



Lipid Metabolism in Tumor-Infiltrating T Cells

10

Shangwen He, Ting Cai, Juanjuan Yuan,
Xiaojun Zheng, and Wei Yang

Abstract

T cells recognize “foreign” antigens and induce durable humoral and cellular immune responses, which are indispensable for defending pathogens, as well as maintaining the integrity and homeostasis of tissues and organs. T cells are the major immune cell population in the tumor microenvironment which play a critical role in the antitumor immune response and cancer immune surveillance. Defective immune response of tumor-infiltrating T cells is the main cause of cancer immune evasion. The antitumor response of T cells is affected by multiple factors in the tumor microenvironment, including immunosuppressive cells, immune inhibitory cytokines, tumor-derived suppressive signals like PD-L1, immunogenicity of tumor cells, as well as metabolic factors like hypoxia and nutrient deprivation. Abundant studies in past decades have proved the metabolic regulations of the immune response of T cells and the tumor-infiltrating T cells. In this chapter, we will discuss the regulations of the antitumor response of tumor-infiltrating T cells by lipid

metabolism, which is one of the main components of metabolic regulation.

Keywords

Tumor-infiltrating T cell · Metabolism · Lipid
Tumor microenvironment · Fatty acid

10.1 Tumor-Infiltrating T Lymphocytes

The degree of T cell infiltration varies across tumor types. Tumors mainly fall into two categories, “hot” and “cold”, which is based on the presence of immune cells in them. Hot tumors have a higher density of tumor-infiltrating lymphocytes (TILs), while cold tumors are defined by the absence of TILs [1, 2]. Moreover, TILs can be further divided into multiple subsets with distinctive functions. Yost et al. have collected 33,106 TILs from the tumor samples of 11 patients with advanced basal or squamous cell carcinoma. By using droplet-based 5' single-cell RNA-sequencing and T cell receptor (TCR)-sequencing libraries, they successfully identified nine distinct T cell clusters in the specimens. The CD4⁺ clusters included regulatory T cells (Treg) cells, follicular helper T (T_{fh}) cells, and T helper 17 cells (Th17) cells. CD8⁺ clusters included naive T cells, memory T cells (T_{mem}), effector memory T cells, activated cells, chronically acti-

S. He · T. Cai · J. Yuan · X. Zheng · W. Yang (✉)
Department of Pathology, School of Basic Medical
Sciences, Southern Medical University,
Guangzhou, China
e-mail: yanglab@smu.edu.cn

vated/exhausted cells, and intermediate exhausted/activated cells [3]. Here, we will review the current understanding of lipid metabolism of the different subsets of tumor-infiltrating T cells.

10.2 Roles of Lipid Metabolism in Tumor-Infiltrating T Cells

10.2.1 CD8⁺ T Cell

CD8⁺ T cells are one of the most important subpopulations of tumor-infiltrating T cells, including cytotoxic effector T cells (T_{eff}) and memory T cells, which both have distinct programs of lipid metabolism. Hypoxia in the tumor microenvironment (TME) induces the metabolic switch from oxidative phosphorylation to glycolysis, which consumes more carbohydrates otherwise lipids. In contrast, the differentiation and function of memory CD8⁺ T cells are mainly relying on fatty acid oxidation and oxidative phosphorylation (OXPHOS) in mitochondria to produce energy [4].

10.2.1.1 CD8⁺ Effector T Cell

Upon tumor antigen engagement, naive T cells are activated and differentiate into effector cells. Quiescent T cells primarily metabolize pyruvate and fatty acids (FAs) via OXPHOS in the mitochondria to produce energy (ATP). Antigen recognition by the TCR and the ligation of co-stimulatory receptors like CD28 and ICOS activate downstream signal pathways, such as phosphatidylinositol-3-OH kinase (PI3K)-protein kinase B (Akt) which correlates with metabolic regulation [5, 6]. After T cell activation, phosphorylation of Akt increases glycolysis and elevates mammalian target of rapamycin (mTOR) signaling, which is central to protein translation regulation. Therefore, the PI3K-mTOR signaling pathway has been suggested to be a regulator of the metabolism of effector T cells, which in turn shuttles carbon derived from glucose into intermediates for the synthesis of lipid and protein [7–9].

As a key metabolism regulator, mTORC1 upregulates fatty acid synthesis (FAS) by regulating the activity of sterol regulatory element-binding proteins (SREBPs) [10]. SREBP1 and SREBP2 are master transcriptional regulators of genes involved in de novo lipid and sterol biosynthesis, which are essential for meeting the heightened lipid requirements during the transition from quiescent to activated state [11]. The inactive precursors of SREBPs reside in the endoplasmic reticulum (ER), where they interact with the sterol cleavage activating protein (SCAP). The SREBP/SCAP complex is processed by proteases in the Golgi to release the N-terminal of the SREBP protein. The activated SREBP then translocates to the nucleus to regulate the expression of genes, which encode the key enzymes of fatty acid and cholesterol biosynthesis, through binding to the promoter that contains sterol regulatory elements (SRE) and E box sequences [12]. Once released from the Golgi, the active form of SREBP1 is susceptible to proteasomal degradation [13]. A recent study found that overexpression of an activated version of Akt led to a rapamycin-sensitive increase in the processed form of SREBP1. Akt induces expression of a number of lipogenic genes, including ATP citrate lyase (ACLY) and fatty acid synthetase (FASN). ACLY converts cytosolic citrate into acetyl-CoA and oxaloacetate, supplying the essential metabolites for lipid biosynthesis. FASN catalyzes the condensation of acetyl-CoA and malonyl-CoA to generate long-chain fatty acids [12, 13].

CD8⁺ T_{eff} cells also adopt lipid catabolism to preserve viability in the tumor microenvironment. For instance, CD8⁺ tumor-infiltrating T cells enhance catabolism of FAs through peroxisome proliferator-activated receptor- α (PPAR- α) signaling. This metabolic switch can partially support the effector function of CD8⁺ TILs in tumor microenvironment in mice model. Further studies use ¹³C₁₆-palmitate to study the change in metabolic pathways. Isotope labeling shows that many amino acids and metabolites in tricarboxylic acid cycle (TCA cycle) including acetyl-CoA are synthesized with FA-derived carbons. Tumor-infiltrating CD8⁺ T cells are also charac-

terized by increased transcription of PPAR- α , which leads to subsequent FA uptake, triglyceride turnover, and peroxisomal and mitochondrial FA catabolism. The same trend is seen in the downstream molecules of PPAR- α , such as acetylcarnitine, palmitoylcarnitine and the ketone body 3-hydroxybutyrate [14]. Bezafibrate, an agonist of PPAR-gamma coactivator 1 α (PGC-1 α)/PPARs axis, could upregulate the expression of PGC-1 α and attenuate tumor progression on hyporesponsive LLC xenograft models. Bezafibrate treatment could improve the survival and functional capacity of tumor-infiltrating cytotoxic T lymphocytes (CTLs). Also, the transcription of PGC-1 α , a gene that regulates mitochondrial biogenesis, was found to be enhanced in tumor-infiltrating T cells. Moreover, the expression of genes related to fatty acid oxidation (FAO), like PGC-1 α , carnitine palmitoyltransferase 1a (CPT1a) and LCAD, was significantly upregulated after treatment with bezafibrate within tumors. Taken together, these findings suggest that bezafibrate can activate PGC-1 α /PPAR and regulate FAO of tumor-infiltrating CTLs to achieve better antitumor efficacy [15].

However, tumor microenvironment may induce the transition of metabolic program of the effector CD8⁺ T cells, mainly the mitochondrial metabolism, to induce hyporesponsiveness of effector CD8⁺ T cells to tumor antigens. The transferred antigen-specific effector CD8⁺ T cells in the TME showed a progression loss of PGC1 α , which is induced by chronic Akt signaling by persistent antigen stimulation in TME [16]. As PGC1 α regulates mitochondrial replication, repression of this protein promotes the loss of mitochondrial mass and eventually mitochondrial function. Metabolic reprogramming of T cells through enforced PGC1 α expression may rescue mitochondrial function and induce superior anti-tumor responses characterized by increased cytokine production and tumor control [17].

10.2.1.2 CD8⁺ Memory T Cells

When encountering stimulatory antigens, the naïve or quiescent CD8⁺ T cells are activated and undergo clonal expansion to boost the population

of antigen-specific T cells. However, after pathogen or antigen clearance, most of the antigen-specific T cells undergo programmed cell death. Only a small fraction of them survive and become long-lived memory T cells [18]. The memory T cells can be divided into two main subtypes: the central memory T cells (T_{cm}) and the effector memory T cells (T_{em}). These cells can be further categorized by more distinctive surface markers. There are many novel populations of T_{mem} cells that were discovered over the years, like tissue-resident memory T (T_{rm}) cells and stem memory T cells (T_{scm}) [19, 20]. In a seminal study, van der Windt et al. have demonstrated that memory T cells engage in lipid metabolism to support survival, which correlates with the longevity of T_{mem} cells. In particular, fatty acid oxidation provides a protective metabolic advantage for the survival of memory T cells so that they can be activated and initiate an immune response more quickly [21, 22]. Unlike CD8⁺ effector T cells or quiescent naïve CD8⁺ T cells, CD8⁺ memory T cells maintained substantial spare respiratory capacity (SRC, a measure of mitochondrial reserve in a cell to produce energy) in their mitochondria, which is important for long-term cellular survival and function [23]. Enhanced SRC in CD8⁺ memory T cells is associated with mitochondrial biogenesis and expression of CPT1a, a protein that is involved in the utilization of fatty acids in the mitochondria. It is suggested that FAO regulated SRC and CD8⁺ memory T cell development [22, 24]. Also, it can't be ignored that at the early stage of T cell activation, the co-stimulatory receptor CD28 upregulates CPT1a to support the generation of T_{mem} through the remodeling of mitochondrial and development of spare respiratory capacity [25].

