept of immunotherapy holds a recent advancement in cancer treatment. A large number of clinical trials are ongoing for effective comparison of different strategies of immunotherapies in different tumor types to potentially overcome the concept of resistance to such therapies. Emerging strategies to enhance the immunotherapeutic responses are discussed as follows: The first strategy that can be used in reducing antitumor immune responses of human leukocyte antigen (HLA) can be **nonspecific immune stimulation** via conduction of interferons (INF-α), cytokines (GM-CSF), interleukins (IL-2, IL-15, IL-12, IL-21), etc. in the form of adjuvant treatment against cancer growth. Recently bacille Calmette-Guérin (BCG) has been used in treatment trials against bladder cancer, which was previously used to treat melanoma and breast cancer with no side effects in humans [300–302]. Next can be **adoptive cell transfer therapy (ACT)**, a specific immune cell-based therapy. This strategy applies infusion of similar immune cells having antitumor properties derived from natural (unchanged) T cells, NK cells, or genetically engineered T cells which recognize either T-cell receptor (TCR) or chimeric antigen receptor (CAR). CAR-T cell immunotherapy on the other hand has gained much success in different cancer types such as lymphoma, B-cell acute lymphocytic leukemia (B-ALL), chronic lymphocytic leukemia (CLL), as well as multiple myeloma. The efficacy of CAR-T immunotherapy can be enhanced via rapid circulation of T cells recognizing specific tumors. One of the major drawbacks of using CAR-T immunotherapy is cytokine release syndrome (CRS) and suppression of tumor-infiltrating lymphocytes due to the release of adenosine. To overcome such limitations CAR-T-engineered T cells are developed that deliver A2A receptor antagonist to tumor-infiltrating T cells, thus enhancing T-cell function. Moreover, using NK ACT therapy, NK cells are stimulated with cytokines (IL-2, IL-12, IL-15, IL-18) and interferons resulting in the highly active NK cells which in turn elevate the expression levels of activating receptors, granzymes, and perforin. Adhesion molecules, FasL, TRAIL, etc. from a clinical trial study on NK-based therapy have been tested in different cancer types with high-risk frequency like renal cell carcinoma, advanced melanoma, AML, lymphoma, gastric cancer, and breast cancer

[177, 180, 303–305]. One more specific strategy can be regulation or **modification in immune checkpoints.** This can be done either by activating the stimulatory checkpoints or by suppressing the inhibitory immune checkpoints: for example, 1) CTLA-4 inhibition via CTLA-4 antibodies (ipilimumab, tremelimumab, etc. are under clinical practice) and 2) PD-1 inhibition via PD-1 or PD-L1 antibodies (pembrolizumab, durvalumab, nivolumab, etc.) showing some promising results in a variety of cancer types such as melanoma, lymphoma, and NSCLC. A great number of other checkpoint inhibitors are under evaluation (LAG-3, OX-40, CD-40, ICOS, GITR, etc.) [181, 262, 306]. Last but not the least, **dendritic cell-based vaccination therapy (DC)** modulates the antigen presentation function of cytotoxic T lymphocytes (CTL) and NK cell immune response within the TME. This strategy can be done via in vivo targeting DCs with cytokines (GM-CSF), antagonistic antibodies (CD-40), and antigen binding to CLR (c-type lectin receptors, DEC-205). In ex vivo targeting immature DCs from monocytes of cancer patients are introduced into mature DCs. A number of clinical trials are underway determining the potential effectiveness in a variety of cancer cells such as ovarian cancer, melanoma, colorectal cancer, and renal cell cancer [307]. In addition to the immunotherapeutic resistance TME and its related physiological conditions like hypoxic environment, affecting the DNA-damaging action of chemotherapeutic agents (drugs) with the generation of oxygen free radicles, are all responsible for chemotherapeutic resistance which can be either acquired or *de novo.* In spite of the fact that the strategy concerned with the treatment is in the form of monotherapies including monoclonal antibodies, the current focus is by and large towards the use of combinational therapy strategies which hold evidence in improved clinical outcomes in comparison to monotherapy. The reason of using combinational therapy approach is that it can inhibit several pathways significantly increasing the efficacy and survival among different cancer patients.

3.5 Conclusion

Cancer metabolism is presently a focus of intense research for cancer biologists due to its importance in combating increasing treatment resistance and discovering new drugs. Because of the disease's complexity, the interconnectedness among signaling and metabolic processes, and the cascading occurrences that convert a normal cell into a tumor cell, a system-wide method is increasingly regarded as crucial in determining the underlying causes of cancer or in determining the best efficient targets for treatment. These comparatively recent discoveries into the molecular mechanisms of immune regulation are having a significant impact on the development of better effective combination immunotherapy treatments for cancer. Besides their diagnostic and prognostic relevance, it has been argued that knowing these rearranged tumor-associated metabolic processes is critical for identifying attractive targets for therapeutic approaches. However, it is crucial to evaluate the potential negative consequences of interfering with these pathways on regularly growing cells. A variety of small-molecule therapeutics that may precisely disrupt critical

metabolic pathways linked with tumor proliferation are now at different phases of development. These includes drugs that block enzymes involved in the glucose absorption and glycolytic pathways; fatty acid, amino acid, and nucleotide biosynthesis; and cancer metabolism modulating signaling pathways. The application of immune checkpoint inhibition to harness the immune system has resulted in spectacular responses across a variety of cancer indications; however, therapies that have the ability to increase these results to a larger number of patients are urgently needed. The metabolic processes used by malignancies to limit T-cell activity are addressed in this chapter. Cancer cells influence the TME's metabolic landscape by limiting accessible metabolites and, as a result, encouraging the buildup of harmful by-products. We will acquire a better knowledge of how cancerous cell metabolism directly regulates T-cell activity by characterizing the tumor cell-intrinsic pathways implicated in these activities. In the future, our findings imply that additional therapies including the reinvigoration of T-cell metabolism or the weakening of cancer cell energetics to supplement our present immunotherapy regimens should be investigated.

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4 Crosstalk of Immuno-Oncology and Metabolism: Influence of *Akkermansia muciniphila* and Personalized Therapy Approach

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Abstract

A healthy cell can turn into a cancerous cell, which requires a complex mechanism, such as genetic and epigenetic modification. This leads to an increase in cell size and growth, differentiation, and uncontrolled proliferation and eventually becomes an immortal cell. These processes are mainly influenced by "reprogramming of cellular metabolism" and this sets up the cancer cells to evade the natural destruction process by lymphocytes (T and B), macrophages, and natural killer cells. Over the last decade, researchers have explored the role of microbiota in regulating cancer immunometabolism, suggesting an unprecedented role in cancer progression and regression. In particular, some microbes act as a probiotic—a live microorganism that produces beneficial effects when administered, mainly *Lactobacillus* and *Bifidobacterium spp*. A new addition to this list is *Akkermansia muciniphila* (*A. muciniphila*). In this review, we emphasize the importance of *A. muciniphila* and the personalized therapy approach in the crosstalk of immuno-oncology and metabolism.

Keywords

Immunometabolism · Glutamine metabolism · Chemotherapy · Radiotherapy · Immune checkpoint therapy · Probiotics · Akkermansia muciniphila

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4.1 Introduction

4.1.1 Immunometabolism in the Cancer Microenvironment

Cancer is a complex disease that results from the combination of many abnormal mutations rather than a single-mutation abnormality. Cancer cells are different from normal cells in numerous ways; mainly they continue to grow and divide, and remain in an immature state (undifferentiated form) due to the rapid growth. Further, they do not undergo apoptosis or repair process; instead, they spread to other parts of the body due to the mutation in adhesion molecules that causes the floating of cancerous cells. The rate of growth and size of the growth would vary, and they remain immortal cells. Additionally, other potential mechanisms by which the cancerous cells grow relentlessly are rapid resistance to the drug treatment and induction of angiogenesis [1].

The normal cells mutate into cancerous cells that can be induced by genetic and epigenetic changes causing uncontrolled growth and reduced or complete differentiation. Sustained growth and division of cancerous cells are mainly influenced by reprogramming of cellular metabolism for neoplastic proliferation and evading the immunological destruction process by T and B lymphocytes, macrophages, and natural killer cells [2]. The term "immunometabolism" has become very popular in cancer immunotherapy treatment that elucidates altered metabolic pathways of immune cells during the cancer progression, especially during their activation and differentiation steps [3]. Typically, mammalian cells survive on the utilization of energy from the catabolic process of various nutrient sources such as glucose, amino acids (primarily glutamine), and free fatty acids [4]. These cellular metabolisms of cancer cells have been thought to play supplementary roles, especially to support cancer cell growth, but the evidence from different cancer studies has suggested otherwise because it also regulates the cancer cell's phenotype actively [5] mainly by metabolic reprogramming of gene and protein expression in cancer immune cells that enhance the uptake of nutrients for the high production of energy which is needed for the uncontrollable growth and proliferation of malignant cells [6, 7].

4.1.2 Glucose Metabolism

Under normal conditions, one mole of glucose could yield 2 moles of pyruvate, ATP, and NADH [5]. The generated pyruvate enters the tricarboxylic acid cycle (TCA cycle) or Krebs cycle, where it undergoes a series of enzymatic reactions to produce 36 ATPs. Besides, pyruvate would be used for the macromolecule biosynthesis using fatty acids and glutamine in the Krebs cycle, which provides continuous replenishment of the carbon skeletons [5]. The activation of T cells is dependent on glycolysis (mainly aerobic) which is cytotoxic in nature to the antigen [8]. But, in the case of cancer cells, they negate the efficient energy-producing pathways; instead, they prefer alternative pathways that utilize less energy but generate more material needed to build newer cells. In the aerobic glycolysis process, cancer cells utilize a

high amount of glucose, comparatively ten times more than non-proliferating normal cells [9], leading to an elevated glycolysis process [10, 11], generating lactate production through the glutaminolysis process, and fatty acid synthesis by preventing the beta-oxidation process in the Krebs cycle [5]. This classic nature of cancer cells is targeted using PET-based imaging which captures the high uptake of radioactive fluorine-labeled glucose analog by cancer cells, to monitor the effectiveness of cancer treatment [12]. This in turn causes glucose deprivation in the body ultimately compromising the cytotoxic effects of activated T cells on cancer cells by delaying one of the key molecules for activated T cells—IFN-γ [13]. In support of this mechanism, Cascone et al. (2018) demonstrated the reduced antitumor activity of T cells when overexpressing the glycolysis-related genes in cancer cells [14]. Moreover, the transport pattern of glucose is significantly perturbated in cancer cells due to oncogenic alterations which are characterized by the overexpression of glucose transporters, GLUT1 to GLU12, GLUT14, and H^+/myoinositol transporter [15], in which the overexpression of GLUT1 transporter has been reported in many cancer types [16, 17]; thus, GLUT1 can be considered an oncogene in the context of cancer cell proliferation [18].

4.1.3 Glutamine Metabolism

Another important nutrient for the proliferation of cancer cells is amino acids. Glutamine is the most abundant circulating amino acid; not only its metabolism involves the energy substrate protein synthesis, but it also plays an essential role in the energy production, synthesis of nucleotides such as purine and pyrimidine [19], and balancing of redox status homeostasis in cancer cells [20]. Glutamine continuously replenishes the Krebs cycle metabolites to build macromolecules and this process is termed as an anaplerosis process, which is essential in cancer cell proliferation [21]. It is considered an essential nutrient despite being a nonessential amino acid for the growth of cancer cells, a process called "glutamine addiction" [21, 22]. Glutamine is produced from glutamate and NH_4^+ by enzymatic reaction of glutamine synthetase (GS), which is either downregulated or completely lost in some of the cancers, such as multiple myeloma, ovarian cancer, and oligodendroglioma cells [23–25].

4.2 Current Treatment Strategy for the Management of Cancer and Metabolism

Since the last two decades, a vast improvement has happened in the therapeutical approach for cancer [26–28]. But the type of treatment strategy varies upon the type of cancer and its state inside the body. In the past, numerous cancer treatments have been practiced, but in recent times targeted therapy has been the most common in practice. This new emerging strategy focuses on understanding the biological process of cancer tissues as the first step, followed by commencing the treatment

procedures to cure it in an efficient and precise way [29–31]. This approach will enhance the survivability and quality of life of patients. Surgery, chemotherapy, radiation therapy, immunotherapy, and precision medicine are currently used to treat cancer patients. In some instances, depending on the state of cancerous tissue, one or a combination of the therapies mentioned earlier will be used.

4.2.1 Surgery

Surgical procedure is one of the gold standard protocols for non-hematological cancers [32]. The surgical procedure provides almost 100% cure to cancer by eliminating the tumors from the body. Application of this procedure may vary from individual to individual based on their health state and tumor condition. It does not apply to the parts where it has progressed to metastasis [33]. Generally, these treatment procedures are used when the tumor is small and localized. It is widely applicable to remove several tumors in different organs/body sites, including breast cancer, brain tumor, prostate cancer, kidney cancer, lung cancer, and liver cancer. The main disadvantage of this method is that it cannot assure 100% cure since recurrence is possible even if a single cancer cell can develop into a tumor.

4.2.2 Chemotherapy

The therapy using drugs to kill or destruct tumor cells is called chemotherapy. These drugs inhibit the growth of tumors and, at the same time, obliterate the cancer cells. It is one of the most used and practical therapeutical approaches but with adverse effects, including toxic effects on healthy cells and tissues. The drug selection or combination of drugs can vary based on tumor, body location, and host response to chemotherapy. The adverse effect of chemotherapy is reversible; it will disappear upon completion of the treatment. The administration of two or more combinations of drugs to treat cancer is called combination chemotherapy [34].

4.2.3 Radiotherapy (RT)

This therapy is one of the standard cancer treatments. It is widely applied in combination with chemotherapy for both complete curative and palliative patients to provide a better quality of life [35]. Though radiotherapy is used for almost 50% of tumor types, it will not be continued for the long term due to its gastrointestinal cytotoxicity reactions, including abdominal pain, tenesmus, rectal bleeding, fecal incontinence, and diarrhea [36–38]. To date, there is no practical approach to predict or to manage actively RT-induced gastrointestinal cytotoxicity.

4.3 Influence of Microbiome for the Regulation of Oncology Therapies

Several research studies revealed the role of host-microbiome interaction in many diseases, including diabetes, IBD, and other liver diseases [39–42]. Despite enormous advances in understanding the microbiome function in human health, it is still unclear how these human microbiomes respond to drug treatment. Multiple researchers have recently reported the interaction between cancer therapeutic drugs and the gut microbiome has spawned a new field of interest that will enhance cancer therapy [43, 44].

4.3.1 Gut Microbiome and Immunotherapy

Immunotherapy is one of the critical treatment strategies in cancer therapy [45]. Harnessing the host immune system is a promising cancer control strategy because of its potential for targeting tumor cells and reducing adverse effects on normal tissues. Since the gut microbiome modulates the host inflammation and immunity, it is highly conceivable that changes in gut microbiome composition will regulate the responses to immunotherapy [46].

4.3.1.1 Cytidyl Guanosyl Oligodinucleotides (CpG ODNs)

These are small oligonucleotides enriched in unmethylated cytidyl guanosyl dinucleotides, mainly used as adjuvants in cancer immunotherapy. It mimics the pathogenic infection and activates TLR9 (Toll-like receptor) and pathogen-associated molecular pattern (PAMP) receptors [47, 48]. In addition to immunostimulatory function, intratumoral administration of CpG ODNs induces an antitumoral immune response in the mice model. A mice model confers that prolonged antitumoral inductive activity is achieved upon treatment with IL-10 (interleukin) antibody to treat lymphoma, colon carcinoma, and melanoma by enhancing TNF production [49]. On the other hand, the efficacy of CpG ODN-based treatment was lower in the germ-free mice model/cocktails of antibiotics-treated mice [49]. A commensal bacterium, *Alistipes shahii*, positively correlates with the tumor necrosis factor in tumor cells. Oral administration of this bacterium restores or induces TNF production in germ-free and antibiotics-treated mice models [49].

4.3.1.2 Immune Checkpoint Therapy

It is another treatment strategy to attain anticancer activity by inhibiting immune checkpoint pathways to arrest the growth of cancerous cells [50]. This strategy mainly mediated between cytotoxic T-lymphocyte-associated protein 4 (CTLA-4), programmed cell death protein-1 (PD-1), and its ligands for the use of immune therapies in lung cancer and melanoma [51, 52]. Immune checkpoint inhibitors (ICI) promote adaptive immune responses, and it is a potent treatment strategy in solid tumors. Still, the mechanism behind the variation immune response by the host is not

clearly understood. Earlier studies indicated that a significant change in the diversity of the gut microbiome was observed in patients who responded positively upon treatment with ICI [53]. *Ruminococcaceae, Faecalibacterium, Clostridiales, Bifidobacterium longum, Collinsella aerofaciens, Enterococcus faecium, and A. muciniphila* were significantly enriched in patients who responded to the therapy. These microbes were collectively called favorable bugs. But *Bacteroidales* increased in nonresponders, so this was hailed as an unfavorable bug. An increase in the effector $CD4^+$ and $CD8^+$ T cells was observed in the patient's blood with a higher abundance of favorable bugs. Patients with a higher amount of "unfavorable bugs" showed higher frequencies of regulatory T cells (Tregs) and myeloid-derived suppressor cells (MDSCs) and diminished cytokine response in the systemic circulation [53]. Reprogramming of the gut microbiome will be considered to improve current therapies and the rational design of combination therapy for cancer.

4.3.2 Gut Microbiome and Chemotherapy

The gut microbiome influences drug-based anticancer therapy. Numerous studies have been conducted to understand the mechanisms of the host response to chemotherapy and their impact on the gut microbiome, including colorectal cancer, lung cancer, and melanoma [54–56]. An interplay between tumor and gut microbiomes regulates the chemotherapy efficacy to control the toxic effects.

4.3.2.1 Gemcitabine

Gemcitabine (2′,2′-difluoro 2′-deoxycytidine, dFdC) is one of the vital cytidine analogs developed next to cytosine arabinoside (Ara-C). It is a potent antitumor drug with distinctive pharmacological and metabolic mechanisms of action. It induces cytotoxic actions against tumor cells by the invasion of the cell membrane through nucleoside transporters and converts into gemcitabine diphosphate (dFdCDP) and triphosphate (dFdCTP) [57]. Several types of tumor cells have been reported with increased infection with *Mycoplasma*, especially with *Mycoplasma hyorhinis* [58]. They mainly interrupt the drug efficacy by expressing nucleoside-catabolizing enzyme analogs. An animal model study revealed that mice injected with *M. hyorhinis* showed gemcitabine resistance by infecting colon cancer cells. They showed the resistance to gemcitabine by deamination active gemcitabine (2′,2′-difluorodeoxycytidine) to inactivate 2′,2′-difluorodeoxyuridine metabolite [59]. Further, other members of gamma proteobacteria other than *Mycoplasma* have also shown resistance to gemcitabine by expressing cytidine deaminase. But this resistant effect was reversed by co-administration of the antibiotic ciprofloxacin to regulate its failed response [59].

4.3.2.2 Cyclophosphamide

It is another alkylating drug that belongs to oxazaphosphorines which mainly acts by stimulating anticancer immunity [60]. A study involving tumor-bearing mice model treated with cyclophosphamide showed the translocation of gut microbes

Lactobacillus johnsonii, L. murinus, and *Enterococcus hirae* into mesenteric lymph nodes and spleen to induce Th1 and Th17 immune responses. In the same study, the germ-free model with tumors failed to show the immune responses and was resistant to cyclophosphamide [61]. Another study disclosed the restoration of cyclophosphamide-mediated immune response by oral administration of *E. hirae* [62]. *L. plantarum, L. casei,* and *L. acidophilus* showed ameliorating effect with cyclophosphamide-mediated immunosuppression in mammary carcinoma mice model [63–65].

4.3.2.3 Fluoropyrimidines

It is a principal drug to treat cancer by targeting pyrimidine and affects DNA synthesis [66]. 5-Fluorouracil (5-FU) and its prodrug capecitabine are uracil analogs, and they are used to treat mainly colorectal cancer (CRC). Their primary mechanism of action is by inhibiting thymidylate synthase from controlling DNA synthesis [67]. Fewer studies have reported that the gut microbiome-mediated metabolism affects the efficacy of fluoropyrimidine through two discrete mechanisms, (a) inhibition of bacterial ribonucleotide metabolism which will antagonize the efficacy and (b) inhibition of deoxyribonucleotide metabolism which will enhance the efficacy. Gut microbial members *E. coli* and *Comamonas* can metabolize 5-fluorouracil into fluorouridine triphosphate (FUTP) which inhibits thymidylate synthase leading to DNA and RNA damage. Any kind of mutation in *E. coli* or *Comamonas* produces fluorouridine monophosphate (FUMP), which decreases the efficacy of 5-FU in the *Caenorhabditis elegans* model [68]. Another gut microbe, *Fusobacterium nucleatum*, promotes CRC chemoresistance to 5-FU. Earlier studies confirmed the higher abundance of *F. nucleatum* in CRC than healthy group and linked it to metastasis [69–71]. Two membrane protein molecules of *F. nucleatum*, Fap2 (Fusobacterium autotransporter protein 2) and FomA (fusobacterial outer membrane protein A), promote inflammatory responses which trigger tumor-immune evasion and progression of tumor growth [69]. It also downregulates miRNA-18a and miRNA-4082 to switch the apoptosis of CRC cells to autophagy and show resistance to 5-FU-based therapy [72].

4.3.2.4 Methotrexate

It is an analog of folate and inhibits folate metabolism by inhibiting dihydrofolate reductase [73]. The adverse reactions, including gastrointestinal toxicity and mechanism of action, are not well known. A mice model treated with methotrexate showed mucosal injury and a significant drop in macrophage ratio. *Bacteroides fragilis* was significantly reduced in the methotrexate group. But another group treated with oral gavage of *B. fragilis* improved macrophage polarization and methotrexate-induced inflammatory reaction [74]. Another study involved in a mice group with gut microbiome depletion using wide-spectrum antibiotics upon treatment with methotrexate exhibited minor intestinal injury, but this injury was reversed by TLR2 ligand administration [75].

4.4 Manipulation of the Gut Microbiome for the Management of Cancer and Metabolism: A Personalized Therapeutic Approach

Several studies have proved that gut microbiome composition is affected not only by diet lifestyle but also by several other factors [76–78]. The earlier section discussed the changes in gut microbiome upon anticancer drug response. But gut microbiome manipulation can be a vital therapeutical approach to enhance the clinical management of cancer using the following concepts.

4.4.1 Probiotics

It is defined as "live microorganisms which, when administered in adequate amounts, confer a health benefit on the host" [79]. To date, scientists have discovered many probiotic microorganisms for treating cancer, of which *Lactobacillus* was the first probiotic studied by Goldin and Gorbach (1980) in colonic cancer [80]. Lactic acid-producing bacteria, including *Lactobacillus* and *Bifidobacterium*, are the most used genera in probiotics. But the use of genera belonging to *Streptococcus, Bacillus,* and *Enterococcus* is limited since some of the strains of these genera are pathogenic. Among the yeast kingdom, Saccharomyces is used as probiotics. The list of probiotics is as follows: the species *polyfermenticus and subtilis* from the genus of *Bacillus*; the species of *lactis and adolescentis* from the genus of *Bifidobacterium*; and the species *acidophilus, casei, fermentum, delbrueckii, helveticus, paracasei, pentosus, plantarum,* and *salivarius* from the genus of *Lactobacillus*, *Clostridium butyricum, Enterococcus faecium Lactococcus lactis, Pediococcus pentosaceus, Propionibacterium acidipropionici,* and *Streptococcus thermophilus* [81]. The recent addition to this long list of probiotics is *A. muciniphila* [82, 83]. The main functions of probiotics are intestinal homeostasis; antimicrobial factors including bacteriocin, defensins, and short-chain fatty acids (SCFAs) to protect from pathogenic entry; colonic enzyme activity regulation; and immune regulatory responses [84–87]. Probiotics can be used to alleviate the adverse effects to enhance chemotherapeutic and immunotherapeutic strategies. Probiotics are used mainly to have beneficial effects when consumed [88], mainly through the modulation of cytokines and the activation of phagocytes to eliminate the early-stage cancer cells and carcinogenesis [89]. Also, they have shown that probiotics modulate cancer cell proliferation and apoptosis [89]. A seminal review paper authored by Slizewska et al. (2021) highlights the important varieties of probiotics in the treatment/management of cancer, especially colonic cancer. The potential benefits of probiotics in cancer were evaluated with many in vitro and in vivo studies and clinical studies [81].

The main hurdle in the treatment of cancer patients is the variation in response from person to person. The patient microbiome mainly plays a vital role in regulating the response to cancer treatment. Though probiotics have been used in the treatment of several diseases including cancer, their supportive role in therapy is still unclear.

A Yakult strain *Bifidobacterium breve* protects from pathogenic infection and exhibits a favorable gut environment in pediatric tumors and immunocompromised patients due to chemotherapy [90]. Another study in a mouse model disclosed that *Bifidobacterium* species have protective anticancer roles by downregulating EGFR, HER-2, and PTGS-2 (COX-2) to have a significant effect against CRC models [91]. Oral mucositis, an adverse effect in neck and head cancer patients who receive chemotherapy, was ameliorated by the promising probiotic *Lactobacillus brevis* [92]. In a lung cancer mice model, the combination of cisplatin with *Lactobacillus acidophilus* showed a significant reduction in tumor size compared to the cisplatin alone-treated model and extended the survival of animals [93]. *A. muciniphila*, a promising immunosuppressant probiotic, showed enhancing efficacy role in PD-1-based immunotherapy against epithelial tumors [94]. Furthermore, this next-generation probiotic microbe showed significant involvement in glucose, lipid metabolism, and intestinal homeostasis [95]. *A. muciniphila* also acts as a regulator of abiraterone acetate in castrate-resistant prostate cancer patients [82]. A membrane protein of *A. muciniphila* or pasteurized *A. muciniphila* regulates colitis and CRC by modulating $CD8^+$ T cells in a mice model [96]. From these examples, it is shown that probiotics can be used to alleviate chemotherapy side effects and improve cancer patients' life quality remarkably. It helps to enhance the efficacy of the therapy by regulating the drug dosage by lowering the toxicity.

4.4.2 Prebiotics

These are non-digestible carbohydrates, including soluble fibers, resistant starch, and oligosaccharides which are utilized by the gut microbiome as a substrate for fermentation [97]. These macromolecules can selectively promote the growth of certain microbes and regulate colonic function. SCFAs are the principal metabolites of the gut microbiome after fermentation of prebiotic fibers, which has a protective role against pathogenesis against tumor cells [98]. A high-fiber diet including inulin with supplementation of *Bifidobacteria* and *Butyrivibrio fibrosol* is associated with lowering of the CRC-related risks in a mouse model [99, 100]. Another well-studied resistant starch favors the growth of butyric acid-producing bacteria and hence involves anti-inflammatory and anticancer properties [101]. Studies showed that beta-galactooligosaccharides increase the intestinal concentration of lactate and short-chain fatty acid, nitroreductase, and β-glucuronidase activities suggesting the potential role in regulating the progression of CRC [102]. Increased intake of marine omega-3 fatty acid (MO3FA) is associated with steady CRC progression due to an increase in the abundances of SCFA-producing bacteria *Lactobacillus* and *Bifidobacterium* and, on the other hand, lowers the richness of *F. nucleatum* and *Akkermansia* [103–105]. It confers the importance of probiotics in a therapeutical approach to treating cancer patients.

4.4.3 Postbiotics

Postbiotics are functionally bioactive compounds produced by the gut microbiome through a fermentation process to improve human health. The important components of postbiotics include metabolites, SCFAs, microbial cell fractions, functional proteins, extracellular polysaccharides (EPS), cell lysates, teichoic acid, peptidoglycan-derived muropeptides, and pili-type structure. Recent research studies showed that postbiotics could regulate the immune-modulatory functions and clinical effects to improve the health of a broad range of diseases, including atopic dermatitis, colic, diarrhea, and cancer [106, 107].

Postbiotics can be used as an efficient strategy for anticancer therapy. In a study to test *S. thermophilus TH-4* on 5-FU-related mucositis, the probiotic supernatant showed the inhibitory effect same as live microorganisms to control mucositis [108]. Also, a rat model study showed the partial protection of its gastrointestinal tract using the supernatants of *E. coli* Nissle 1917 and *L. fermentum* BR11 from 5-FU-induced mucositis [109]. An in vitro study on colon cancer cells confirmed that 5-FU cytotoxic activity was controlled by the supernatant from *L. plantarum*. This control is supported by apoptosis and deduction in the survival rate of cancer cells [110]. This evidence addresses the potent role of postbiotics in improving chemotherapy efficacy and masking its adverse effects.

4.4.4 Antibiotics (Ab)

These are molecules that inhibit the growth or replication of bacteria within the body. Even though Ab affects gut microbiome diversity, it can be a vital cog to treat tumor-related bacterial infections. As mentioned earlier, gemcitabine resistance-associated bacteria antagonize its effect by the production of cytidine deaminase. But gemcitabine therapy in combination with ciprofloxacin improves the efficacy of the drug [59, 111]. Likewise, levofloxacin is administered with irinotecan to overcome the irinotecan-related adverse diarrheal effects in treating patients with metastasis of CRC [112].

