METABOLISM OF CANCER CELLS AND IMMUNE CELLS IN THE TUMOR MICROENVIRONMENT

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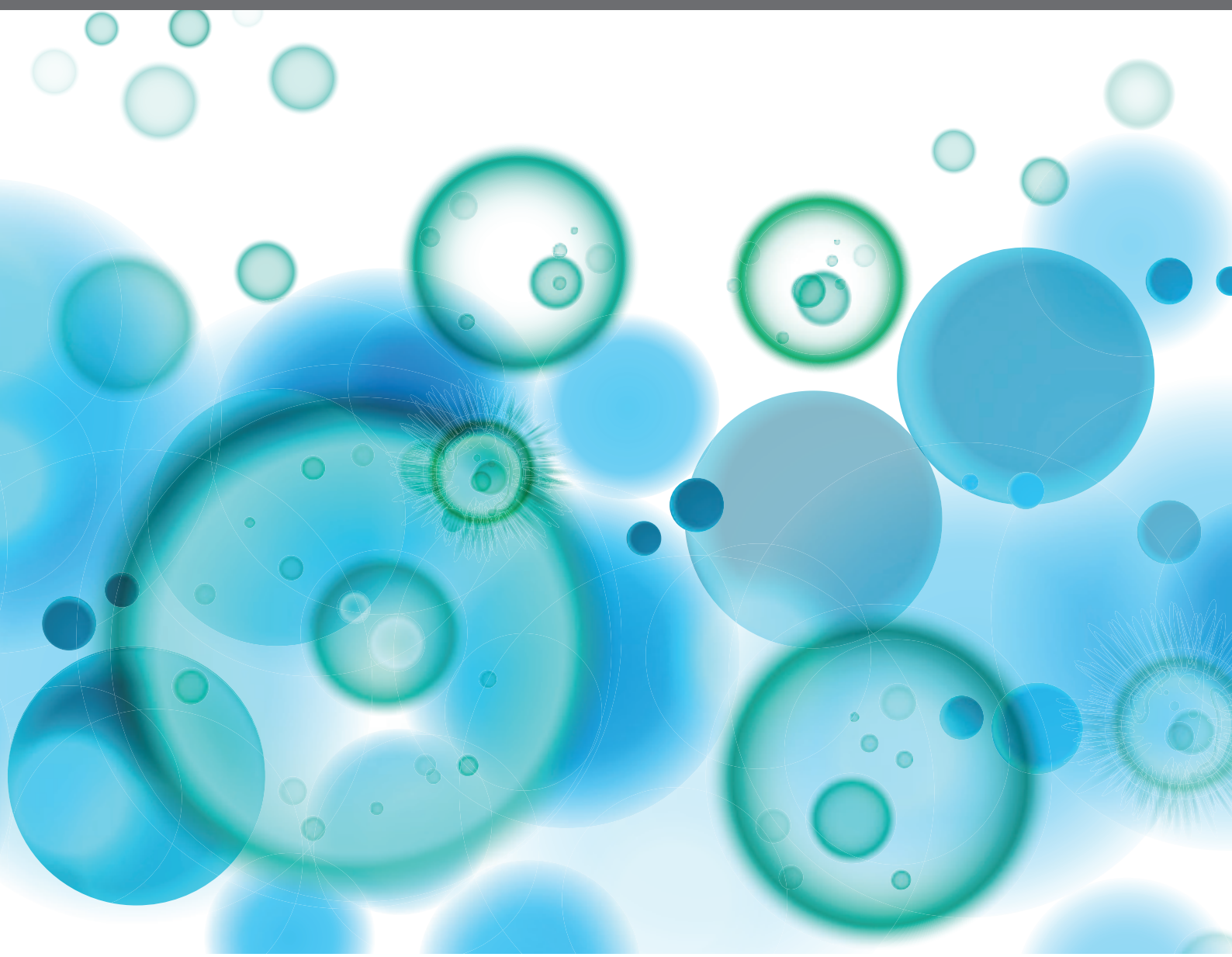

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METABOLISM OF CANCER CELLS AND IMMUNE CELLS IN THE TUMOR MICROENVIRONMENT

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Metabolism of glucose, lipids, amino acids, and nucleotides represents the fundamental capability of host to utilize distinct nutrients and energy to support diverse function of different cell lineages. Cancer cells undergo the Warburg Effect to adapt to the microenvironment composed by stromal cells and immune cells. The crosstalk among cancer cells and immune cells orchestrate tumor progression. In the tumor microenvironment, immune cells also show metabolic reprogramming. For example, naive or memory T cells switch from the oxidation of fatty acids to glycolysis and glutaminolysis after activation; meanwhile massive glucose and glutamine are transported into cells to meet their metabolic demands. Defective glucose or glutamine metabolism impairs the differentiation and expansion of helper T cells.

The molecular pathways that control immune cell metabolism and function are intimately linked. Understanding such metabolic reprogramming of immune cells in the tumor microenvironment could offer new directions in manipulation of peripheral immune responses. Recent findings in immune cell metabolism hold the promising possibilities by metabolic manipulation of immune cells towards clinical therapeutics for treating cancer. This Research Topic includes updated findings and views in the metabolism of cancer cells and immune cells in the tumor microenvironment.