In terms of the source of FA for FAO, rather than acquiring extracellular FA directly, memory T cells use extracellular glucose to support FAO and OXPHOS. This phenomenon indicates that lipids need to be synthesized to satisfy the need for FAO. It is found that CD8⁺ effector T cells acquire more long-chain FA (LCFA) from their surroundings than CD8⁺ memory T cells. As O'Sullivan et al. found non-accumulation of lipid droplets in T_{mem} cells, it is speculated that

cellular FAs were converted to triacylglycerol (TAG) in ER. Then the TAG would directly undergo lysosomal hydrolase lysosome acid lipase (LAL)-mediated lipolysis to generate FA for FAO [26]. Under optimal conditions, lipolysis is not the necessary metabolic event for T cells. However, upon nutrient deprivation, lipolysis could provide a large amount of energy to T cells in the tumor microenvironment. Lipolytic machinery in T_{mem} cells differs from those in adipocytes, which involves critical enzymes like adipose triglyceride lipase (ATGL) and hormone-sensitive lipase (HSL) [27]. More evidences have also displayed that the transcription factor forkhead homeobox type protein O1 (FoxO1) promotes T_{mem} differentiation by increasing lipolysis [26]. Processes of lipolysis or autophagy can degrade lipids not only to fuel OXPHOS but also for the generation of lipid signaling molecules, such as lipid ligands that activate the PPAR pathway [28, 29]. This futile metabolic cycling of FAS and FAO, which has been documented in muscle and adipose tissue, confers lymphocytes the advantage of extremely rapid activation [27].

The importance of fatty acid metabolism in $CD8^+$ memory T cells can also be found in mice that lack tumor necrosis factor receptor-associated factor 6 (TRAF6), which shows compromised $CD8^+$ T_{mem} generation but normal activation and expansion of $CD8^+$ T_{eff} . TRAF6 mainly stimulates AMP-activated protein kinase (AMPK) and inhibits mTOR signaling in $CD8^+$ T cells, thereby increasing fatty acid oxidation [28]. AMPK is a cellular energy sensor and controller of FAO; it inhibits acetyl-CoA carboxylase 2 (ACC2) by phosphorylation, thus promoting the oxidation of long-chain fatty acid. These studies suggested that FAO is instrumental in the generation of $CD8^+$ memory T cells. Without FAO, the differentiation of T_{mem} cells will be hindered [30]. However, Raud et al. noticed that at high concentration (40–200 μM), etomoxir, a widely used inhibitor of long-chain fatty acid oxidation by CPT1a, may have an off-target effect as affecting the abundance of TCA cycle intermediates [31]. Thus, it reminds us that attention should be paid in the concentration of chemicals used in metabolic research.

T_{mem} cells also obtain lipids to sustain FAO in the long term, though in a relatively smaller amount compared with $CD8^+$ T_{mem} cells [26]. Consistent with recent findings, IL-7 induces the expression of aquaporin 9 (AQP9), which transports glycerol into the cells. Elevated expression of AQP9 leads to glycerol uptake and lipogenesis, sustaining the level of TAG in memory $CD8^+$ T cells. Exogenous glycerol serves as potential fuel sources for the synthesis of triacylglycerides and ATP and for the activation of mTORC1 and expression of c-Myc [32].

The difference of mitochondria between T_{mem} and T_{eff} cells may also be the mechanism underlying the distinct lipid metabolism of T_{mem} . T_{eff} cells have morphologically distinctive mitochondria, which are scattered in the cytoplasm, while the mitochondria of T_{mem} cells maintain densely packed and transform into fused tubes. Through the study of *Opal* floxed mouse model, fusion protein *Opal* is found to be instrumental in T_{mem} generation after infection but not to T_{eff} cells [33]. Inducing mitochondrial fusion in T_{eff} cells makes it more similar to T_{mem} cells, improving their anti-tumor function. Also, $CD8^+$ T_{mem} cells have more mitochondrial mass. Therefore, when $CD8^+$ T_{mem} cells differentiate into secondary T_{eff} cells, they have higher levels of ATP than primary effector T cells and develop robust immune response [21]. It is also suggested that the difference in cristae morphology between T_{mem} and T_{eff} is linked to their metabolism adaption. Fusion of mitochondria in T_{mem} cells remodels the electron transport chain (ETC) complex to favor OXPHOS and FAO. In contrast, fission of mitochondria in T_{eff} cells leads to ETC inefficiency, increasing aerobic glycolysis [33, 34].

The tissue-resident $CD8^+$ memory T cells (T_{rm}) are a recently identified subpopulation of memory $CD8^+$ T cells that do not circulate in the blood and are primarily inhabited in nonlymphoid tissues, such as the skin and intestine. The infiltration of T_{rm} leads to better clinical outcomes in various types of human cancers, including lung cancer. T_{rm} cells appear to play a role in tumor-specific T cell response [35]. Elevated level of lipid in the external environment of T_{rm} cells greatly influences their metabolism. Several

metabolism-related surface molecules like low-density lipoprotein receptor (LDLR), ApoE, scavenger receptor CD36, and fatty acid binding proteins (FABP) 4 and 5 are highly expressed on intestinal T_{m} cells to transfer lipids from the extracellular space to cytosol. FABPs regulate fatty acid influx and transfer free fatty acid (FFA) to mitochondria, providing the substrate for the FAO. Furthermore, FABP4 reduces cholesterol ester accumulation via inhibition of PPAR γ pathways. Both FABP4 and FABP5 have a critical role in the maintenance, longevity and function of $CD8^+$ T_{m} cells [36]. All the evidence suggest that T_{m} have specialized lipid metabolism when compared with traditional T_{mem} [37–39].

10.2.2 $CD4^+$ T Cells

The proper differentiation of $CD4^+$ T cells into effector cells and suppressive cells provides a balance between protective immune response and excessive inflammation. $CD4^+$ T cells differentiate into effector T cells, i.e., Th1, Th2 and Th17, or inducible regulatory T cell (Treg), providing protection against a wide range of pathogens and “nonself” cells, including tumor cells [40].

The differentiation of $CD4^+$ T cells is finely tuned by a complex network including cytokine signaling, transcription factors, as well as metabolic programs. The differentiation of $CD4^+$ T cells to T_{eff} and Treg cells shows distinct metabolic programs. Th1, Th2 and Th17 cells are highly glycolytic, while Treg cells favor enhanced lipid oxidation rates [41]. Changes in metabolic program as enhanced glycolysis or lipid oxidation can affect the differentiation of $CD4^+$ T cells. In a recent study, Michalek et al. found the cytokine production of Th1, Th2 and Th17 is inhibited by promoting lipid oxidation with the addition of exogenous fatty acid during initial T cell activation. Instead, Treg cells have slightly increased expression of lineage-specific transcription factors Foxp3. Exogenous FA reduced the number of viable Th1, Th2 and Th17 cytokine production cells. The compromised function of $CD4^+$ T cells cannot be res-

cued by adding cytokines like IL-12, IL-4, or TGF- β , respectively. This might be explained by the different metabolic programs in $CD4^+$ T cell subsets; T_{eff} cells mainly utilize glucose as energy source, while Treg cells more favor lipid as energy source [41].

The cellular fatty acid level also controls the differentiation of Th17 and Treg cells. Cellular fatty acids are mainly from de novo synthesis and exogenous uptake. Inhibition of ACC1 not only blocks de novo fatty acid synthesis but also interferes with Th17 differentiation. Th17 cells rely on ACC1 for the production of phospholipids, a critical component of cellular membranes. Meanwhile Treg cells use exogenous fatty acids for phospholipid supply. Evidence suggests that in the tumor tissue, where most of the fatty acids are consumed by tumor cells, exogenous fatty acid deficiency impedes Treg response and promotes Th17 response [4, 7, 42].

AMPK/mTOR signaling axis have been previously demonstrated to regulate the metabolism and differentiation of $CD4^+$ T cells into T_{eff} or Treg cells. T cell activation increases glycolysis and reduces FAO with mTOR signaling [41, 43]. Inhibition of mTOR signaling results in elevated oxidation of fatty acid [44] and promotes Treg differentiation. In the presence of TGF- β , rapamycin resulted in an elevated level of Treg cells. Further study showed that etomoxir, a CPT1a inhibitor, reduced rapamycin induction of Treg cells. Upon mTOR inhibition, the differentiation of Treg subset requires lipid oxidation to support increased energy requirement [41]. As illustrated in mTOR-deficient mice, lack of mTOR severely impairs the differentiation of effector T cell subsets. Both natural and inducible Treg cells exhibited elevated levels of AMPK phosphorylation and activation compared with T_{eff} and naive $CD4^+$ T cells [45, 46]. Metformin is the activator of AMPK, the administration of which leads to increased FAO and increased Treg cells [30]. Together, co-treatment of $CD4^+$ T cells with rapamycin and metformin to inhibit mTOR and activate AMPK results in increased lipid oxidation [41].

Non-alcoholic fatty liver disease (NAFLD) is an important risk factor for predicting hepatocel-

lular carcinoma (HCC), which is characterized by the accumulation of lipid. It is found that increased fat deposition in the liver may induce selective apoptosis of CD4⁺ T cells. Lipid-laden hepatocytes release linoleic acid (C18:2), which is taken up by CD4⁺ T cells and upregulates CPT1a expression [47]. By feeding mice with a diet rich in linoleic acid, the same phenomenon can be seen in CD4⁺ T cells inhabited in the liver. Additionally, it has been demonstrated that the level of PPAR- α mRNA increases in human hepatocellular carcinoma. Studies also show PPAR- α , together with PGC-1, directly upregulate the transcription of CPT1a. There is no difference in the uptake of linoleic acid between CD4⁺ and CD8⁺ T cells. Nevertheless, the upregulation of CPT1a increases the transportation of linoleic acid into mitochondria, disrupting electron transport chain. Leakage of electron increased reactive oxygen species (ROS) led to cell death of CD4⁺ T cells. Taken together, release of linoleic acid leads to the loss of CD4⁺ T cells that facilitate tumorigenesis. Therefore, inhibiting PPAR- α may reduce the expression of CPT1a and subsequent CD4⁺ T cell apoptosis to prevent HCC induced by NAFLD [48, 49].