But in fewer cases, the therapy with single or combination multiple antibiotic therapies worsened the state of cancer in patients. Treatment of cisplatin with an antibiotic cocktail of vancomycin, ampicillin, and neomycin in lung cancer mice model boomeranged by increasing tumor size and lower survival rate than cisplatin therapy alone since it induced dysbiosis of the microbiome [93]. Another study on mice models of sarcoma, melanoma, and colon cancer showed a diminished anticancer effect in antibiotics cocktail including ampicillin, colistin, and streptomycin or beta-lactamase inhibitor imipenem alone [113]. However antibiotic administration can lead to worsening of the therapeutic outcomes in some instances. But the two key benefits to terminally ill patients are prolongation of survival and relief from symptoms, which draw attention to the use of antibiotics in manipulating the gut microbiome to control cancer and metabolism.

4.4.5 Phage Therapy

Bacteriophages are viruses that infect and kill bacteria [114]. Before the invention of antibiotics, phages were used to treat bacterium-related infections in the early 1900s. In the current scenario, due to the rise in antibiotic resistance, the topic of phage therapy is drawing attention because of its antibacterial role. Host-microbial communities can be reprogrammed to create a favorable commensal environment using phage therapy by selectively killing the pathogenic bacteria in the gut microbiome. The relationship between bacteriophage and gut microbiome will have a possible effect on cancer therapy to regulate tumorigenesis.

Our gut microbiome trains our T-cell-related immune responses by the host, and thus this consortium has an essential role in inducing immunity against tumors. A group of French investigators found that cross-reactivity between MHC class I-restricted antigens and microbial antigens was reported which was associated with tumor cells. They found that a gut microbe *Enterococcus hirae* harbors a bacteriophage that modulates immune responses by restricting CD8+ T-lymphocytes upon immunotherapy with cyclophosphamide in mouse models. In renal and lung cancer patients, bacteriophage was associated with increased endurance of cells after PD-1 immunotherapy. In patients with melanoma, a small percentage of human T cells specific for naturally processed prophage epitopes could recognize microbial peptides [115]. This "molecular mimicry" induced by gut phage may represent cross-reactivity between tumors and microbial antigens in cancer therapy.

4.5 A Newer Probiotic: Genus *Akkermansia*

Akkermansia spp. belongs to a Verrucomicrobia family; until today only two species were identified, namely *A. muciniphila* and *Akkermansia glycaniphila*. Both species are considered as intestinal mucin-degrading bacterium; the former was originally isolated from the fecal samples of healthy Caucasian females in 2004, whereas the latter was isolated from the fecal samples of reticulated python, *Malayopython reticulatus* in 2016 [116, 117]. *A. muciniphila* is an oval-shaped, nonmotile, and gram-negative anaerobic bacteria, which grows well with an optimum temperature at 37 °C and with an optimum pH of 6.5 [116]. Studies have suggested that *A. muciniphila* is present in wild animals, mice, hamsters, and humans, predominantly in herbivores [118–121]. It has been reported that the abundance level of *A. muciniphila* in the human feces sample is approximately 3–4%, and in rare conditions, the level can be up to 5%. *A. muciniphila* is considered a potential probiotic due to its nature that can effectively use the gastrointestinal tract (GI) mucin [121]. Moreover, it is believed that its abundance level is modulated not only by the dietary pattern but also by other changes in the mucin level due to its unique way of survival mechanism—degradation of gastrointestinal mucin from the host, causing the release of carbon and nitrogen sources for its survival [116, 122].

4.5.1 Mechanistic Evidence of *A. muciniphila* in Cancer Treatment

Moreover, it promotes the growth of other bacteria through a cross-feeding mechanism, and mainly releases amino acids and sugars during the GI mucin degradation process. It has been extensively studied in major diseases, and differential expressions have been found in its abundance level that can relate to the pathophysiology of such diseases, mainly diabetes mellitus, obesity, cardiovascular diseases, immune disorders, pregnancy complications, cancer, tumor, brain disorders, liver diseases, and kidney diseases [96, 123–134]. It has been reported that the drugs that we use to treat these diseases also have an impact on the abundance of *A. muciniphila*, mainly metformin [135], gemcitabine [136], paclitaxel [137], anti-PDI therapy [94], and some phytochemicals, such as andrographolide [138], puerarin [139], Bofutsushosan or Kampo [140], and resveratrol [141]. Additionally, *A. muciniphila* augments some of the actions of the drugs, mainly the cisplatin—an anticancer drug—in lung cancer mice [141] and anti-PDI therapy through the production of CD4+ T cells [94]. Moreover, *A. muciniphila* has been found to mediate the beneficial effects of metformin, such as glucose tolerance and glucose metabolism via secretion of glucose-regulating peptides and SCFA production in mice and humans [135, 142, 143]. Though *A. muciniphila* is considered a potential probiotic and is involved in the complex network and vicious cycle, its abundance level could determine the outcome of the effect.

The classical role of *A. muciniphila* is to degrade the mucin, thereby getting its carbon and nitrogen source and supplying to the goblet cells which induces the secretion of mucin. The secreted mucin would play a crucial role in maintaining the gut barrier integrity through the reduction of circulatory lipopolysaccharide synthesis, inflammatory cytokines, and white blood cell counts. A higher abundance of *A. muciniphila* may be detrimental because of the higher degradation of mucus that damages the mucosal barrier leading to a greater level of translocation for endotoxin, causing the activation of inflammatory reaction. In the context of cancer, colitis is one of the potential risk factors for colon cancer, which could be driven by the presence of a higher abundance of *A. muciniphila*. It has been reported that cancer chemotherapy consists of antibiotics that increased the abundance of macrophages and inflammatory cytokines because the antibiotic treatment (for example, rifaximin and vancomycin) indirectly enriches the abundance of *A. muciniphila*. Also, *A. muciniphila* (higher level) causes increased metastatic dissemination of tumor cells into the blood, lymph nodes, and lungs. Thus, it is evident that a higher abundance of *A. muciniphila* is associated with disease conditions that progress to carcinoma where an inflammatory-mediated pathway plays a central role because *A. muciniphila* could differentially affect the gut ecosystem depending on the presence of inflammation [144]. Moreover, Farhana et al. (2018) have observed a difference in the abundance of *A. muciniphila* between African Americans and Caucasian Americans affected with CRC. Thus, the abundance of *A. muciniphila* could depend on the genetic factor as well [145]. Furthermore, Lapidot et al. (2020) and Snider et al. (2019) have found a higher abundance of *A. muciniphila*, in conditions like cirrhosis to carcinoma and esophageal adenocarcinoma

[146, 147]. On the contrary, studies on various cancers, such as nasopharyngeal cancer, obesity-associated breast cancer, CRC, and intestinal tumor, have found that the abundance of *A. muciniphila* is reduced [148–152].

Also, *Akkermansia* has been found to promote the immune response mainly by maintaining the gut barrier integrity more robust through different mechanisms, such as (1) *Akkermansia*-derived extracellular vesicles [153]; (2) *Akkermansia*'s outer membrane protein—Amuc_1100 [126] and recombinant protein Amuc_1434 [154], which activate Toll-like receptor 2 and TRAIL-mediated apoptotic pathways, respectively; and (3) induction of dendritic cells to secrete IL-12 that recruits CCR9+CXCR3+CD4+ T lymphocytes in the tumor microenvironment, which in turn promotes the abundance of *A. muciniphila*, and an increased secretion of IL-12 enhances the efficacy of ICI [155]. In addition to this, *A. muciniphila* enhances the efficacy of cisplatin drug through (a) downregulation of Ki-67, p53, and FasL proteins, and the suppression of CD4+CD25+Foxp3+ Treg expression; (b) upregulation of Fas proteins, IFN-γ, IL-6, TNF-α, IFI27l2, and IGFBP7 proteins in cancer cells; and (c) activation of the cytokine-cytokine receptor interaction, JAK-STAT signaling pathway, Th17 cell differentiation, FOXO signaling pathway, and PI3K-Akt signaling pathways in cancer cells [83]. Another mechanism of action could be through the secretion of SCFAs, such as acetate and propionate, through hydrolysis and fermentation processes [156, 157]. Both these SCFAs act through the G protein-coupled receptors, such as GPR41 and GPR43, especially through GPR43, to mediate the SCFA apoptosis of cancer cells [158]. Propionate produced by *A. muciniphila* inhibits the histone deacetylases (HDACs) mainly HDAC6 and HDAC9, which induce histone hyperacetylation and activation of mTOR-S6K and STAT3 pathways and promote the generation of Th17, Th1, FoxP3+, and IL10+ T cells and expression of IL-10, IFN-g, and IL-17 in CD8+ T cells in both Tc1- and Tc17-cell subsets leading to the activation of regulatory T cells and migration of T cells, eventually causing cancer cell apoptosis [158–160] (Fig. 4.1).

4.5.2 *A. muciniphila*, a Key Player in Cancer Immunotherapy

Considering all this evidence, *A. muciniphila* could play an unprecedented role in cancer immunotherapy. This probiotic involves a complex and vicious cycle, where it modulates the efficacy of cancer drugs while at the same time being modulated by a few compounds; also overabundance and less prevalence might be detrimental to the gut barrier function. So, it is evident that the maintenance of its optimal level is very crucial in the treatment of cancer. For example, supplementation of natural compounds, such as Sini decoction and Huoxue Yiqi Recipe-2 (traditional Chinese medicines), and yogurt could increase the abundance of *A. muciniphila*, thereby activating its immune response in treating CRC, lung cancer, and metastatic renal cell carcinoma [161–163]. Synthetic compounds, such as abiraterone acetate, anti-PD-1 immune checkpoint inhibitors, vancomycin, rifaximin, vitamin D, metformin, and androgen receptor axis therapy, enhance the abundance of *A. muciniphila*, thereby improving immune response in different types of cancer [82, 155, 164–

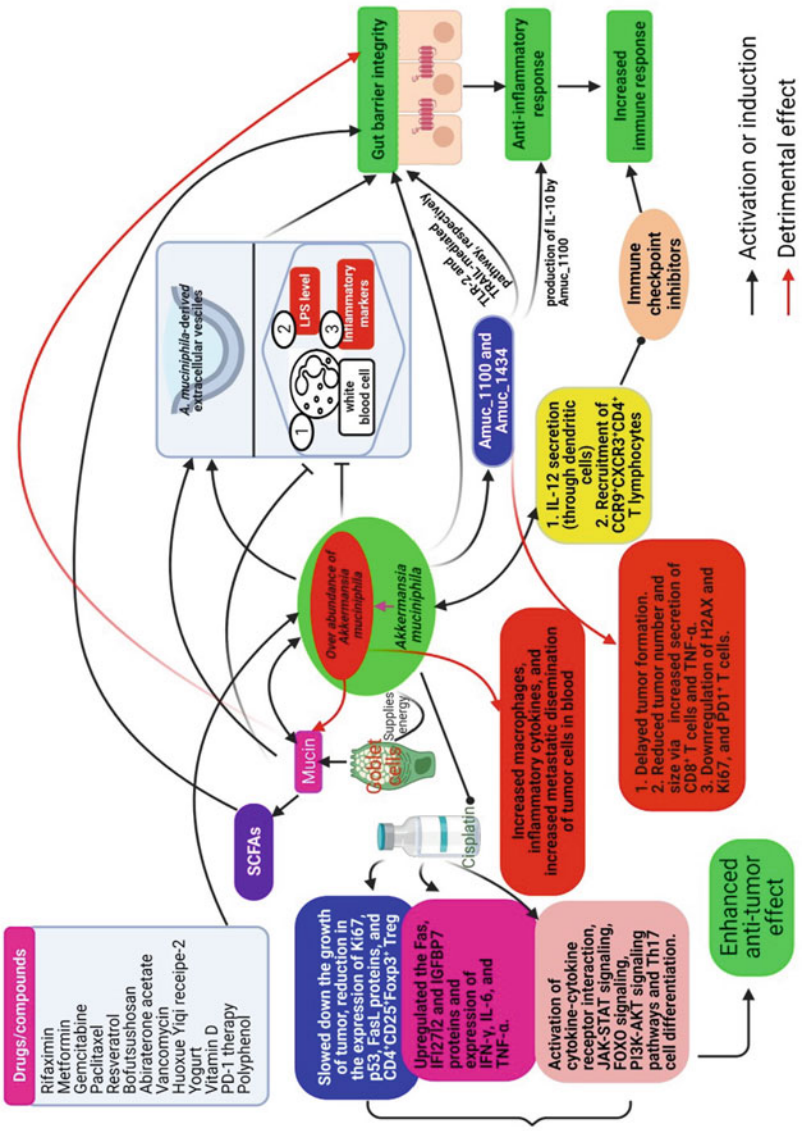

Fig. 4.1 A vicious cycle of *Akkermansia muciniphila* in the crosstalk of immuno-oncology and metabolism

168]. Oral supplementation of *A. muciniphila* conducted in clinical and preclinical studies has demonstrated the direct beneficial effects on cancer treatment, including potentiation of cisplatin drug efficiency and activation of various immune responses to counteract the cancer cell progression and differentiation [83, 169–171]. Interestingly, it has been demonstrated that supplementation of pasteurized *A. muciniphila* is more effective than the live *A. muciniphila* against cancer cells [169, 171]. In addition to this, outer membrane protein derived from *Akkermansia* (Amuc_1100) and recombinant protein (Amuc_1434) protects against cancer progression, mainly through the activation of the TLR2 pathway and TRAIL-mediated apoptotic pathway [133, 154, 170] (Table 4.1 and Fig. 4.1).

4.5.3 Crosstalk of *A. muciniphila* in Immuno-Oncology and Cancer Metabolism

There is no direct study (both clinical and preclinical) that establishes the direct relationship of *A. muciniphila* on glucose metabolism in a cancerous cell. However, Green et al. (2016) have demonstrated that *A. muciniphila* is required for interferon-gamma (IFN-γ) to mediate its negative effects through the regulation of the Irgm1 gene on glucose tolerance [173]. IFN-γ is a key cytokine from the immune system for regulating glucose metabolism because of its sensitive nature to cellular metabolic state [174], and it is a pleiotropic molecule with antiproliferative [175], antitumor [175], and pro-apoptotic characters [176]. IFN-γ inhibits the cancer progression possibly through various processes, such as inhibition of metastasis, angiogenesis, and M2 macrophage polarization and induction of Treg fragility, tumor senescence, apoptosis, and dormancy [176]. Conversely, there are reports which suggest that IFN-γ can mediate the tumorigenesis process through the activation of the STAT1 signaling pathway [177] and $CD4^+$ T cells [178]. These discrepancies might be due to the acute and chronic activation of IFN-γ, the amount of IFN-γ secretion, and most importantly the type of cancer development [176]. Also, Chen et al. (2020) have demonstrated that *A. muciniphila* enhanced the antitumor effect of cisplatin through the elevation of IFN-γ level in lung cancer [83]. Thus, *A. muciniphila* might exert its anticancer effect through the optimal level of IFN-γ secretion, rather than an abnormal level of IFN-γ secretion. This hypothesis warrants further study.

Inosine, a purine metabolite, is generated from adenosine intracellularly and extracellularly by the deamination process [179]. Interestingly, it was found to be derived from the role of the gut microbiome, mainly by *A. muciniphila*, *Bifidobacterium*, and others [180]. This inosine has been identified as an important modulator of response to immune checkpoint blockade (ICB) therapy and plays a key mechanistic role in T-cell activation in a different type of cancer [180]. Moreover, co-administration of inosine with immune checkpoint inhibitors promoted the infiltration of IFN-γ+ CD4+ and IFN-γ+ CD8+ T cell in tumor cells, thereby promoting their antitumor efficacy. Due to the aerobic glycolysis process, the cancer cells consume an enormous amount of glucose for their energy expenditure, leading

Table 4.1 Preclinical and clinical evidence for the role of *A. muciniphila* in the cross talk of immune oncology and metabolism

Drug/natural compounds	Type of cancer	Type of studies	Effect
Sini decoction	Azoxymethane/ DSS-induced CRC	Mice	Increased the relative abundance of *A. muciniphila* [161]
Oral administration of *A. muciniphila* (pasteurized)	FMT from cancer patients	Mice	Increased the antiaging and anticancer metabolites, such as spermidine, spermine, propionate, butyrate, 2-hydroxybutyrate, and bile acids [169]
Abiraterone acetate (AA)	Castrate-resistant prostate cancer patients	In vitro	Preferentially enriches *A. muciniphila* [82]
Bifidobacterium-containing yogurt	Metastatic renal cell carcinoma	Clinical study	Increased relative abundance of *A. muciniphila* in the clinical benefit group [162]
Supplementation of *A. muciniphila*	CRC	Mice	Enhanced antitumor immune response and tumor clearance through the activation of Toll-like receptor 2 (TLR2) [170]
Oral administration of *A. muciniphila*	Lung cancer	Mice	Enhances the efficacy of cisplatin [83]
Anti-PD-1 immune checkpoint inhibitors	Metastatic renal cell carcinoma	Clinical study	Increased relative abundance of *A. muciniphila* in the clinical benefit group (nivolumab plus ipilimumab) [155]
Huoxue Yiqi Recipe-2	Lung cancer	Mice	Increased the abundance of *A. muciniphila* which was thought to enhance the therapeutic effect of PD-L1 antibodies [163]
Vancomycin	MSS-type CRC	Mice	Increased the relative abundance of *A. muciniphila* and modulated the glycerophospholipid pathway [164]
Recombinant protein Amuc_1434	CRC	In vitro	Suppressed the cell viability of LS174T cells via TRAIL-mediated apoptotic pathway [154]
Live or pasteurized *A. muciniphila*	Pancreatic islet cancer	In vitro	*A. muciniphila* can promote the expression of insulin secretion-related genes in INS-1 cells and inhibit the apoptosis process [171]
Vitamin D supplementation	CRC	Mice	Increased the relative abundance of *A. muciniphila* [165]
PD-1 therapy	Hepatocellular carcinoma	Clinical study	*A. muciniphila* enriched in PD-1 therapy responder group [172]
Androgen receptor axis-targeted therapies	Prostate cancer in men	Clinical study	*A. muciniphila* enriched in ATT group than the healthy volunteer [166]
Metformin	CRC	Clinical study	Increases the abundance of *A. muciniphila* [167]

to glucose deprivation. Strikingly, Wang et al. (2020) have demonstrated that inosine is an alternative carbon source for $CD8^+$ T-cell function under glucose deprivation [181]. Thus, the cancer cells can compete with T cells for the utilization of inosine and their proliferation is blocked by the presence of inosine. In addition, glutamine is an important carbon and nitrogen donor of activated T cells. Inosine did not promote the T-cell proliferation through the glutaminolysis process; instead, its presence is needed for the inosine-dependent T-cell proliferation, which establishes the potential role of inosine in cancer metabolism [181].

4.6 Summary and Future Directions

In summary, metabolic reprogramming in the cancer microenvironment plays a vital role in determining the progression or regression of cancer. Recent advancements in exploring the role of microbiota in the cancer microenvironment suggest its potential beneficial effects in preventing cancer progression. For example, the supplementation or addition of probiotic—*A. muciniphila*—could be useful in treating the cancer disease in various organs, through various potential mechanisms, but the researchers/clinicians should be careful about the abundance of *A. muciniphila* which depends on the genetic background, cancer type, and level of inflammation while considering for the treatment of cancer either in animal models or in a clinical setting, because of its differential role in cancer progression. Drugs used in cancer treatment including antibiotics can muddle the gut microbiome structure and cause collateral damage in cancer patients. Nevertheless, using novel approaches, such as targeted drug-carrying phages, and using phages to target pathogenic bacteria will reduce the perturbation at the microbiome-cancer interface to ensure a healthy environment for the host.

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Targeting the Immuno-Oncology Metabolism in Cancer

5

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Abstract

Cancer is a disparate disease and tumor cells requires continuous reprogramming of metabolic and signaling pathways to sustain growth and survival. Interplay between tumor and its microenvironment has a major role in defining the metabolic reprogramming, which also supports immune evasion of cancer cells. Cancer cells also modulate the metabolism of immune and stromal cells in their microenvironment. The role of metabolic crosstalk between tumor and immune cells and the mechanism related to change in immune cell function has not been sufficiently explored. Each metabolic pathway may have synergistic or adverse effects on cancer cells and immune cells depending upon the nutrient availability and metabolic waste. Immuno-oncology metabolism has immense potential to complement current treatment modalities and enhance therapeutic outcomes. In this book chapter, we describe the different metabolic pathways and their impact upon immune-oncology metabolism along with the possible therapeutic opportunities.

Keywords

Cancer · Metabolism · Metabolites · Metabolic reprogramming · Immune cells · Tumor immunology · Immuno-oncology

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5.1 Introduction

Cancer cells are well known to modulate and reprogram the cellular signaling pathways and sustain their proliferation, growth, survival, and malignant transformation. These reprogramming can be at genetic and epigenetic levels to sustain the malignant transformation. Cellular metabolism plays a central role in regulating and sustaining this malignant growth. Metabolomic reprogramming is easily induced by various pathways and is tightly linked to oncogenic signaling. There are several oncogenic drivers (p53, KRAS, MYC) which are involved in this reprogramming apart from their well-known functions in senescence, cell cycle regulation, and DNA repair [1]. These changes get exemplified via oncometabolites such as succinate, which are not "bystanders" but have a direct effect on cancer progression. Succinate can accumulate due to loss-of-function mutations in succinate dehydrogenase (SDH). This tricarboxylic acid (TCA) cycle intermediate can inhibit α-ketoglutarate-dependent dioxygenases, which induces a malignant phenotype [2, 3]. Mortality related to cancer is an outcome of malignant transformation due to genetic and epigenetic changes leading to hallmarks of cancer. In recent years, there has been an increased appreciation for metabolic reprogramming along with immune evasion as prominent hallmarks of cancer (sustaining proliferative signaling, resisting cell death, evading growth suppressors, inducing angiogenesis, enabling replicative immortality, and activating invasion and metastasis) [4]. Moreover, it is also becoming more evident that alterations in tumor metabolism and constituents of the tumor microenvironment are linked and work in unison to promote malignancy [5, 6].

Evasion from the immune system and metabolic reprogramming are always interlinked as the tumor cells modulate the microenvironment to control the metabolic intermediates that may suppress immune responses. This interplay between tumor, its microenvironment, and immune cells is commonly known as immunometabolism [7]. In addition to the cellular heterogeneity of cancer cells, a wide variety of immune populations also exist within the tumor microenvironment. In this book chapter, we describe the interplay between cancer cells and tumor immunophenotypes and highlight the therapeutic opportunities related to these interactions.

5.1.1 Interaction Between Immune Cells, Cancer Cells, and Tumor Microenvironment: At a Glance

The tumor microenvironment is a heterogeneous mixture of cancer cells, tumor-associated stromal cells, as well as non-stromal factors (i.e., extracellular matrix proteins and other soluble factors) that orchestrate cancer cell heterogeneity and clonal evolution and drive cancer cell progression and, ultimately, metastasis. Most of the solid tumors are characterized by a tumor microenvironment that is nutrient poor, hypoxic, and acidic on account of higher levels of lactate, a by-product of glycolysis [8]. Increased levels of extracellular lactate are associated with tumor

progression, increased tumor vascularization, and tumor immune suppression [9]. Moreover, several *in vitro* and *in vivo* investigations have demonstrated that depletion of glucose in the tumor microenvironment and accumulation of its downstream catabolite lactate inactivate infiltrating and tumor-resident immune cells [10, 11]. Adenosine released by tumor cells under hypoxic conditions also modulates the extracellular environment in favor of cancer cells by promoting anti-inflammatory activities. The oncometabolites, lactate, adenosine, and anti-inflammatory cytokines along with hypoxia and nutrient deprivation create a hostile condition for various immune cells to survive within the tumor microenvironment. These adverse conditions mostly favor co-inhibitory molecules and inhibit immune cell proliferation and production of various enzymes including perforins and granzymes [8]. T cells infiltrating the tumors have to deal with glucose and amino acid deprivation as well as simultaneous accumulated toxic catabolic by-products. Reactive nitrogen species accumulated during arginine and tryptophan catabolism induce dormancy in tumor-infiltrating lymphocytes and adversely affect T cell-mediated immunity [12].

A well-regulated and effective response against tumor growth can be driven by the interaction of innate and adaptive immunity. Tumor microenvironment harbors infiltrating T cells and B cells of adaptive immunity along with innate immune cells such as macrophages, dendritic cells, and natural killer cells. T cells can either interact directly to tumor tissues or engage and stimulate the other cells in the tumor microenvironment. B cells have their pro- and antitumor functions which are yet to be explored [13]. On the other hand, macrophages can be polarized in two different forms, i.e., pro-inflammatory (M1) and anti-inflammatory (M2) phenotype, based on the stimulation provided inside the tumor microenvironment [14]. Interferon-γ (IFN-γ) and toll-like receptor (TLR) ligands favor M1 phenotype, whereas IL-4 and IL-1 induce M2 phenotypes with distinct metabolic demands. M1 macrophages mostly depend on anabolic pathways including anaerobic glycolysis, pentose phosphate pathway activation, and fatty acid synthesis, whereas M2 phenotype utilizes mostly oxidative phosphorylation (OXPHOS) to meet its metabolic needs [15]. Therapeutic modalities to date against these immune adversaries have limited success in the clinic, as they mostly have not taken the metabolic switching into account while being developed.

5.2 Carbohydrate Metabolism and Its Impact on Immuno-Oncology Metabolism

The major metabolic pathway for carbohydrate metabolism is glycolysis. This cycle begins with the binding of glucose to its transporters (e.g., GLUT1). The first step involves an ATP-dependent reaction catalyzed by hexokinase to produce glucose-6-phosphate (G6P). Glycolysis provides a net gain of 2 ATP and pyruvate as the end product [16]. G9P can also be utilized for NADPH production and ribose synthesis in the cytosol via the pentose phosphate pathway (PPP). NADPH also serves as an important reducing equivalent for glutathione (GSH), a key antioxidant, and is

integral in protecting pro-inflammatory immune cells, fatty acid synthesis, and phagocytic function [17, 18]. Another metabolic product of PPP, ribose-5-phosphate, serves as the key percussor for nucleic acid synthesis. On the other hand, pyruvate is converted to acetyl-CoA in mitochondria by pyruvate dehydrogenase. Acetyl-CoA acts as a key component of the tricarboxylic acid (TCA) cycle, which generates important intermediates participating in various metabolic processes. TCA along with the electron transport chain also provides a net gain of 36 ATP molecules. Citrate, a TCA cycle intermediate, helps in the production of various modulators of inflammatory response such as nitric oxide, ROS, and prostaglandin E2 (PGE2) [19]. Another product of the TCA cycle, succinate, can modulate the HIF1α pathway and hence increase glycolysis during inflammation [20].

Highly proliferating cells like cancer cells consume a high amount of glucose and produce an increased amount of lactate [21]. Glucose metabolism in cancer cells is regulated by various oncogenes and growth factors such as c-Myc, p53, and hypoxia-inducible factor (HIF) 1α [22]. This metabolic reprogramming further involves various signaling pathways, e.g., AMP-activated protein kinase (AMPK), phosphoinositide-3-kinase (PI3K), Notch, Akt, and mammalian target of rapamycin (mTOR) [23]. c-Myc can promote mitochondrial biogenesis, increasing the number of mitochondria as well as induction of enzymes related to the glycolytic pathway in cancer cells [24, 25]. During tumor metabolic reprogramming, p53 regulates glycolysis via induction of glycolysis and apoptosis regulator (TIGAR) and inhibition of glucose transporters GLUT-1 and GLUT-4 [22, 26]. c-Myc can induce glutaminolysis, whereas p53 can limit the same pathway in response to cellular stress and DNA damage [27, 28].

Glucose can also be stored in its polymerized form known as glycogen [29]. Glucose-6-phosphate transporter (G6PT) subunits help in mediating the regulation of the glycogen-glucose homeostasis. Deficiency of G6PT can lead to myeloid progenitor dysregulation, neutrophilia, inflammatory bowel disease (IBD), and autoimmune endocrine disorders [30, 31]. Glycogen can upregulate iNOS expression by interacting with TLR2 to induce the production of NO and cytokines, e.g., IL-6 and TNF [32]. In mouse intestinal macrophages, glycogen is also inversely correlated with the regulation of cytokine secretion and oxidative stress [33].

Galactose, a monosaccharide hydrolyzed from lactose, enters the Leloir pathway to generate glucose-1-phosphate which can be further utilized in glycolysis after its conversion to G-6-P [34]. This pathway can modulate the immune cell functioning in the tumor microenvironment [35, 36].