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EDITORIAL
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Editorial: Metabolism of Cancer Cells and Immune Cells in the Tumor Microenvironment

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Keywords: metabolism, immune cells, cancer, tumor microenvironement, immune editing

Editorial on the Research Topic

Metabolism of Cancer Cells and Immune Cells in the Tumor Microenvironment

Immune editing orchestrates the tumor initiation and progression. The crosstalk between tumor cells and immune cells in the tumor microenvironment (TME) manipulates the development of tumors. Although the recent advancement of immunotherapy was encouraging, and countless patients have achieved significant benefits, some patients still do not respond to immunotherapy, due to the complexity and diversity of the TME. Exploring the underlying mechanisms of TME-driven tumorigenesis and progression are essential for developing potential precise approaches for cancer treatment.

Cells require energy to maintain their survival, and various metabolites are also bioactive. It has now been recognized that metabolism regulates the phenotype and biological function of cells. In the TME, tumor cells and immune cells reprogram their metabolic patterns to adapt to the hypoxic, acidic, and low-nutrition microenvironment. For example, tumor cells display enhanced aerobic glycolysis but reduced oxidative phosphorylation (OXPHOS). Macrophages tend to be M2 polarized, exhibit upregulated fatty acid synthesis and β-oxidation. Cytotoxic T lymphocytes show dampened glycolysis but enhanced OXPHOS. Therefore, the metabolic reprogramming of various cells in the tumor microenvironment is bound to be of great significance for tumor immune editing. Understanding the metabolic reprogramming of tumor cells and immune cells will provide a new direction for regulating tumor immunity.

In this context, the goal of this research topic was to bring together a collection of thoughtful papers that review the advancement and prospect of the metabolism in cancer cells and immune cells and to inspire the researchers for future studies on the tumor immunity and metabolism, as well as to provide clues for clinical cancer therapy.

Hypoxia contributes to oncogenes activation and loss of tumor suppressors that constitute major regulators of Warburg effect and many other metabolic pathways such as glutaminolysis. The hypoxia-inducible factors promote angiogenesis via increasing vascular endothelial growth factors and modulate the cell phenotypes in the TME. Sormendi and Wielockx summarized the current knowledge of hypoxia-reprogrammed metabolism during cancer development and the mechanisms in cancer cells and immune cells in the TME. Endothelial cells (ECs) conduit for oxygen and nutrient delivery to tumor tissues. Zecchin et al. discussed how the ECs adapt their metabolism to form vessels in the TME.

Immunity and mitochondria are closely interlinked with each other. The mitochondria are the most important organelles for cell energy metabolism. They regulate activation, differentiation, and

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survival of immune cells, as well as release signals such as mitochondrial DNA (mtDNA) and mitochondrial ROS (mtROS) to regulate the transcription in immune cells. Angajala et al. discussed the underlying mechanism by which mitochondria coordinate to drive distinct immune responses.

Mevalonate metabolism is always fueled by glycolysis. It is a critical pathway for cancer stem cells and immune cells and governs immune surveillance. Gruenbacher and Thurnher discussed how activation and differentiation-induced metabolic reprogramming affects the mevalonate pathway for cholesterol biosynthesis in immune and cancer cells. They concluded that while inhibition of mevalonate metabolism in tumor cells may attenuate growth and proliferation, mevalonate pathway in innate immune cells such as macrophages may contribute to trained immunity.

The aryl hydrocarbon receptor (AhR) is an important cytosolic, ligand-dependent transcription factor and plays critical roles in the initiation, promotion, progression, invasion, and metastasis of cancer. Interestingly, a correlation between AhR and immune system has been recognized and suggested as an immunosuppressive effector. Xue et al. reviewed the role of AhR in tumor immunity and its potential mechanism in the TME.

T cells are major components for anti-tumor immunity. Their dynamic program of metabolism determines the differentiation, activation, and function. Manipulating the reprogramming of T-cell metabolic pathways is a therapeutic approach, in particular, for antitumor immunity. Kouidhi et al. illustrated some potential cell metabolism pathways involved in shaping T lymphocyte function and differentiation. They also demonstrated subsets of T cells have specific metabolic requirements and signaling pathways that contribute to their respective function.

In summary, the eight articles composing this research topic provide insights into key and complementary mechanisms underlying metabolism in cancer cells and immune cells in the TME. This issue will inspire researchers to explore questions on metabolic immunology and be beneficial for developing efficient strategies in clinical cancer therapy.

AUTHOR CONTRIBUTIONS

YL wrote the manuscript. BZ contributed to the discussion.

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REVIEW
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Hypoxia Pathway Proteins As Central Mediators of Metabolism in the Tumor Cells and Their Microenvironment

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Low oxygen tension or hypoxia is a determining factor in the course of many different processes in animals, including when tissue expansion and cellular metabolism result in high oxygen demands that exceed its supply. This is mainly happening when cells actively proliferate and the proliferating mass becomes distant from the blood vessels, such as in growing tumors. Metabolic alterations in response to hypoxia can be triggered in a direct manner, such as the switch from oxidative phosphorylation to glycolysis or inhibition of fatty acid desaturation. However, as the modulated action of hypoxia-inducible factors or the oxygen sensors (prolyl hydroxylase domain-containing enzymes) can also lead to changes in enzyme expression, these metabolic changes can also be indirect. With this review, we want to summarize our current knowledge of the hypoxia-induced changes in metabolism during cancer development, how they are affected in the tumor cells and in the cells of the microenvironment, most prominently in immune cells.