10.2.2.1 CD4⁺ Regulatory T Cells

Accumulating evidence has revealed that regulatory T cell is an important part of TME in various types of cancers, such as skin, pancreas, breast and ovarian tumors in both humans and mice [50]. In contrast to the activated CD4⁺ effector T cells, nutrient-restrictive TME does not seem to impair the functions of Treg cells. As previously reported, regulatory T cell favors FAO and has a heightened level of AMPK [24]. Consistent with previous findings, inhibition of fatty acid transport and oxidation, using sulfo-*N*-succinimidyl oleate (SSO) and etomoxir, results in a significant loss of Treg cells. Apart from the decrease of number, treatment with etomoxir and SSO also significantly suppresses the expression of markers of Treg cells, like Granzyme B, CD39 and NRP1 [51]. These results suggest that free FA may suppress immune response within tumors.

The increase in the production of FA by cancer cells and cancer-associated adipocytes makes lip-

ids the available fuel for Treg cells in the TME. The differentiation and survival of Treg cells are supported by FA uptake and catabolism [7]. In a mouse tumor model, Pacella et al. stained tumor-infiltrating Treg cells with BODIPY, a fluorescent dye that enables the detection of lipids and other lipophilic compounds. It showed that increased intracellular lipid content accumulated in tumor-infiltrating Treg cells. It seems like the Treg cells rely on FAS, rather than uptake of fatty acid, to build up the lipid storage, which is similar to T_{mem}. Recent studies found that tumor-infiltrating Treg cells prefer to use glycolysis to fuel fatty acid synthesis. Gene set enrichment analysis showed that genes associated with glycolysis and lipid biosynthesis have heightened expression in tumor-infiltrating Treg cells extracted from liver cancer [52].

Other bioactive lipids, such as steroids, sphingolipids and fat-soluble vitamins, can also affect the function of Treg cells [50, 53–55]. Sphingosine 1-phosphate (S1P) is an important regulator of many biological processes. Both the number of tumor-infiltrating Treg cells and the expression of sphingosine 1-phosphate receptor 1 (S1P1, one of the five G protein-coupled receptors of S1P) were substantially enhanced in bladder cancer tissues. There is a strong and positive correlation between the expression of S1P1 and the number of tumor-infiltrating Treg cells. S1p1 induced the secretion of TGF- β and IL-10 and promoted the generation of cancer-associated inducible Treg (iTreg) and recruitment of natural regulatory T cells (nTreg). Survival analysis showed that increased levels of both S1P1 and Treg cells are linked to shorter overall survival and poor prognosis in bladder cancer patients [56, 57].

10.2.2.2 CD4⁺ Th17 Cells

CD4⁺ T helper 17 cells are a subset of the pro-inflammatory T cells which are characterized by RAR-related orphan receptor gamma (ROR γ). ROR γ is the master transcriptional regulator of Th17 cells. Th17 cells exhibit great diversity in the roles they play in the immune system, most prominently serving as a controversial double-edged sword in cancer. For one thing, Th17 cells are responsible for impairing tumor surveillance,

and inducing immunosuppression and tumor growth. For another, they can also mediate protective antitumor immunity through recruiting immune cells into tumors, which shows great advantage for cancer immunotherapy like adoptive cell transfer (ACT) [58].

As a regulator of lipid metabolism, CD5L is found to be the switch that controls the transition between pathogenic and nonpathogenic Th17 cells [59]. Pathogenic Th17 cells express more pro-inflammatory genes, while nonpathogenic Th17 cells mainly express immunosuppressive genes [60]. CD5L is primarily expressed in nonpathogenic Th17 cells. And the knockdown of CD5L skewed Th17 cells phenotype toward disease-inducing Th17 cells by regulating the lipidome of Th17 cells. As a result of CD5L deletion, the lipid profile of Th17 cells changes remarkably, increasing the level of cholesterol ester and the ratio of saturated fatty acid (SFA). CD5L restricts the synthesis of cholesterol, which has been linked to the production of endogenous ROR γ t ligand. Moreover, CD5L manipulated the binding of ROR γ to the promoter of different genes by altering the composition of lipids in the cell. SFA increased ROR γ binding to *Il17* and *Il10* gene locus, while polyunsaturated fatty acid promoted ROR γ binding to *Il10* CNS-9 locus. Therefore, CD5L regulated the expression of IL-17, IL-23, and IL-10. Also, CD5L controlled the cholesterol and long-chain fatty acid biosynthetic pathways, subsequently affecting the transcriptional activity of ROR γ and pathogenicity of Th17 cells [61].

In mice fed with a high-fat diet (HFD), Th17 cells showed significant upregulation of genes in the lipid metabolic pathways in gene ontology analysis. HFD induces the expression of genes related to FAS, including *Acaca*. *ACC1* is encoded by *Acaca*, which is found to control the differentiation of Th17 cells. The differentiation of Th17 cells was significantly declined when *ACC1* was inhibited by 5-(tetradecyloxy)-2-furoic acid. It was found that *ACC1* modulated the specific binding of ROR γ t to control the functions of ROR γ t in Th17 cells [62].

Interestingly, liver X receptors (LXR) suppress Th17 differentiation. LXR ligands nega-

tively regulate mouse [44] and human [46] Th17 differentiation. This is in part due to the LXR promoting the expression of sterol regulatory element binding protein 1 (SREBP1). SREBP1 is subsequently recruited to the E-box element of the *Il17* promoter. Aryl hydrocarbon receptor (AHR) also binds to the promoter of *Il17*, enhancing Th17 polarization [46]. SREBP1 interferes with the binding of AHR and *Il17*. It appears that LXR mediates the suppression of Th17 cells through the interference with AHR [63, 64].

The crosstalk between the gut microbiome and bile acid (BA) has emerged as a critical regulator of T cell function in the digestive tract. Primary bile acids are derivatives of cholesterol synthesized by the hepatocyte, and secondary bile acids are converted by microbes from primary bile acids. According to recent studies, gut microbiota has high prognostic value in predicting the risk and progression of gastrointestinal cancers [65, 66]. MDR1 (ABCB1) is widely recognized to be responsible for the drug resistance to chemotherapy. MDR1 is also widely expressed in intestinal T_{eff} cells. Bile acid could be toxic to T cells at high concentrations. In order to adapt to the environment of the intestine, T_{eff} cells inhabiting in the ileum upregulate *Mdr1* to maintain homeostasis in the face of conjugated bile acids. T_{eff} cells lacking *Mdr1* display mucosal dysfunction in the ileum and displayed increased TNF and IFN- γ expression [66].

Lithocholic acid (LCA) is a secondary bile acid formed in the intestine. In a screening of more than 30 kinds of bile acid metabolites, two types of lithocholic acid were found to affect the differentiation of Th17 and Treg cells. 3-OxoLCA inhibited the differentiation of Th17 cells, reducing the expression of IL-17a by interacting with ROR γ t ligand-binding domain. Additional isoalloLCA led to increased production of mitoROS to enhance the differentiation of Treg cells, through increasing *Foxp3* expression [67].

10.2.2.3 CD4⁺ Effector Memory T Cells

Compared with the central memory T cells mentioned above, CD4⁺ effector memory T cells have limited ability to conduct FAS and FAO. In order to simulate the tumor microenvironment, research-

ers have created a special medium, which is chemically defined as serum-free. The activated CD4⁺ T cells grown in optimal glucose have a large number of lipid droplets. When subsequently placed in low-glucose conditions, lipid droplets are consumed as fuel for CD4⁺ T cells. However, things are a little different for T_{em} cells, which do not upregulate FAS in optimal glucose concentration and possess much fewer lipid droplets. Also, inhibition of fatty acid synthesis and uptake produced little or no change in T_{em} expansion [26]. It suggests that the expansion and function of T_{em} are largely independent of fatty acid metabolism. In low glucose, T_{em} cells could convert some of the glutamine imported from the medium into fatty acids. Using citrate labeled with four heavy carbon isotopes to examine how glutamine is utilized, it is discovered that a very low level of glutamine is routed to FAS in effector memory T cells [68].

Apart from the ability to expand, Ecker et al. noticed a strong correlation between the effector activity of T_{em} cells and fatty acid metabolism. Upon glucose deprivation, naive T cells and T_{em} cells increased FAS, which resulted in a drop of IFN- γ secretion. If the FAS of naive T cells was inhibited, naive T cells dramatically upregulated IFN- γ . As mentioned above, the lipid metabolism of T_{em} cells remained largely unchanged when they were facing glucose starvation. At the same time, T_{em} cells maintained a relatively high level of IFN- γ , which further illustrated the limited ability of T_{em} cells to metabolize fatty acid [68].

10.2.3 Exhausted T Cells

T cells usually drift into exhausted status in the tumor, which is represented by a group of T cells with impaired function and sustained expression of inhibitory receptors [69, 70]. For instance, the expansion ability of CD8⁺ T cells was severely impaired because of chronic infection [70]. The dysfunction of exhausted T cells is related to the co-expression of multiple inhibitory receptors, including cytotoxic T lymphocyte antigen 4 (CTLA-4), lymphocyte activation gene 3 (LAG-3), T cell immunoglobulin and

mucin-containing gene 3 (Tim-3) and programmed death-1 (PD-1). A good example is that the CD8⁺ TILs isolated from diet-induced obese (DIO) mice express more PD-1, Tim-3, and Lag3 [71]. The upregulation of these molecules also regulates the lipid metabolism of exhausted T cells (T_{ex}) in the tumor [72, 73].

PD-1 expression is one of the characteristics of T cell exhaustion; its signaling favored the oxidation of fatty acid for T cells while inhibiting the transport and utilization of amino acid and glucose in vivo. Similar to the long-lived CD8⁺ T_{mem}, T cells with PD-1 ligation had a substantial mitochondrial reserve to produce more energy, as shown by higher level of spare respiratory capacity. To be more specific, PD-1 inhibited PI3K activity, thereby inducing the expression of CPT1a [74]. Moreover, the level of FASN was greatly increased since the stimulation of PD-1. Therefore, T cells have altered lipid metabolism after incubation with PD-1, which is characterized by elevated lipolysis and FAO [75].

Meanwhile, PD-1 can promote the differentiation of Treg cells, which suppress T_{eff} functions [5, 72, 76]. PGC-1 α expression is repressed by PD-1 through PI3K-Akt and mTOR pathways on the initial stage of the infection of the *lymphocytic choriomeningitis virus* (LCMV), leading to the mitochondrial depolarization. PGC-1 α expression was enhanced in the absence of PD-1, and retroviral expression of PGC-1 could partly rescue the mitochondrial function of T_{eff} [29].