An increased dietary intake of fructose uniquely contributed to obesity and obesity-related cardiometabolic complications [37]. Obesity is one of the risk factors for cancer and recent pieces of evidence suggest that increased fructose intake also promotes cancer cell proliferation [38]. Fructose metabolism generates dihydroxyacetone phosphate (DHAP) and glyceraldehydes-3-phosphate (GA3P) which further feeds into glycolysis [39]. High fructose levels can lead to the accumulation of macrophages and increased levels of inflammatory cytokines, i.e., TNFα, IL-6, NO, and IL-1 [38]. IL-6 and IL-1 shift the metabolic switch of dendritic cells towards increased glycolysis [40].

5.2.1 T Cells

Cancer cells are eliminated and destructed by the effector immune cells such as activated cytotoxic T cells, which themselves undergo metabolic reprogramming to perform cancer-eliminating functions. Aerobic glycolysis is necessary for T-cell function and proliferation; however, it is not critical for their activation and survival [10, 36, 41]. During antigenic stimulation, T cells increase their glucose uptake and glycolysis by upregulating the glucose transporter GLUT-1 [42, 43]. However, anaerobic glycolysis is required for T cells to perform effector functions, which is not sustainable as a permanent state. Hence, after an initial temporary rise in effector T cells during acute infection, the memory T-cell population increases which is mostly dependent on the mitochondrial respiration rather than aerobic glycolysis [44, 45].

In contrast, highly proliferating cancer cells consume a high amount of glucose, thereby restricting glucose availability to other cells, such as CD8+ T cells, in the tumor microenvironment [46–48]. A key feature of T cells is to utilize the glucose metabolism as per their state of activation. To this end, naïve T cells require glucose to produce ATP via the tricarboxylic acid (TCA) cycle and OXPHOS [49], whereas activated T cells switch metabolically from OXPHOS to aerobic glycolysis [49–51]. Limited availability of glucose in TME decreases glycolytic activity and cytokine production (IFNs) in CD8+ T cells [11]. On the other hand, Tregs get benefited from this glucose-depleted TME as their metabolic needs can only be met by OXPHOS [52, 53]. IFN-γ excretion from tumor-infiltrating CD8+ T cells is directly inhibited by glucose deprivation [11]. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH), a key glycolytic enzyme, is responsible for this decreased production of T-cell effector molecule by inhibiting the translation of IFN-γ under low glucose flux [36]. Phosphoenolpyruvate (PEP), a glycolytic product, sustains Ca2+ and nuclear factors of activated T cells (NFAT) by blocking endoplasmic reticulum Ca2+-ATPase. However, low glycolytic flux reduces the PEP levels which in turn suppresses T-cell receptor (TCR)-dependent activation of Ca2+ and NFAT signaling [54]. Interestingly, recent articles and clinical data suggest an inverse relationship between tumor glucose metabolism and T cell-mediated tumor rejection [55]. Tumor-infiltrating Treg cells support tumor progression by suppressing inflammation and antitumor immunity. These cells survive in hypoxia and do require glucose for their function and survival [56]. Forkhead box protein P3 (FOXP3), a lineage-defining transcription factor, is reported to modulate the expression of genes involved in the remodeling of Treg cell metabolism. It downregulates the genes for glycolytic enzymes by inhibiting the PI3K-Akt-mTORC1 signaling pathway and upregulates the genes related to fatty acid and amino acid metabolism [57].

5.2.2 B Cells

Antibody-producing B cells are also metabolically active as T cells. However, glucose utilization varies according to the B-cell developmental stage. Pre-B cells

require low glucose whereas naïve N cells increase their glucose uptake during proliferation [58, 59]. In a hypoxic environment, B cells require an increased glycolytic activity in germinal centers to maintain the growth and proliferation of B cells [60]. A hypoxic tumor microenvironment in combination with glucose deprivation hence in turn creates an immunosuppressive milieu that favors certain phenotypes of T cells and B cells.

5.2.3 Natural Killer Cells

Similar to Teff cells, natural killer (NK) cells also utilize glucose via glycolysis and OXPHOS for their growth and cytokine production [61]. Increased levels of IL-15 boost the mTOR activity to further facilitate glucose uptake and bioenergetic metabolism [62, 63]. Altered activity of the mTOR signaling pathway or glycolytic pathway impairs the cytotoxic activity in NK cells, thus promoting cancer growth and proliferation [64]. Sterol regulatory element-binding protein (SREBP) transcription factors help in rewiring the metabolic reprogramming and the production of IFNγ and granzyme B in NK cells. Inhibiting SREBP has been documented to affect the NK cell-mediated cytotoxicity [63]. However, the effect of tumor metabolic rewiring upon SREBP-mediated NK cell function has still been unknown.

5.2.4 Macrophages

Within the tumor microenvironment, polarization also alters the metabolic profile of macrophages. While pro-inflammatory M1-like macrophages require high glycolytic activity, the immunosuppressive and anti-inflammatory M2-like macrophages rely on OXPHOS [65, 66]. Generation of lactate from glucose produces NADH which is required for various biosynthetic pathways to activate M1 macrophages, phagocytosis, and cytokine production [67, 68]. HIF-1 which is heterodimeric can stimulate various genes related to glycolytic pathway enzymes, e.g., hexokinase, pyruvate kinase, GLUT-1, and lactate dehydrogenase [69, 70]. HIF-1 protein synthesis is regulated by phosphatidylinositol 3-kinase (PI3K) and ERK mitogen-activated protein kinase (MAPK) pathways [71]. Activation of HIF-1α which regulates inflammatory pathways is induced by TLR-mediated signaling in macrophages [20]. TLR-stimulated macrophages increase their reverse electron transport chain and suppress mitochondrial oxidative processes in response to increased glucose dependency [72, 73]. Macrophages also ramp up the pentose phosphate pathway via modulation of carbohydrate kinase-like protein (CARKL) to support the increasing demand for NADPH, nucleotide synthesis, and fatty acid biosynthesis [74, 75]. Succinate, a TCA cycle intermediate, can regulate M2 polarization by modulating the HIF1-α signaling pathway [76]. Another intermediate, itaconate, can regulate the levels of type 1 interferons and anti-inflammatory response via alkylation of KEAP1 and hence activating NRF2 [77]. In M2 macrophages, αKG generated through TCA cycle or glutaminolysis acts as a cofactor for enzymes engaged in epigenetic

processes and is essential for mitochondrial integrity as well as macrophage activation [75, 78]. An alternative route for αKG production is serine biosynthesis, which is reported to play a key role in T-cell functions [79].

5.2.5 Dendritic Cells

Upregulation of the glycolysis pathway, increased glucose uptake, and lactate production are also key features of activated dendritic cells (DCs) [80]. mTORC1-regulated Akt signaling and HIF1α pathway positively modulate DC activation and cytokine secretion [81]. Along with the secretion of cytokines responsible for T-cell differentiation, the motility of DCs and their migration to lymph nodes are also highly dependent on glycolytic metabolism [82, 83]. During glucose deprivation or the early phase of activation, DCs maintain their immune effector function by utilizing stored glycogen to meet their glycolytic needs [84].

5.3 Targeting the Carbohydrate Metabolism

Glucose deprivation in the tumor microenvironment results from the increased glycolytic activity of cancer cells in association with poor vasculature transport [85]. Cancer cells metabolically compete with the antitumor immune cells and create a nutrient-deprived niche, which hinders the ability of effector immune cells to secrete cytokines [11, 36]. T cells deprived of glucose can undergo exhaustion which limits their expansion and effector functions [86]. Glucose deprivation shifts T-cell differentiation from effector to regulatory phenotype by inducing FOXP3 expression [43]. Increased secretion of granulocyte-macrophage colony-stimulating factor (CSF) and macrophage CSF by cancer cells is a direct result of increased glucose uptake which further promotes MDSCs and suppresses effector T cells [87]. Targeting cancer-associated glucose metabolism can be achieved by either inhibiting the key regulatory enzymes of the glycolytic pathway or using a competitive inhibitor of glucose, e.g., 2-DG (Fig. 5.1 and Table 5.1).

Such therapies may help in inhibiting tumor growth; however, they may also suppress the effector functions and proliferation of antitumor immune cells [8, 54, 112–115]. Metabolic reprogramming of immune cells is also regulated by immune checkpoints including CTLA-4, PD-L1, and PD-1 which inhibit glycolytic pathway and increase fatty acid catabolism [116, 117]. Immune checkpoint inhibitors thus rescue the effector functions of immune cells and cytokine production in part by restoring the glycolysis and other anabolic pathways [11]. Interestingly, inhibition of myeloid cell-specific PD-1 is highly effective in reducing cancer cell proliferation compared to inhibition of T cell-specific PD-1 [118]. However, all these current strategies have been most effective against tumors with a high glycolytic index or high neoantigen load [119, 120]. Tumors with low glycolysis flux, minimal neoantigen load, and increased oxidative phosphorylation do not respond well to immune checkpoint inhibitors [120]. To increase the efficacy of these checkpoint

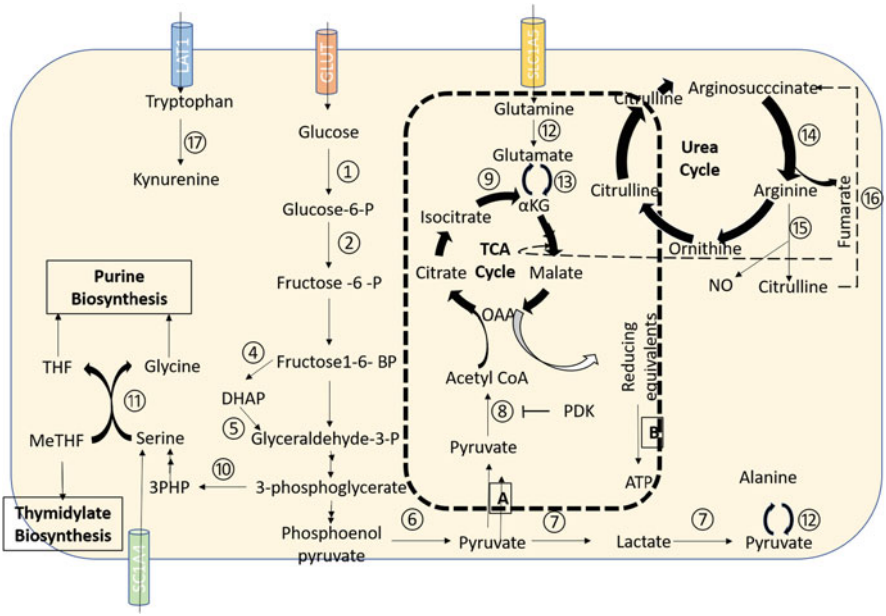

Fig. 5.1 Targets for cancer therapy in carbohydrate and amino acid metabolism: ① hexokinase, ② phosphoglucose isomerase, ③ phosphofructokinase, ④ aldolase, ⑤ triose phosphate isomerase, ⑥ pyruvate kinase, ⑦ lactate dehydrogenase, ⑧ pyruvate dehydrogenase, ⑨ isocitrate dehydrogenase, ⑩ phosphoglycerate dehydrogenase, ⑪ serine hydroxymethyl transferase, ⑫ transaminase, ⑬ glutamate dehydrogenase, ⑭ arginosuccinate lyase, ⑮ nitric oxide synthase, ⑯ arginosuccinate synthase, ⑰ IDO/TDO, ⑱ *PDK* pyruvate dehydrogenase kinase, *A* mitochondrial pyruvate carrier, *B* electron transport chain

inhibitors across all cancer types, combinatorial approaches with various pathway inhibitors including metabolic interventions will likely be needed.

As mentioned above, a consequence of increased glycolytic activity is the high amount of lactate that promotes an acidic environment under hypoxic conditions [121–123]. This lactate buildup and acidic conditions favor an immunosuppressive environment by inhibiting the T-cell function, differentiation, and cytokine production [124, 125]. Low pH also promotes monocyte-derived dendritic cells with increased oxidative phosphorylation and reduced glycolytic activity [126]. Neutralization of this acidic environment using bicarbonate can improve cancer treatment efficacy. In combination with anti-PD-1 antibodies and adoptive T-cell therapy, oral bicarbonate was shown to inhibit tumor growth and increased survival in a melanoma mouse model [113, 127]. Recently, the V-domain Ig suppressor of T-cell activation (VISTA) has been a new addition to immune checkpoint proteins. An acidic pH select antibody, PSGL-1, was shown to inhibit VISTA-mediated immunosuppression of T cells under an acidic condition in in vivo. This study also revealed a promising combinatorial approach of VISTA and PD-1 that exploits the acidic tumor microenvironment to suppress tumor growth in the MC38 mouse model

Table 5.1 Summary of anticancer drugs targeting carbohydrate metabolism

Target	Inhibitors	Cancer type	Refs
Glucose transporters (GLUTs)	2-Deoxyglucose (2DG), Phloretin, Silybin, Glutor, STF-31, WZB117, Fasentin	Breast cancer, colon cancer, lung cancer, lymphoma, osteosarcoma, pancreatic cancer	[88–96]
Hexokinase (HK)	2-Deoxyglucose (2DG), 3-bromopyruvate, lonidamine, methyl jasmonate	Breast cancer, colon cancer, lymphoma, neuroblastoma, pancreatic cancer	[88, 91, 92, 94, 97–99]
Glyceraldehyde 3-phosphate dehydrogenase (GAPDH)	3-Bromopyruvate, ornidazole, a-chlorohydrin	Liver cancer, lymphoma	[88, 94, 100]
Phosphofructokinase (PFK)	3-(3-Pyridinyl)-1-(4-pyridinyl)-2-propen-1-one (3PO)	Breast cancer, lymphoma, melanoma	[88, 92]
Pyruvate kinase-M2 (PK-M2)	TLN-232/CAP-23, Shikonin, Alkannin, TEPP-46, DASA-58, ML-265, oleanolic acid (OA), dimethylaminomicheliolide	Breast cancer, glioblastoma, liver cancer, lung cancer, melanoma, renal cell carcinoma	[88, 91, 99–106]
Lactate dehydrogenase (LDH)	2,3-Dihydroxynaphtalen-1-carboxylic acid, N-hydroxy-2-carboxy-substituted indoles, oxamate, 3-hydroxyisoxazole-4-carboxylic acid, FK866, AZD3965, AR-C155858, quercetin	Glioblastoma, lymphoma, pancreatic cancer	[88, 93, 99, 100, 107–110]
Isocitrate dehydrogenase (IDH)	AGI-5198, AGI-6780	Glioblastoma, leukemia	[104, 111]
Pyruvate dehydrogenase kinase (PDK)	PDK tyrosine kinase inhibitors, dichloroacetate	Breast cancer, glioblastoma, lung cancer	[25, 89, 104, 107]

[128]. Additionally, increased lactate accumulation in the tumor microenvironment can promote tumor immunosuppression through inhibition of NK cells and macrophage, DC differentiation, monocyte activation, Treg survival, and increasing of MDSC proliferation [129–132]. A G protein-coupled receptor (GPR81)-mediated signaling pathway in immune cells and endothelial cells also gets activated during increased lactate production by cancer cells. Inhibition of the GPR81-mediated signaling pathway can lead to impaired Tregs and low levels of IL10 secretion [133, 134]. Another approach can be to inhibit the regulatory enzyme, i.e., lactate dehydrogenase (LDH) or the lactate transporters. However, targeting LDH can have differential effects on immune cells along with its anticancer effect [135]. For example, inhibition of LDH reduces tumor growth and can also ameliorate T-cell

proliferation as well as cytokine production [107, 113]. Hence, clinical evaluation and the effect on immune function must be considered before using these therapeutic approaches. In contrast, inhibitors to lactate transporters monocarboxylate transporter (MCT) 1 and/or 4 can lead to cancer cell death by reducing the rate of glycolysis while keeping the T cell intact with increased IL-2 and IFN-γ production [107, 113].

5.4 Amino Acid Metabolism and Its Impact on Immuno-Oncology Metabolism

In addition to carbohydrates, amino acids are also important nutrients associated with immuno-oncology metabolism [136]. Amino acid metabolism serves as an integral building block for protein synthesis, nucleic acid synthesis, production of neurotransmitters, conversion to glucose and lipids, epigenetic modification, detoxification, and maintenance of intracellular redox status.

5.4.1 Glutamine

Of the amino acids, glutamine, a nonessential amino acid, has long been associated with cancer development. Glutamine is the most abundant amino acid in circulation and is used as a major source of nitrogen and carbon for nucleotide synthesis, energy generation, nitric oxide production, and various biosynthetic pathways involved in cellular proliferation [137, 138]. Within the tumor microenvironment, glutamine also plays a key role in cellular metabolism related to innate and adaptive immunity [73, 139]. Any alteration in glutamine metabolism has a severe effect on the activation and development of various immune cells such as macrophages, Th17, regulatory T (Treg), and B cells. Glutamine can be derived either through de novo synthesis or through uptake from the extracellular space using amino acid transporters such as SLC1A5 [140]. Glutamine-specific antiporters shuttle glutamine out of the cell in exchange for other nutrients, such as leucine, that cannot be synthesized de novo [141]. De novo synthesis of glutamine takes place in mitochondria catalyzed by glutamine synthase in an ATP-dependent manner using ammonia and glutamate and can be further hydrolyzed to form glutamate which enters the TCA cycle [142, 143]. Cancer cells harbor mutated Myc gene, which transcriptionally promotes mitochondrial glutaminolysis and enhanced glutamine intake from the extracellular space [144, 145]. Glutamine uptake by immune cells depends on neutral amino acid transporter type 2 (ASCT2), whose deficiency impairs the activation of Th1 and Th17 helper T cells [146]. Glutamine deprivation in the tumor microenvironment promotes Treg differentiation, which can be countered by supplementing with α-ketoglutarate and inducing mTORC1 signaling via Tbet, a Teff cell transcription factor [147]. B-cell proliferation and differentiation into plasma cells also require a glutamine pool [148]. M2 macrophages upregulate glutamine synthetase to induce glutamine synthesis from glutamate.

Tumor-associated macrophages also rely on increased glutamine synthetase expression and its inhibition decreases M2 phenotype, thus reducing in vivo tumor metastasis [149, 150]. Thus, glutamine deprivation or pathways related to glutamine within the tumor microenvironment are being explored as a therapeutic target for cancer treatment. Glutamine metabolism is also linked with NO production along with arginine metabolism [151].

5.4.2 Arginine

Arginine, a nonessential amino acid, serves as a precursor for ornithine, citrulline, and nitrite and can differentially regulate immune cell functions in the tumor microenvironment [152]. As mentioned above, NO is also generated during the arginine-to-nitrite pathway [153]. Intracellular arginine pools are maintained CAT-1 transporters that promote extracellular uptake of arginine or through de novo synthesis utilizing citrulline and aspartate [154, 155]. Arginine de novo synthesis requires a lot of energy expenditure in the form of ATP molecules. Arginase-1 (Arg1) further catabolizes arginine into ornithine and urea, whereas iNOS converts arginine into NO and citrulline. Arg1 and iNOS are key enzymes in mediating immune response through the activation of macrophages under physiological and pathological conditions [73, 156]. Myeloid-derived suppressor cells (MDSCs) specifically have a metabolic shift towards increased amino acid metabolism which depletes arginine in addition to tryptophan, and cysteine from the tumor microenvironment, thereby inhibiting T-cell effector functions [157]. HIF1α has been reported to upregulate the expression of Arg1 and iNOS to induce increased levels of NO in macrophages and MDSCs to sustain their phagocytic and immunosuppressive activity, respectively [155]. Additionally, upregulated Arg-1 leads to enhanced arginine degradation by tumor M2 macrophages or MDSCs and hence results in arginine deprivation, reduced expression of the CD3ζ chain, cell cycle arrest, as well as a compromised antigen-specific T-cell response [158, 159]. Increased generation of NO via arginine metabolism by iNOS impairs T-cell effector function and leads to cell death [159]. Activated T cells are highly reliant on arginine and tryptophan to maintain their status and function [160]. Arginine can induce a metabolic switch from glycolysis to OXPHOS and hence can generate central memory T cells with enhanced antitumor activity [161]. This is achieved by arginine's direct effect on conformational and structural changes on T-cell nuclear proteins (BAZ1B, PSIP1, and TSN). Tumor-derived PGE2 induces a tolerogenic phenotype in the dendritic cell by upregulating the Arg1 expression [162]. IL-6 also contributes towards downregulating MHCII and dysregulated tumor immunity by increasing Arg1-expression in dendritic cells [163].

5.4.3 Serine and Glycine

Serine and glycine have recently been identified as important contributors to immune cell function and tumorigenesis. To date, the role of serine in modulating the immune response has been largely in the context of the adaptive immune response, with less focus on the innate immune response. Serine is essential for purine biosynthesis that is required for T-cell proliferation. Impairment in serine/glycine metabolism can also inhibit the activation of naïve T-cell activation [79, 164]. Very little is known about the role of serine and glycine so far in innate immune cells. Inside mitochondria, serine acts as the precursor for glycine which is a key molecule for one-carbon metabolism related to the methionine cycle leading to methylation reactions, folate cycle, purine synthesis, and redox homeostasis. Apart from its extracellular acquisition, serine can be synthesized de novo during glycolysis by enzymatically converting 3-phosphoglycerate to serine within immune cells [165].

5.4.4 Tryptophan

Apart from other amino acids discussed so far, tryptophan is an essential amino acid that can only be acquired through dietary intake. Tryptophan contributes as a precursor for the synthesis of biologically essential metabolites via the kynurenine and serotonin pathways [166, 167]. Serotonin is a well-studied compound in neurological research, whereas recently kynurenine has gained importance as a potent T and NK cell immunosuppressant in cancer biology. Tryptophan catabolism involves the enzyme indoleamine 2,3-dioxygenase (IDO), which converts it to N-formylkynurenine and further into kynurenine. Increased IDO in several immune cells including macrophages and MDSCs, coupled with enhanced kynurenine production, modulates the T-cell behavior and acts as an immunosuppressant to promote tumor progression [168]. IDO overexpression in cancer cells and extracellular depletion of tryptophan are mediated by cyclooxygenase-2 and prostaglandin E2 via PKC and PI3K signaling [169]. Another enzyme, tryptophan 2,3-dioxygenase (TDO), is also observed to be overexpressed and induces immune dysfunction in various human malignancies. The mechanism behind this function is suggested to be similar to IDO, i.e., through tryptophan depletion or kynurenine production [170, 171]. Tumor-associated macrophages also express higher amounts of IDO and TDO and further decrease tryptophan availability and promote immunosuppressing immune cells in the tumor microenvironment [172]. Low levels of tryptophan and its metabolite kynurenine also promote Treg development and impaired Th17 differentiation and dendritic cell priming [173, 174]. The T-cell function is also inhibited by elevated uncharged transfer RNA (tRNA) which activates a stress response kinase and general control nonderepressible 2 (GC2) in response to tryptophan deprivation [175].

5.5 Targeting Amino Acid Metabolism

Amino acids provide critical components for various cellular processes and can modulate tumor progression and immunity. As discussed, glutamine, arginine, and tryptophan majorly play key roles in these processes. Deprivation of the metabolites or inhibition of key regulatory enzymes associated with the amino acid metabolism thus is of immense interest to curb the tumor growth (Fig. 5.1 and Table 5.2).

Glutamine is one of the most consumed and abundant amino acids in circulation which serves as fuel for the TCA cycle and lipid biosynthesis helps in maintaining mitochondrial integrity and cancer cell survival [200, 201]. Glutaminase (GLS) is one of the key enzymes which convert glutamine to glutamate and is used as a potential therapeutic target to suppress tumor growth [201, 202]. Inhibition of GLS can induce mitochondrial stress which leads to increased aspartate dependence and decreased glycolytic activity in cancer cells [203]. Interestingly cancer and immune cells differentially utilize glutamine for cellular processes. Inhibition of GLS in a breast cancer model decreases MDSC infiltration and promotes the proliferation of M1 macrophages, hence reducing tumor growth [204]. Inhibition of glutamine

Table 5.2 Summary of anticancer drugs targeting amino acid metabolism

	Target	Inhibitor	Cancer type	References
Glutamine metabolism	GLS1	CB-839 BPTES	B-cell lymphoma, hepatocellular carcinoma, myeloma, non-small cell lung cancer, pancreatic cancer	[176–179]
Serine and one-carbon metabolism	PHGDH SHMT1/ 2 RNR DHFR, TYMS, MTHFR	cbr-5884 nct-503 shin1 gemcitabine, 5-FU, methotrexate, pemetrexed, pralatrexate	Acute leukemia, breast cancer, colorectal cancer, diffuse large B-cell lymphoma, lung cancer, melanoma, pancreatic cancer, peripheral T-cell lymphoma	[180–188]
Arginine metabolism	Arginine deprivation, ARG1	ADI-PEG20, CB-1158	Advanced and metastatic solid tumors, hepatocellular carcinoma	[189, 190]
Tryptophan metabolism	IDO1 TDO	Epacadostat navoximod, pembrolizumab, HTI-1090	Lung cancer, melanoma, solid tumors	[19, 191, 192]
Amino acid transporters	ASCT2, LAT1	GPNA	Colon cancer, glioblastoma, neuroblastoma, oral cancer, T-cell lymphoblastic lymphoma	[193–199]

synthetase also activates HIF-1α and promotes inflammatory M1-like macrophages [149, 201]. Targeting GLS leads to metabolic reprogramming in T cells to regulate their effector function and survival. Whereas Th1 and cytotoxic T cells upregulate their glycolysis rate, Th17 cells get suppressed during GLS inhibition [205–207]. In combination with anti-PD-1 immunotherapy glutamine inhibition showed promising results by promoting T effector functions and reducing tumor metabolism [208].

Arginine depletion has been shown to reduce tumor burden and is well tolerated with low side effects. However, to compensate for the arginine deprivation, cancer cells use an alternative approach of arginine synthesis from citrulline via activating the arginine-succinate synthetase (ASS1) pathway [209, 210]. On the other hand, arginine starvation can lead to an immunosuppressive microenvironment through increasing MDSCs to suppress T-cell responses [211]. Inhibition of Arg1 within the acidic tumor microenvironment results in tumor regression and improved T effector function through the decrease in tumor-associated macrophage secretion of tumor growth factors [212, 213]. Within a pancreatic neuroendocrine mouse model, co-transfer of cytotoxic T cells with iNOS-expressing macrophages induced polarization of M2- towards M1-like macrophages; upregulated IFNγ, TNF, and IL-12; decreased angiogenesis; increased T-cell homing; and reduced tumor growth [214, 215].

Rate-limiting enzymes of tryptophan metabolism, IDO, and TDO may suppress antitumor immunity in various cancers. A key feature of tumor metabolism in cancer patients with a poor prognosis is depleted tryptophan and increased kynurenine [216, 217]. Preclinical data suggested improved immune function by inhibiting IDO and reducing kynurenine accumulation within the tumor microenvironment; however, these inhibitors failed miserably in clinical trials [218]. Interestingly, when used in combination with other treatment modules like chemotherapy, radiotherapy, or immunotherapy, IDO inhibitors showed beneficial outcomes [219]. For inoperable melanoma cases, IDO inhibitor combined with anti-PD-1 exhibited objective response rates [220]. In combination with a dendritic cell vaccine, IDO inhibitors were able to convert Treg cells into Th17 phenotype, hence supporting cytotoxic T cells to kill cancer cells [221, 222]. There is still a lot of work that needs to be done to identify the suitable conditions where IDO and TDO inhibitors can be utilized for high therapeutic efficacy.

5.6 Lipid Metabolism and Its Impact on Immuno-Oncology Metabolism

Carbohydrates and amino acids are largely involved in energetics, reducing equivalents, and as constituents for macromolecules; however, lipids are well recognized for their role as a highly diverse biological molecule responsible for efficient energy storage, crucial structural components of biological membranes, and metabolic signaling molecules as few to mention [223]. The adipocytes are often referred to as an endocrine organ owing to complex metabolic processes being regulated by the stored and active lipid components [224]. Neoplastic growth is

favored by and metabolically precipitates factors that are responsible for sustaining growth and metastasis. These factors invariably need to address the increased energy requirements, structural demands, and reactive oxygen species (ROS) being generated by deranged metabolic arithmetic in rapidly growing tissue. Lipids are present in various forms such as fatty acids (FA), triacylglycerols (TAGs), and cholesterol and are interlinked with each other considering their natural role [225]. This hypothesis is strongly supported by various studies which explored the role of obesity and thus the adipocytes in various cancers and their course. Obesity (BMI >40 kg/m^2) has been found to be associated with >1.5-fold increased risk of mortality of neoplastic cause [226]. It increases the risk of other diseases, viz. type 2 diabetes mellitus, hepatic steatosis, and biliary stones, and precipitates the pro-inflammatory conditions, thus culminating into a microenvironment favorable to tumorigenesis as well as progression [227]. The mechanisms enlisted to favor tumorigenesis include promotion of angiogenesis (by generating hypoxic environment), augmentation of pro-inflammatory cytokines (interleukin-6, TNF-α, vascular endothelial growth factor, prostaglandins, leukotrienes, etc.), de novo synthesis of fatty acids (to curb the damage by ROS and thus evade cell death), increased fatty acid oxidation (to provide energy by mitochondrial β-oxidation), and altered lipid storage (lipid droplets) [228–231].