Keywords: oxygen sensors, hypoxia, immunity, hypoxia-inducible factor prolyl hydroxylases, glycolysis, lactate

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INTRODUCTION

Metabolism is the set of chemical processes by which energy homeostasis is maintained, allowing cells to adjust to the needs that the surrounding environment demands. By adjusting their metabolic pathway network, cells are able to adapt to nutrients and deprived oxygen availability, as well as to adequately respond to different cell signals. During the past few years, the importance of the metabolic state of a cell and how this exerts differentiation and functionality during physiological and pathological processes has become evident. Indeed, metabolic reprogramming is considered a hall-mark of cancer progression (1, 2). Recently, new progresses in molecular biology and high-throughput molecular analyses revealed that many of the signaling pathways, which are altered by gene mutations can regulate cell metabolism. However, the oncogenic transformation process not only involves cancer cells, but it also alters their tumor microenvironment (TME), which includes stromal and infiltrating immune cells (3). Although in this context their metabolism has received less attention, they signify a rich cell population in many solid tumors. Moreover, the metabolic changes that these cells endure have been shown to have a great impact on their contribution during tumor development. These metabolic changes not only translate in different cell functionality, but they are also important in establishing a pro-tumoral "metabolite crosstalk." According to this idea, it has been shown that specific excreted metabolites, including lactate (4) are exploited or signal to particular cells. Also the connection between metabolism and signal transduction within

the neoplastic area is known. We will, therefore, summarize the most essential and relevant studies in the field of cancer-related metabolism, highlighting the regulating properties of hypoxia pathway proteins.

Hypoxia Pathway Proteins in Cancer

Solid tumors are characterized by rapid cell growth that is not equally compensated with a functionally effective and efficient vasculature. This poor vessel irrigation leads to a highly heterogeneous tumor mass with variable oxygen pressure and nutrient levels that cancer cells as well as TME cells need to overcome. Vaupel and colleagues recognized that the partial pressure of oxygen (pO_2) within human cancers is significantly lower than in surrounding tissue. This so-called intra-tumoral hypoxia is associated with increased risk of local spread, metastasis, and patient mortality (5). Indeed, a complex pathway exists that regulates the adaptive response to hypoxia. The master regulators of the cellular response to hypoxia constitute a heterodimeric complex formed by a constitutively expressed nuclear HIFβ, and a cytoplasmic oxygen-dependent HIFα (HIF-1α, HIF-2α, and HIF-3α) subunit. Stabilization of HIFα is regulated by a group of oxygen and iron dependent enzymes, known as hypoxia-inducible factor (HIF)-prolyl hydroxylase domain enzymes (PHD1–3). Therefore, under physiological oxygen concentrations PHDs hydroxylate two prolyl residues of HIFα, which allows binding of the Von Hippel–Lindau tumor-suppressor protein, leading to subsequent ubiquitination and proteasomal degradation of this alpha subunit. However, in hypoxia PHDs are much less active, allowing gene transcription regulation by the HIF isoforms, with overlapping, distinct or even opposite roles (6) (**Figure 1**). Since its discovery, regulation of the hypoxia pathway has been strongly related to cancer development. It cannot only modulate survival and proliferation of cancer cells, activation of this pathway can induce angiogenesis, escape from immune-surveillance, epithelial-to-mesenchymal transition, and even distant metastasis (7, 8). Due to the central regulatory role of PHD2 (9) in the hypoxia pathway, several studies have focused on this isoform. In this regard, we were able to demonstrate that loss of PHD2 in tumor cells leads to decreased tumor growth, depending on an anti-proliferative effect of TGFβ activation through matrix metalloproteinases, but not HIF-1α (10, 11). More recently, using a spontaneous breast cancer mouse model, Kuchnio and colleagues showed that PHD2 haplo-deficiency in cancer cells reduce metastasis *via* two mechanisms: (1) by