Tnfrsf9 (4-1BB or CD137) is expressed after T cell activation, which stimulates cell differentiation and survival [77]. LAG-3 functions as an inhibitory receptor. In humans, tumor-infiltrating CD8⁺ T cells separated from melanoma co-expressed 4-1BB and LAG-3 [78]. Activating 4-1BB with agonistic anti-4-1BB antibody demonstrated potent antitumor effects in murine tumor models [79]. The stimulation of 4-1BB increased the metabolism of glucose and fatty acids, which were important for cell proliferation. The signaling pathway liver kinase B1 (LKB1)-AMPK-ACC was activated by anti-4-1BB treatment, which supported the energy need of T cells [80].

10.2.4 $\gamma\delta$ T Cells

$\gamma\delta$ T cells are a unique subset of T cells, which are usually regarded as innate immune cells. $\gamma\delta$ T cells have been reported to play a controversial role in different types of cancer [81]. In humans, the majority of $\gamma\delta$ T cells express the V γ 9V δ 2⁺ TCR [82]. $\gamma\delta$ T cells can recognize and respond to the presentation of lipid antigens [83], cancer cell-derived antigens [84] and mevalonate metabolites in tumor cells [85, 86]. Non-peptide antigens like phosphoantigens (such as isopentenyl pyrophosphate, IPP), which are derivatives of the metabolites of bacterial isoprenoid biosynthesis or the mevalonate pathway, can selectively activate V γ 9V δ 2⁺ T cells [87]. V γ 9V δ 2⁺ T cells are also activated by accumulated phosphorylated mevalonate metabolites due to dysregulated mevalonate pathway in tumor cells [86].

LDL cholesterol has been identified as a risk factor for breast cancer proliferation and invasion [88]. Rodrigues et al. showed that upon activation, up to 30% of V γ 9V δ 2⁺ T cells expressed LDLR to internalize LDL cholesterol. Although about 30% V γ 9V δ 2⁺ T cells express LDLR upon activation in tumor microenvironment, the accumulation of cholesterol inhibits activation and antitumor function of V γ 9V δ 2⁺ T cells, including the expression of IFN- γ . As shown in a tumor-bearing mouse model, LDL-cholesterol compromised the capacity of V γ 9V δ 2⁺ T cells to control the growth of breast cancer [89].

27-hydroxycholesterol (27HC) is oxysterol, which serves as an endogenous estrogen receptor. 27HC has been found to be a predictive marker of breast cancer progression [90]. 27HC could promote metastasis in several models of mammary cancer. Inhibition of CYP27A1, a rate-limiting enzyme in 27-hydroxycholesterol biosynthesis, led to less metastasis by decreasing 27HC. In the presence of polymorphonuclear neutrophils (PMNs), 27HC stimulated the proliferation of $\gamma\delta$ T cells. Therefore, more PMN and $\gamma\delta$ T cells infiltrated in tumors, facilitating further recruitment and creating positive feedback. Also, PMN and $\gamma\delta$ T cells suppressed the tumor-infiltrating CD8⁺ T cells within tumors and metastatic lesions [85].

10.3 Cholesterol Metabolism of Tumor-Infiltrating T Cells

Cholesterol is a key component of the cell membrane and a precursor for steroid hormone [91, 92]. Cholesterol metabolism is mainly regulated by two transcription factors: one is SREBPs, which induce the expression of proteins that promote cholesterol uptake and synthesis, such as LDLR and hydroxymethylglutaryl coenzyme A reductase (HMGCR), and the other one is LXRs, which control the efflux of cholesterol. LXRs regulate the synthesis of ATP-binding cassette transporter ABCA1, ABCG1, and inducible degrader of LDLR (Idol). LXRs inhibit the LDLR pathway through transcriptional induction of Idol, which triggers the ubiquitination and subsequent degradation of LDLR [93].

It is widely known that the activation and expansion of T cells require increased uptake and de novo synthesis of cholesterol for membrane formation. Therefore, cholesterol efflux pathways can limit cell expansion [94]. Bensinger et al. reported that the activation of T cell induced the expression of oxysterol-metabolizing enzyme sulfotransferase family 2b member 1 (Sult2b1) to suppress LXR pathway [95]. Oxidized cholesterol derivatives (oxysterols) can activate LXRs [96], and Sult2b1 promoted the sulfation of oxysterols, thus reducing the available ligand of LXR. For further study, they used radioactive isotope to label the substrate to detect cell division. T cells with LXR β knockdown displayed higher division speed compared with wild-type T cells. A similar phenomenon can be found in lymphocytes lacking LXR β expression that showed increased homeostatic proliferation [64]. As discussed above, LXR depended on ABCG1 to regulate sterol trafficking. In order to suppress cell proliferation, LXR β stimulated ABCG1 and subsequently reduced cholesterol level in T cells. Therefore, Sult2b1-LXR-ABCG1 axis plays an important role in the proliferation of T cells by manipulating cellular cholesterol metabolism.

As mentioned above, LDLR mediates cholesterol uptake of tumor-infiltrating T cells [97]. In the tumor microenvironment, T cells increased the capacity of cholesterol uptake. Surprisingly,

an elevated level of cholesterol in tumor-infiltrating CD8⁺ T cells was linked to the expression of inhibitory receptors, such as PD-1, 2B4, TIM-3, and LAG-3 [98]. Ma et al. treated CD8⁺ T cells with different concentrations of cholesterol and supernatant of tumor culture. Consistent with previous findings, cholesterol induced immune checkpoint expression in a dose-dependent manner. Addition of cholesterol increased ER stress and disrupted lipid metabolism in T cells. In response to that, T cells upregulated the transcription of XBP1, an ER stress sensor, and that of PD-1 and 2B4. XBP1 was also a transcription factor, binding to the promoter of *pdccl1* and *CD244*. Overexpression of XBP1 led to a higher level of PD-1, 2B4, TIM-3, and LAG-3. So reasonably, inhibiting XBP1 restored the antitumor activity of CD8⁺ T cells, so was reducing cholesterol [99].

CD8⁺ T cells that are skewed toward the phenotype of producing IL-9 (Tc9) have been recognized as a target for adoptive cancer immunotherapy [100]. The mechanism underlying the antitumor function of Tc9 cells may be the low concentration of cholesterol in Tc9 compared with Tc1. As already noted, Tc9 cells had considerably lower expression of genes involved in cholesterol synthesis, such as *Hmgcr*. However, Tc9 cells had an active expression of cholesterol efflux genes like *Abcg1* and *Abca1*. The expression of IL-9 was important for Tc9 cells to maintain the antitumor activity. Cholesterol in Tc9 cells significantly affected IL-9 expression and the antitumor function. Further studies revealed that all oxysterols, except for 22(*S*)-hydroxycholesterol, inhibited IL-9 expression by activation and sumoylation of LXR. LXR sumoylation inhibited the binding of p65, a transcriptional factor, to *Il9* promoter. In summary, cholesterol adversely affected the antitumor function of Tc9 cells through modulating IL-9 [101].

However, increasing the membrane cholesterol level could augment the antitumor function of CD8⁺ tumor-infiltrating T cells. ACAT1 and ACAT2 are key enzymes of cholesterol esterification. Inhibition of ACAT1 and ACAT2 or ACAT1 alone in T cells resulted in enhanced

effector function, characterized by increased cytotoxicity. Further studies conducted in genetically engineered mice showed similar results in CD8⁺ but not CD4⁺ T cells, which may be owing to different metabolic programs. In the melanoma model, ACAT1-deficient CD8⁺ tumor-infiltrating T cells generated potent antitumor activity. This was due to the increase of the plasma membrane cholesterol level of CD8⁺ T cells, which causes enhanced TCR clustering and signaling as well as a more efficient formation of the immunological synapse [102, 103]. Therefore, the inhibition of ACAT1 strengthened the antitumor function of CD8⁺ T cells through elevated membrane cholesterol content [104].

10.4 Oxygen Deprivation in Tumor Affects Lipid Metabolism in Tumor-Infiltrating T Cells

Recent studies suggested that the nutrient-restrictive TME dampens lymphocyte's viability and regulates their differentiation. Hypoxic areas are common within solid tumors, as angiogenesis often lags behind the rapid expansion of tumors. The average oxygen level in tumors ranges from 0.5 to 5%, which is lower than that in most healthy organs. Worse still, lack of O₂ prevents T cells from producing energy through OXPHOS [8]. As a consequence, some T cells like Treg cells increase lipid metabolism.

Hypoxia-inducible factors (HIF-1 and HIF-2) are key transcriptional factors induced by hypoxic pressure. The expression of HIF-1 increased exponentially at low oxygen levels [105, 106]. HIF-1 and HIF-2 have been reported to mediate hypoxic responses, driving angiogenesis, glycolysis and tumor invasion [72].

Recently, some studies have demonstrated that HIF-1 α served as a metabolic checkpoint to regulate the differentiation of CD4⁺ T cells. Although differentiated from the same precursor, naive CD4⁺ T cells, Th17 and Treg cells expressed distinct transcriptional regulators (ROR γ t versus Foxp3, respectively), leading to their opposing functions (pro-inflammatory versus anti-inflam-

matory). The precursor of these T cells co-expresses ROR γ t and Foxp3. Upon activation, molecular cues such as HIF transform these cells until one of them becomes the dominant transcriptional regulator. Foxp3 activates a large bank of genes that mediate the suppressive phenotype of Treg [51, 107]. ROR γ t regulates the development of Th17 cells, which is linked to many autoimmune disorders [108].