Various pathways of lipid metabolism converge around acetyl-CoA primarily synthesized from citrate by the action of ATP citrate lyase (ACLY) during aerobic metabolism of glucose. Acetyl-CoA is a precursor for the synthesis of 16 carbon fatty acids (palmitate) by a sequential action of acetyl-CoA carboxylase and fatty acid synthase (FASN). Further, palmitate is elongated by microsomal elongation and desaturated by desaturase enzyme (stearoyl-CoA desaturase or fatty acid desaturase) to synthesize other nonessential fatty acids [232].

Cholesterol synthesis also begins with the condensation of acetyl-CoA to form hydroxymethyl glutaryl-CoA (HMG-CoA). Further action of HMG-CoA reductase forms mevalonate (committed step in cholesterol synthesis) which undergoes various reactions through stages of 5 carbon isoprenoid units (isopentenyl pyrophosphate and dimethyl allyl pyrophosphate), geranyl pyrophosphate, farnesyl pyrophosphate, and squalene to form cholesterol. The pathway is regulated transcriptionally by sterol regulatory element-binding protein (SREBP). The latter is held in the endoplasmic reticulum (ER) membrane by SREBP-cleavage-activating protein (SCAP) which in turn is maintained in an inactive state by INSIG in the sterol-rich membrane. On depletion of cholesterol, SCAP is activated by dissociation from INSIG and cleaves SREBP free of endoplasmic reticulum which can now move to the nucleus to act on sterol regulatory element (SRE) in the nucleus and enhances the synthesis of HMGCoA reductase [233]. Further, the cellular cholesterol efflux contributing to reverse cholesterol transport is mediated by anion-binding cassette (ABC) type of channels ABCA1 as well as its isoforms and cellular uptake is mediated by LDL receptors [233]. Fatty acids are oxidized to generate reducing equivalents and acetyl-CoA (fated to enter Krebs cycle to generate reducing equivalents). Fatty acids are first converted to fatty acyl-CoA by enzyme fatty acid-CoA synthase and then transferred to mitochondrial by carnitine

palmitoyltransferase (CPT). Once inside mitochondria, it undergoes sequential reactions to generate reducing equivalents and acetyl-CoA [234].

5.7 Targeting Fatty Acid Metabolism

As mentioned above, cancer cells along with their glycolytic flux also increase the uptake and biosynthesis of lipids and cholesterol by upregulating various enzymes related to these pathways [28, 235]. Cancer cells store these excess amounts of lipids as lipid droplets which have been correlated with cancer malignancy [235]. Targeting the lipid metabolism using various inhibitors of key regulatory enzymes has shown promising results in suppressing tumor growth (Fig. 5.2 and Table 5.3).

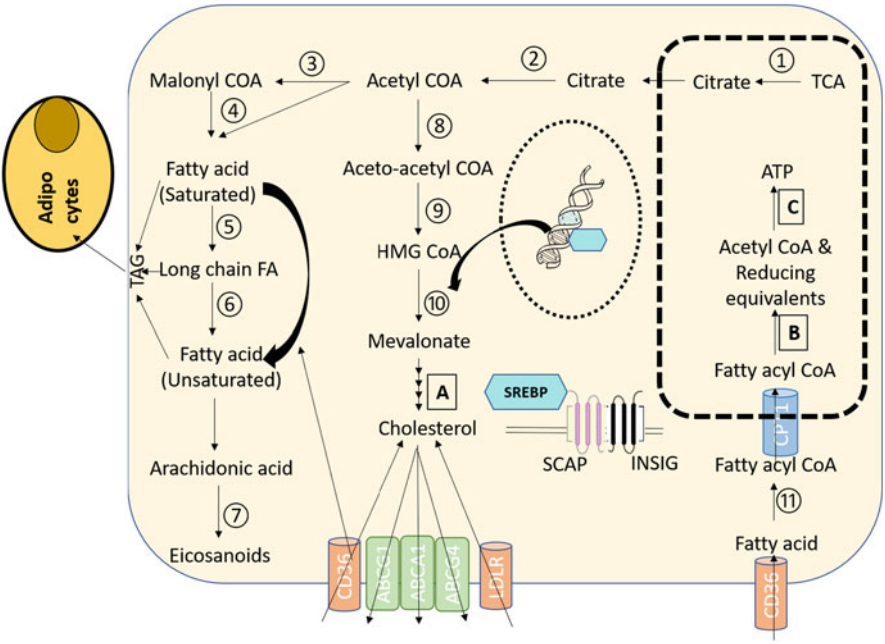

Fig. 5.2 Overview of fatty acid metabolism. *TCA* tricarboxylic acid cycle, ①: citrate synthase, ②: ATP citrate lyase, ③: acetyl-CoA carboxylase, ④: fatty acid synthase, ⑤: elongase, ⑥: desaturase, ⑦: cyclooxygenase (1 and 2) ⑧: acetyl-CoA acyl transfers, ⑨: HMG CoA synthase, ⑩ HMG CoA reductase, ⑪: fatty acyl CoA synthase. A: cholesterol synthesis, B: β-Oxidation, C: oxidative phosphorylation. *SREBP* sterol regulatory element-binding protein, *SCAP* SREBP cleavage-activating protein

Table 5.3 Summary of anticancer drugs targeting lipid metabolism

Target	Inhibitors	Cancer type	Refs
FASN	C75, cerulenin, orlistat, triclosan, EGCG, TVB-3166, and amentoflavone	Breast cancer, endometrial cancer, glioblastoma, lung cancer, melanoma, mesothelioma, ovarian cancer, prostate cancer, renal cell carcinoma	[236–248]
ACLY	SB-204990 LY294002	Lung cancer	[249–251]
ACC	TOFA, metformin, AICAR ND-654, and ND-646	Cervix cancer, colon cancer, head and neck cancer, hepatocellular carcinoma, lung cancer, ovarian cancer, prostate cancer, renal cell carcinoma	[252–260]
CPT1	Etomoxir, ranolazine, and perhexiline	Breast cancer, glioblastoma, lymphocytic leukemia, prostate cancer	[261–267]
HMGCR	Statins and lipophilic statins	Colorectal cancer, melanoma, multiple myeloma, prostate cancer	[268–270]
ACAT1	Avasimibe, avasimin, and bitter-melon extract	Breast cancer, colorectal cancer, prostate cancer	[87, 271, 272]

5.7.1 Fatty Acid Synthesis

Fatty acid synthase (FASN) is a key regulatory enzyme that has been studied as a therapeutic target to inhibit fatty acid metabolism in cancer cells [273, 274]. Augmented synthesis of FASN enzyme has been reported in almost all tumors and is hypothesized as a contributor to poor prognosis. This effect has been attributed to the association of enzyme with metastasis to lymph node and liver as well as the stage in case of colorectal cancer, breast cancer, and prostate cancer [275, 276]. FASN expression is also related to poor prognosis in pancreatic, cervical, and renal cancer. Triple-negative breast cancers showed relatively higher expression of this enzyme and single-nucleotide polymorphism associated with FASN is associated with the recurrence of non-small cell lung cancer [277]. FASN is rampantly explored as a target using C75, cerulenin, orlistat, and IPI-9119 as its inhibitors [278]. Blocking FASN can inhibit cancer cells and on the other hand it protects T cells from apoptosis due to repeated stimulation of T-cell receptors and its activation [279].

Along with FASN, ATP citrate lyase (ACLY) and acetyl-CoA carboxylase (ACC) are also rate-limiting enzymes of fatty acid metabolism whose inhibition can lead to decreased proliferation and growth of cancer cells [280, 281]. ACLY is a crucial link between metabolic pathways of carbohydrates and lipid metabolism. Its expression has been independently associated with worse prognosis in non-small cell lung cancer, specifically in elderly subjects [282] and enhanced metastasis in colon cancer [277]. ACC protein naturally occurs in two active forms α and β and is correlated with the occurrence and progression of various malignancies. Whereas phosphorylated α form indicated better prognostic outcomes in adenocarcinoma lung, its low levels are associated with worse outcomes in colorectal carcinoma

[283, 284]. Also, β form is noted to play a favorable role in the renal and pancreatic tumor which is in contrast to its α counterpart [285].

Inhibiting ACC and ACLY using their inhibitors ND-654 and SB-204990, respectively, has shown antitumor efficacy in various cancers including liver, lung, glioblastoma, and breast [249, 252, 286, 287]. Accumulation of fatty acids and lipids generally has immunosuppressive effects; however, the effect varies for each immune cell subtype. Lipid accumulation increases oxidative phosphorylation in myeloid cells and promotes their immunosuppressive function, whereas bone marrow-derived myeloid cell differentiates into immunosuppressive M2-like macrophages in the presence of fatty acid oleate [288, 289]. Dendritic cells are responsible for antigen presentation which primes the T cell and helps in their activation. Lipid accumulation in the tumor microenvironment and its uptake by these infiltrating dendritic cells impair the antigen presentation and hence activation of the T cells [290, 291]. SREBP, as earlier discussed, not only influences cholesterol synthesis but also regulates fatty acid synthesis on multiple metabolic crossroads by transcriptional regulation of all three enzymes being discussed. Despite being such a versatile target, SREBP being a transcriptional agent is hardly amenable to pharmacological intervention. However other factors associated with SREBP, viz. SCAP, INSIG, and SRENP transporters, can be prospective pharmacological targets. Fatostatin is one of such agents inhibiting SREBP-SCAP interaction, which has shown potential by curbing the growth and dissemination of prostate cancer [292].

Targeting fatty acid synthesis has shown beneficial outcomes in preclinical studies by ameliorating lipid accumulation in mouse models. Inhibition of fatty acid synthesis either by TOFA, and acetyl-CoA carboxylase (ACC) inhibitor, or by FASN inhibitors has been shown to rescue dendritic cell function in murine models [290, 291].

5.7.2 Fatty Acid Uptake

Cancer cells also enhance their uptake of fatty acids from within their microenvironment. Both LDL receptors and cluster of differentiation 36 (CD36) protein are effectors of cellular cholesterol uptake and thus amenable targets. The absence of LDL receptors has been cited as markers of negative prognosis in colorectal cancer and the presence of same has been demonstrated to cause better survival in small-cell lung cancer [293]. However, there are reports of adverse outcomes with the same in the case of CA pancreas, renal carcinoma, and urinary bladder cancer. Tumors with CD-36 expression have shown an increased propensity to metastasize. Further, it is noted that attenuation of CD36 can be targeted to impair tumor migration. CD36 is also known to promote survival and Treg function within the tumor microenvironment [294]. Inhibiting fatty acid-binding protein (FABP5) can prevent the accumulation of Treg cells and ameliorate its function as well [56, 295, 296].

5.7.3 Fatty Acid Oxidation

Alternatively, inhibition of fatty acid oxidation can inhibit M2 macrophage-mediated secretion of IL-1β, hence limiting cancer cell migration as well as enhancing antitumor efficacy of T cells [297]. Fatty acid oxidation is essential for CD8+ memory T-cell development as well as it helps in the differentiation of Tregs [298]. Carnitine palmitoyltransferase 1 (CPT1) is a rate-limiting step of the preceding pathway ensuring entry of fatty acids in mitochondria for degradation. Amelioration of cancer progression has been noted in studies targeting CPT1 by either knockout or inhibition [261, 299]. This impact is precisely noted in malignancies related to the c-Myc pathway. CPT1 inhibitors such as ST1326, etomoxir, and ranolazine have also shown good efficacy in cancer while blocking fatty acid oxidation [261, 262].

5.7.4 Cholesterol Biosynthesis and Its Derivatives

Targeting cholesterol metabolism also inhibits cancer cell proliferation, metastasis, and survival through suppressing nuclear hormone production and lipid raft formation [300]. Statins that inhibit HMGCR, a rate-limiting enzyme of the mevalonate pathway, reduce cholesterol biosynthesis and have shown improved prognosis in cancer patients [301, 302]. Molecules targeting downregulation of lipid biosynthesis not only will limit tumor cells of energy source but also are proposed to dampen its evasion from other therapies which induce damage by ROS. These targets have been listed in the form of structural agents helping to build lipid droplets, viz. lipid droplet-associated proteins and enzymes (e.g., lysophosphatidylcholine acyltransferase) [303]. Mobilization of stored lipids is processed by regulated mechanisms involving different lipases. Of these, monoacylglycerol lipase (MAG-L) is reported to be upregulated in several cancers and leads to adverse outcomes. Thus, attenuation of MAG-L using both knockout and inhibition (by agent JZL184) has shown promise in melanoma and ovarian cancer [304].

ACAT1 is a proposed marker for a good outcome in breast, liver, colorectal, and renal cancer [305]. However, the adverse outcome is noted in endometrial and renal tumors with increased expression of ACAT2 [306]. Targeting cholesterol esterification using ACAT-1 inhibitors such as avasimibe along with anti-PD-1 immunotherapy or chemotherapeutic agents has shown better efficacy as compared to monotherapies in reducing tumor burden by reducing the Treg population and increasing CD8+ T cells within the tumor microenvironment [87, 307–309]. Inhibiting fatty acid and cholesterol synthesis and their uptake via targeting the rate-limiting enzyme regulators or transcription may pave way for the inhibition of tumor growth and novel anticancer therapeutics.

5.8 Concluding Remarks

All these above mentioned studies point towards a strong interaction and relation between immune cell and cancer cell metabolism. Immune cell metabolism is vastly modulated by cancer cells and stromal cells in their microenvironment. Carbohydrate and amino acid metabolism along with lipids drive the expression of key genes which are responsible for the phenotypic switch during cancer progression. Deprivation of nutrients or inhibition of key enzymes related to the pathways utilizing these metabolites can lead to impaired cellular and effector functions. A significant amount of research has been done to understand the role and regulation related to metabolic cross talk between cancer and immune cells; however, still many questions remain unanswered. Further studies will be needed to understand the signaling axis which regulates the gene expression and effector functions. In conclusion, immuno-oncology metabolism promises to identify novel therapeutic targets and mechanistic insights into cancer progression. It has become an area of great interest as a targeted approach to intervening the metabolic pathways has immense potential to complement current treatment modalities and enhance therapeutic outcomes.

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Immune Cell Metabolites as Fuel for Cancer Cells

6

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Abstract

Immune cells exhibit continuous adaptative and dynamic metabolic adaptations and generate immune response to neoplastic cells. In view of metabolic adaptations coupled to the development of tumor immunity, immune cell metabolites are excellent models to study the functional outcomes of cellular metabolism. These metabolic adaptations guide differentiation of immune cells into distinct cellular states and activation of T cells and macrophages. Understanding these intricacies will help us understand how differential metabolic regulation of immune cell metabolites works through signaling pathways. The surrounding tumor microenvironment (TME) also influences the adjacent neoplastic cells, and cross talk between these facilitates tumor progression. Glucose metabolism is the key moiety which regulates metabolic adaptations and directs the activity of immune cell metabolites. It is well understood that the immune cell metabolites are the key molecules driving rapid adaptation in metabolic pathways. This is a dynamic yet closely coupled mechanism in cancer cells and the surrounding TME and the precise mechanism of how bidirectional interactions manifest in driving tumor progression still needs further evaluation.

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Metabolic reprograming also affects B cells, T cells, dendritic cells, macrophages, and myeloid-derived suppressor cells (MDSCs) among other immune cells.

The role of these immune cells in TME is mainly dependable on the concentration and nature of numerous factors, like diffusible metabolites (i.e., lactate), reactive oxygen species (ROS), cytokines, and growth factors. In this chapter, we highlight the role of immune cell metabolites as fuel for driving oncogenic progression. We have summarized the key moieties of immune cells and representatives of lymphoid and myeloid lineages and linked them to understanding how they drive metabolic changes directing towards cancer progression. We also emphasize on the potential role of TME interactions in driving metabolic adaptations and how targeting metabolic reprogramming is being explored as a potential therapeutic strategy for anticancer treatment in conjunction with established chemotherapies and immunotherapies.

Keywords

Metabolism reprogramming · Immune cells · Cancer · Immune cell metabolites · Tumor microenvironment · Metabolic targets

6.1 Introduction

The complex metabolic metamorphosis in cancer is heterogenous and has been continuously evolving over the time period. This is mainly attributed to metabolic adaptations directing metabolic reprogramming of immune cell metabolites and plasticity of metabolic metabolites. These metabolic adaptations drive tumor progression through the interaction of immune cell metabolites with immune stroma [1, 2]. This involves transitioning the metabolic switch towards the "*Warburg effect*," and this has been reported to induce immune suppression. Hence, it is very important to have an in-depth understanding of the signaling pathways and associated molecular mechanisms driving tumor metabolism in the TME, which can also be helpful in designing novel targeted therapies. Based on the present treatment options available for cancer treatment, targeted immunotherapy has been extensively explored to target immune cells in the TME in cancer patients [3, 4]. Despite ongoing research and clinical trials, the percentage of patients which respond to these treatments is very small in view of which there is still need to explore the effective targets for improving treatment response in cancer patients. In view of this, metabolic targets are being extensively explored as a therapeutic approach which is mainly driven by the interdependence of metabolic status of neoplastic cells and immune cells in the TME [5–8]. It has been well envisaged that, during the initial cancer development, immune cells activate the defense mechanism and suppress tumor growth; however, as the tumor progresses and becomes established, metabolic switch drives tumor progression [9] and eventually supports the development of resistance to therapy [10, 11]. Here, we describe the

role of metabolic switches in immune cells and its metabolites to understand TME interactions driving cancer progression. We also highlight the targeted therapy directed towards key immune cell metabolites and how they can help improve treatment strategies in conjunction with established chemotherapeutic and immunotherapy regimen.

6.2 Metabolic Reprogramming of Cancer Cells

6.2.1 Glycolysis and Oxidative Metabolism

Glycolysis and oxidative metabolism are the two key processes which drive metabolic reprogramming of cancer cells and effect immune response [12, 13]. In proliferating neoplastic cells, glucose is converted to lactate under aerobic environment. This oxygenated form of glycolysis is a well-established phenomenon known as the "*Warburg effect*" [14]. This increased consumption of glucose is driven by glucose transporter 1 (*GLUT1*) [15] which activates aerobic glycolysis. "*Warburg effect*" has long been known to be the key marker of hypoxic neoplastic cells which are actively proliferating cells, e.g., activated effector T cells, B cells, and natural killer (NK) cells [16, 17]. In addition to the *Warburg effect*, metabolic switch in tumor cells to glycolysis has been reported to promote cancer cell survival under hypoxic conditions. Glycolysis is a high-energy consuming process and is coupled with elevated ROS levels which makes neoplastic cells more susceptible to oxidative stress [18–21]. This induction of aerobic glycolysis induction is mediated by hypoxia-inducible factor 1-α (*HIF1-α*) [22], which in turn upregulates lactate dehydrogenase A (*LDHA*) and phosphoinositide-dependent kinase-1 (*PDK1*). These genes are key modulators for diverting pyruvate towards aerobic glycolysis [23]. Genetic disruption or pharmacological inhibition of glycolysis or its metabolites has been shown to perturb its classical activation [22, 24–26]. This has been effectively demonstrated using mechanistic target of rapamycin complex-1 (*mTORC1*), a regulator of HIF1-α that has been effective in modulating microbial ligand β-glucan-driven activation of glycolysis in macrophages [24]. Targeting *GLUT1* and glycolytic enzymes (pyruvate kinase M1/2 (*PKM2*) and glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*)) or regulators of glycolysis (*HIF1-α* and *mTORC1*) has been shown to deplete glucose consumption, thereby downregulating aerobic glycolysis [22, 24–26].

Another hallmark of metabolically active cells is oxidative metabolism. Elevated oxidative metabolism is also directly correlated to cytokine production in tumor cells [27–30]. Bacterial infection triggers mobilization of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase—Src proto-oncogene non-receptor tyrosine kinase (*SRC*) axis which activates components of electron transport chain (ETC) [27]. Peroxisome proliferator-activated receptor-γ (*PPAR-γ*) has also been reported to upregulate oxidative metabolism and mitochondrial biogenesis through an interleukin 4 (IL-4)-inducible transcriptional coactivator 1β (*PGC-1β*) [31]. Numerous other carbon substrates have also been linked to enhanced oxidative metabolism.

Glucose also serves as a substrate for fatty acid synthesis (*de novo* lipogenesis). The metabolites of fatty acid synthesis also fuel oxidative metabolism, e.g., by β-oxidation [32]. Enhanced oxidation of glutamine has also been shown to elevate oxidative metabolism [28]. Elevated oxidative metabolism also increases acetyl-CoA (Ac-CoA) production, which is a metabolic substrate for histone acetylation. This increases transcriptional induction of IL-4-inducible genes along with histone acetylation. IL-4 activates protein kinase B (*PKB*) and *mTORC1* pathways which enhances the uptake of glucose and its oxidation [33, 34]. This activation of IL-4 coupled to AKT-mTORC1 axis is the key process which modulates the activity of ATP citrate lyase (ACLY) [33]. As is evident, oxidative metabolism is a complex process and activates multiple alternative pathways in neoplastic cells.

6.2.2 Lipid Metabolism

The cycle of lipid synthesis and metabolism is efficiently coupled to energy demand. In tumor cells, high energy requirements initiate metabolic reprogramming of key lipid metabolism molecules which in turn affect the function of immune cells [35, 36]. In response to lipid metabolism reprogramming of molecules in tumor cells, there is an elevated expression of lipid cell metabolites, namely fatty acid synthase (FASN), monoacylglycerol lipase, and acetyl-CoA carboxylase (ACC) to name a few. All these molecules play a key role in regulating lipid metabolism and are coupled to the effector function of immune cells.

Of these, ACLY is a well-established molecule, the elevated expression of which is directly correlated with cholesterol synthesis, a key process in tumor metabolism [35]. The fatty acid (FA) synthesis, an important metabolic process, regulates lipid-derived membrane structures in tumor cells. This process of FA synthesis is vital for cell proliferation and activation of effector T cells. This process of FA synthesis is effectively coupled to catabolic FA oxidation, which provides the necessary ATP supply for sustaining energy needs of memory T cells and Tregs [37, 38]. This intricate balance between the FA synthesis and FA oxidation also modulates the differentiation of T-cell subsets. FA synthesis and oxidation have also been reported to promote the immunosuppressive role in tumor-infiltrating cells, such as tumor-associated macrophages (TAMs), MDSCs, and Tregs. Tregs, the immune-suppressive cells, have been reported to rely mainly on FA oxidation to meet the energy needs and express low concentrations of the *GLUT1* [39]. This process of FA oxidation is mainly coupled to the constitutive activation of AMP-activated protein kinase (*AMPK*) pathway which drives FA oxidation [18]. This activation of AMPK pathway mainly inhibits anabolic processes which orchestrates metabolic responses through FA synthesis and concomitant activation of FA autophagy and oxidation. Additionally, the process of FA oxidation is also coupled to the inhibition of *mTOR* pathway [18]. This concomitant coupling of FA oxidation to the activation of AMPK pathway and inhibition of *mTORC1* pathway stimulates lipid oxidation in Tregs. This coupled lipid oxidation of Tregs has been explored from a therapeutic viewpoint to pharmacologically inhibit *mTORC1* using everolimus and activate *AMPK*

pathway using metformin, an antidiabetic compound. This resulted in the activation or stimulation of Tregs, thereby restraining T cell-mediated immune responses [39].

Another molecule, prostaglandin (PE), secreted from tumor cells, has been shown to recruit MDSCs and trigger TAM polarization towards M2 phenotype [19]. The precursor of PE, arachidonic acid (AA), plays an important role in modulating antitumor immunity along with the development of systemic immunity [20]. Of these PE, prostaglandin E2 (PGE2), in particular, has been reported to play a crucial role in reprogramming M1 macrophages (antitumor) to M2 macrophages (pro-tumor). In addition, PGE2 has also been demonstrated to elevate the activation of signal transducer and activator of transcription 3 (*STAT3*), which in turn induces the polarization of M1-to-M2 macrophage [21]. This M1-to-M2 macrophage polarization has been shown to induce the expression of forkhead box P3 (*FOXP3*) in naïve T cells and suppress the production of cytokines by NK cells. This leads to acquisition of Treg-associated immunosuppression [40]. PGE2 has also been shown to promote both tumor metastasis and immunosuppression when coupled to sphingolipid molecule sphingosine-1-phosphate (S1P) in tumor cells and TAMs [20, 35, 41]. Given the role of PGE2 in M1/M2 polarization and tumor progression, PGE2 has been extensively studied for therapeutic targeting. In a colon cancer model, blocking PGE2-producing enzyme, cyclooxygenase-2 (COX-2), and microsomal PGE2 synthase 1 (mPGES1) facilitated reversion of M2-to-M1 polarization in TAMs [40]. In addition in bladder cancer, COX-2 inhibition has also been shown to reduce PD-L1 expression [42]. In addition to targeting PGE2 biosynthesis, inhibitors of FA beta-oxidation, e.g., molecules targeting carnitine palmitoyltransferase 1 (CPT1), such as COX2 inhibitors, are also being explored for targeting lipid metabolism to boost the immune system response.

Interestingly, malignant cells also harness additional energy demands from the nearby adipocytes which facilitate energy needs of metabolically high tumor cells. In adipocytes of metastatic ovarian cancer cells, triglycerides (TGs) were reported to convert to free FA in response to metabolic reprogramming [36]. In addition, leptin, an adipocyte-related hormone, has also been reported to regulate systemic lipid metabolism and immune response [43]. This leptin moiety has been illustrated to promote pro-inflammatory cytokine production in cancer cells and T-cell immune response. Additionally, leptin has also been reported to modulate phagocytic functions of macrophages [43]. Deficiency of leptin has been shown to be directly coupled to loss of innate and adaptive immunity [44]. This indicates the crucial role of leptin in systemic metabolism of immune response, the imbalance of which directly correlates to poor antitumor immunity.

Besides the above lipid metabolites, polyunsaturated fatty acids (PUFA), including alpha-linolenic acid (ALA), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA), have been associated with anti-inflammatory effects [45–49]. Also, DHA, EPA, and omega-3 (n-3) PUFAs have been shown to compete for enzymes for the conversion of n-6 PUFA into immune-suppressive and pro-inflammatory PGEs. These PGEs then reprogram M1-to-M2 macrophage polarization. Hence, increased uptake of absolute or relative (n-3/n-6 ratio) DHA or DHA and EPA, individually or

in combination with PGE2 inhibitors, has been reported to enhance antitumor immunity [44, 50].

Given the important role of high energy demand in lipid metabolic reprogramming to modulate oncogenic processes, lipid moieties have emerged as interesting therapeutic targets for anticancer therapy to augment the efficacy of established chemotherapy regimens. Targeting specific molecules of lipid pathway mediators or lipid metabolic pathways plays a critical role in activating or suppressing immune cells that are summarized above. These molecules are being investigated from a clinical perspective as potential targets for pharmacological inhibition.

6.2.3 Amino Acid Metabolism

The biosynthesis of polyamines and nonessential amino acids requires a supply of reduced nitrogen [51]. Of all the amino acids, glutamine is the primary source of nitrogen source. Glutamine molecule harbors two amide groups, both of which are synergistically used for the biosynthesis of amino acids. Glutamate is formed from the transfer of the amide nitrogen moiety of glutamine and also plays a role in the biosynthesis of hexosamines and asparagine [52, 53]. Deamination of glutamine by glutaminases to glutamate releases ammonia group and the resulting glutamate moiety rewires transamination reactions directing the production of α-ketoglutarate and nonessential amino acids. The proliferating neoplastic cells mainly rely on the uptake of glutamine to maintain anabolic growth [54, 55]. This acute nitrogen balance plays a major role in tumor cell proliferation. This has successfully been demonstrated in epithelial cells. During epithelial cell proliferation, glutamate aminotransferases are mainly used to maintain cell proliferation phase [56]. In this process nitrogen molecule generated from glutamate transamination is transferred to keto acids such as oxaloacetate, pyruvate, and 3-phosphohydroxypyruvate [56]. This coupled reaction has been clearly demonstrated in cancer cells, where glutamine deprivation in T cells delayed T-cell activation and led to a proliferative block [57]. In addition, glutamine deficiency has also been reported to induce cell cycle block in K-ras-transformed fibroblasts [58].

Besides glutamine, arginine is yet another nonessential amino acid which plays an important role in the biosynthesis of creatine, proline, polyamine, and glutamate, and also nitric oxide production. Downregulation of arginosuccinate synthetase 1 (ASS1), an arginine biosynthesis enzyme, along with arginosuccinate lyase and/or ornithine transcarbamylase, triggers susceptibility to arginine-depletion therapies. This has been successful in cancers like melanoma, prostate, hepatocellular carcinoma, and renal cell carcinoma [59, 60]. This specific depletion of arginine in ASS1-deficient neoplastic cells induced synthetic lethality and decreased polyamine levels [61]. On similar lines, ASS1 has also been reported to play a critical role in T cells, where deficiency of ASS1 was associated with reduced peripheral T cells and T-cell differentiation [62]. This ASSI deficiency elevated the sensitivity of T cells to arginase. Also, in the absence of glutamine, asparagine is reported to take

over and plays a major role in promoting cell proliferation and cell survival in cancer cells. A key molecule, asparagine synthetase (ASNS), is reported to be commonly elevated in neoplastic cells [63–65] and decreased expression of ASNS has been documented to remodel the tumor cells to being self-reliant directing them to exogenous asparagine in acute lymphoblastic leukemia (ALL) [66, 67]. This metabolic vulnerability has been exploited for patient treatment in ALL wherein bacterial asparaginase has been shown to downmodulate blood asparagine levels, thereby inducing apoptosis in neoplastic cells [66].