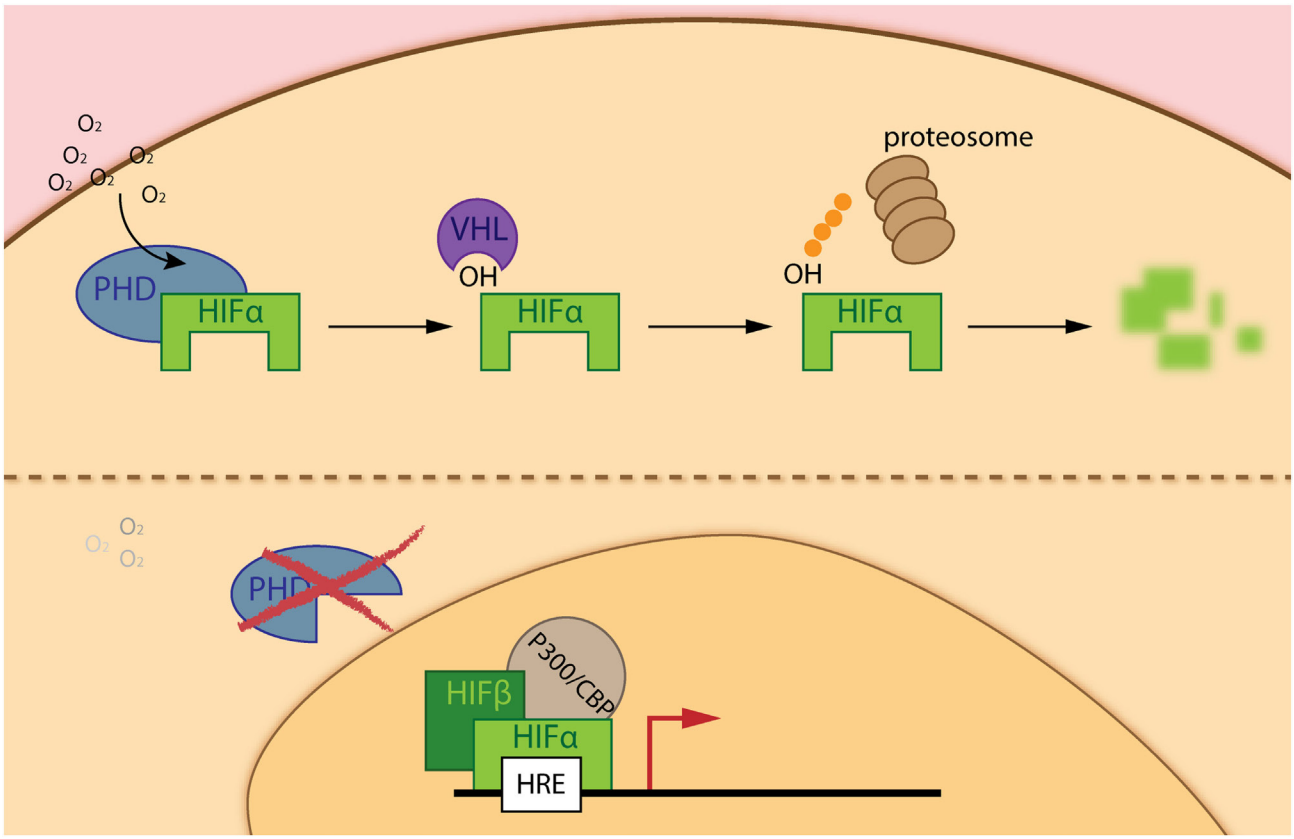

FIGURE 1 | The hypoxia inducible factor (HIF) pathway. Under adequate oxygen pressure, prolyl-hydroxylase domain enzymes (PHDs) hydroxylate two prolyl residues on the α-subunit of the hypoxia-inducible transcription factors (HIF-α). The hydroxylated residues are recognized by the ubiquitin ligase Von Hippel–Lindau, leading to subsequent degradation of HIF-α *via* the proteasome (upper part of the figure). However, during hypoxia (deprived oxygen pressure), PHDs are inactive and HIF-α is able to translocate into the nucleus, interacts with the HIF-β subunit and P300/CBP enabling binding to hypoxia-responsive elements (HRE) in the promotor region of genes implicated in the hypoxia response (lower part of the figure).

decreasing cancer-associated fibroblasts (CAF) activation due to a reduced secretion of TGFβ by cancer cells, matrix production and contraction by CAFs and (2) by improving vessel normalization (12). As mentioned earlier, newly formed vessels are often disorganized, immature, and leaky. Mice heterozygous for PHD2 are protected from distant metastasis due to endothelial normalization in a HIF2-α-dependent manner (13). Branco-Price and colleagues described that deficiency of HIF-1α in the endothelium diminishes NO synthesis, resulting in retarded tumor cell migration and consequent tumor cell metastasis. However, loss of HIF-2α had a reversed effect (14).

The HIF-pathway proteins not only regulate growth and dissemination of cancer cells, but they also control the tumor-associated immune cells (15, 16). Therefore, many different groups have been focusing on what role the hypoxia pathway proteins play in the two major contrasting forces throughout tumor development: (1) anti-tumor defense and (2) suppression by the immune system. Concerning the latter, tumor-associated macrophages (TAMs) or pro-tumoral macrophages help the tumor to grow. Actually, most of the studies performed relate to the role of the HIF-pathway proteins in TAMs during cancer development. The first studies on this were focused on the role of the HIF transcription factors, revealing that loss of HIF-1α in TAMs increases M2 polarization and pro-angiogenic responses. Moreover, these TAMs overexpress HIF-2α, which correlates with poor patient prognosis (17). In line with this, HIF-2α deficiency in macrophages reduces TAM infiltration into hepatocellular carcinoma in mice (18). In addition, in a transgenic mouse model of breast carcinoma development (MMTV-PyMT), Doedens and colleagues demonstrated that targeted deletion of HIF-1α in macrophages leads to reduced breast tumor growth. Indeed, their work strongly proposes a HIF1-α-dependent macrophage-mediated T cell suppression (19). Furthermore, our research group demonstrated that PHD2 deficiency in myeloid and T cells is a pre-requisite to diminish tumor volume due to increased death of cancer cells (20). Nevertheless, Clever et al. recently reported for the first time a clear role for PHDs in regulating T cell anti-tumoral response. In this study, wild-type and PHD1–3 T cell triple knock-out mice showed similar subcutaneous B16 tumor growth, while the triple PHD KO mice were significantly protected from tumor colonization in the lung (21).

Since hypoxia constitutes one of the hallmarks of solid tumors, and oxygen availability has a direct effect on cell metabolism, it is not surprising that numerous authors have described the reciprocal regulation that HIFs exert on metabolic reprogramming of cancer cells and immune response in the TME and *vice versa* (22–25). In this regard, oxygen not only regulates PHD activity directly (6), CO_2 production during mitochondrial respiration through the TCA cycle can also suppress HIF activity in high concentrations. The mechanism behind this process still needs to be clarified, but it seems that acidification inhibits protein synthesis (mTOR inhibition) and HIF1 α is extremely sensitive to protein synthesis (26). In addition, ROS production during oxidative metabolism influences HIF activity (27, 28), as well as accumulation of specific immunometabolites such as α-ketoglutarate (α-KG), fumarate, and succinate (29–32).