In a HIF-1 α knockout mouse model, it is found that the transcription and translation of ROR γ t were upregulated in T cells to promote Th17 differentiation. In addition, HIF-1 α inhibited the differentiation of Treg through degrading Foxp3 protein rather than Foxp3 mRNA [109]. Other studies have also proved that HIF-1 α promoted the differentiation of Th17 cell through affecting their metabolism. Shi et al. found that deficiency in HIF-1 α resulted in the diminished glycolytic activity. It was indicated that HIF-1 α mediated the increase in glycolytic activity, promoting Th17 differentiation [110]. On the other hand, some studies suggest that HIF-1 α may protect Treg cells from glucose deprivation in tumor, as HIF-1 α promotes tumor-infiltrating Treg cells to utilize free fatty acid [24]. Treg cells will utilize lipid metabolism to thrive in glucose deprivation environments, suggesting the metabolic shift may be responsible for their survival within tumors. For example, under a hypoxic situation, the transcription of Foxp3 in naive CD4⁺ T cells upregulated more than tenfold [111]. Miska et al. reveal that the suppressive function of Treg cells is maintained by the lipid uptake and oxidation program under hypoxia. Hif1 α ^{-/-} Treg cells have a higher basal oxygen consumption rate (OCR), maximal OCR, and ATP-linked respiration than wild-type Treg cells after incubation in 1% oxygen. However, there were no significant differences in the composition of the metabolite of freshly sorted Treg cells from both types of mice. It suggested that the metabolic change in Hif1 α ^{-/-} Treg cells was due to the hypoxic environment. Further studies showed that tumor-infiltrating Treg cells dramatically increased the uptake of fatty acid compared with glucose within the glioma environment. HIF-1 α is known to transport intracellular free fatty acid into lipid droplets.

Inhibition of lipid metabolism using etomoxir abolished immunosuppression in Hif1 α ^{-/-} Treg cells. After the injection of Hif1 α ^{-/-} and wild-type Treg cells in tumor-bearing mice, the authors found that the ability of Hif1 α ^{-/-} Treg cells to infiltrate the tumor was ablated. And Hif1 α ^{-/-} tumor-bearing mice had prolonged survival. In conclusion, this suggested that HIF-1 α promotes lipid metabolism in Treg cells, which is significant for its suppressive capability in the tumor [51]. However, there are conflicting views on the impact of HIF on Treg cells. Dang et al. showed that HIF-1 α induced the degradation of Foxp3 protein, inhibiting Treg differentiation, while knockout of HIF-1 α restored the Foxp3 stability [109]. It has been well established that HIF-1 α degrades in an ubiquitin-dependent manner via proline hydroxylation at amino acid positions 402 and 564 by prolyl hydroxylases (PHDs). Researchers co-transfected 293T cells with Foxp3 and a HIF-1 α mutant in which prolines 402 (p402A) and 564 (p564A) were mutated to alanines. In this way, 293T cells expressed HIF-1 α protein that was resistant to posttranslational modification and subsequent degradation. The expression of mutant HIF-1 α was incompetent to induce Foxp3 degradation in 293T cells [107]. Therefore, the normal expression of HIF-1 α in T cells mediated the suppression of the development of Treg through the degradation of Foxp3. Inhibition of protease increased Foxp3 protein stability, suggesting that HIF-1 α mediates the proteasomal degradation of Foxp3 [112].

10.5 Tumor Cell-Derived Lipids

Tumor cells constantly interact with the surrounding environment in order to sustain their survival and growth. Some types of tumors can release large quantities of prostaglandin and other kinds of lipids into TME [113, 114]. These metabolites can greatly influence TME and induce the loss of function in TILs. For example, in a study including 22 breast cancer patients, high levels of unbound free fatty acids (FFAu) are found in cancer tissue. To be more precise, the concentration of FFAu increased to at least

25 nM in most of the samples and exceeded 100 nM in 11 patients. The rise of free fatty acids in plasma membrane decreases membrane fluidity, and disrupts lipid raft and immune synapse formation [114]. The IC_{50} for unbound oleate is 125 ± 30 nM, 200-fold greater than normal plasma levels, which is lower than that in some of the samples. In vitro studies showed that elevated levels of FFA are released from tumor cells within minutes of CTL attack [115, 116]. Another study showed that with the treatment of oleic acid or linoleic acid, proliferation of lymphocytes was enhanced at 25 mM, and inhibited at higher concentrations (75 and 100 mM). Both fatty acids promoted cell death at 200 mM concentration [117].

What's more, cancer cell is not the only source of the FFAu in tumor tissue. In order to promote their own growth, tumor cells function as metabolic parasites to extract energy from tumor stromal cells, such as fibroblasts and adipocytes [118, 119]. Cancer-associated adipocytes exhibited a constant loss of lipid content following the initial crosstalk with tumor cells. FFAs could be released from these adipocytes through lipolysis. Then FFAs were transferred to cancer cells and stored in the lipid droplets as a source of energy. The transfer of lipids from host cells can be seen in both prostate [120] and ovarian cancer [121, 122].

Considering all the negative effects of FFAu, it comes as no surprise that obesity also impairs the function of TILs. To investigate the effect of obesity, mice were divided into two groups. In the control group, mice were fed with a diet containing 10% fat, in contrast to a 60% fat diet in diet-induced obese (DIO) group. Tumor-infiltrating T cells in DIO mice had higher expression of PD-1, causing T cell dysfunction. Consistent with that, the expression of CPT1a was also upregulated in the CD8⁺ T cells of DIO mice. CPT1a was upregulated in early exhausted T cells after infection, regulating fatty acid oxidation [29]. Similar results were observed with 4T1 breast carcinoma cells in DIO mice, demonstrating that the correlation between obesity and T cell dysfunction exists across strains and tumor types [71].

10.5.1 Prostaglandins (PG)

Tumor cell-derived lipid mediators such as prostaglandin also serve to build an immunosuppressive TME. Arachidonic acid, the precursor of prostaglandin (PG), is converted by cyclooxygenase (Cox) enzymes Cox-1 and Cox-2 to prostaglandins, prostacyclins, and thromboxanes. COX-2/PGE₂ can affect tumor progression through interaction with tumor-infiltrating Treg and Th17 cells.

There is a metabolic crosstalk between tumor cells and Treg through tumor-derived prostaglandins. High level of Cox-2 expression is correlated with tumorigenesis and tumor neovascularization in human prostate cancer [123, 124]. Cox-2 expression is enhanced in diverse tumor entities, such as bone, stomach, pancreatic, lung, breast, and colorectal cancers and ductal pancreatic adenocarcinomas (PDAC) [125–128]. Overexpression of Cox-2 in the tumor results in increased production of PGE₂, which in turn increased the suppressive ability of Treg [129]. Furthermore, tumor-derived COX-2/PGE₂ promoted tumor-infiltrating Treg cell activity by inducing the expression of Foxp3 [130]. The accumulation of Foxp3-positive Treg cells led to immune suppression in gastric tumors. Moreover, elevated Foxp3 expression in Treg cells was linked to the advanced stage of gastric cancer [131].

These tumor-infiltrating Treg also suppressed the antitumor function of CD8 $\alpha\beta$ T cells, as shown by the decrease of viability in CD8⁺ T cells [128]. Moreover, in response to the cytotoxic activity of $\gamma\delta$ T cells, PDAC cells released PGE₂, which binds to prostaglandin E₂ and E₄ receptors on the $\gamma\delta$ T cells. PGE₂ inhibited $\gamma\delta$ T cells by cyclic adenosine monophosphate (cAMP)-mediated protein kinase [132–135]. Through the PGE₂-cAMP pathway, PGE₂ also induced the expression of Tim-3, which is an inhibitory receptor of exhausted T cells [136]. Also, PGE₂ has been found to stimulate the differentiation of Treg from naive CD4⁺ T cells [130]. Therefore, inhibiting the production of PGE₂ abrogated the suppression of tumor-infiltrating Treg and decreased the size of tumor.

Additionally, Treg cells are found to be effective producers of PGE₂ themselves [137, 138]. iTreg cells generated in PGE₂-secreting tumor also released PGE₂. These iTreg cells were strongly immunosuppressive and produced higher levels of PGE₂ and adenosine [139].

Th17 cells have been recognized to cause chronic inflammation in the tumor microenvironment. Qian et al. found that Th17 cells were increased in the tumor tissues of mammary gland tumor-bearing mice. IL-23 has two subunits, p19 and p40, which are important for the formation and maintenance of Th17 cells. Data indicated that tumor-secreted PGE₂ enhanced the expression of IL-23. PGE₂ is bound to the cAMP-response element in the p19 gene promoter, promoting p19 gene transcription and therefore the increase of Th17 cells [140].

10.5.2 Polyunsaturated Fatty Acids

Apart from prostaglandin, lipid-containing food like fish oil can also affect the lipid metabolism of TILs. Intake of fish oil, which is rich in marine ω -3 polyunsaturated fatty acids (PUFAs), including eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), and docosapentaenoic acid (DPA), modulates T cell activity in the TME. PUFAs can alter the immune system through multiple ways, for example, by changing membrane lipid composition and signaling protein posttranslational lipidation like palmitoylation, myristoylation and prenylation. In addition, dietary DHA blunted the production of intracellular second messengers, like diacylglycerol and ceramide [141].

Omega-3 polyunsaturated fatty acid supplementation can modify the immune response and mammary carcinogenesis in murine models. In the control group, mice were fed on a diet with 11% of corn oil (CO) compared with the experimental diet containing 10% of menhaden fish oil (FO) and 1% of corn oil. FO significantly reduced the incidence and multiplicity of tumors in HER-2/neu mice. In comparison, FO-fed PyMT mice did not show any significant difference in the incidence and multiplicity of tumors.

Similarly, lymphocytes in FO-fed HER-2/neu mice had greater ability to infiltrate the tumor, but not in FO-fed PyMT mice. The antitumor effect that fish oil possessed in HER-2/neu mice can be attributed by the increase in lymphocytes and tumor-infiltrating lymphocytes in FO-fed HER2/neu mice [142].

The positive effect of PUFA on immunity is further supported by human data. Two prospective cohort studies among 121,700 participants in the Nurses' Health Study (NHS) (1984–2010) and 51,529 participants in the Health Professionals Follow-Up Study (1986–2010) showed that a high dose of marine ω -3 polyunsaturated fatty acids was associated with a lower risk of CRC accompanied with high-level Foxp3⁺ T cell infiltration. Intake of marine ω -3 polyunsaturated fatty acids downregulated the suppressive activity of tumor-infiltrating Treg cells [143].