6.3 Metabolic Programming of Immune Cells and Tumor Microenvironment Cross Talk

A tumor mass consists of many cell populations that cross talk with each other and contribute to the sustenance of the tumor. Apart from cancer cells, fibroblasts, endothelial cells, and a variety of immune cells lead to the development of a conducive tumor microenvironment that benefits the growth and proliferation of cancer cells [68, 69]. The development of a heightened inflammatory microenvironment is a prominent feature of most aggressive cancer types, indicating a prominent role of immune cells in cancer progression [70]. The inflammation is mounted by the collective action of one or multiple types of immune cells, which are assigned to the function of monitoring the body for different types of infection or aggressive conditions like cancer [71]. Interestingly the TME remains highly nutrient deprived. Therefore, multiple adjustments in the metabolic profile of most of the immune cell types are required for their continuous persistence in the TME [72, 73]. Similarly, hypoxia is another major factor that promotes TME, primarily by upregulating the glycolysis-associated genes [74, 75]. Besides, *HIF1* also suppresses the citric acid (TCA) cycle [76]. However, how hypoxia affects the proliferation of individual immune cell types is intriguing and needs further investigation.

This relationship of the immune system with cancerous cells is an exciting question that has intrigued scientists for decades. However, multiple technological advances and the advent of omics approaches have cemented the belief that immune cells also provide the required supplements to drive the disease progression. The tumors represent a highly specialized ecosystem that witnesses a large landscape of adaptive strategies. The one most well known is the anaerobic glycolysis (known as the Warburg effect). However, less in ATP yield, this alteration in the energy production scheme provides an opportunity to survive in oxygen deprivation [77]. Similarly, multiple alterations and shifts in the metabolism of immune cells are observed that provide essential supports to the survival of neoplastic cells [78, 79]. The other key example of metabolic rewiring is the production of a large number of oncometabolites that may further support the tumorigenicity [80]. Immune cells take determining roles in this reprogramming of TME metabolism (discussed in the next section). They do so by establishing multiple lines of interaction networks, which are later exploited by transformed cells. Cancerous tissues are highly

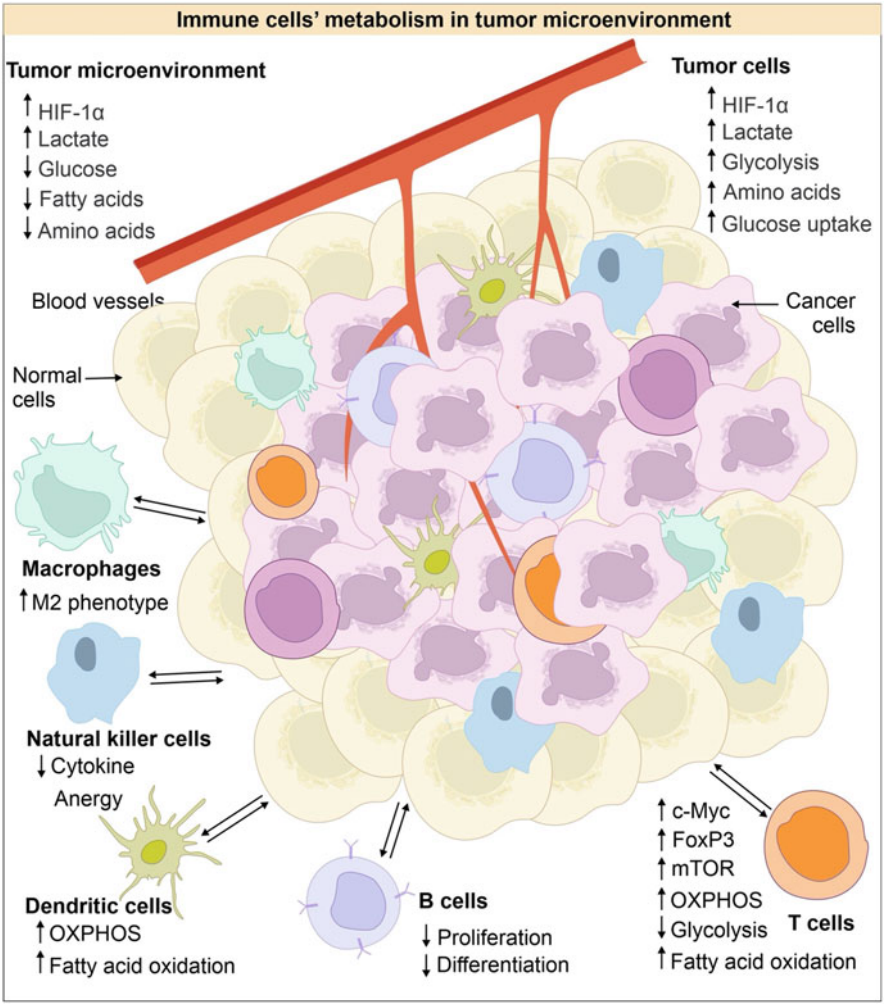

Fig. 6.1 Metabolic alterations in tumor microenvironment: A representative schematic showing various cell types infiltrating the tumor through blood vessels (center). The cross talk with the cancer cells modulates the metabolic profile of other cell types in vicinity. As shown, the rewiring of metabolic pathways tends to alter the proliferation and activity of the immune cells, and instead of removing the transformed cells, they tend to support their growth and proliferation. *c-Myc* cellular Myc, *FOXP3*, forkhead box protein 3, *HIF1-α* hypoxia-inducible factor-1 alpha, *mTOR* mechanistic target of rapamycin, *OXPHOS* oxidative phosphorylation

heterogeneous in their constituting cell population. As shown in Fig. 6.1, a large number of immune cells can infiltrate, reside, and interact with other cells in the vicinity.

Innate immunity provides the first line of surveillance and defense in the body, and its primary function is to identify and curb growing infections by phagocytosing

any dying cells or debris [81]. However, cancerous cells can reprogram them by altering their metabolism, further supporting the tumor's proliferation and growth [82]. The tumor-associated macrophages apply a distinct metabolic route to adapt to an anti-inflammatory (M2) form rather than the pro-inflammatory (M1) type [83]. Both these populations have their metabolic preferences; for example, oxidative phosphorylation (*OXPHOS*) and fatty acid oxidation (FAO) are preferred by M2, while M1 subpopulations rely mostly on anaerobic glycolysis, FA synthesis, and pentose phosphate pathway (*PPP*) [84]. The chemokines and cytokines play essential roles in this M1-to-M2 transition [85]. M2 subpopulations also contribute to matrix remodeling, repair, and blood vessel formation [86]. Semaphorin 3A-mediated phosphorylation of vascular endothelial growth factor (VEGF) receptor 1 tempts M2 macrophages towards a hypoxic environment [87]. Later, these cells contribute to abnormal angiogenesis and metastasis by upregulating the development and DNA damage responses 1 (*REDD1*) and suppressing *mTOR* signaling pathway [88]. They also contribute to tumor survival by secreting matrix metalloproteases (*MMP 1* and *7*), thus helping cell proliferation [89].

High glycolysis is observed in the neutrophils, the other primary innate immunity cell type [90]. However, mitochondrial functions are not very prominent for these cells except in forming neutrophil extracellular traps (*NETs*), mediating chemotaxis, and initiating apoptosis pathways [91, 92]. Despite being part of innate immune defense, neutrophils may also play supportive roles in tumor progression by promoting invasion and metastasis [93, 94]. They do so primarily by increasing the production of reactive oxygen species (ROS) and downplaying the activities of the T lymphocytes and NK cells [95, 96]. NK cells are crucial for natural defense and are currently targeted for their potential as a probable immunotherapeutic target [97]. In the normal NK cells, IL-15- and mTOR-mediated increased glucose uptake regulates the expressions of CD98 (an amino acid transporter chaperone) and a transferrin receptor CD71, which are necessary factors for the division and activity of these cells [98]. Transcription factor sterol regulatory element-binding protein (*SREBP*)-mediated cytokine-dependent signaling also contributes to NK cells' metabolic rewiring, causing upregulation in OXPHOS and glycolysis [99]. However, suppression of glucose metabolism, downregulation of *SREBP*, and *mTOR*-mediated downstream pathways are reported in cancer-associated NK cells that might weaken the cell lytic activity of these cells [16, 97].

Effector T ($T_{effector}$) cells reside in a nutrient-rich condition of lymph nodes and spleen. Nevertheless, they face a drastic shift once they reach a metabolism-restricted tumor site, e.g., an increase in glycolysis, pentose phosphate pathway, and glutaminolysis [57, 100]. In contrast to the naïve T cells that rely on OXPHOS and FAO pathways, the activated (encountered with antigen) $T_{effector}$ cells have upregulated the expression of glucose transporters and increased the aerobic glycolytic flux for their proliferation in a CD28-, HIF1-α-, and Myc-mediated manner [57, 101]. The long-lived quiescent population of memory T cells embraces a similar way of survival like the other less active cells (e.g., naïve T cells) do. They prefer catabolic ways, like OXPHOS, as compared to other biosynthetic pathways [102]. Increased mitochondrial mass in these cells supports their rapid response

while countering the reinfections by providing the bioenergetic advantage to fuel in quick ATPs [38]. In TME, the rapidly dividing cancer cells take up most of the nutrient and energy supplies, imposing metabolic restriction on surrounding T cells. In such conditions, a state of anergy is created due to the T cells' inability to produce cytokines, which may probably occur because of the suppression of the glycolytic machinery [103]. Under such conditions, T cells may also see a decreased secretion of a pro-inflammatory molecule, interferon-gamma (*IFN-γ*), a prominent antitumor cytokine molecule [104]. The nutrient sensor molecule *AMPK* and *GAPDH*, a glycolytic enzyme, play critical regulatory roles in these metabolic alterations [104, 105]. Glucose deprivation may further cause phosphoenolpyruvate-mediated suppression of the nuclear factor of activated T cells (*NFAT*) and calcium signaling [106].

The regulatory T cells (T_{reg}), in contrast to $T_{effector}$ cells, have a different response to the glucose availability due to the low expression of *GLUT1* [39]. These cells mostly hang on OXPHOS and lipid oxidation pathways for the energy requirements. The transcription factor, *FOXP3*, suppresses the pro-glycolytic *PI3K/AKT/mTORC1* signaling and induces genes required for lipid peroxidation [107, 108]. The antibody-producing B lymphocytes are metabolically active cell types, while naïve B-cell activation can be observed through the upregulation of lactate synthesis and glucose uptake [109]. Oxidative phosphorylation and tricarboxylic acid cycle support their activation, and contrary to T-cell types, they do not show compromised growth and functioning under glucose deprivation [109]. The effect of hypoxic conditions on T-cell proliferation is often debated as it has been shown to increase or curb cell proliferation in different studies. One study shows that HIF1-α does not affect T-cell proliferation, whereas a different study on Lck-Cre/HIF1-floxed mice suggests that under HIF1-α deficiency, $CD4^+$ and $CD8^+$ both secrete increased cytokine *IFN-γ* and show high proliferation [110, 111]. Similarly, one study suggests that hypoxia may suppress the CD28-mediated T-cell proliferation, while another finds an increased expression of CD137, which increases the infiltration of T cells in the tumor [112, 113]. ROS generation may also have similar contrasting effects on the proliferation and functioning of T cells [114, 115].

Tumor-associated B and T cells may also take advantage of amino acid metabolic pathways for their functioning and proliferation. T cells utilize glutamine to synthesize pyruvate to balance the energy requirements [105]. Glutamine deprivation may result in increased differentiation into T_{regs} and prevention of Th1 and Th17 activation [116, 117]. Mutations in glutaminolysis-favoring MYC gene may further add to the immune-deprived environment by inducing the addiction of cancer cells to glutamine [118]. The cancer cells also express specific amino acid-catabolizing enzymes, which may lead to the depletion of some crucial amino acids (e.g., tryptophan) that support the T-cell differentiation and activation [73, 119]. Tryptophan diminution also causes an increase in the levels of stress-sensing general control nonderepressible 2 (*GCN2*) kinase using transfer RNAs [120]. This may lead to impaired Th1 differentiation while promoting the Treg development and suppressing the activation of the T cell [121].

A similar effect can be observed following the extracellular depletion of cysteine and arginine. Antigen-presenting cells (APCs) maintain a high cysteine in the extracellular environment that is utilized by T cells for their activation [122, 123]. However, the dependence of T cells on cysteine is not established yet due to several conflicting reports. Tumor cells also utilize the high cysteine levels in the TME to maintain sufficient amounts of glutathione synthase (*GSH*) to survive oxidative stress conditions [124, 125]. Likewise, arginine is vital for the survival and antitumor properties of T memory cells. In lack of extracellular arginine, downregulation of cytokine production compromised aerobic glycolysis pathway, and impaired activation of T cells is reported [126, 127]. Similar roles of serine were highlighted in a study when *in vitro* culture of T lymphocytes failed to proliferate in the absence of extracellular serine, which could probably be linked to low serine-mediated perturbation in the purine biosynthesis pathway [128]. Alanine-free media may also suppress the activities of cultured T cells, which is marked with the low secretion of cytokines. This observation is supported by the proteomic studies that indicated that low alanine might cause compromised protein synthesis in the T cells [129].

These observations indicate an intense competition among neoplastic cells and tumor-residing immune cells for resources, metabolites, and energy. Most of the cancers have shown modulation of activities of different immune cell types in such a manner that instead of fighting and removing the transformed cells from the vicinity, our immune cells start supplementing their growth and proliferation in multiple ways.

6.4 Effect of Immune Cell Metabolites on Cancer Progression

The development of cancer begins with tumor initiation; being a multistep process, the cells acquire desirous state via silencing of tumor-suppressor genes and via oncogene activation [53, 130]. The role of immune cells in acting against cancerous cells is well known; however, recent years have seen a great expansion in our knowledge of the dual roles played by immune cells in cancer progression and suppression [130, 131]. Numerous studies have provided crucial insights into mechanisms through which different metabolites of immune cells promote cancer progression [132–134]. The complex nature of immune system in modulating malignancy in the recent years has been well appreciated [135]. An insight into the role of different immune cells in promoting cancer has been well discussed in the following sections:

6.4.1 Role of T Cells and B Cells in Cancer: A Love-Hate Relationship

The dual role of the immune system in cancer has been well established [132]. On the one side the immune cells suppress tumor development as well as their progression by destroying/inhibiting the growth of cancerous cells whereas on the other side

it promotes tumor progression by creating a conducive TME facilitating growth and metastasis [135]. In-depth studies on the role of immune cells as T and B cells have demonstrated their contradictory role in tumors [136]. Studies have shown that not all T cells depict antitumor response; certain subpopulation of T cells such as CD 4+-expressing markers, i.e., CD 25, and Foxp3+ known as regulatory T cells (Tregs) play an important role in driving tumor progression via inhibiting immune response against cancer [137]; in normal conditions they are present in low number; however, they are extensively increased in nearly all types of cancer [138]. Different studies have depicted the pivotal role of Tregs in tumor metastasis; their role in carcinogenesis in humans has been implicated in recent studies. The mechanism of action of Tregs in tumor promotion has been depicted in many studies; it is seen that Treg results in immunosuppression via numerous pathways such as production of suppressive cytokines like transforming growth factor–β (*TGF-β*) and pro-inflammatory cytokines, e.g., interleukin 10 (*IL-10*), which directly suppress immune response [138–142]. Few studies have also demonstrated the role of Tregs in cancer metastasis via RANKL-RANK signaling pathway. Studies have demonstrated the role of STAT signaling pathway activated by Tregs in immunosuppression, metastasis, and angiogenesis of cancer cells; it has also been observed that via STAT pathways Tregs interact with many cells to exert their effect. Studies have also depicted the role of Tregs in suppressing nonregulatory CD4+T cells and inhibiting the proliferation and antitumor capacity of CD8+ T cells to promote cancer progression. The role of Treg cells in metastasis is well established [143–145]. The role of B cells in cancer is ill defined; many studies are being carried out to elucidate the role of B cells in cancer [146]. B cells being the main humoral immune cells play an important role in the body and against tumor. Studies have shown that the role of circulating immune complexes is driven by the production of B cells, which induces chronic inflammation. This activates myeloid cells and FcR engagement [147, 148]. In addition, tumor-associated CD19+ B cells have also been demonstrated to activate signal transducers that lead to increased angiogenesis, thereby supporting tumor progression; however, in the recent years the role of B-cell subset represented by regulatory B cells (Bregs) has been implicated in tumor progression. The role of these Bregs has also been investigated in modulating TME which is summarized herewith. Studies have shown the diverse role of B cells in TME; it is seen that besides secreting antibodies and cytokines, they also modulate innate immune responses. These cells primarily maintain and mediate immune tolerance. Prominent markers differentiating Bregs are CD19+ and CD24hi CD27+ CD38hi; it is seen that Bregs stimulate the production of suppressive cytokines such as interleukin 35 (*IL-35*), IL-10, and *TGF-β*; they also induce high level of expression of negative immune checkpoint molecules. It has been observed in different studies that Bregs besides inhibiting the proliferation of CD4+ T cells promote the transformation of CD4+ T cells to Tregs and the Tregs secreting IL-10; further in many studies it was found that Bregs result in loss of function of T cells followed by their apoptosis; further Bregs promote tumor by acting on other immune cells such as NK [149–

154]. The need of an hour is to devise potential strategies and mechanisms for the applications of tumor-infiltrating B cells in tumor therapy.

6.4.2 Tumor-Associated Macrophages

TAMs are the population of macrophages encircling the tumor microenvironment; numerous studies have demonstrated the prominent role of TAMs as promoters of metastasis [155]. TAMs act via a cascade of steps such as promoting angiogenesis and lymphangiogenesis, remodeling of extracellular matrix, producing proteolytic enzymes and growth factors, inducing various inhibitory checkpoint proteins in T cells, and secreting cytokines that impair the activity of T cells resulting in immune suppression [156, 157]. TAMs play an important role in the progression and promotion towards tumor metastasis starting with the invasion of tumor cells to establishing pre-metastasis [155, 158, 159]. It has been well established that metastatic tumor cells become invasive and escape the confinements of cell membrane through interaction with the surrounding stroma. This phenomenon is called "*epithelial-mesenchymal transition*" (EMT). Studies have also shown that TAMs regulate the EMT transition process. Numerous nonclinical studies have demonstrated that TAMs show an elevated expression of N-cadherin/Snail, which are hallmarks of mesenchymal transition, whereas the expression levels of E-cadherins are downregulated [158, 160, 161]. It is seen that TAMs participate in the EMT process via secretion of many pro-inflammatory cytokines such as IL-8 and TNF-α. TAMs have also shown to promote ECM remodeling via secreting different proteolytic enzymes such as cathepsins and matrix metalloproteinases. It is also illustrated that once these tumor cells break away from the constraint of ECM networks they behave in an unregulated fashion. Besides promoting tumor invasion TAMs have shown to play an important role in vascularization, intravasation, and extravasation of tumor cells via various mechanisms [160]. It has been well established that tumor vasculature plays an important coherent role in driving the metastasis of malignant tumor. TAMs have been reported to play a crucial role in the regulation of angiogenesis during tumor progression. Tumor cells are reported to form tumor clusters in the intra-tumoral regions and other invasive fronts. These areas are considered as the hotspots of angiogenesis and metastasis. Further, TAMs also stimulate the new tumor vessel formation and also stimulate the remodeling process of already established vasculature to a more leaky form to favor tumor spread with the coordination of different tumorigenic molecules [162, 163]. Studies have shown that TAMs account for lymphangiogenesis, via modulation of VEGF receptors and ligands, thereby promoting lymphangiogenesis either directly or indirectly. TAMs have also been shown to play an important role in tumor cell intravasation via tripartite arrangement of neoplastic cells, TAMs, and endothelial cells and via activation of different signaling pathways; further the role of TAMs in promoting tumor cell survival while in circulation has also been established [158]; it is generally seen that TAMs in TME lack the activities depicted by macrophages in general immune response elicited against tumor cells, thereby eliciting an

immunosuppressive response. Studies have shown that TAMs modulate host immune response via increased expression of different cytokines, chemokines that alter the function of APCs, as well as effector immune cells [155, 164]. Few studies have also depicted the role of TAMs in promoting the extravasation of tumor cells which upon settling in the capillaries of the targeted organs extrude and attach through vessel walls; further it is seen that TAMs prepare sites for tumor cells to establish pre-metastatic niches with the help of various tumor-secreted factors, such as CSF-1, CCL2, VEGF, TNF-α, PLGF, and TGF-β, and also via attenuating the tumoricidal activities of TH-1 cells and dendritic cells [165]. With extensive information available on the mode of action of TAMs the need of the hour is to focus on strategies targeting TAMs' elimination.

6.4.3 Role of Dendritic Cells and Granulocytes in Cancer Progression

Dendritic cells (DCs) are professional cells derived from bone marrow which perform antigen-presenting functions; they are scattered throughout antigen-exposed tissues and their draining lymph nodes (LNs) [166–168]. They are seen to regulate the crucial balance between peripheral and adaptive immune response. Under normal conditions, DCs are the key decision makers, determining the activation of the adaptive arm of immune system. Besides playing an important role in T-cell activation and differentiation they play an important role in the mode of action of NK cells and B cells [169, 170]. DCs are known to exhibit functional plasticity and are distinguished into different subsets based on their phenotype, tissue distribution, and molecular signatures [169, 170]. The antitumor immunity of DC subsets has been clearly established [171]; however, their role in favoring tumor growth and progression has recently been debated; they are known to exhibit this role via promoting immune tolerance. Studies in the recent past have provided mechanistic insights on how tumor induction transforms tumor-associated DCs (tDCs) from a potentially immunostimulatory to an immunosuppressive (regulatory) cell type and how tumor-induced signals block the generation of mature, immunocompetent DCs in some tumors. RegDCs are often referred to as tolerogenic DCs belonging to a family of regulatory myeloid cells, displaying a variety of tumor-specific markers, e.g., PD-L2, B7-H3, B7-H4, and CD103, which can be identified using a combination of immunomodulatory molecule expression, cytokine production, surface marker expression, and functional regulatory or suppressive properties [171–174]. Different studies have depicted different mechanisms of action of RegDCs in terms of their immune-suppressive potential. Plausible mechanism of action of RegDCs reported with respect to immune suppression is via IDO, a catabolic enzyme, which specifically targets molecules involved in nitric oxide (NO) production, e.g., arginase, tryptophan, and iNOS. In addition, cytokines, such as TGF-β and IL-10, are also produced by RegDCs, which play an important role in suppressing the effector T-cell activity or altering T helper cell polarization. Upon induction of RegDCs, they not only inhibit the T-cell proliferation but also induce the production of Treg

cells which play an important role in tumor metastasis [172–175]. Studies have implicated the role of DC-derived macrophages in suppressing T-cell responses through the production of immunosuppressive molecules such as nitric oxide, arginase, and IL-10; more insights/studies into the role of RegDCs and effective strategies for them are needed to devise suitable therapeutic options for different types of cancers [176–178].

Granulocytes play an important role in activating immune responses. Along with neutrophils, granulocytes constitute an important first line of defense mechanism against the infection in the human circulatory system. These are constantly replenished through granulopoiesis in the bone marrow, from where they mobilize to peripheral circulation to defend against invading pathogens. They are recruited to the infection sites via chemokines where they elicit their response [179, 180]. Since decades the central function of neutrophil granulocytes as effector cells of the innate immune system has been known because of their ability to rapidly recognize, take up, and eliminate pathogens; however, in the recent past there has been growing evidence suggesting the role of neutrophils in promoting tumor growth and metastasis [181, 182]. Cancer research has thrown light on the diverging roles of neutrophils; studies have shown that upon polarization of neutrophils divergent phenotypes are produced, depending on the action-specific tumor-derived factors such as TGFβ, IFNβ, and G-CSF [183, 184]. Neutrophils either promote or limit tumorigenesis via various mechanisms; they are thought to play an important role in transformation to a cancerous cell via production of reactive nitrogen species (RNS) or ROS and proteases; these molecules play an important role in epithelial mediated damage and subsequent tumor-promoting inflammation [157, 185]. Studies have demonstrated that neutrophils play an important role in tumor initiation and progression via conversion of senescent cancer cells into proliferating cancer cells through the expression and action of different receptor antagonists. Further proliferation of cancerous cells is directly stimulated by the transfer of neutrophil elastase (NE) to cancer cells via activation of PI3K signaling and degradation of insulin receptor substrate 1 (IRS1). Different studies have reported that neutrophils express inducible arginase 1 (ARG1) or nitric oxide synthase (iNOS) which suppresses CD8+ T cell-mediated antitumor immune response, thereby promoting tumor progression [186, 187]. Immunosuppression is also achieved by TGF-β-mediated signaling in neutrophils, which remodels extracellular matrix (ECM) via different signaling pathways and induces angiogenesis. Studies have shown the role of neutrophils in several steps of metastasis such as their recruitment and migration of cancer cells leading to enhanced metastasis. Studies have also demonstrated the role of Breg in instructing neutrophils to elicit metastatic response. Neutrophils are also stated to induce leaky vasculature which supports the extravasation of disseminated neoplastic cells by the expression of MMPs [185–188]. Despite promising advances the exact pathways dissecting the role of neutrophils in cancer progression are still debated [189].

6.4.4 Myeloid-Derived Suppressor Cells (MDSCs)

One of the important components of TME includes MDSCs that are accumulated during chronic inflammation and tumor progression [190]. Studies have shown that one of the hallmarks of cancer is abnormal differentiation and function of myeloid cells; it is seen that MDSCs depict a potent immune-suppressive activity and have shown to play an important role in tumor progression and chronic inflammation [191]. The genesis of MDSCs occurs in bone marrow led by migration to peripheral lymphoid organs and tumor tissues; the entire process is regulated via a complex set of signals induced by different chemokines. It is generally seen that in peripheral lymphoid organs MDSCs differentiate into dendritic cells and macrophages; however, in the tumor sites they exhibit a modified/altered differentiation pattern by differentiating into tumor-associated macrophages [192–194]. MDSCs exhibit tumorigenic and immune-suppressive response via various mechanisms such as deprivation of essential amino acids arginine and cysteine, which play an important role in T-cell proliferation and antitumor reactivity, via production of nitric oxide (NO) and ROS, resulting in the nitration of T-cell receptors (TCR) and chemokines important for T-cell migration; further it is observed that MDSCs induce excessive production of interleukins such as IL-10 and other transforming growth factors such as TGF-1, thereby inhibiting immune effector cell functions [195–197]. Once differentiated MDSCs result in the upregulation of expression levels of apoptotic markers such as programmed death-ligand 1 (PD-L1) which can further markedly downregulate T cell-mediated antitumor reactivity via interaction with PD-L1-specific receptors expressed on T cells. Studies have shown that MDSCs promote the secretion of angiogenic factors which play a significant role in tumor neovascularization; they also promote the production of growth factors, matrix metalloproteinases, and cytokines which play a crucial role in stimulating tumor growth and also skewing immune reactions towards Th2 type and activation of regulatory T cells (Tregs). MDSCs have also been shown to promote/induce apoptosis of T cells and NK cells [9, 193, 197, 198]. Many supportive studies have therefore concluded that MDSCs are not only activated and recruited by tumorigenic factors but also directly support tumor initiation, development, neovascularization, and metastasis and may be considered as one of the major players in tumor-medicated immunosuppression [199].

6.5 Therapeutic Implication: Metabolic Regulation in Cancer Immunotherapy

The established chemotherapeutic regimes have helped in the management of cancer patients. However, a major challenge is the treatment of refractory, relapsed patients and the patients who do not respond to established treatment protocols. This gap is being extensively explored using the targeted immunotherapies to potentiate treatment efficacy of cancer treatments [200]. In the way forward, novel approaches are being deciphered to generate tumor-specific T effector cells which can potentiate the

development of T memory cells [82, 201]. This is mainly directed to having elevated plasticity and viability upon re-exposure to cancer antigens for attaining timely differentiation of T effector cells. This will help generate long-lasting immune-mediated antitumor response in comparison to transient antitumor effect [200]. As T-cell differentiation is driven and coupled to metabolic process, combining immune cell metabolite-targeting drugs with checkpoint inhibitors is a viable option from a therapeutic viewpoint [82]. Targeting immune cell metabolite can help generate T effector cells and long-term memory cells which can in turn prevent accumulation of exhausted T cells (Table 6.1).