Cancer Cell Metabolism

The Warburg effect is found to be one of the most striking metabolic shifts that healthy normal cells undergo during tumorigenesis (33). This effect of aerobic glycolysis, described by Warburg already in 1920s still forms a hot-topic of tumor metabolism nowadays. This process defines that cancer cells predominantly obtain their energy (in terms of ATP production) through the glycolytic pathway rather than the TCA cycle, even in the presence of adequate oxygen levels (33). But why would cancer cells use glycolysis when energy production is inefficient? Despite the low amount of ATP produced by glycolysis (2 ATP molecules per glucose molecule in glycolysis versus 36 molecules of ATP in TCA), the efficiency of this process relies on faster kinetics of glycolysis, producing a comparable amount of ATP by either form of glucose metabolism during the same period of time (34). This also implies that nutrients are conserved for biosynthesis of nucleic acids, lipids, and amino acids to support cell growth, rather than oxidized in mitochondria for maximal ATP production (35–41). Moreover, this high glycolytic rate entails a great lactate excretion, leading to increased TME acidosis, which alters the tumor stroma interface allowing enhanced invasiveness (42, 43). The presence of variable levels of lactate and hypoxia constitutes one of the main reasons for tumor heterogeneity. Indeed, "metabolic symbiosis" among hypoxic and aerobic cells within the tumor mass has been demonstrated. Lisanti and coworkers described "the Reversed Warburg effect" in which CAFs perform aerobic glycolysis and provide cancer cells with metabolites for oxidative phosphorylation (OxPhos) (44, 45) (**Figure 2**). In this pro-tumoral "metabolite crosstalk," lactate produced by hypoxic cells is taken up by aerobic cells, which use it as their principal substrate for OxPhos. Lactate recycling is not new, and is well known from the Cori cycle in the liver (46). Sonveaux et al. showed that human cancer cells cultured under hypoxic conditions convert glucose to lactate and excrete it, while aerobic cancer cells take this lactate back up *via* monocarboxylate transporter 1 (MCT1) and utilize it for OxPhos (4). Another important glycolysis-related enzyme is pyruvate kinase (PK), which catalyzes the final glycolytic reaction. Therefore, reduction of PK activity causes a build-up of glycolytic intermediates that are redirected toward biosynthesis. Elevated expression of the isoform PKM2 has been demonstrated in several types of cancer, including colon, kidney, lung, and breast (47). Several studies have shown that PKM2 directly regulates the Warburg effect, since the knock-out of this enzyme reduces glucose uptake and lactate production, increasing oxygen consumption, and finally reducing tumorigenesis (48–50). In addition to this, Luo et al. reported that hydroxylation of PKM2 by PHD3 allows its binding to HIF-1α, enhancing expression of HIF-1α targeted genes (51). In addition, HIF-1α restricts OXPHOS and regulates the expression of pyruvate dehydrogenase kinase (PDK), an enzyme that phosphorylates and inactivates pyruvate dehydrogenase. The latter limits pyruvate utilization for OxPhos (52). Furthermore, active Akt2 accumulates in the mitochondria during hypoxia and phosphorylates pyruvate dehydrogenase kinase 1 (PDK1) on Thr346 to inactivate the pyruvate dehydrogenase complex (53). Regarding the HIF pathway, cancer cells present frequent activation of the PI3K–mTOR axis, which functions as a nutrient