Although EPA and DHA can reduce inflammation associated with obesity and diabetes, high dose of EPA and DHA can be deleterious possibly by generating a hyporesponsive host environment. Treatment of DHA and EPA at high concentration (100 μ M) suppressed the proliferation and cytokine secretion of Th1 cells in a PPAR-dependent pattern. Pre-treatment of PPAR-gamma antagonist restored the function of Th1 cells [144].

10.6 Conclusion

The tumor microenvironment imposes metabolic hurdles, such as hypoxia and glucose deprivation, to the survival and development of tumor-infiltrating lymphocytes, causing metabolic reprogramming in them. In response to these challenges, T cells fine-tune their metabolism to restrict tumor progression. Lipid metabolism is of great importance to regulate the function and cell fate decisions of T cells (Table 10.1).

Quiescent cells like naive T cells utilize fatty acids and amino acids interchangeably for energy. Notably, upon antigen recognition and co-stimulation, naive T cells are activated and differentiate into effector T cells. It is clear that

Table 10.1 Impact of lipids in T cells

Lipid	Cell type	Impact	Molecular mechanism	References
Fatty acid	CD4 ⁺ T cells	Inhibit Th1, Th2, Th17 cell differentiation	Decrease expression of lineage-specific transcription factors	[41]
Linoleic acid	CD4 ⁺ T cells	Induce selective apoptosis of CD4 ⁺ T cells	Increase CPT1a expression, resulting in increased leakage of electron and reactive oxygen species	[47–49]
Conjugated bile acid	CD4 ⁺ T cells	Maintain T cell homeostasis in the ileum	MDR1 alleviates oxidative stress	[66]
Cholesterol	CD8 ⁺ T cells	Increase ER stress, induce T cell exhaustion	Increase XBP1 to upregulate expression of the inhibitory receptors PD-1 and 2B4	[99]
27-Hydroxycholesterol (27-HC)	CD8 ⁺ T cells	Decrease the number of cytotoxic CD8 ⁺ T cells in the tumor	Not fully illustrated	[85]
Fatty acid	Treg cells	Promote Treg differentiation	Increase expression of FoxP3	[41]
Sphingosine 1-phosphate	Treg cells	Promoted generation of cancer-associated inducible Treg and recruitment of natural Treg cells	Induce secretion of TGF- β and IL-10	[57]
5-(tetradecyloxy)-2-Furoic acid	Th17 cells	Inhibit Th17 cell differentiation	Impair the functions of ROR γ t through inhibiting ACC1	[62, 63]
3-OxoLCA	Th17 cells	Inhibit Th17 cell differentiation	Reduce IL-17a expression by interference with ROR γ t ligand-binding domain	[67]
IsoalloLCA	Th17 cells	Promote Th17 cell differentiation	Increase Foxp3 expression	[67]
27-Dihydroxycholesterol (27-OHC)	Th17 cells	Promote Th17 T cell differentiation	Increase IL-17 production dependent on ROR γ t	[63]
27-Hydroxycholesterol (27-HC)	$\gamma\delta$ T cells	Promote $\gamma\delta$ T cell proliferation in the presence of PMNs to enhance tumor metastasis	Not fully illustrated	[85]
22(R)-Hydroxycholesterol	Tc9 cells	Inhibit IL-9 production	Increase activation and sumoylation of LXR to repress IL-9 expression	[101]

each T cell subset utilizes a metabolic program that is adapted to its function. Within the TME, CD8⁺ effector T cells significantly upregulate de novo fatty acid synthesis and uptake. With the contraction of the primary immune response, decreased glycolysis and increased lipid oxidation can drive the enrichment of long-lived CD8⁺ memory cells [41].

CD4⁺ Th1, Th2 and Th17 cells expressed high surface levels of the glucose transporter Glut1 and were highly glycolytic. The addition of lipids

strongly inhibited the production of Th1, Th2 and Th17 cytokines. In comparison, Treg cells have activated AMPK signaling and rely on lipid oxidation as a main energy source [41].

In order to escape immunosurveillance, tumor cells and cancer-associated adipocytes can generate an immunosuppressive environment through hypoxia and lipid secretion. High levels of FFA, prostaglandin and oxygen deprivation in the TME will dampen the antitumor activity of T cells.

Considering the fact that hypoglycemia and hypoxia are common in the TME, lipid metabolism will play a more significant role in TILs. Extensive researches prove a synergistic antitumor effect by combination of targeting lipid metabolism with traditional immunotherapy. Inhibitors of lipid metabolism, like statin and avasimibe, show great potential to reboost the antitumor ability of TILs in animal models. However, further research and clinical trials are needed to clarify the precise mechanism of these drugs. In summary, we believe that targeting lipid metabolic pathways will be a promising therapy with the prospect for further application.

References

- van der Woude LL, et al. Migrating into the tumor: a roadmap for T cells. *Trends Cancer*. 2017;3(11):797–808.
- Palucka AK, Coussens LM. The basis of oncoimmunology. *Cell*. 2016;164(6):1233–47.
- Yost KE, et al. Clonal replacement of tumor-specific T cells following PD-1 blockade. *Nat Med*. 2019;25(8):1251–9.
- Chalmin F, et al. Regulation of T cell antitumor immune response by tumor induced metabolic stress. *Cell Stress*. 2018;3(1):9–18.
- Patsoukis N, et al. Immunometabolic regulations mediated by coinhibitory receptors and their impact on T cell immune responses. *Front Immunol*. 2017;8:330.
- Frauwirth KA, et al. The CD28 signaling pathway regulates glucose metabolism. *Immunity*. 2002;16(6):769–77.
- Lochner M, Berod L, Sparwasser T. Fatty acid metabolism in the regulation of T cell function. *Trends Immunol*. 2015;36(2):81–91.
- Zhang Y, Ertl HC. Starved and asphyxiated: how can CD8(+) T cells within a tumor microenvironment prevent tumor progression. *Front Immunol*. 2016;7:32.
- Jiang S, Yan W. T-cell immunometabolism against cancer. *Cancer Lett*. 2016;382(2):255–8.
- Raud B, et al. Fatty acid metabolism in CD8(+) T cell memory: challenging current concepts. *Immunol Rev*. 2018;283(1):213–31.
- Kidani Y, et al. Sterol regulatory element-binding proteins are essential for the metabolic programming of effector T cells and adaptive immunity. *Nat Immunol*. 2013;14(5):489–99.
- Porstmann T, et al. SREBP activity is regulated by mTORC1 and contributes to Akt-dependent cell growth. *Cell Metab*. 2008;8(3):224–36.
- Duvel K, et al. Activation of a metabolic gene regulatory network downstream of mTOR complex 1. *Mol Cell*. 2010;39(2):171–83.
- Zhang Y, et al. Enhancing CD8(+) T cell fatty acid catabolism within a metabolically challenging tumor microenvironment increases the efficacy of melanoma immunotherapy. *Cancer Cell*. 2017;32(3):377–391.e9.
- Wan H, et al. PGC-1 α activator-induced fatty acid oxidation in tumor-infiltrating CTLs enhances effects of PD-1 blockade therapy in lung cancer. *Tumori*. 2019;106(1):55–63. <https://doi.org/10.1177/0300891619868287>.
- Fernandez-Marcos PJ, Auwerx J. Regulation of PGC-1 α , a nodal regulator of mitochondrial biogenesis. *Am J Clin Nutr*. 2011;93(4):884S–90S.
- Scharping NE, et al. The tumor microenvironment represses T cell mitochondrial biogenesis to drive intratumoral T cell metabolic insufficiency and dysfunction. *Immunity*. 2016;45(2):374–88.
- Cui W, Kaech SM. Generation of effector CD8+ T cells and their conversion to memory T cells. *Immunol Rev*. 2010;236:151–66.
- Enamorado M, et al. Enhanced anti-tumour immunity requires the interplay between resident and circulating memory CD8(+) T cells. *Nat Commun*. 2017;8:16073.
- Steinert EM, et al. Quantifying memory CD8 T cells reveals regionalization of Immunosurveillance. *Cell*. 2015;161(4):737–49.
- van der Windt GJ, et al. CD8 memory T cells have a bioenergetic advantage that underlies their rapid recall ability. *Proc Natl Acad Sci U S A*. 2013;110(35):14336–41.
- van der Windt GJ, et al. Mitochondrial respiratory capacity is a critical regulator of CD8+ T cell memory development. *Immunity*. 2012;36(1):68–78.
- Henson SM, et al. p38 signaling inhibits mTORC1-independent autophagy in senescent human CD8(+) T cells. *J Clin Invest*. 2014;124(9):4004–16.
- Gupta S, Roy A, Dwarakanath BS. Metabolic cooperation and competition in the tumor microenvironment: implications for therapy. *Front Oncol*. 2017;7:68.
- Klein Geltink RI, et al. Mitochondrial priming by CD28. *Cell*. 2017;171(2):385–397.e11.
- O’Sullivan D, et al. Memory CD8(+) T cells use cell-intrinsic lipolysis to support the metabolic programming necessary for development. *Immunity*. 2014;41(1):75–88.
- Zechner R, et al. FAT SIGNALS—lipases and lipolysis in lipid metabolism and signaling. *Cell Metab*. 2012;15(3):279–91.
- Pearce EL, et al. Enhancing CD8 T-cell memory by modulating fatty acid metabolism. *Nature*. 2009;460(7251):103–7.
- Bengsch B, et al. Bioenergetic insufficiencies due to metabolic alterations regulated by the inhibitory receptor PD-1 are an early driver of CD8(+) T cell exhaustion. *Immunity*. 2016;45(2):358–73.