This is well supported by clinical trials, which have tried to harness the delicate metabolic balance coupled to immune cell function. The use of metabolic enzymes targeting specific immune cell metabolite and oncogenes has provided valuable insights into metabolic regulation and immunomodulation [201]. The initial results from targeting immune cell metabolites *in vivo* have been promising in view of which the immune cell metabolites have been explored in clinical trials [51]. The metabolite programming is mainly directed towards targeting HIF1, lactate, AMPK, and mTOR pathway (Fig. 6.2). TLN-232, a PKM2 inhibitor, has been used for clinical trials in refractory renal cell carcinoma (NCT00422786) patients. TLN-232 phosphorylates mTOR inhibitor, AKT1S1, and activates mTORC1 signaling pathway [202]. In addition to mTORC1 pathway, AMPK activators and mTOR inhibitors are also being extensively tested in preclinical and clinical settings for cancer patients [203]. AMPK activity regulators also include metformin and 5-aminoimidazole-4-carboxamide ribonucleotide which have been successfully evaluated for antitumor effects in preclinical models and clinical trials [204, 205]. Metformin has also been reported to activate AMPK pathway, which in turn directly affects antitumor response via increased differentiation of CD8+ memory T cells [206]. AMPK also plays an important role in the metabolic adaptation of T cells for generating effector T cells [207] and Tregs along with the reduction of Th1 and Th17 cells [39]. In addition, metformin also mediates inhibition of the rate-limiting enzyme HK2 of glycolysis in neoplastic cells [208], which abrogates the complex I of mitochondrial electron transport chain [209].

As already highlighted in the text above, AMPK activation is coupled to the inhibition of mTORC1 for effective metabolic effects in cancer cells [168, 210]. This coupled mechanism was shown to deplete Glut1 expression, thereby increasing Tregs. This indicates the potential role of AMPK activation as a checkpoint for immune response [211]. Also, inhibition of mTOR using REDD1 repolarizes M1-M2 macrophages and inhibition of REDD1 has also been shown to activate glycolysis in TAMs. This potentiates competitive challenge between endothelial cells and TAMs for glucose, thereby preventing hyperactivation of vascular junctions in TME [212]. REDD1 inhibitor has been explored in phase 2 clinical trial (NCT00713518) for the management and treatment of neovascularization in AMD patients. Denitrosylation of HIF1-α is also being explored for inhibiting the activity of glycolytic enzymes which in turn alleviates M1 phenotype [211]. Monocarboxylate transporters (MCTs), a family of transmembrane proteins (MCT1, MCT2, MCT3, and MCT4) which mediate bidirectional proton-lined

Table 6.1 Effect of micro- and macronutrients targeting immune cell metabolites and effect on immune system

Cancer	Clinical trial	Phase	No. of patients	Treatment targets	Parameters analyzed	Findings	Ref
Breast cancer	Brazilian Clinical Trials Registry (REBEC): RBR-2b2hqh	Double blinded, randomized	45	n-3 fatty acid	Immune cells	Stable high-sensitivity C-reactive protein (hsCRP) in fatty acids final median 0.3 ($p = 0.510$, IQR 0.0–0.7) versus increased hsCRP in placebo group final median 0.2 ($p = 0.024$, IQR 0.1–0.3)	[47]
	Clinical trial registered in Tabriz University of Medical Sciences (IR201212172017N10)	Randomized, double blinded, placebo controlled	30	Beta-glucan	Immune cells	Increased global health status/ quality of life (QoL) for beta-glucan group ($p = 0.023$). Symptom scales\item score significant in beta-glucan group as compared to baseline score ($p = 0.012$)	[218]
Renal cell carcinoma	Not available	Observational	54	Glycolysis	Immune cells	MitoTracker Green staining and decreased peripheral blood mononuclear cells (PBMC) PD-1 low CD8+ T cell cytoplasmic reactive oxygen species (ROS) ($p < 0.05$)	[219]
Colorectal and melanoma	NCT01195311	Phase 1	52	Indoleamine 2,3 dioxygenase 1 (IDO1) inhibitor	Immune cells	IDO1 inhibitor was well tolerated. Stable disease lasting $\geq$16 weeks in 7/52 patients	[220]
Head, neck, and esophageal cancer	NCT00333099	Double blinded	28	Fatty acids, arginine, glutamine	Immune cells	Maintained CD4/CD8 lymphocyte count ratio (2.47 ± 0.31 vs. 1.95 ± 0.20) antibiotics versus no antibiotics. Shorter	[49]

						progression-free survival [$p < 0.01$, HR 3.1, 95% CI 1.4–6.9], increased risk of progressive disease ($p < 0.01$, 75% versus 22%), and shorter overall survival ($p = 0.03$, HR 3.5, 95% CI 1.1–10.8)	
Esophageal cancer	UMIN000004732	Randomized	71	Eicosapentaenoic acid (EPA), arginine, docosahexaenoic acid (DHA), nucleotides	Immune cells	Decreased levels of C-reactive protein levels (CRP) ($p = 0.001$) and tumor necrosis factor (TNF) ($p = 0.014$)	[48]
Head and neck cancer	Not available	Observational	31	Fatty acids, vitamins, amino acids, antioxidants, ribonucleic acids	Immune cells	Decreased hsCRP levels ($p = 0.002$, 9.8 vs. 3.2) and α-1 acid glycoprotein levels ($p = 0.020$, 1.2 vs. 1.0)	[45]
Gastric and esophageal cancer	Not available	Randomized	22	Fatty acids, arginine, nucleotides	Immune cells	Elevated Th17 levels (9.0 ± 2.2 versus $14.4 \pm 3.5\%$)	[46]

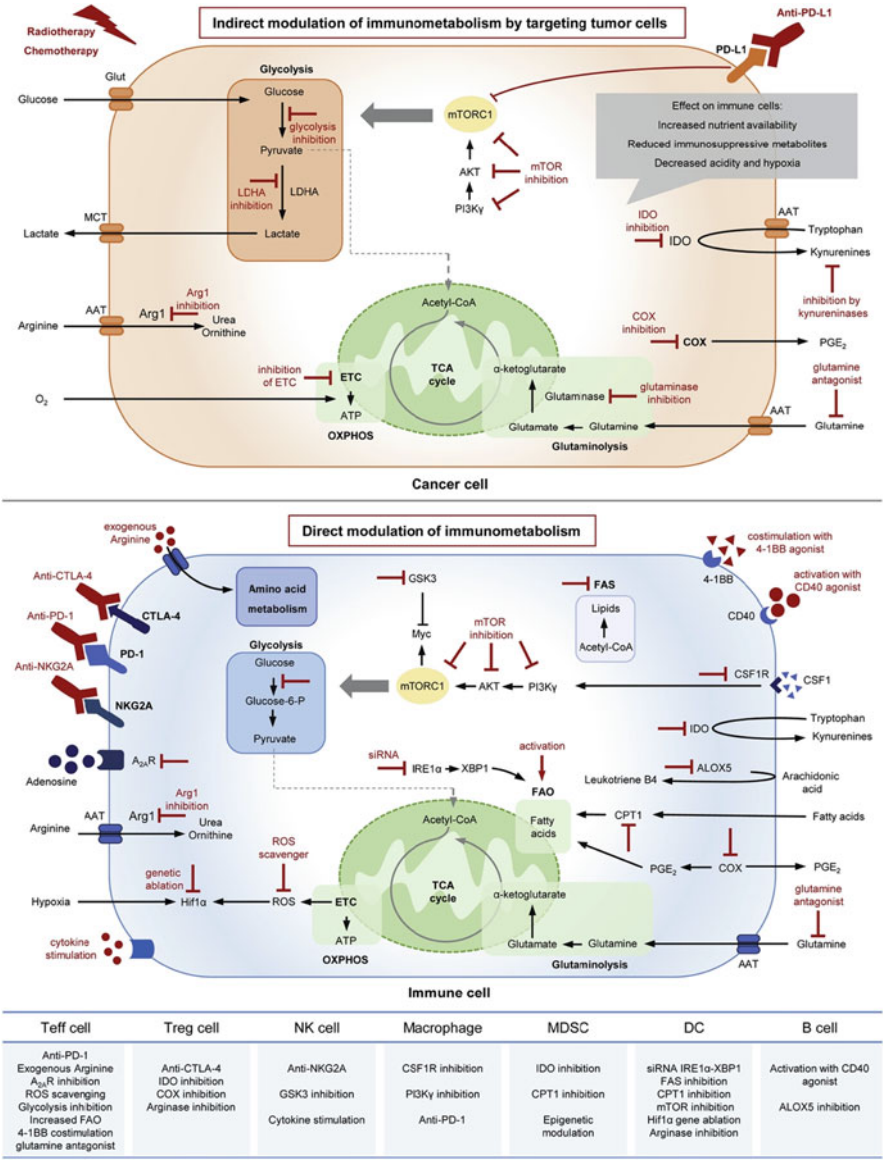

Fig. 6.2 Strategies for targeting immune cell metabolites for targeted therapies (CC-BY-4.0 (https://creativecommons.org/licenses/by/4.0/))

movement of metabolites like lactate, branched-chain ketoacids, and ketone bodies, are also being explored from therapeutic targeting in cancer [213]. The MCT1 inhibitors (AZD3965 and SR12800) [214, 215] and AR-C155858 [216], a dual-MCT1/MCT2 inhibitor, are being tested in clinical trials.

Currently, immune checkpoint inhibitors are being extensively explored for therapeutic modalities of which atezolizumab, nivolumab, durvalumab, avelumab, and pembrolizumab are anticipated to be the effective treatment choices for cancer patients in the near future (Table 6.2) [217]. Given the closed rewiring of metabolic pathways to immune cells in neoplastic progression, targeting of immune cell metabolites may be the candidate immunomodulator for reprogramming T-cell metabolism in the years ahead. Co-administration of immune checkpoint inhibitors along with metabolic targeting agents can be effectively used for differentiation and activation of T cells into effector and memory cells for long-term antitumor immunity. Repurposing chemotherapy regimens, with immunotherapies and tumor vaccines along with metabolic targets, opens up avenues for improving targeted cancer therapy in the management of patients (Fig. 6.3).

6.6 Conclusion and Future Perspective

The coupled interactions between immune cell metabolites and metabolic reprogramming are well-established innate immune response to oncogenic transformations. These immune cell metabolites and metabolic transformations work in conjunction to contribute significantly to the functioning and driving of tumor-specific immunity. These immune cell metabolites have been reported to play a crucial role in suppressing and promoting disease-specific tumor progression and repolarizing immune cells. This has in turn highlighted these immune cell metabolites as novel therapeutic approaches. The metabolic reprogramming of immune cells also drives metabolic plasticity of immune cells, e.g., T cells and macrophages, which is being explored extensively from a therapeutic viewpoint to develop targeted therapies in tumor immunology. This persistent reprogramming of immune cell metabolites is also driven by metabolic and epigenetic changes in T cells and macrophages, which highlights the importance of exploring therapeutic immune strategies. In addition to immune cells, this work also emphasizes the importance of cross talk between immune cells, tumor cells, and stromal cells which have been reported to regulate the metabolic requirements of cancer cells with respect to proliferation, invasion, metastasis, survival, and immune surveillance. We also summarize the immune cell metabolites specific to immune cells which play a major role in directing metabolic adaptations and immune response within tumor cells and in response to TME interactions. This is interesting in view of the complex competition for resources during metabolic adaptations between the immune cells, neoplastic cells, and stromal cells, which competitively determines the fate and function of individual cell subsets. This also emphasizes the importance of identifying specific niches in the TME which can modulate metabolic adaptations and impede immune cells. Successful clinical trials involving immune cell metabolites have presented the strength of exploring immune metabolic pathways and how they are coupled to the biological process. These immune cell metabolites are also a key determinant of the immune cell response and antitumor immunity. Hence, the mechanistic understanding of these immune metabolic processes will

Table 6.2 Ongoing clinical trials targeting immune cell metabolites in cancer

S. No.	Cancer	Clinical trial	Phase	Treatment
1.	Non-small cell lung cancer	NCT03048500	Randomized, Phase 2	Metformin hydrochloride + nivolumab; metformin + nivolumab
2.	Advanced or metastatic solid tumor	NCT03314935	Phase 1/2	INCB001158 (arginase inhibitors) + chemotherapy
3.		NCT02903914	Phase 1	INCB001158 (arginase inhibitors) ± immune checkpoint therapy
4.	Melanoma	NCT03311308	Randomized double blind	Metformin + pembrolizumab vs. pembrolizumab
5.		NCT03047928	Phase 1/2	PD-L1/IDO vaccine + nivolumab
6.		NCT01604889	Phase 1/2 randomized, blinded	Epacadostat + ipilimumab
7.	Colon cancer	NCT03072641	Randomized	Probiotics
8.		NCT02861300	Phase 1/2	CB-839 (oral glutaminase inhibitor) + capecitabine
9.	Gastrointestinal stromal tumor	NCT03291054	Phase 1/2	Epacadostat + pembrolizumab
10.	Renal cell carcinoma	NCT03428217	Phase 2, double-blind randomized	CB-839 (oral glutaminase inhibitor) + cabozantinib vs. cabozantinib

PD-L1 programmed death-ligand 1, *IDO* indoleamine 2,3-dioxygenase

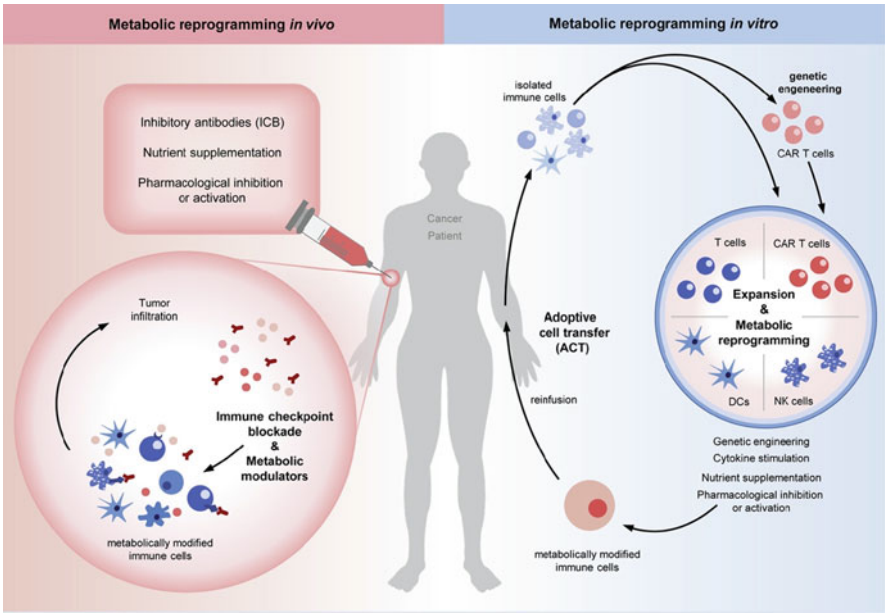

Fig. 6.3 Strategies for metabolic reprogramming of immune cells for treatment in cancer patients (CC-BY-4.0 (https://creativecommons.org/licenses/by/4.0/))

help us better address the gaps in the development of novel therapeutic targets for improving T-cell immune function.

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Conflict of Interest The authors declare no conflict of interest.

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7 Timing of the Major Metabolic Switches in Immune Cell Activation and Differentiation During Cancer Development

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Abstract

The immune system is divided into two parts: innate and adaptive. Immune cells derived from myeloid and lymphoid progenitor cells play a critical role in combating infection, cell stress, cancer, and autoimmune responses by mounting appropriate and robust immune responses. According to the evidence, an immune cell's nutrient uptake and utilization are critical for governing their proliferation and differentiation and influencing metabolic pathways that lead to immune cell fate. Cancer development is characterized by altered metabolism. Metabolism and immune system are inextricably linked, and multi level interactions between them shape immune responses. This chapter deliberates potential metabolic switches in immune cells which modulate the activation, differentiation, and response to fight against a tumor. We believe that it is possible to design new treatments for cancer therapy to steer a specific immune cell metabolism.

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Keywords

Cancer · Immune cell metabolism · T-lymphocytes · Macrophages · MDSC · DCs · NK cells · Tumor cell metabolism · Hypoxia · Tumor microenvironment · Cancer therapy

Abbreviations

A2b	Adenosine receptor
ACC1	Acetyl coenzyme A carboxylase
AKT	AKT serine threonine kinase
AMP	Adenosine monophosphate
AMPK	5′-Activated protein kinase
APCs	Antigen-presenting cells
Arg-1	Arginase-1
COX10	Cytochrome C oxidase assembly factor heme A: farnesyltransferase COX10
COX2	Cyclooxygenase 2
CTLs	Cytotoxic T lymphocytes
DAMP	Damage-associated molecular pattern
DCs	Dendritic cells
ERK	Extracellular signal-regulated kinases
ETC	Electron transport chain
FADH2	Flavin adenine dinucleotide 2
FAO	Fatty acid oxidation
FOXP3	Forkhead box P3
G6P	Glucose-6-phosphate
GAPDH	Glyceraldehyde-3-phosphate dehydrogenase
GCN2	General control nonderepressible 2
GLUT1	Glucose transporter 1
HIF1α	Hypoxia-inducible factor 1
IC	Immune complexes
IDO	Indoleamine 2,3-dioxygenase
IFN-γ	Interferon gamma
IL-1	Interleukin-1
IL-10	Interleukin-10
IL-12	Interleukin-12
IL-13	Interleukin-13
IL-17	Interleukin-17
IL-4	Interleukin-4
IL-6	Interleukin-6
IL-7	Interleukin-7
iNOS	Inducible nitric oxide synthase
JAK	Janus kinase

LDHA	Lactate dehydrogenase A
LPS	Liposaccharide
M1/M2	Macrophages
MDSCs	Myeloid-derived suppressor cells
MHC-I/-II	Major histocompatibility complex I/II
MMPs	Metalloproteases
mROS	Mitochondrial reactive oxygen species
MSR1	Macrophage scavenger receptor 1
mTOR	Mammalian target of rapamycin
mTORC1	mTOR complex 1
mTORC2	mTOR complex 2
MΦs	Macrophages
NADH	Nicotinamide adenine dinucleotide
NETs	Neutrophil extracellular traps
NFAT	Nuclear factor of activated T cells
NF-κB	Nuclear factor kappa B
NK	Natural killer
NOS2	Nitric oxide synthase 2
OXPHOS	Oxidative phosphorylation
PAMPs	Pathogen-associated molecular pattern
PD-1	Programmed cell death protein 1
PD-L1	Programmed death-ligand 1
PDPK1	Phosphoinositide-dependent protein
PEP	Phosphoenolpyruvate
PEPCK1	Phosphoenolpyruvate carboxykinase 1
PGC-1β	Proliferator-activated receptor-γ coactivator-1 beta
PI3K	Phosphoinositide 3-kinase
PKM2	Pyruvate kinase M1/2
PPAR-γ	Peroxisome proliferator-activated receptor gamma
PPP	Pentose phosphate shunt/pathway
PTEN	Phosphatase and tensin homolog
RNS	Reactive nitrogen species
RORγ	RAR-related orphan receptor gamma
ROS	Reactive oxygen species
S-2HG	S-2-hydroxyglutarate
SHTM2	Serine hydroxymethyltransferase 2
SIRT	Sirtuin
SREBP	Sterol regulatory element-binding protein
STAT	Signal transducer and activator of transcription
STAT6	Signal transducer and activator of transcription 6
TADCs	CCR7, tumor-associated DCs
TAG	Triacylglycerol
TAMs	Tumor-associated macrophages
TANs	Tumor-associated neutrophils
TCA	Tricarboxylic acid

TCR	T-cell receptor
TGF-β	Transforming growth factor beta
TME	Tumor microenvironment
TNFα	Tumor necrosis factor alpha
Tregs	T regulatory cells
TSC-1	Tuberous sclerosis 1
VEGF	Vascular endothelial growth factor
VHL	Von Hippel-Lindau

7.1 Introduction

Cancer is an unrestrained growth of abnormal cells in the body. It may occur anywhere in our body, and it progresses when the body's standard control mechanism does not respond. Therefore, instead of undergoing apoptosis, cells become oncogenic and continuously grow and form abnormal, unhealthy cells. These dividing cells form a mass of tissue called a tumor, or it also occurs in suspension like in leukemia; it depends on the type of cancer. Currently, there are more than 200 types of cancer identified to date. Cancer is caused by many factors, including chemical or toxic compound exposure, ionizing radiation, pathogens, and human genetic changes. Cancer is the second leading cause of death worldwide, accounting for nearly ten million deaths in 2020 (WHO). Cancer accounts for about 15–20% of deaths worldwide, significantly injuring the healthcare system. There are several notable differences between cancer and healthy cells. Unfortunately, only a few of these key differences have been identified, while others have been discovered but are poorly understood.

Metabolites are small molecules (<1500 Da) made or used when our body breaks down the food, chemicals, and drugs to generate the energy needed for body function. The study of such metabolites is called metabolomics, the emerging field of measuring all small molecules. Typically, healthy and uncontrolled malignant cells show distinct cell metabolism; cancer cell metabolism refers to the variations in cellular metabolism pathways apparent in cancer cells compared with most normal tissue cells, a hallmark of cancer progression [1–3]. The metabolic status of cells affects their long-standing decision-making and potential to influence other cells' destiny in their locality. As we understand, uncontrolled malignant cells need more and more nutrients or metabolites for their growth than healthy cells. Our immune system is vital to control cancer or any unwanted infection. Therefore, metabolic reprogramming in uncontrolled cancer cells and flexibility for the adaption in the tumor microenvironment (TME) are considered the central mechanisms of cancer treatment combat [4]. TME encompasses tumor-associated stromal cells, the extracellular matrix, a wide variety of metabolites, and chemokine/cytokines, affecting our immune system energy consumption and metabolic switches and disturbing immunity, inducing immune cells to become tolerogenic and ineffective against a

tumor. The following sections discuss how modulating the immune cell metabolism can improve the immune responses against cancer. Responses of T cells, B cells, natural killer (NK/NKT) cells, dendritic cells (DCs), macrophages (M1/M2), neutrophils, and myeloid-derived suppressor cells (MDSCs) are observed in the TME during the initiation and progression of cancer [5, 6]. Over the last three decades, a thorough understanding of immune cell interactions with cancer has emerged. The abnormal metabolic activity of uncontrolled cancer cells influences the immune cell's nutrient states and metabolic fitness within the TME. Most of the time, metabolic triggers observed in cancer cells cause immune cells to activate and differentiate [7–9].

As a result, immune and cancer cells have similarities in using nutrients and engaging in metabolic regulation to sustain proliferation and survival [10]. Advances are the fascinating possibility that "metabolic competition" within the TME may allow cancer cells to suppress antitumor immunity effectively. The following section focuses on how immune metabolic regulations fine-tune immune cell activation and antitumor immunity, including a metabolic switch to the *Warburg effects* that occurs in conjunction with immune inhibition. As a result, identifying the detailed molecular pathways and signaling cascades that regulate metabolism in the TME is critical for developing novel therapies. Here we emphasize the focus of future medicinal innovations on the key metabolism switches, notably in TME immune cells. We gather proof in this chapter to show how changes in these metabolites influence the functioning of immune cells over the course of cancer.

7.2 Immune Metabolism and Cancers

Many recent results of immune metabolism have shown that the metabolic condition of the immune cells may control several immunological responses. Immune cells have surprisingly different activities and physiological actions linked to specific metabolite needs. The end product is pyruvate, which is used to divide the single glycolytic route into two ATP molecules. In addition, pyruvate transforms in mitochondria to carbon dioxide, NADH, and FADH2. These reduction partners, i.e., NADH and FADH2, stimulate the production of extra ATP molecules for energy needs through oxidative phosphorylation (OXPHOS). Cancer cells use a special metabolism in contrast to healthy somatic cells to withstand distinct anabolism requirements. Therefore, the change in metabolism leads to acidic, hypoxic, and degrading essential nutrients in TME, which are mostly needed through the activity of immune cells. The mitochondrial OXPHOS of nutrients, counting glucose, amino acids, and fatty acids, goes through the tricarboxylic acid (TCA) cycle. The electron transport chain (ETC) benefits ATP production for naive, polarized/differentiated immune cells. However, cells convert to a different aerobic glycolysis mechanism during high-proliferation conditions such as following immune cell activation or cancer cell transformation. Although there is a small number of PTAs during aerobic glycolysis, around ten times more glucose can create lactate than normal tissue [11].

Meanwhile, pyruvate is metabolized into lactate and "secreted outside" of cells, allowing increased glucose inflow via NAD+ production. As a result of the substantial buildup of glycolytic intermediates, biomass synthesis is enabled, which is required for quickly proliferating cancer cells. The detection and significant recurrence of the *Warburg effect*, the primary glycolytic pathway, and somewhat reduced OXPHOS were later thought to limit cancer cells. However, recent research has shown that *Warburg effect*-like metabolic reprogramming occurs in continually reproducing immune cells, most notably macrophages and/or T cells, and controls the activity of immune cell subsets in inflamed tissue or cancer (Fig. 7.1).

7.2.1 Metabolic Switches in Lymphoid Cells Associated with Cancer Progression

Lymphoid cells are created from lymphoid progenitor stem cells; they provide lifelong immunity against infection and play an essential role in autoimmunity and cancer therapies. The size divides lymphoid cells into small (T and B) and large granular (NK cells). Lymphocytes are also called white blood cells ($CD45^+$ leukocytes) that provide an immune response against the autoimmune cell or foreign substances like pathogenic antigens. There are several key classes of lymphocytes; one of them is B cells, which are generated and are maturated in the bone marrow, whereas T lymphocytes are generated in the bone marrow and mature in the thymus. Lymphocytes are adaptive immune system cells after creation and maturation and produce antigen-specific immune responses to malignancies by recognizing the presence of tumor antigen in cancer cells or cells with antigen (APCs). These lymphocytes effectively respond to antigen or foreign protein encounters, differentiating themselves into the active form as effector phenotype and the reservation form as memory cells. The active T effector cells can make cytotoxic granules or produce unique signals for other immune cells to achieve the adaptive immune response. A few activated effector T cells do not experience cell death, leading to memory T-cell phenotype that persists in the peripheral lymphoid organs and remains potent to antigen recalls.

In contrast, B cells encounter an antigen; they differentiate into antibody-producing plasma cells and memory cells. Memory cells stay longer and produce additional antibodies for future encounters with the same antigen. Thus, NK cells fit the subtype of lymphoid cells dealing primarily with cytotoxic responses.

7.2.2 T Cell and Its Subsets

T cells play a crucial role in the eradication of pathogens and the scrutiny of pathological cells. T cells are of two major subsets founded on their unique T-cell receptor (TCR) chain, cytokine expression profile, and co-receptor expression. Based on TCR, they can discriminate between self- and non-self-antigens. During T-cell development in the thymus, its precursors obligate into two T-cell lineages

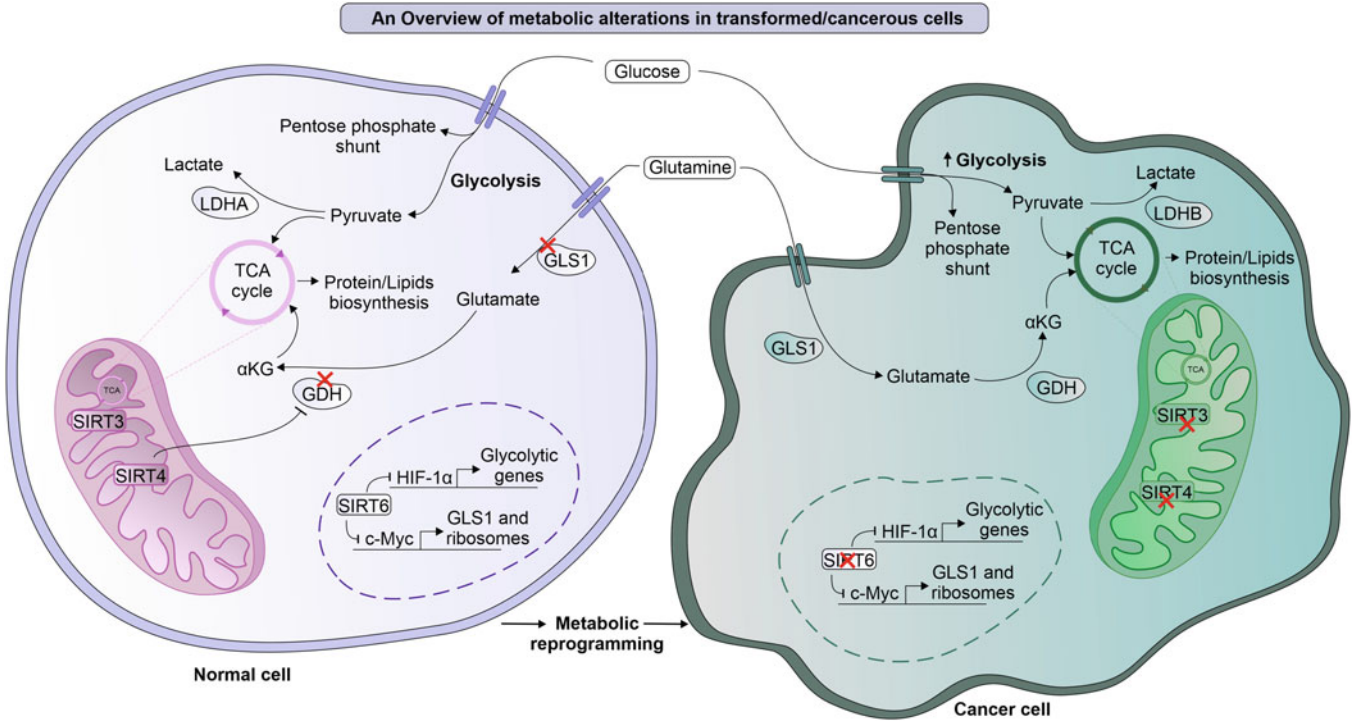

Fig. 7.1 An overview of metabolic alteration in transformed/cancerous and normal cells. Glucose is generally divided into pyruvates by the route of glycolysis. The process for the generation of ribose-5-phosphate can be retransferred to glycolysis intermediate-6-phosphates (G6P) and NADPH, which were most needed for nucleotide synthesis redox balance maintenance and fatty acid synthesis. Glycolysis intermedium (PPP) (FAS). Pyruvate can either be metabolized and released to lactate or enter the mitochondria and supplement the tricarboxylic acid (TCA) cycle. Glutamine and FA can also survive anaplerotic processes and lead to glutaminolysis and FAO in the TCA cycle. The TCA cycle creates equivalent reduction (NADH, FADH2) pairs, which can move into the electron transport chain (ETC) and contribute to energy production as ATPs and mitochondrial reactive oxygen species (mROS). In addition, the TCA cycle also serves as a source of metabolic intermediates such as citrate, shuttled via the cytoplasm from the mitochondria to the FAS

based on expressing different TCR chains, either αβ T cells or γδ T cells. According to their co-receptor expression, most human T cells are αβ T cells (~95%), further subdividing into CD4 T cells and CD8 cytotoxic T lymphocytes (CTLs). These effector CD8 and CD4 T cells' activation happens upon cognate TCR ligation to antigens presented on MHC-I and MHC-II molecules by APCs. Depending on the cytokine environment, CD4 T cells further differentiate into Th1, Th2, Th17, or Treg subsets, which have different functions and cytokine expression profiles [12]. While other helper T-cell subsets (such as Th3, Th9, and Th22) have recently been discovered, their precise function in cancer remains unknown and is not covered in this chapter.