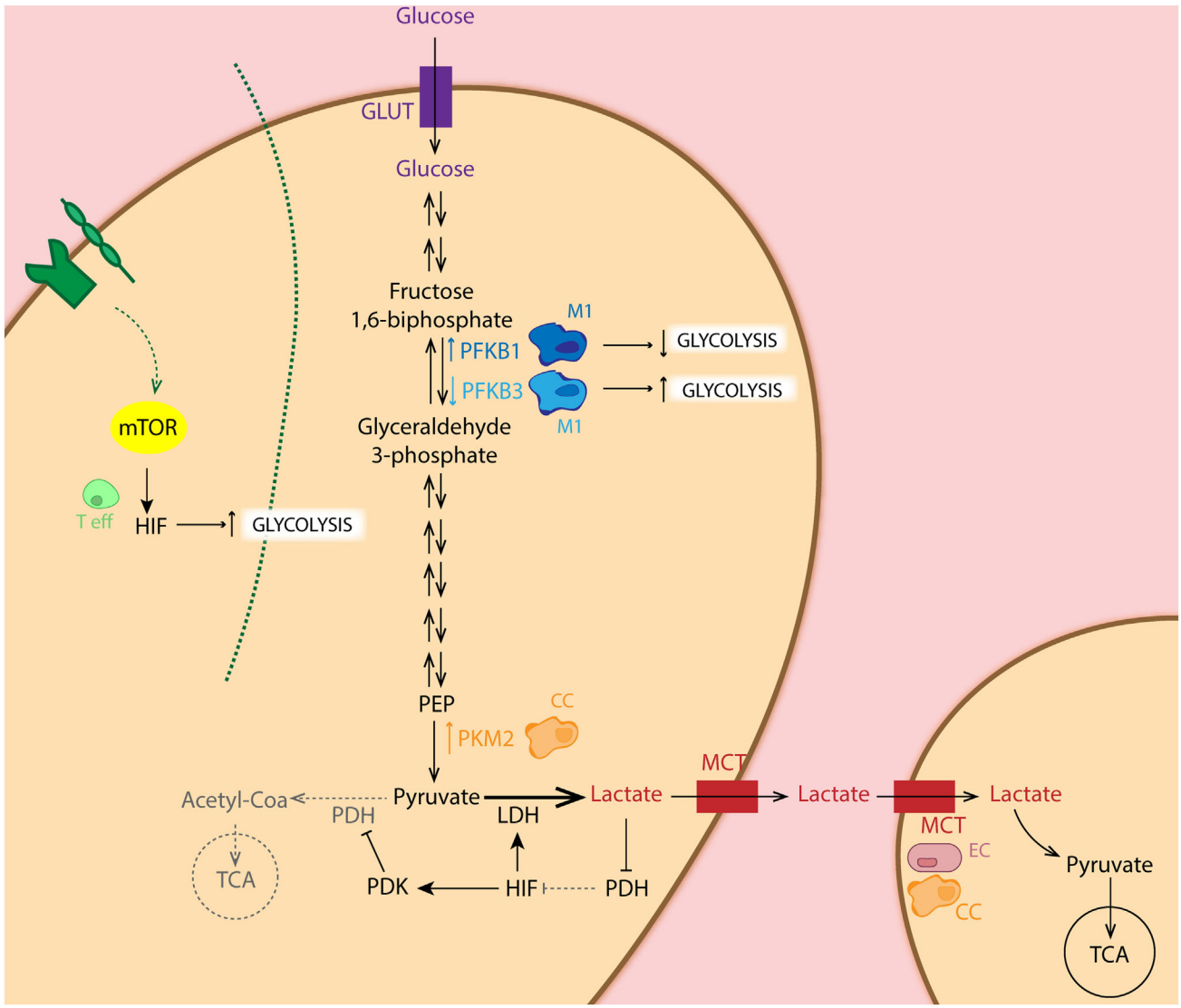

FIGURE 2 | Schematic representation of the glycolytic pathway in different cell types. Metabolic intermediates such as lactate inhibit the PHD function, leading to accumulation of hypoxia-inducible factor (HIF) which regulates expression of several glycolytic enzymes. Importantly, HIF balances glucose metabolism: (1) by inducing expression of LDH, leading to conversion of pyruvate to lactate, (2) as well as dampening the entry of pyruvate in the TCA cycle through inhibition of PDH by pyruvate dehydrogenase kinase (PDK) overexpression. Particularly, cancer cells (CC: orange) show increased expression of PKM2, the limiting rate enzyme of glycolysis, favoring glucose metabolism. CCs as well as endothelial cells (ECs: pink) show increase expression of MCT, facilitating lactate uptake for oxidative phosphorylation. Pro-tumoral M2 macrophages (light blue) present increased expression of phosphofructo-2-kinase/fructose-2,6-biphosphatase 3 (PFKFB3) isoform, enhancing glycolysis in these cells, whereas anti-tumoral M1 (dark blue) express the low activity isoform PFKFB1. Activation of T cells (green) *via* TCR-CD28 leads to enhanced glycolysis essential for their effector functions in an mTOR/HIF-dependent manner.

sensor pathway. mTOR activation favors HIFα activity and promotes tumor angiogenesis. Thus, it has been shown that loss of the mTOR inhibitor TSC2 (tuberous sclerosis complex 2 protein) results in the accumulation of HIF-1α and increased expression VEGF (54). Another study relates mTOR-mediated regulation of HIF-1α to the pathogenesis and increased angiogenesis in chronic myelogenous leukemia (55) (**Figure 2**).

Previous studies have shown that specific metabolites are able to directly regulate the hypoxia pathway. Therefore, loss-of-function mutations of the tumor suppressor genes encoding the succinate dehydrogenase complex and fumarate hydratase lead to the accumulation of succinate or fumarate, resulting in HIF stabilization through inhibition of PHDs (56). Also other intracellular metabolites, such as pyruvate, lactate and oxaloacetate block PHD-mediated inhibition of HIF-1α underlying its prominent basal activity, commonly seen in many highly glycolytic cancer cells. This suggests that enhancement of HIF-1 by glucose metabolites may constitute a feed-forward signaling mechanism involved in malignant progression (57). In addition, isocitrate dehydrogenases 1 and 2 (IDH1 and IDH2) are

frequently mutated in cancer. These enzymes function at the intersection of different processes, including oxygen-sensing signal transduction, cellular defense against oxidative stress, oxidative respiration, and cellular metabolism in lipid synthesis. The mutated forms of IDHs (58) produce 2-HG instead of α-KG essential for PHD function (59). Indeed, it has been shown that 2-HG can either inhibit or activate PHD-driven hydroxylation of HIF in an enantiomer-specific way (60).

Since the discovery of the Warburg effect, mitochondrial dysfunction was designated as a metabolic hallmark of cancer cells. However, earlier studies provided genetic evidence that mitochondrial metabolism is essential for tumorigenesis (61–63). Indeed, cancer cells generate an abundant amount of NADPH in the mitochondria and the cytosol to sustain high antioxidant activity and prevent the build-up of potentially detrimental ROS (64, 65).