30. Byersdorfer CA. The role of fatty acid oxidation in the metabolic reprogramming of activated t-cells. *Front Immunol.* 2014;5:641.
31. Raud B, et al. Etomoxir actions on regulatory and memory T cells are independent of Cpt1a-mediated fatty acid oxidation. *Cell Metab.* 2018;28(3):504–515.e7.
32. Cui G, et al. IL-7-induced glycerol transport and TAG synthesis promotes memory CD8+ T cell longevity. *Cell.* 2015;161(4):750–61.
33. Patten DA, et al. OPA1-dependent cristae modulation is essential for cellular adaptation to metabolic demand. *EMBO J.* 2014;33(22):2676–91.
34. Buck MD, et al. Mitochondrial dynamics controls T cell fate through metabolic programming. *Cell.* 2016;166(1):63–76.
35. Mami-Chouaib F, et al. Resident memory T cells, critical components in tumor immunology. *J Immunother Cancer.* 2018;6(1):87.
36. Pan Y, et al. Survival of tissue-resident memory T cells requires exogenous lipid uptake and metabolism. *Nature.* 2017;543(7644):252–6.
37. Beura LK, et al. T cells in nonlymphoid tissues give rise to lymph-node-resident memory T cells. *Immunity.* 2018;48(2):327–338.e5.
38. Fahrner AM, et al. Attributes of gammadelta intraepithelial lymphocytes as suggested by their transcriptional profile. *Proc Natl Acad Sci U S A.* 2001;98(18):10261–6.
39. Konjar S, Veldhoen M. Dynamic metabolic state of tissue resident CD8 T cells. *Front Immunol.* 2019;10:1683.
40. Perucha E, et al. The cholesterol biosynthesis pathway regulates IL-10 expression in human Th1 cells. *Nat Commun.* 2019;10(1):498.
41. Michalek RD, et al. Cutting edge: distinct glycolytic and lipid oxidative metabolic programs are essential for effector and regulatory CD4+ T cell subsets. *J Immunol.* 2011;186(6):3299–303.
42. Berod L, et al. De novo fatty acid synthesis controls the fate between regulatory T and T helper 17 cells. *Nat Med.* 2014;20(11):1327–33.
43. Wieman HL, Wofford JA, Rathmell JC. Cytokine stimulation promotes glucose uptake via phosphatidylinositol-3 kinase/Akt regulation of Glut1 activity and trafficking. *Mol Biol Cell.* 2007;18(4):1437–46.
44. Brown NF, et al. The mammalian target of rapamycin regulates lipid metabolism in primary cultures of rat hepatocytes. *Metabolism.* 2007;56(11):1500–7.
45. O'Sullivan D, Pearce EL. Fatty acid synthesis tips the TH17-Treg cell balance. *Nat Med.* 2014;20(11):1235–6.
46. Pollizzi KN, Powell JD. Integrating canonical and metabolic signalling programmes in the regulation of T cell responses. *Nat Rev Immunol.* 2014;14(7):435–46.
47. Ma C, et al. NAFLD causes selective CD4(+) T lymphocyte loss and promotes hepatocarcinogenesis. *Nature.* 2016;531(7593):253–7.
48. Brown ZJ, et al. Carnitine palmitoyltransferase gene upregulation by linoleic acid induces CD4(+) T cell apoptosis promoting HCC development. *Cell Death Dis.* 2018;9(6):620.
49. Sugiura A, Rathmell JC. Metabolic barriers to T cell function in tumors. *J Immunol.* 2018;200(2):400–7.
50. Wang H, Franco F, Ho PC. Metabolic regulation of Tregs in cancer: opportunities for immunotherapy. *Trends Cancer.* 2017;3(8):583–92.
51. Miska J, et al. HIF-1alpha is a metabolic switch between glycolytic-driven migration and oxidative phosphorylation-driven immunosuppression of Tregs in glioblastoma. *Cell Rep.* 2019;27(1):226–237.e4.
52. Pacella I, et al. Fatty acid metabolism complements glycolysis in the selective regulatory T cell expansion during tumor growth. *Proc Natl Acad Sci U S A.* 2018;115(28):E6546–55.
53. Campbell DJ, Koch MA. Phenotypical and functional specialization of FOXP3+ regulatory T cells. *Nat Rev Immunol.* 2011;11(2):119–30.
54. Liu G, et al. The receptor S1P1 overrides regulatory T cell-mediated immune suppression through Akt-mTOR. *Nat Immunol.* 2009;10(7):769–77.
55. Jeffery LE, et al. 1,25-Dihydroxyvitamin D3 and IL-2 combine to inhibit T cell production of inflammatory cytokines and promote development of regulatory T cells expressing CTLA-4 and FoxP3. *J Immunol.* 2009;183(9):5458–67.
56. Blaho VA, et al. HDL-bound sphingosine-1-phosphate restrains lymphopoiesis and neuroinflammation. *Nature.* 2015;523(7560):342–6.
57. Liu YN, et al. Sphingosine 1 phosphate receptor-1 (S1P1) promotes tumor-associated regulatory T cell expansion: leading to poor survival in bladder cancer. *Cell Death Dis.* 2019;10(2):50.
58. Bailey SR, et al. Th17 cells in cancer: the ultimate identity crisis. *Front Immunol.* 2014;5:276.
59. Kurokawa J, et al. Macrophage-derived AIM is endocytosed into adipocytes and decreases lipid droplets via inhibition of fatty acid synthase activity. *Cell Metab.* 2010;11(6):479–92.
60. Wu X, Tian J, Wang S. Insight into non-pathogenic Th17 cells in autoimmune diseases. *Front Immunol.* 2018;9:1112.
61. Wang C, et al. CD5L/AIM regulates lipid biosynthesis and restrains Th17 cell pathogenicity. *Cell.* 2015;163(6):1413–27.
62. Endo Y, et al. Obesity drives Th17 cell differentiation by inducing the lipid metabolic kinase, ACC1. *Cell Rep.* 2015;12(6):1042–55.
63. Soroosh P, et al. Oxysterols are agonist ligands of RORgammat and drive Th17 cell differentiation. *Proc Natl Acad Sci U S A.* 2014;111(33):12163–8.
64. Fessler MB. The intracellular cholesterol landscape: dynamic integrator of the immune response. *Trends Immunol.* 2016;37(12):819–30.
65. Jia W, Xie G, Jia W. Bile acid-microbiota crosstalk in gastrointestinal inflammation and carcinogenesis. *Nat Rev Gastroenterol Hepatol.* 2018;15(2):111–28.

66. Cao W, et al. The xenobiotic transporter Mdr1 enforces T cell homeostasis in the presence of intestinal bile acids. *Immunity*. 2017;47(6):1182–1196.e10.
67. Hang S, et al. Bile acid metabolites control TH17 and Treg cell differentiation. *Nature*. 2019;576:143.
68. Ecker C, et al. Differential reliance on lipid metabolism as a salvage pathway underlies functional differences of T cell subsets in poor nutrient environments. *Cell Rep*. 2018;23(3):741–55.
69. Speiser DE, et al. T cell differentiation in chronic infection and cancer: functional adaptation or exhaustion? *Nat Rev Immunol*. 2014;14(11):768–74.
70. Man K, et al. Transcription factor IRF4 promotes CD8(+) T cell exhaustion and limits the development of memory-like T cells during chronic infection. *Immunity*. 2017;47(6):1129–1141.e5.
71. Wang Z, et al. Paradoxical effects of obesity on T cell function during tumor progression and PD-1 checkpoint blockade. *Nat Med*. 2019;25(1):141–51.
72. Xia A, et al. T cell dysfunction in cancer immunity and immunotherapy. *Front Immunol*. 2019;10:1719.
73. Wherry EJ. T cell exhaustion. *Nat Immunol*. 2011;12(6):492–9.
74. Deberardinis RJ, Lum JJ, Thompson CB. Phosphatidylinositol 3-kinase-dependent modulation of carnitine palmitoyltransferase 1A expression regulates lipid metabolism during hematopoietic cell growth. *J Biol Chem*. 2006;281(49):37372–80.
75. Patsoukis N, et al. PD-1 alters T-cell metabolic reprogramming by inhibiting glycolysis and promoting lipolysis and fatty acid oxidation. *Nat Commun*. 2015;6:6692.
76. Francisco LM, et al. PD-L1 regulates the development, maintenance, and function of induced regulatory T cells. *J Exp Med*. 2009;206(13):3015–29.
77. Eun SY, et al. 4-1BB ligand signaling to T cells limits T cell activation. *J Immunol*. 2015;194(1):134–41.
78. Gros A, et al. PD-1 identifies the patient-specific CD8(+) tumor-reactive repertoire infiltrating human tumors. *J Clin Invest*. 2014;124(5):2246–59.
79. Chester C, Ambulkar S, Kohrt HE. 4-1BB agonism: adding the accelerator to cancer immunotherapy. *Cancer Immunol Immunother*. 2016;65(10):1243–8.
80. Choi BK, et al. 4-1BB signaling activates glucose and fatty acid metabolism to enhance CD8(+) T cell proliferation. *Cell Mol Immunol*. 2017;14(9):748–57.
81. Li L, et al. Microenvironmental oxygen pressure orchestrates an anti- and pro-tumoral gammadelta T cell equilibrium via tumor-derived exosomes. *Oncogene*. 2019;38(15):2830–43.
82. Muto M, et al. Myeloid molecular characteristics of human gammadelta T cells support their acquisition of tumor antigen-presenting capacity. *Cancer Immunol Immunother*. 2015;64(8):941–9.
83. Cheng HY, et al. Increased cholesterol content in gammadelta (gammadelta) T lymphocytes differentially regulates their activation. *PLoS One*. 2013;8(5):e63746.
84. Peng G, et al. Tumor-infiltrating gammadelta T cells suppress T and dendritic cell function via mechanisms controlled by a unique toll-like receptor signaling pathway. *Immunity*. 2007;27(2):334–48.
85. Baek AE, et al. The cholesterol metabolite 27-hydroxycholesterol facilitates breast cancer metastasis through its actions on immune cells. *Nat Commun*. 2017;8(1):864.
86. Gober HJ, et al. Human T cell receptor gammadelta cells recognize endogenous mevalonate metabolites in tumor cells. *J Exp Med*. 2003;197(2):163–8.
87. Thurnher M, Gruenbacher G. T lymphocyte regulation by mevalonate metabolism. *Sci Signal*. 2015;8(370):re4.
88. dos Santos CR, et al. LDL-cholesterol signaling induces breast cancer proliferation and invasion. *Lipids Health Dis*. 2014;13:16.
89. Rodrigues NV, et al. Low-density lipoprotein uptake inhibits the activation and antitumor functions of human Vgamma9Vdelta2 T cells. *Cancer Immunol Res*. 2018;6(4):448–57.
90. Nelson ER, et al. 27-hydroxycholesterol links hypercholesterolemia and breast cancer pathophysiology. *Science*. 2013;342(6162):1094–8.
91. Dessi S, et al. Cholesterol content in tumor tissues is inversely associated with high-density lipoprotein cholesterol in serum in patients with gastrointestinal cancer. *Cancer*. 1994;73(2):253–8.
92. Simons K, Ikonen E. How cells handle cholesterol. *Science*. 2000;290(5497):1721–6.
93. Zelcer N, et al. LXR regulates cholesterol uptake through idole-dependent ubiquitination of the LDL receptor. *Science*. 2009;325(5936):100–4.
94. Glass CK, Saijo K. Immunology: oxysterols hold T cells in check. *Nature*. 2008;455(7209):40–1.
95. Bensinger SJ, et al. LXR signaling couples sterol metabolism to proliferation in the acquired immune response. *Cell*. 2008;134(1):97–111.
96. Janowski BA, et al. An oxysterol signalling pathway mediated by the nuclear receptor LXR alpha. *Nature*. 1996;383(6602):728–31.
97. Luo J, Yang H, Song B-L. Mechanisms and regulation of cholesterol homeostasis. *Nat Rev Mol Cell Biol*. 2019;21(4):225–45.
98. Murai T. Cholesterol lowering: role in cancer prevention and treatment. *Biol Chem*. 2015;396(1):1–11.
99. Ma X, et al. Cholesterol induces CD8(+) T cell exhaustion in the tumor microenvironment. *Cell Metab*. 2019;30(1):143–156.e5.
100. Lu Y, et al. Tumor-specific IL-9-producing CD8+Tc9 cells are superior effector than type-I cytotoxic Tc1 cells for adoptive immunotherapy of cancers. *Proc Natl Acad Sci*. 2014;111(6):2265–70.
101. Ma X, et al. Cholesterol negatively regulates IL-9-producing CD8(+) T cell differentiation and antitumor activity. *J Exp Med*. 2018;215(6):1555–69.
102. Molnár E, et al. Cholesterol and sphingomyelin drive ligand-independent T-cell antigen receptor nanoclustering. *J Biol Chem*. 2012;287(51):42664–74.