Immune cells, especially T cells, are vital players in the immune system to fight against cancer because they mount antigen-specific responses against tumor cells. A few tumor-specific activated T cells directly destroy cancer cells by various mechanisms, (a) secreting cytotoxic molecules and (b) releasing signaling molecules, such as cytokines, that prime and activate other types of immune cells. The metabolic switches are crucial in the activation and differentiation of T cells from emerging evidence. The metabolism-based approaches for treating cancer appear to be the best strategy, and identifying new metabolite targets may help immuno-based therapy. This section emphasizes recent advances in metabolic switches that affect the T cells and their subtype proliferation, differentiation, and execution of effector functions. T-cell activation, expansion, and ultimately differentiation into different subsets like cytotoxic, Tregs, and helper T cells (Th1, Th2, Treg, and Th17) occur after engaging with the first signal through T-cell receptor (TCR) and second as co-stimulatory signals [13, 14]. In cancer cells, activated cytotoxic CD8+ T cells use a specific and effective cytotoxic activity.

Activated helper CD4+ T cells, on the other hand, differentiate into effector phenotypes that either support or suppress tumor progression. The best way to convey this is as follows: the Th1 subset ($CD4^+IFN\gamma^+$) secretes the anticancer IFN-γ cytokine, which leads to stimulation of the NK and macrophages, and further initiates the anticancer immune response, while the Th2 subset ($CD4^+IL4^+$) and CD4 Treg subset ($CD4^+CD25^+FoxP3^+/CD127^-$) suppress the immunity against the tumor or pathogens. The other subsets of helper CD4 T cells, Th17 cells ($CD4^+IL17^+$), can either support or stop tumor growth [15].

7.2.2.1 The Quiescent T-Cell Metabolism

T cells in naïve or quiescent states restrict their proliferation; they use nutrients for homeostasis and care but not for biosynthesis. These metabolically quiescent T cells primarily utilize glycolysis, FAO, and amino acids to fuel OXOPHOS, as shown in Fig. 7.2 [16–18]. Naïve T cells reduce the rates of glycolysis and glutaminolysis pathways; they are sustained mostly via the extracellular signaling of cytokines like IL4 and IL7 [19, 20]. In the absence of these extracellular signaling cytokines, naïve T cells will die due to bioenergetic decline and lessened expression of Bcl-2 [21]. The quiescence state of T cells is preserved by inhibiting the TCR signaling and Akt pathway by environmental sensors. Researchers showed that tuberous sclerosis 1 (TSC-1) acts as an inhibitor for the AKT pathway, resulting in a

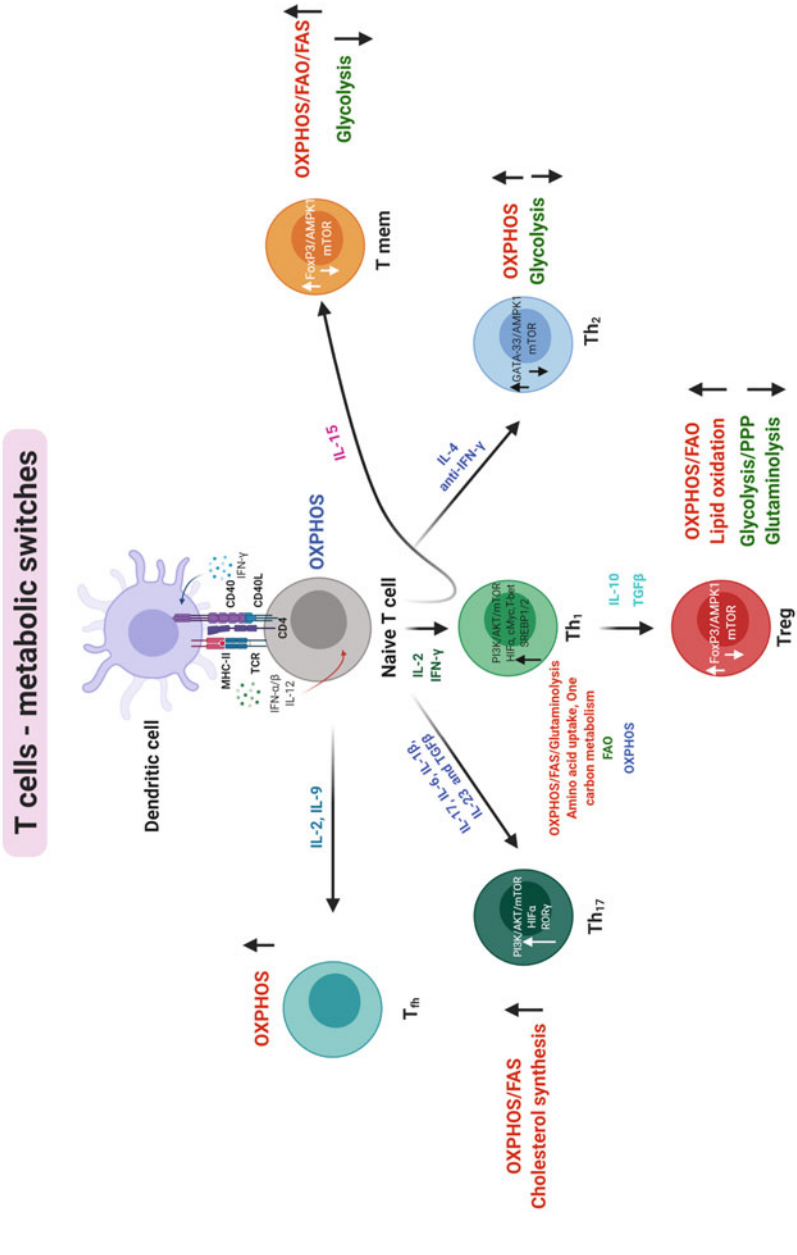

Fig. 7.2 T-cell activation results in metabolic rewiring. Other metabolic states exist in naive T cells that have developed into distinct subsets, such as Th1, Th2, Th17, Tfh, Treg, and Tmem cells (T memory cells). In addition, T-cell subsets have critical metabolic switches that are either activated or deactivated, as illustrated by red and green colors

premature withdrawal from quiescence [22], implying that the TSC-1-reliant switch of mammalian target of rapamycin (mTOR) is critical in maintaining T-cell quiescence.

7.2.2.2 Metabolic Reprogramming in T-Cell Activation

The transition from a relaxing naïve T cell into triggered and highly proliferative effector T cells requires considerable metabolic switch alteration. T-cell activation increases the bioenergetic and biosynthetic needs to support their proliferation and differentiation. Activated T cells facilitated increased uptake of fatty acids, suppressed FAO, and promoted lipid synthesis [16]. These metabolic switches are composed of a cascade downstream of TCR and a co-stimulatory signal via CD28 and other cytokine receptors. For example, the PI3K-AKT-mTOR pathway leads to the expression of transcription factors like hypoxia-inducible factor 1 (HIF1α) and c-Myc regulating T-cell metabolic plans' functional fortunes [23]. This metabolic reprogramming consequence shifts toward aerobic glycolysis (contains the mitochondrion-independent glucose metabolism into pyruvate and its following conversion into lactate, generating insufficient ATP, i.e., only two molecules of ATP per glucose). On the other hand, OXPHOS generates up to 36 ATP molecules on behalf of one glucose molecule with an upregulation of the OXOPHOS.

Glutaminolysis is a process that produces ATP as well as an increase in biomass such as amino acids, lipids, and nucleic acids to supply essential building blocks for growing cells [24]. Thus, T cells switch to anabolism to incorporate nutrients into biomass and sustain speedy proliferation and functionality after activation. Thus, tumor cells and activated T-cell metabolism are analogous to engaging a *Warburg*-like metabolic rewiring. In hypoxic conditions, antigen prime T effector cells are mainly reliant on aerobic glycolysis for their energy requirement, simultaneously raising the lactate production [25–27]; this process is led by HIF1α [28, 29]. HIF1α induces signaling, and molecular events influence GLUT1 (glucose transporter), rate-limiting enzymes of glycolysis pathway hexokinase 2, and amino acid transporters facilitating glycolysis and glutaminolysis to fulfill activated T cells' needs while suppressing FAO [30, 31]. Notably, many of the molecular alterations caused in rapidly proliferating T cells are analogous to the signaling and metabolic changes that drive cancer cell reprogramming. Inhibiting or restricting glucose availability decreases T-cell activation, differentiation, and cytokine production [7, 18].

Furthermore, GLUT-1 inhibition, whether pharmacological or genetic, impairs T cell activation and function [32–34]. In contrast, overexpressing GLUT1 in mice increased glycolytic flux in T cells [31]. Unlike cytotoxic and effector T cells, Tregs and memory CD8+ T cells rely on OXOPHOS and FAO for survival, differentiation, and function.

The active T-cell state maintains and employs mitochondrial OXPHOS or aerobic glycolysis depending on the dietary shortage. On the other hand, aerobic glycolysis appears to be required for optimum metabolite and chemokine/cytokine synthesis [31, 34]. Following TCR activation, mTOR complex 1 (mTORC1) was activated, which resulted in mitoribosome production and mitochondrial complex IV activity.

In addition, T-cell dormancy recovery is linked to COX10 expression [35]. According to certain studies, glutamine can be used as an alternative energy supply pathway [14, 36]. As a result, glutaminolysis is essential for T-cell activation and function [37, 38], and glutamine deficiency inhibits T-cell proliferation and cytokine production.

Furthermore, activated T cells raised glutamine levels in a CD28-dependent manner, which was accompanied by enhanced glutamine transporter expression. Removing these receptors induces a faulty shift to the effector T-cell phenotype [33, 36], which is linked to extracellular signal-regulated kinase (ERK) activity, implying a biological connection to the TCR cascade. FAS upregulation is another essential metabolic mechanism that permits T-cell effector differentiation and expansion, namely through acetyl coenzyme A carboxylase (ACC1), which is necessary for the conversion of acetyl coenzyme A to malonyl coenzyme A [39].

7.2.2.3 Effector T-Cell Metabolic Switch

A metabolic transition occurs at two distinct levels: transcriptional programming (c-Myc and estrogen-related receptors, ERRs) and posttranscriptional modification (AMPK activation and HIF-1 stabilization) [40]. The c-Myc expression has various functional roles in cell metabolism, including glycolytic programming and support for aerobic glycolysis. It also stimulates glutaminolysis, resulting in more ketoglutarate to feed the TCA cycle. Furthermore, c-Myc prefers the creation of citrate from glucose for use in fatty acid synthesis [14]. Finally, c-Myc's genetic deletions of c-Myc in mouse models and cancer cells lead to T-cell inhibition of TCR-induced glycolysis and glutaminolysis [41].

ERRs are also required for the regulation of metabolic processes [42]. ERR regulates metabolic pathways essential for T-cell activation, differentiation, and effector activity, and ERR levels grow during these processes. An ERR-deficient animal model, for example, revealed that ERR deficiency decreases GLUT-1 overexpression, glucose absorption, and mitochondrial activity, eventually limiting T-cell growth and proliferation [42].

Several signaling pathways and transcription factors drive metabolic reprogramming in activated T cells. A key regulator of T-cell metabolism is an mTOR, which integrates the signals provided by nutrient stress and T cells' energy. There are two distinct complexes, mTORC1 and mTORC2, that differ in their regulation and downstream targets [43]. The PI3K/Akt/mTOR pathway is involved in co-stimulatory CD28-dependent signaling to enhance glucose metabolism and avoid T-cell anergy [44]. PI3K activation recruits Akt isoforms 1–3 and 3-phosphoinositide-dependent protein (PDPK1) to the cell membrane, where it can then activate Akt through phosphorylation; this cascade activates mTORC1 [7]. In addition, Akt and mTORC1 stimulate GLUT-1 translocation to the cell surface and prevent it from being internalized, leading to increased amino acid transporter synthesis and phosphorylation of glycolytic enzymes, which increases glycolytic flux and therefore promotes anabolism [40, 45]. Furthermore, via phosphorylating 4EBP and p70S6K, mTORC1 increases protein translation [46]. Conversely,

inhibiting mTORC1 decreases glycolysis following T-cell activation, resulting in T-cell anergy [45].

Adenosine monophosphate (AMP) activated the protein kinase (AMPK), which is another master regulator of T-cell activation (Fig. 7.2). In contrast to mTORC1, AMPK promotes catabolic metabolic programming in response to stress-induced energy restriction [47]. As a result, its activity relies on the cellular AMP/ATP ratio, promoting mitochondrial ATP production while suppressing energy-burning mTORC1-dependent anabolic pathways [47, 48]. MPK activation is required for memory T-cell development [49], which is consistent with the main mitochondrial network of these cells. It is also necessary to grow effector T cells and metabolic flexibility in humans [50]. AMPK-knockout T cells have a high level of mTORC1 at the basal state, a high glycolysis rate, and a decreased ability to respond to metabolic stress and switch toward catabolic metabolism [48].

The transcription factor HIF1α is also important in metabolic reprogramming, allowing T cells to adapt to hypoxic environments (Figs. 7.1 and 7.2). The presence of HIF1α increases glucose absorption and allows the metabolic switch from OXPHOS to aerobic glycolysis [29]. HIF1α levels depend on mTORC1 action to sustain glycolytic metabolism and regulate transcriptional programming required for T-cell proliferation. T-cell glycolysis and effector responses are enhanced when its negative regulator, von Hippel-Lindau (VHL), is deleted [51]. HIF1α also promotes the metabolite S-2-hydroxyglutarate (S-2HG) buildup, which controls epigenetic modifications in activated T cells that regulate immunological signal expression supplementation. After adoptive transfer, CD8+ T cells increased proliferation, persistence, and antitumor efficacy [52].

7.3 Exact Metabolic Alterations and Subsets of T Cells

These Th1, Th2, and Th17 T-cell subsets generally use glycolysis via mTORC1 signaling, whereas Tregs primarily engage mostly FAO [25] (Fig. 7.2). Therefore, anti-mTOR (rapamycin) or HIF1α inhibition results in the production of Treg cells, even in cell culture medium enriched with Th17-polarizing cytokines in vitro [53, 54]. Th17 cells rely on mTOR- and HIF1α-dependent glycolysis; blocking glycolysis in HIF-deficient mice leads to the inhibition of Th17 differentiation [55]. In mice models, Treg has shown high levels of AMPK, and stimulation of AMPK tends to decrease GLUT-1 expression [25]. Thus, blocking glycolysis inhibits Th17 differentiation and promotes Treg generation [25, 56]. Furthermore, signaling through mTORC1 leads to quiescent CD4+ T-cell activation into Th1 cells, whereas activation of mTORC2 leads into Th2 phenotype [57, 58]. Constant with this, lactate dehydrogenase A (LDH-A), which supports aerobic glycolysis in Th1 polarization, is encouraged in T-cell activation and promotes IFN-γ expression by maintaining high acetyl-CoA concentrations, thereby enhancing histone acetylation and transcription of *Ifng* [59].

In addition to LDH-A, glyceraldehyde 3-phosphate dehydrogenase (GAPDH) expression glycolytic enzyme binds to AU-rich regions in IFN mRNA 3′UTR when

the enzyme is not involved at a high glycolytic rate which controls IFN mRNA stability [34]. Ho et al. investigated the role of glycolysis in Th1 polarization and discovered that the glycolytic metabolite phosphoenolpyruvate (PEP) supports Ca^{+} $^{+}$- and NFAT-mediated IFN production. Furthermore, in a melanoma mouse model, PEP supplementation or overexpression of PEP carboxykinase 1 (PEPCK1) in CD4+ T cells increased IFN levels and anticancer activity [60].

A study that used mass spectrometry to enumerate protein dynamics to assess the proliferation and persistence of activated $CD4^{+}$ T lymphocytes identified the mitochondrial proteome's fast remodeling with a diversified metabolic signature by one-carbon metabolism [61]. Serine accumulates due to an enhanced glycolytic rate, which feeds purine and thymidine synthesis, allowing T-cell proliferation and survival. The mitochondrial serine hydroxymethyltransferase 2 (SHTM2) gene deletion reduced the frequency of antigen-specific T-cell abundance in vivo and the generation of inflammatory cytokines IL-17 and IL-6, but not IFN or TNF. As a result, mitochondrial function is accomplished through one-carbon metabolism. As a result, mitochondrial activity via one-carbon metabolism is required for T-cell proliferation, while glycolysis is required for IFN production. This nucleotide metabolism is also important in achieving macrophages' innate immunological memory state after TLR stimulation [62]. Using unbiased proteomics, variations in metabolic requirements for Tregs were discovered between in vitro-cultured cells (glycolysis and FAO) and isolated ex vivo cells (only glycolytic).

7.3.1 Regulatory T-Cell Metabolism

Regulatory T cells (Tregs or Treg cells), formerly known as suppressor T cells, are T-cell subsets that regulate the immune system, maintain tolerance to autoantigens, and prevent autoimmune illness. Tregs are immunosuppressive cells that suppress or inhibit the induction and proliferation of effector T cells [63]. Tregs reduce defense against tumors or infections by expressing the surface biomarkers CD4 and CD25 and the intranuclear transcriptional factor FoxP3 ($CD4^{+}CD25^{+}FoxP3^{+}/CD127^{-}$). This suppressor cell's metabolism is mostly based on glycolysis-driven lipogenesis with high lipid oxidation rates [25], and it plays an important role in the mevalonate pathway [64]. Furthermore, like effector T cells, Tregs are highly glycolytic, with enhanced glucose absorption [60], but are less glutaminolytic [65]. Glycolysis has an important role in Foxp3 expression and, as a result, Treg immunosuppressive effects. Furthermore, Tregs have high AMPK expression and activity levels at the molecular level and low mTOR activation levels via phosphatase and tensin homolog (PTEN) [66].

7.4 Cross Talk Between Immune and Cancer Cells Regulates T-Cell Function in the TME: An Immunometabolism Perspective

Cancer cells' metabolic characteristics are distinctive in that they satisfy their demands by utilizing favorable molecular and biochemical systems that allow food consumption in a more specialized manner than non-tumor cellular counterparts. These modifications allow for growth programs, adaptability to various microenvironmental conditions with little harm, and survival in the face of stress and/or restricted food supply. Such aberrations supporting malignant cell development and creating metabolic products change the microenvironment and impact the fate and function of our fighter cells, i.e., immune cells living in TME.

The cancer cells have higher metabolic activity due to their proliferative capacity. The inadequate vasculature, which affects the blood circulation in the TME, can cause a nutrient deprivation TME and regulate immune cells' fate (Figs. 7.2 and 7.3). Low blood circulation in TME can affect TCR signaling, glycolytic metabolism, amino acid absorption, and overall metabolism, all of which are characteristics of T effector cells, resulting in decreased antitumor efficacy of tumor antigen-specific T cells. CD4 Treg cells, on the other hand, which rely mostly on FAO [7, 25], may survive in these conditions and demonstrate biological activity, i.e., immunosuppressive effects on antitumor T cells, which are favorable for tumor clearance. New research linked Treg cell growth in the TME to the activation of AMPK, a sensor of food restriction and metabolic stress [67].

Furthermore, metabolic waste produced by hypermetabolic cancer cells, such as lactate and kynurenine, might impair antitumor T-cell activity and promote the growth of suppressive immune cells, i.e., Tregs [5, 23]. Additionally, TME hypoxia promotes HIF1 expression, which aids in the formation and maintenance of Treg cells [68] and increased expression of PD-L1 in MDSC, therefore mediating strong inhibitory effects in tumor-specific T-cell activity [69]. Overall, the metabolic and nutritional changes that define the TME modify metabolic reprogramming and play an important role in T-cell proliferation/differentiation by inhibiting T effector cell differentiation and boosting various immunosuppressive pathways (Fig. 7.3).

In general, metabolic pathways govern T-cell growth, differentiation, and function in the TME. Furthermore, because activated T cells and tumor cells have comparable metabolisms, we explain the influence of the TME on T-cell metabolic changes dependent on the situation, which may give methods for maximum anticancer effects and enhanced T-cell immunity. As a result, investigations on T lymphocyte metabolism can help with fundamental research on immune metabolism as well as give prospective targets for drug development and innovative clinical cancer therapy techniques.

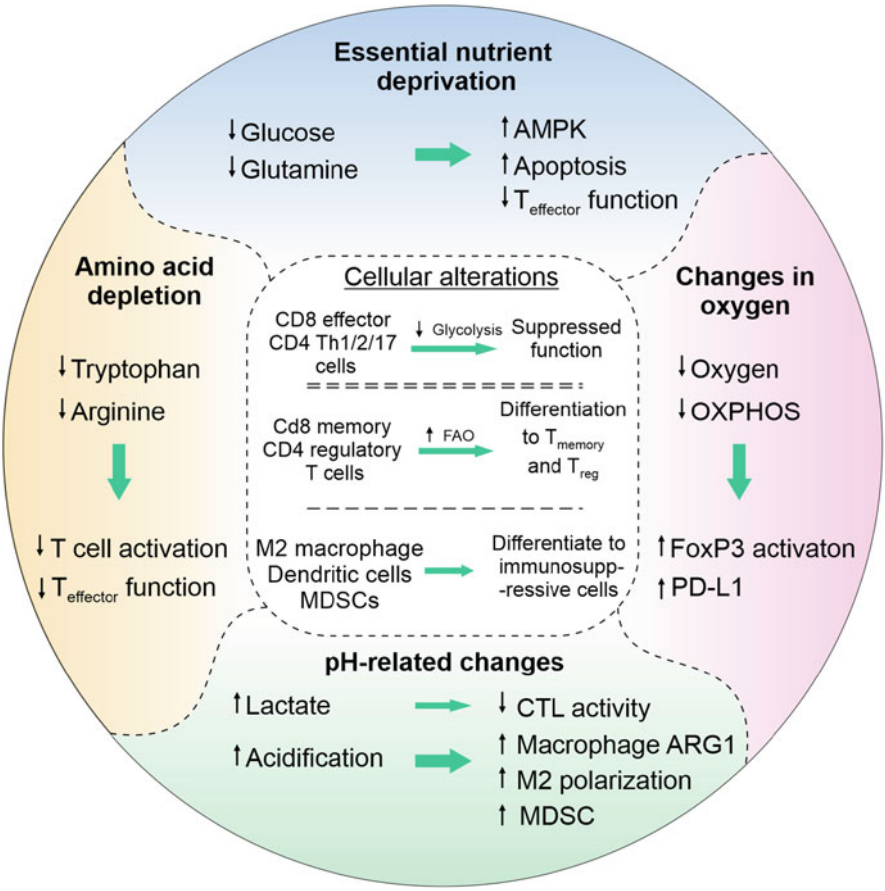

Fig. 7.3 The impact of metabolic alterations in the tumor microenvironment (TME) on immune cell activity when essential nutrients are depleted. Metabolic change or critical nutrient deprivation in the TME induced by cancer cells enhanced metabolic activity and influenced the differentiation program of myeloid cells and T cells

7.5 Myeloid Cells' Metabolic Link

Myeloid cells play an important role in homeostasis by encouraging lymphocytes to react to pathogens [70], removing foreign bodies, and phagocytizing dead cells in the system. Furthermore, myeloid cells are thought to be facilitators of the immune system's innate arm, which fights pathogenic components, cell stress, and tissue damage by expressing various sensors such as pattern recognition receptors (PRR), which detect danger signals that initiate the appropriate immune response. Undeveloped myeloid cells are generated in the bone marrow and differentiate/proliferate

into granulocytes, mast cells, macrophages, or dendritic cells in response to environmental stimuli.

Myeloid cells, once formed, migrate from the bone marrow to tissues. These cells will shift toward an immunosuppressive phenotype in cancer, with the generation of M2-like tumor-associated macrophages (TAM) and myeloid-derived suppressor cells (MDSCs) [71], which are primarily triggered or dependent on microenvironmental signals such as cytokines/chemokines and other factors that promote immune suppression.

7.5.1 Macrophages

Macrophages (MΦs) are innate immune cells that develop in response to a pathogenic infection or the accumulation of cell debris. MΦs are big, terminally differentiated specialized immune cells with strong secretory, phagocytic, and antigen-presenting capacities that also contribute to host defense, homeostasis, and disease. Despite their morphological and functional variety, MΦs are generally classified into two kinds based on preliminary studies: (1) MΦs with M1 phenotype and (2) MΦs with M2 phenotype.

M1 phenotypes are characterized by the production of inflammatory cytokines such as IL-12, tumor necrosis factor (TNF), IL-6, IL-1, reactive nitrogen species (RNS), reactive oxygen species (ROS), and microbicidal functions in response to inflammatory stimuli such as IFN-γ + LPS, whereas M2 phenotypes are characterized by anti-inflammatory stimuli such as IL-4, IL-13, and IL-10 (MMPs). Furthermore, the M2 phenotype promotes immunosuppression, extracellular matrix remodeling, tumor cell extravasation, and metastasis in established malignancies and regulates chemotherapy response [6, 72, 73]. However, in vivo, such distinct phenotypes are frequently muddled [72].

MΦs characterize significant lymphoreticular infiltrates in solid tumors and play a crucial role in cancer progression [72, 74, 75]. Therefore, we discuss the metabolic switch associated with tumor-associated MΦs (TAM) during cancer progression and development [73, 76]. TAMs are mostly immunosuppressive and polarized toward M2-phenotype MΦs, which promote angiogenesis and tissue remodeling in advanced tumors. However, in a few cases, an increase in TAM in the TME was associated with a better prognosis. Tumor-bearing mice who were injected with more TAMs that were present in the niche at the time of initiation had smaller tumors [76]. Later, myeloid cell migration from the blood and differentiation into TAMs may result in cancer regression [76]. TAM-produced ROS and RNS cause cancer cell death, resulting in tumor regression or genetic instability that promotes malignant transformation. Furthermore, M2-phenotype TAMs decrease antitumor adaptive immunity while releasing growth factors and matrix proteinases that inhibit tumor advancement; hence, MΦs are said to be double-edged swords in tumor progression and regression [77]. Through metabolic changes in the TME, tumor cells play an important role in converting TAM to M2-phenotype MΦs (Fig. 7.4).