Although anaerobic glycolysis is an acclaimed feature of cancer cells, this is not the only metabolic alteration in the transformed cells. In fact, for tumor cells to proliferate, fatty acid (FA) synthesis (for membrane biogenesis) as well as glutaminolysis (for amino acid precursors) has been reported to be affected during tumorigenesis (37, 66, 67). It has been shown that lipid production is critical for cancer cell survival, while the expression of the central lipogenic enzyme fatty acid synthase (FASN) is strongly correlated with cancer progression (68, 69). FAs used for cancer cells during lipogenesis can be endogenously derived from citrate in the TCA cycle, but they can also be seized from exogenous sources. To obtain free FA from circulation, lipoprotein lipase (LPL) hydrolyzes circulating triglycerides. Then, free FAs are imported into the cell *via* the FA translocase CD36. Both proteins LPL and CD36 are widely expressed in breast, liposarcoma, and prostate tumor samples (70) (**Figure 3**). In addition to this, lipid

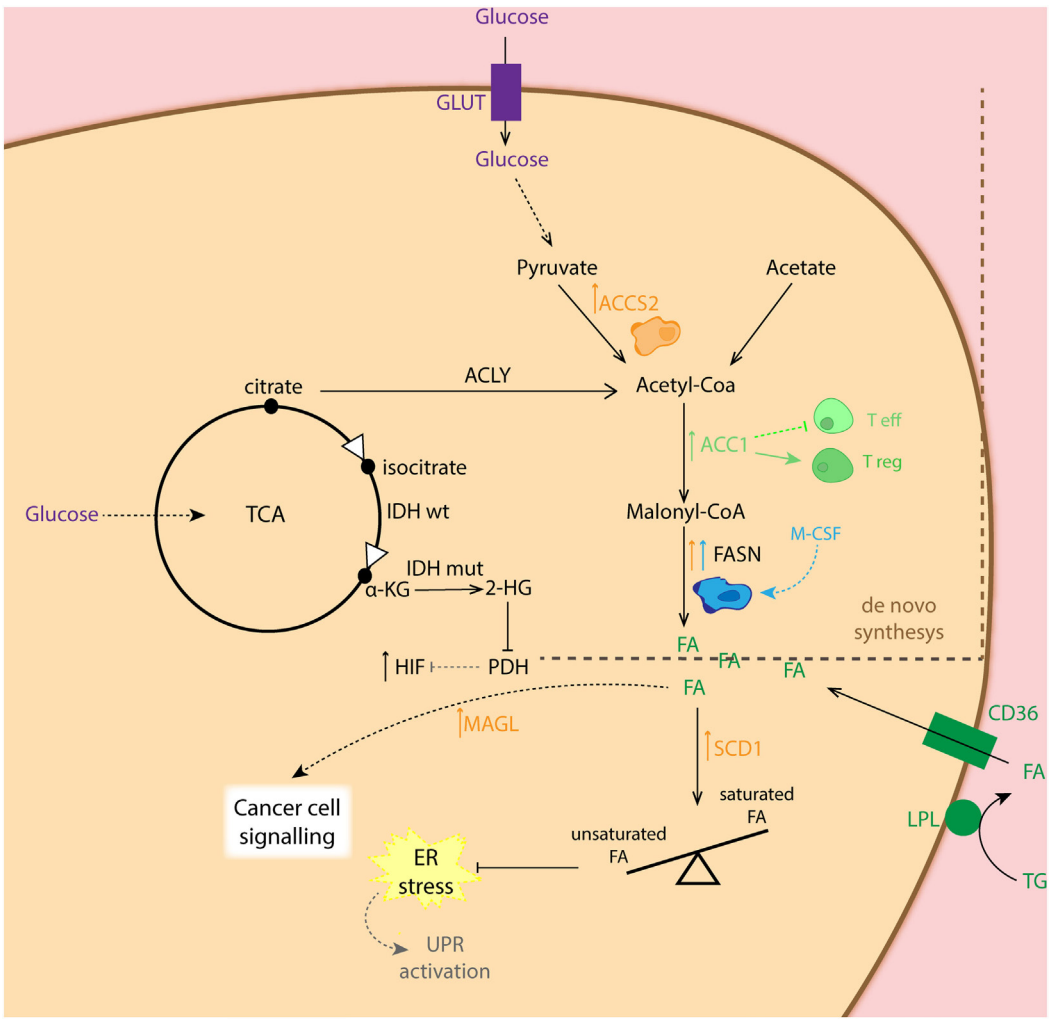

FIGURE 3 | Schematic representation of the fatty acid (FA) synthesis in different cell types. Cancer cells (CC: orange) show increased expression of ACCS2 favoring conversion of pyruvate to acetyl-coA as well as fatty acid synthase (FASN) for conversion of malonyl-coA to FA. The latter, is also overexpressed in M2 macrophages (blue). CCs can also obtain FA from exogenous sources *via* converting triglycerides (TG) into FA, which are taken up by the CD36 transporter. Overexpression of monoacylglycerol lipase (MAGL) in CC is related to synthesis of lipids involved in cell signaling. Increased expression of SCD1 in CC balances higher presence of unsaturated FA essential for maintenance of ER homeostasis. Enhanced expression of ACC1 shifts T cell differentiation toward T regulatory cells (Treg: dark green).