103. Zech T, et al. Accumulation of raft lipids in T-cell plasma membrane domains engaged in TCR signaling. *EMBO J*. 2009;28(5):466–76.
104. Yang W, et al. Potentiating the antitumor response of CD8(+) T cells by modulating cholesterol metabolism. *Nature*. 2016;531(7596):651–5.
105. Nakamura H, et al. TCR engagement increases hypoxia-inducible factor-1 alpha protein synthesis via rapamycin-sensitive pathway under hypoxic conditions in human peripheral T cells. *J Immunol*. 2005;174(12):7592–9.
106. Semenza GL. Hypoxia-inducible factors in physiology and medicine. *Cell*. 2012;148(3):399–408.
107. Semenza GL. Hypoxia-inducible factor 1 (HIF-1) pathway. *Sci STKE*. 2007;2007(407):cm8.
108. Zhang Y, et al. ROR nuclear receptors: structures, related diseases, and drug discovery. *Acta Pharmacol Sin*. 2015;36(1):71–87.
109. Dang EV, et al. Control of T(H)17/T(reg) balance by hypoxia-inducible factor 1. *Cell*. 2011;146(5):772–84.
110. Shi LZ, et al. HIF1alpha-dependent glycolytic pathway orchestrates a metabolic checkpoint for the differentiation of TH17 and Treg cells. *J Exp Med*. 2011;208(7):1367–76.
111. Clambey ET, et al. Hypoxia-inducible factor-1 alpha-dependent induction of FoxP3 drives regulatory T-cell abundance and function during inflammatory hypoxia of the mucosa. *Proc Natl Acad Sci U S A*. 2012;109(41):E2784–93.
112. Hsiao HW, et al. Deltex1 antagonizes HIF-1alpha and sustains the stability of regulatory T cells in vivo. *Nat Commun*. 2015;6:6353.
113. Muenst S, et al. The immune system and cancer evasion strategies: therapeutic concepts. *J Intern Med*. 2016;279(6):541–62.
114. Cohen JJ, Blasberg R. Impact of the tumor microenvironment on tumor-infiltrating lymphocytes: focus on breast cancer. *Breast Cancer (Auckl)*. 2017;11:1178223417731565.
115. Kleinfeld AM, Okada C. Free fatty acid release from human breast cancer tissue inhibits cytotoxic T-lymphocyte-mediated killing. *J Lipid Res*. 2005;46(9):1983–90.
116. Richieri GV, Kleinfeld AM. Free fatty acids are produced in and secreted from target cells very early in cytotoxic T lymphocyte-mediated killing. *J Immunol*. 1991;147(8):2809–15.
117. Gorjao R, et al. Regulation of human lymphocyte proliferation by fatty acids. *Cell Biochem Funct*. 2007;25(3):305–15.
118. Martinez-Outschoorn UE, Sotgia F, Lisanti MP. Power surge: supporting cells “fuel” cancer cell mitochondria. *Cell Metab*. 2012;15(1):4–5.
119. Martinez-Outschoorn UE, et al. Energy transfer in “parasitic” cancer metabolism: mitochondria are the powerhouse and Achilles’ heel of tumor cells. *Cell Cycle*. 2011;10(24):4208–16.
120. Gazi E, et al. Direct evidence of lipid translocation between adipocytes and prostate cancer cells with imaging FTIR microspectroscopy. *J Lipid Res*. 2007;48(8):1846–56.
121. Nieman KM, et al. Adipocytes promote ovarian cancer metastasis and provide energy for rapid tumor growth. *Nat Med*. 2011;17(11):1498–503.
122. Wang YY, et al. Mammary adipocytes stimulate breast cancer invasion through metabolic remodeling of tumor cells. *JCI Insight*. 2017;2(4):e87489.
123. Howe LR. Inflammation and breast cancer. Cyclooxygenase/prostaglandin signaling and breast cancer. *Breast Cancer Res*. 2007;9(4):210.
124. Miller AM, Pisa P. Tumor escape mechanisms in prostate cancer. *Cancer Immunol Immunother*. 2007;56(1):81–7.
125. Miao J, et al. Prostaglandin E2 and PD-1 mediated inhibition of antitumor CTL responses in the human tumor microenvironment. *Oncotarget*. 2017;8(52):89802–10.
126. Alzabin S, et al. Hematopoietic progenitor kinase 1 is a critical component of prostaglandin E2-mediated suppression of the anti-tumor immune response. *Cancer Immunol Immunother*. 2010;59(3):419–29.
127. Krysan K, et al. COX-2-dependent stabilization of survivin in non-small cell lung cancer. *FASEB J*. 2004;18(1):206–8.
128. Karavitis J, et al. Regulation of COX2 expression in mouse mammary tumor cells controls bone metastasis and PGE2-induction of regulatory T cell migration. *PLoS One*. 2012;7(9):e46342.
129. Bergmann C, et al. Expansion of human T regulatory type 1 cells in the microenvironment of cyclooxygenase 2 overexpressing head and neck squamous cell carcinoma. *Cancer Res*. 2007;67(18):8865–73.
130. Sharma S, et al. Tumor cyclooxygenase-2/prostaglandin E2-dependent promotion of FOXP3 expression and CD4+ CD25+ T regulatory cell activities in lung cancer. *Cancer Res*. 2005;65(12):5211–20.
131. Yuan XL, et al. Elevated expression of Foxp3 in tumor-infiltrating Treg cells suppresses T-cell proliferation and contributes to gastric cancer progression in a COX-2-dependent manner. *Clin Immunol*. 2010;134(3):277–88.
132. Gonnermann D, et al. Resistance of cyclooxygenase-2 expressing pancreatic ductal adenocarcinoma cells against gammadelta T cell cytotoxicity. *Oncotargets Ther*. 2015;4(3):e988460.
133. Wong JL, et al. Synergistic COX2 induction by IFNgamma and TNFalpha self-limits Type-1 immunity in the human tumor microenvironment. *Cancer Immunol Res*. 2016;4(4):303–11.
134. Gupta RA, Dubois RN. Colorectal cancer prevention and treatment by inhibition of cyclooxygenase-2. *Nat Rev Cancer*. 2001;1(1):11–21.
135. Dannenberg AJ, Subbaramaiah K. Targeting cyclooxygenase-2 in human neoplasia: rationale and promise. *Cancer Cell*. 2003;4(6):431–6.
136. Yun SJ, et al. Regulation of TIM-3 expression in a human T cell line by tumor-conditioned media and

- cyclic AMP-dependent signaling. *Mol Immunol.* 2019;105:224–32.
137. Mandapathil M, et al. Adenosine and prostaglandin E2 cooperate in the suppression of immune responses mediated by adaptive regulatory T cells. *J Biol Chem.* 2010;285(36):27571–80.
138. Mandapathil M, Whiteside TL. Targeting human inducible regulatory T cells (Tr1) in patients with cancer: blocking of adenosine-prostaglandin E(2) cooperation. *Expert Opin Biol Ther.* 2011;11(9):1203–14.
139. Whiteside TL. Regulatory T cell subsets in human cancer: are they regulating for or against tumor progression? *Cancer Immunol Immunother.* 2014;63(1):67–72.
140. Qian X, et al. Increased Th17 cells in the tumor microenvironment is mediated by IL-23 via tumor-secreted prostaglandin E2. *J Immunol.* 2013;190(11):5894–902.
141. Chapkin RS, et al. Immunomodulatory effects of (n-3) fatty acids: putative link to inflammation and colon cancer. *J Nutr.* 2007;137(1 Suppl):200s–4s.
142. Turbitt WJ, et al. Fish oil enhances T cell function and tumor infiltration and is correlated with a cancer prevention effect in HER-2/neu but not PyMT transgenic mice. *Nutr Cancer.* 2015;67(6):965–75.
143. Song M, et al. Marine omega-3 polyunsaturated fatty acid intake and risk of colorectal cancer characterized by tumor-infiltrating T cells. *JAMA Oncol.* 2016;2(9):1197–206.
144. Jaudszus A, et al. Evaluation of suppressive and pro-resolving effects of EPA and DHA in human primary monocytes and T-helper cells. *J Lipid Res.* 2013;54(4):923–35.