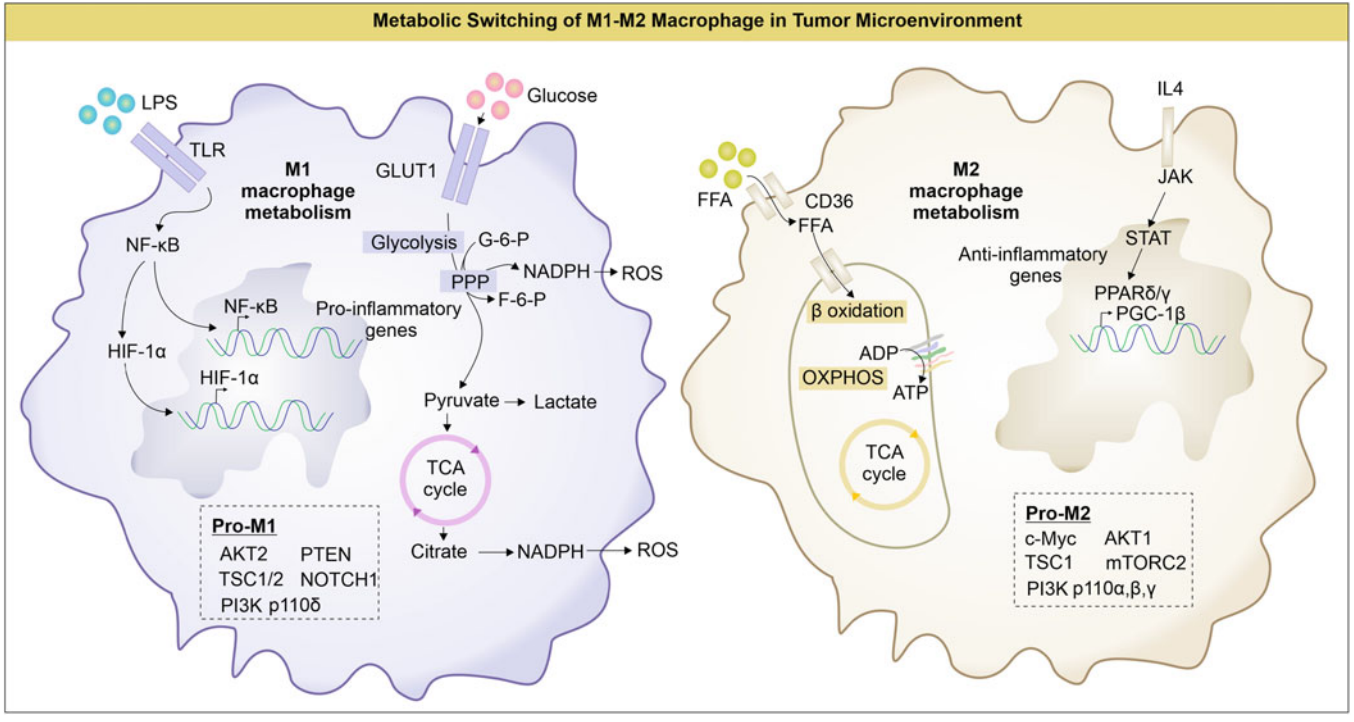

Fig. 7.4 M1-M2 macrophage metabolic switching in the tumor microenvironment. LPS-induced M1 macrophages are primarily inflammatory, producing pro-inflammatory soluble cytokines via the transcriptional factors NF-B and HIF activation. M1-like macrophages increased glucose breakdown into lactate and decreased OXPHOS. Soluble IL4 cytokine-polarized M2 macrophages, on the other hand, engage JAK-STAT to promote PPAR and PGC-1, boosting metabolic and anti-inflammatory genes. As a result, M2-like macrophages have greater FAO and mitochondrial OXPHOS levels

The polarized-condition macrophages display distinct features; for example, M1-phenotype MΦs have higher glycolysis, whereas M2-phenotype MΦs show higher oxidative phosphorylation [78–80]. Likewise, when human monocytes stimulated with β-glucan switched to a glycolytic mode, this shift happened via the AKT-mTOR-HIF1α pathway [81]. In murine model, MΦs encouraged with LPS shift to glycolysis and accumulation of the TCA cycle intermediate, succinate, which via the HIF-1α induces the inflammatory cytokine IL-1β [79]. A higher level of glycolysis can stimulate TNF in MΦs [82]; these observations suggest that glycolysis regulates the inflammatory phenotype of MΦs. The spatial organization of TAM populations is based on the hypoxia and lactate levels in the TME [83]. Indeed, hypoxia endorses the M2 phenotype of TAMs characterized by low expression of class II MHC [84]. Highly proliferating cells require an abundant supply of oxygen and nutrients. However, tumor masses frequently grow faster than the vascular, resulting in an oxygen-deficient environment, known as hypoxia [85].

TAMs with high arginase-1 (Arg-1) and mannose receptor C type 1 (CD206) levels cluster in regions with insufficient blood and oxygen supply (Fig. 7.4). TAMs in these regions express hypoxia-responsive factor-1 (HIF-1) and shift their metabolism to glycolytic fermentation. In the TME, TAMs are exposed to a high concentration of tumor cell-derived lactic acid. Surprisingly, this tumor-derived lactic acid stabilizes HIF1 expression in TAM under hypoxia and normoxic settings. Lactic acid also promotes polarization toward M2-like TAMs, as seen by increased Arg-1 and VEGF production [86]. Cancer cells, therefore, initiate a vicious cycle by producing lactate, which boosts acidic pH by triggering hypoxia responses in TAMs.

TAMs also release a high level of anti-inflammatory cytokines in response to lactic acid, which suppresses the immune system. Lactate dehydrogenase stimulates a strong immune response against cancer. Seth et al. have demonstrated that ablation of lactate dehydrogenase A (LDH-A) and subsequent lactate in myeloid cells leads to lung cancer development [87]. These findings show the broad impact of TME metabolic reprogramming of TAMs away from M2 polarization. Several metabolic genes have been linked to the regulation of M polarization and the control of TAM phenotype [88]. PKM2, for example, suppresses LPS-induced IL-1 production, restricting the M1 phenotype [89]. Furthermore, O'Neill et al. demonstrated that blocking PKM2 reduced PD-L1 expression on TAMs, dendritic cells, T cells, and tumor cells [90]. This is accomplished by direct binding of PKM2 and HIF1 to the PD-L1 promoter.

Surprisingly, the study discovered that LDH-A/lactate also increased PD-L1 expression in the TME [87]. Although the mechanism of lactate-induced PD-L1 expression is unknown, HIF1α may play a role [69]. In addition to the glycolytic switch, the glutamine-glutamate route is an important metabolic process in macrophages. Macrophages have high glutamine utilization rates and high glutaminase levels, an enzyme involved in glutamine metabolism. The TME is largely dependent on this glutamine-glutamate pathway whether M is resting or present [91]. Furthermore, by generating a high ketoglutarate/succinate ratio, glutaminolysis is required to polarize M to M2 phenotype in the TME [92].

Lipid metabolism also altered in the macrophages in response to distinct microenvironmental stimuli [93]; mouse macrophages activate and take up fatty acid using Th2 cytokine IL4. At the same time, this is suppressed in the M1 phenotype [94]. TAG uptake and subsequent lysosomal lipolysis were required for FAO and M2 activation in these IL4-treated macrophages [95]. Few studies have shown that IL-4 PPAR through STAT6 changes to mitochondrial respiration and FAO [94].

Iron homeostasis is a critical metabolic process in macrophages. Iron metabolism in polarized macrophages differs; the M1 phenotype expresses low amounts of iron exporter and ferroprotein but large amounts of H-ferritin, which is implicated in iron storage [96]. On the other hand, M2 macrophages treated with IL4 exhibit modest amounts of H-ferritin but large levels of ferroprotein. According to this profile, M1 macrophages prefer iron sequestration and restrict bacterial and tumor development, whereas M2 macrophages prefer iron release and promote tissue healing and tumor cell proliferation [96, 97].

7.6 Myeloid-Derived Suppressor Cells (MDSCs)

One of the critical T-cell activation and function regulators in the TME is myeloid-derived suppressor cells (MDSC), which form one of the significant innate cellular mechanisms. MDSCs are heterogeneous types of immature myelomonocytic cells that suppress the activity of T cells [98]. These cells proliferated rapidly in tumor carriers and were originally identified in mice as having a dual-positive $CD11b^{+}Gr1^{+}$ phenotype. MDSCs are divided into two types: monocytic MDSCs and granulocytic MDSCs, both of which can inhibit antigen-specific $CD8^{+}$ T-cell activation (Fig. 7.5). Amino acid metabolism and oxidative stress play critical roles in mediating the suppressive impact of MDSCs on effector T-cell function, primarily through the depletion of necessary amino acids for T cells and the production of reactive species [99]. MDSCs can reduce L-arginine levels by metabolizing it via Arg-1 expression.

Similarly, by sequestering L-cysteine, MDSCs can produce L-cysteine deficiency [100]. When these amino acids are depleted, the chain of the TCR is downregulated, and T cell and NK cell function is inhibited, whereas support Treg cells are activated (Fig. 7.5). In addition, MDSCs, like macrophages and DCs, contain the inducible enzyme IDO, which catalyzes the conversion of tryptophan to kynurenine [101, 102]. As a result, IDO uses its negative influence on T cells via tryptophan shortage to drive Treg cell growth [103, 104], as seen in Fig. 7.5.

By expressing NOS2, Arg-1, and NADPH oxidase, MDSC subsets produce RNI (NO, peroxynitrite) and ROI (peroxide), which inhibit TCR and IL-2 receptor signaling and limit T-cell activation and proliferation [99]. The importance of nitrogen metabolism in controlling MDSC immunosuppressive activities in tumors is well recognized, but less is known about the other metabolic switches in MDSCs. During MDSC maturation, there was an increase in glycolysis, glutaminolysis, TCA activity, and interaction with arginine metabolism. However, its association with elevated AMPK and SIRT expression, both of which are known to interfere with glycolysis (Fig. 7.1), needs to be explored in detail [103]. Recently, increased fatty

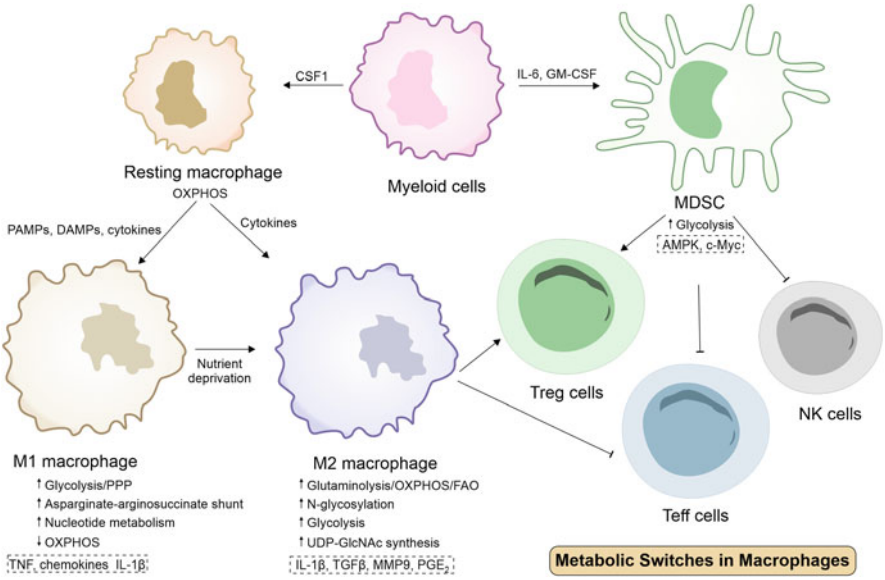

Fig. 7.5 Macrophage metabolic switches. Based on the metabolic switches and cytokine availability indicated in the picture, resting macrophages develop into M1, M2, or MDSCs. The main metabolic pathways that are elevated, downregulated, or unaltered for each immunological phenotype are highlighted. Arrows denote the key metabolic indicators involved in these pathways. For example, MDSC can suppress NK and Teff cells while assisting Treg cells

acid absorption and FAO were found to control the immunosuppressive function of tumor-infiltrating MDSCs [104].

7.6.1 Dendritic Cells

These cells are traditionally described as professional antigen-presenting cells (APC) that link between innate and adaptive arm of immunity. They also play an important role in lymphocyte priming. Immature DCs get activated and undergo maturation after receiving danger signals from the niche (pathogen-associated molecular pattern (PAMP) or damage-associated molecular pattern (DAMP) molecules). DC maturation involves upregulation of antigen-presenting and -associated markers such as MHC class II, CD80, CD86, CD40, IL-12, and CCR7. Once activated, matured DCs traveled to the lymphoid organs to efficiently present antigen/epitope and induce the antigen-specific immune responses. However, in some circumstances, these antigen-presenting DCs encourage immune tolerance and immune evasion when DCs present in the tumor environment (i.e., tumor-associated DCs, TADCs) demonstrate a diminished ability to induce immunity. At the same time, they promote immunosuppression [105, 106]. Inactivated DCs gather a higher frequency of functional, less mature DCs, and a decreased number of functionally competent DCs have been

reported for numerous murine and human cancers [99]. Although the precise processes behind the reduced performance of TADCs remain unknown, current studies on metabolic control of DC activities provide a new viewpoint on the problem [107, 108].

7.7 Metabolic Reprogramming in DCs

DCs primarily employ OXPHOS as an energy source under resting or quiescent state (Fig. 7.6), but after stimulation with the pathogen or other molecules, DCs shift to glycolytic metabolism [109]. In early activation through TLR ligands, glycolysis is essential. For example, the expression of CD40 and CD86 characterizes its activated state and IL-12p40 upregulation, while in later stages, glycolysis is only crucial for its survival [109]. TLR-stimulated DCs exhibit increased glycolysis while decreasing OXPHOS, which is thought to be triggered by NOS2-induced NO production (which inhibits cytochrome c oxidase, a key enzyme of ETC) and activation of the PI3K-AKT pathway (which inhibits AMP-activated protein kinase, a key regulator of OXPHOS). These data point to glycolysis as a critical metabolic switch in DC

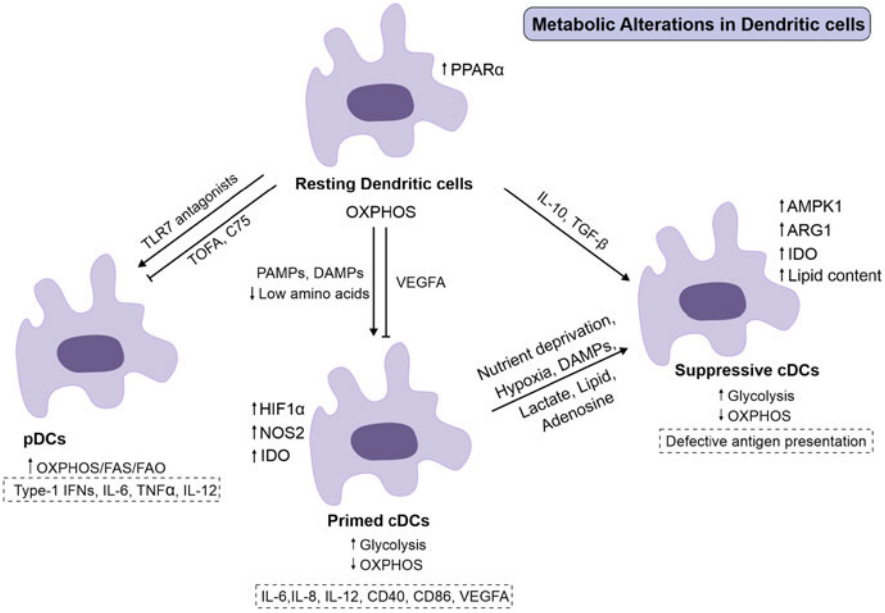

Fig. 7.6 Metabolic alteration in dendritic cells. Dendritic cells (DC) that are dormant are converted into plasmacytoid DCs (pDC), primed DCs, or suppressive DCs. The accompanying graphic depicts the major metabolic routes for each subgroup of DC cells. Arrows show the primary metabolic indicators implicated in these changes. Several metabolites or the regulation of metabolic gene expression inside DCs can skew immune cell phenotypic and polarization, therefore altering immune activities

activation. Furthermore, by preserving mitochondrial membrane potential and preventing cytochrome c release, this metabolic shift creates ATP, which enhances their durability after activation.

Numerous factors may influence the DC (TADC) activation, maturation, and function; the hypoxic condition in TME effect on these TADC is not fully understood. However, in preclinical mice, the study observed that this hypoxia enhanced the TDDC maturation [109]. This is preferred by activation of the HIF1 and PI3K/Akt pathways, which results in higher glucose uptake via the glucose transporter GLUT-1, increased activity of glycolysis's rate-limiting enzymes particularly regarding hexokinase and phosphofructokinase, increased lactate production, and decreased mitochondria OXPHOS and oxidation [109]. Conversely, restricting HIF1 in mouse models reduces glucose uptake, inhibits maturation, and impairs T-cell priming capacity [110].

Furthermore, iNOS promotes OXPHOS reduction, which inhibits mitochondrial NADH reoxidation via NO generation, as well as activation of the PI3K/Akt pathway, which inhibits AMPK (a master regulator of OXPHOS). On the other hand, hypoxia inhibited the development and migration of human monocyte-derived DCs [111]. Hypoxia causes metabolites such as adenosine and lactic acid to accumulate in the TME, negatively influencing DC activation. Indeed, hypoxia enhanced adenosine receptor (A2b) expression on human DCs, prompting them to adopt a Th2-promoting phenotype [112]. The interaction of adenosine and adenosine receptors affects DC differentiation and function. Such DCs have lower stimulatory activity and higher levels of IL-6, COX2, TGF-β, IL-10, IL-8, and VEGFA expression, and promote tumor development [113]. IL-10 and TGF-β inhibitory cytokines, like tumor-conditioned DCs, would help Treg cells [114].

TADCs have been shown to increase the expression of the immune checkpoint receptor PD-1 and its ligand PD-L1 [115]. Because PD-L1 is a HIF1 target gene [69], it is not unreasonable to believe that tumor hypoxia would induce PD-L1 on DCs and promote immune evasion. Furthermore, lactic acid, like TADCs, encourages an impaired phenotype in DCs [116]. Again, rapidly growing tumor cells in the TME impose nutrient competition and accumulation of metabolites such as adenosine, which may activate the metabolic sensor AMPK in TADCs. AMPK has been shown to promote oxidative phosphorylation while inhibiting glycolysis. This might explain why TADC activation is hindered metabolically. Tolerogenic DCs have a metabolic hallmark of enhanced OXPHOS, which controls their tolerogenic activity [107]. Future metabolic investigations in TADCs will need to determine if they truly transition from glycolysis to OXPHOS as the tumor progresses.

Immunosuppression is mediated by TADCs through the production of amino acid metabolism enzymes such as ARG1, NOS2, and indoleamine 2,3-dioxygenase (IDO) (Fig. 7.6). Furthermore, ARG1 and IDO expression in TADCs, like macrophages and MDSCs, depletes arginine and tryptophan in the tumor microenvironment, which inhibits CD8+ T-cell response and survival [115]; see MDSC section for more details. Furthermore, it was recently discovered that vitamin A

metabolism to retinoic acid in TADCs drives Treg cell and tolerogenic melanoma response [117].

Lipid metabolism, such as FAS regulation in DC activation, impacts ER and Golgi enlargement, which affects their antigen-presenting potential [118]. In addition, MSR1, a scavenger receptor identified in TADCs, facilitates lipid absorption and accumulation [119]. Thus, the DC antigen presentation and T-cell responsiveness are harmed by lipid buildup. In the nutrient-deficient microenvironment of established tumors, metabolic switching of TADCs from glycolysis to OXPHOS may favor FAS and lipid accumulation, contributing to their tolerogenic state (Fig. 7.6). However, nutrient deficiency in the TME also causes ER stress, which is linked to ovarian cancer progression [120]. Despite their involvement in tumor development, DCs have been widely investigated in the context of tumor vaccines to increase an antitumor response due to their natural ability to deliver antigen and stimulate an immune response [121]. Furthermore, vaccinations have recently been demonstrated to increase the expression of the nutrition sensor GCN2 in DCs in order to trigger a $CD8^+$ T-cell immunological response [108]. Thus, it would be fascinating to investigate how vaccine-mediated regulation of DC metabolism may contribute to their antitumor response in the future.

7.8 Metabolic Alteration in Neutrophils

Neutrophils are white blood cells or granulocytes that help us fight against pathogens and other diseases, representing 40–70% of the total circulating leukocytes in our body. These are the first immune cells to be exposed to a bacterial infection. Typically, our bodies utilize energy created by glycolysis of glucose, mitochondrial respiration (oxidative phosphorylation) in the presence of oxygen, and TCA cycle to produce pyruvate, glutamine, or free fatty acids. Neutrophils in a naive stage primarily use glucose for fuel and contain few mitochondria. However, other metabolic pathways in neutrophils have been identified, including the PPP, TCA, OXPHOS, and FAO [122]. Tumor-associated neutrophils (TANs) are neutrophils with both pro- and antitumor activities that are attracted by tumor-released chemokines. TGF-β, an immune-suppressive cytokine, regulates the TAN; in the absence of TGF-β, TANs help cytotoxic CD8+ T-cell responses and anticancer responses, but in the presence of TGF-β, they promote tumor development. TANs can also generate additional factors that promote tumor development, angiogenesis, and metastasis, including arginase 1, ROI, cathepsins, MMPs, and pro-angiogenic cytokines.

Because neutrophils have few mitochondria, they are highly committed to aerobic glycolysis and PPP as the primary energy metabolism during rest. In an activated state, neutrophils perform a variety of important functions, including degranulation, phagocytosis (in both major metabolic pathways, glycolysis), NET formation (in both major metabolic pathways, glycolysis), ROS production (PPP and glutaminolysis), and chemotaxis/migration (glycolysis, mitochondrial metabolism), as illustrated in Fig. 7.7. Furthermore, the PPP pathway produces NADPH, which

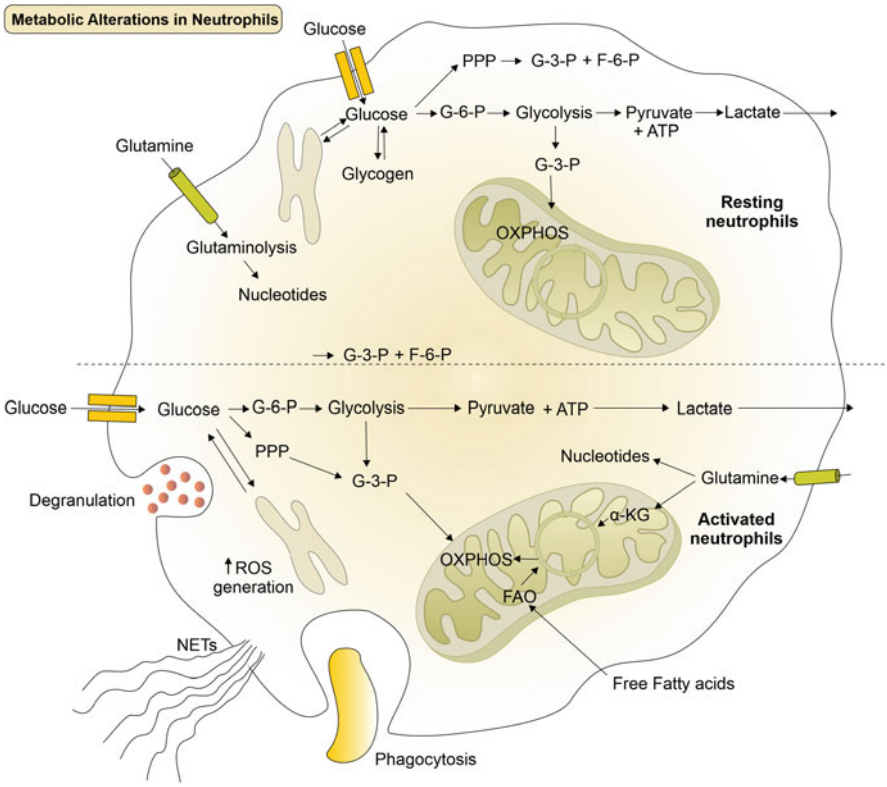

Fig. 7.7 Metabolic alteration in resting and activated neutrophils. The metabolic pathway in resting (upper panel) and activated neutrophils (lower panel); the activated neutrophils needed more energy and generated more reactive oxygen species (ROS)

acts as a cofactor for the crucial enzyme NADPH oxidase, which is involved in neutrophil microbicidal activities. Another essential role of neutrophils in the formation of neutrophil extracellular traps is a combination of DNA, histones, and antimicrobial peptides that capture and destroy bacteria (NETs). The uptake of glucose, glycolysis, and a metabolic shift toward PPP are all required to create NETs. Furthermore, neutrophils are commonly associated with antitumoral activities such as direct tumor cell killing and antigen presentation, which boosts cytotoxic T lymphocyte-mediated antitumor immunity. As a result, two primary neutrophil subpopulations in the TME have been actively researched: tumor-associated neutrophils (TANs) and myeloid-derived suppressor cells (MDSCs), which are the most studied neutrophil-like cell population in cancer progression [121, 123].

Neutrophils, like macrophages (M1 for antitumor, M2 for pro-tumor), are classified as N1 (antitumor/anti-inflammatory) and N2 (pro-tumor/pro-inflammatory) [124]. Even though N1/N2 neutrophils are phenotypically different, there is no

current marker to distinguish them in the TME. N2 TANs, or pro-inflammatory/pro-tumor neutrophils (N2), are a kind of neutrophils that promotes inflammation and tumor development. TANs are characterized by increased ROS release, which promotes cancer development in a variety of ways. Because increased ROS stabilizes HIF1, it increases the synthesis of VEGF and MIF, which are crucial in cancer development and chemotherapy resistance [125].

N2 TANs are currently thought to be a viable therapeutic target since they promote cancer spread to distant tissues. At the moment, nothing is known regarding the energy metabolism of TANs. NETs play an important role in the sequestration of circulating cancer cells and the promotion of metastasis [126]. These findings show that a metabolic difference in neutrophils, which regulates activities such as NETs, may contribute to tumor development. More research is critically needed to completely understand neutrophil activity in cancer and their control by metabolic changes, which affect cancer treatment.

7.9 NK/NKT

Natural killer (NK) and natural killer T (NKT) cells play an essential role in cancer cell growth regulation by secreting antitumor cytokines such as IFN-γ and cytotoxic chemicals such as perforin/granzymes. Mouse NK cells primarily employ oxidative phosphorylation [127]. However, when IL-15 was continuously stimulated, it demonstrated increased glycolytic activity [128]. Thus, glycolysis's primary job in inactivated NK cells is to control cytotoxic NK cell markers such as granzyme B and IFN-γ, which are important actors in anticancer responses.

7.10 Summary and Outlook for the Future

Altered metabolism is a hallmark of cancer progression; mapping it is not a simple process. Cancer cell metabolic rewiring promotes its growth, survival, proliferation, resistance, and long-term maintenance. We gathered together some elements from immune cells and the TME metabolism part of the cancer hallmark. In conclusion, the function of every immune and other cells that appear in the TME is supported by metabolism. Immunometabolic pathways provide a crucial determinant of myeloid and T cell's efficient fate and control their quantitative, qualitative, and fitness program, ultimately maintaining tumor immunity. Seeing the significant influence of immune cell functions in encouraging and repressing various cancer progression, altering immune cell metabolic states shows an ample possibility of becoming novel therapeutic targets against cancer. Overall, we concluded that when immune cells are metabolically fit, they can destroy cancer. Conversely, immune cells are suppressed in cancer's metabolic hijack, as depicted in Fig. 7.8. There is, therefore, a novel treatment strategy based on the current evidence. In addition, metabolic targeting, along with approved blocks of immunological control points, can be a unique approach to facilitating long-term recovery or therapy for cancer.

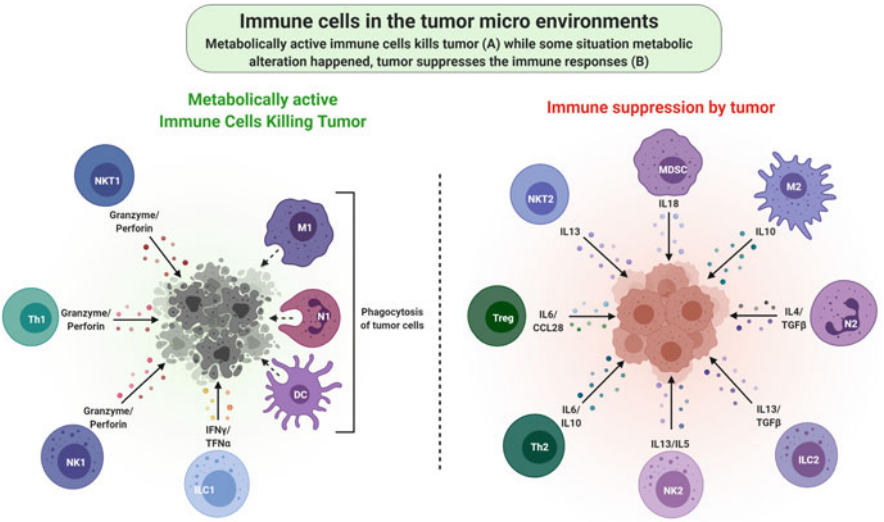

Fig. 7.8 Immune cells are metabolically active and inactive in tumor microenvironments. In general, metabolically active immune cells destroy tumor cells (**a**), but when there is a metabolic change, the tumor inhibits immune responses (**b**)

Acknowledgments Figure prepared by using Biorender and AI.

Author Contributions R.S. conceived and authored the chapter's first draft and provided little information. The chapter was critically reviewed, and an abbreviation list was created by A.V. and C.R. Most numbers of figures were supplied by U.A. All authors read and approved the final draft of the chapter.

Conflict of Interest The authors declare no conflict of interest.

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