metabolism plays an important role in preventing ER stress, as it appears that balancing saturated and unsaturated lipid species are required, due to the lipotoxic effects of the former. Therefore, desaturation of *de novo* synthesized lipids by oxygen-dependent stearyl-Coenzyme A (CoA) desaturases (SCDs) plays a critical role for cancer cell survival. In this regard, SCD1-mediated lipid desaturation has been found to be a critical determinant of cancer cell survival downstream SREBP transcription factors (which are regulated by the mTORC1 pathway) (71). In fact, unsaturated lipid deficiency of hypoxic cells has been shown to cause cell death by ER stress and activation of the unfolded protein response in an mTORC1-dependent manner (72, 73). Another important lipid-metabolism-related enzyme is acetyl-CoA synthase 2 (ACSS2). ACSS2 converts acetate to acetyl-CoA, which is used as a nutritional source by cancer cells supporting biosynthesis of membrane phospholipids. Moreover, it is an epigenetic regulator in histone acetylation. It was also shown that hypoxia enhances the expression of ACSS2, which has been related to poor prognosis in breast cancer patients (74). The importance of lipid metabolism alterations in cancer cells relays not only on the role of lipids for biogenesis but also on their capacity to signal. In this respect, it has been shown that in human cancer cells as well as primary tumors, monoacylglycerol lipase (MAGL) is vastly overexpressed. This enzyme regulates a FA network that drives oncogenic signaling lipids, which promotes migration, invasion, survival, and *in vivo* tumor growth (75).

As mention before, tumors are glutamine addicted. Cancer cells display high rates of glutaminolysis in order to obtain several precursors needed for supporting robust proliferation. In this regard, c-Myc has been shown to directly upregulate glutamine-metabolizing enzymes, such as glutaminase, which leads to fast integration of nitrogens and carbons in the anabolic network (76–78). Interestingly, it has been reported that the c-Myc function is directly regulated by both HIF-1α and HIF-2α (79, 80). The reductive metabolism of glutamine, mediated by IDH1, adds extensively to lipogenesis in cancer cells (81) (**Figure 4**), and which is partially facilitated by an increase in PDK1 (53, 82) and c-Myc (76, 83) in a HIF1-dependent manner, but it is primarily determined by the relative abundance of citrate and α-KG (83, 84). In addition, Kynurenine is another oncometabolite, which was defined as a tryptophan metabolite made from indoleamine-2,3-dioxygenase (IDO) (85), and known for its robust immunosuppressive effects (86) (**Figure 4**).

In addition to this, metabolic fitness relates to an increased HIF signaling in tumor cells, which permits the cancer cells to strive better for crucial metabolites, such as glucose and glutamine, than the stromal cells (87). This competition for nutrients has been demonstrated in the exhaustion of tumor-associated lymphocytes, suggesting a metabolic associated immune suppression (88, 89).

Tumor Microenvironment

Although tumor cells have been the main subject of study in cancer research, stromal cells and infiltrating immune cells have gained great interest, over the past years. Furthermore, the intriguing crosstalk of tumor cells with the TME or even different components of the TME among each other (as introduced in the previous section), regulate a vast amount of processes during tumor development (3). In this section, we will discuss several metabolic adaptations of different cell types of the TME (**Figure 5**).

Stromal Cells

Endothelial Cells (ECs)

Endothelial cells are the best characterized cells of the TME. Despite the fact that ECs dispose of immediate access to oxygen in the blood, ECs are highly glycolytic, generating up to 85% of their ATP *via* glycolysis. Indeed, their glycolytic rate is comparable to that of cancer cells, and increases even during proliferation (90). Rather than acting as a bioenergetic power source, it has been reported that through the production of pro-angiogenic reactive oxygen species (ROS) mitochondria in ECs have a signaling function (91). In this regard, also the important role of the pentose phosphate pathway (PPP) as a weight against oxidative stress is noteworthy, as it controls redox homeostasis through NADPH production, together with ribose-5-phosphate for synthesis of lipids nucleotides and amino acids (92). Although glutamine and FA metabolism for ATP production in ECs is still under debate, glutaminolysis has been described to be essential for EC proliferation, as inhibition of glutaminase induces their senescence (93, 94). Also cholesterol synthesis has been shown to be crucial for vessel sprouting, as it enables the development of lipid rafts required for proper membrane localization and signaling of the VEGF receptors (95). At this point, it is important to note that VEGF creates a tip cell signal, required for vessel sprouting, a process during which ECs display exclusive patterns of cellular metabolism with high rates of glycolysis and reliant on FAO for nucleic acid synthesis and proliferation (94, 96). Since tip cells are located far from functional vessels, their activity is also regulated *via* the hypoxia pathway (97). In this regard, it has been shown that deletion of endothelial HIF-2α enhances angiogenesis, although the vessels were more disorganized and hypoxic (98, 99). In addition, PHD2+/− mice showed increased HIF-α stabilization, and normalization of the endothelium, increased oxygenation and reduced secondary metastasis (13). On top of this, it has been reported that the HIF subunits have opposing roles when it comes to the permeability of the endothelium. HIF-1α deficiency in ECs hampers tumor cell migration through endothelial layers, while loss of HIF-2α enhances tumor cell migration and metastasis (14). These contrasting effects may be directly related to the dramatic differences in inducible nitric oxide synthase (iNOS) expression in both deficient lines. In addition to this, a pro-tumoral "metabolite crosstalk" between EC and cancer cells has been reported. In this regard, Boidot et al. identified a direct link between the function of p53 and MCT1 expression, regulating the influx of lactate produced by cancer cells into EC (100). Later studies from the same group showed that internalized lactate through MCT1 by EC promote tumor angiogenesis through PHD2 inhibition, and activating HIF1 (101, 102). The same mechanism has been reported to trigger IκBα degradation, stimulating an autocrine pro-angiogenic NFκB/IL-8 pathway, and finally driving cell migration and tube formation (103). Furthermore, the receptor tyrosine kinases AXL, TIE2, and VEGFR-2 is activated by lactate in order to promote angiogenesis (104).

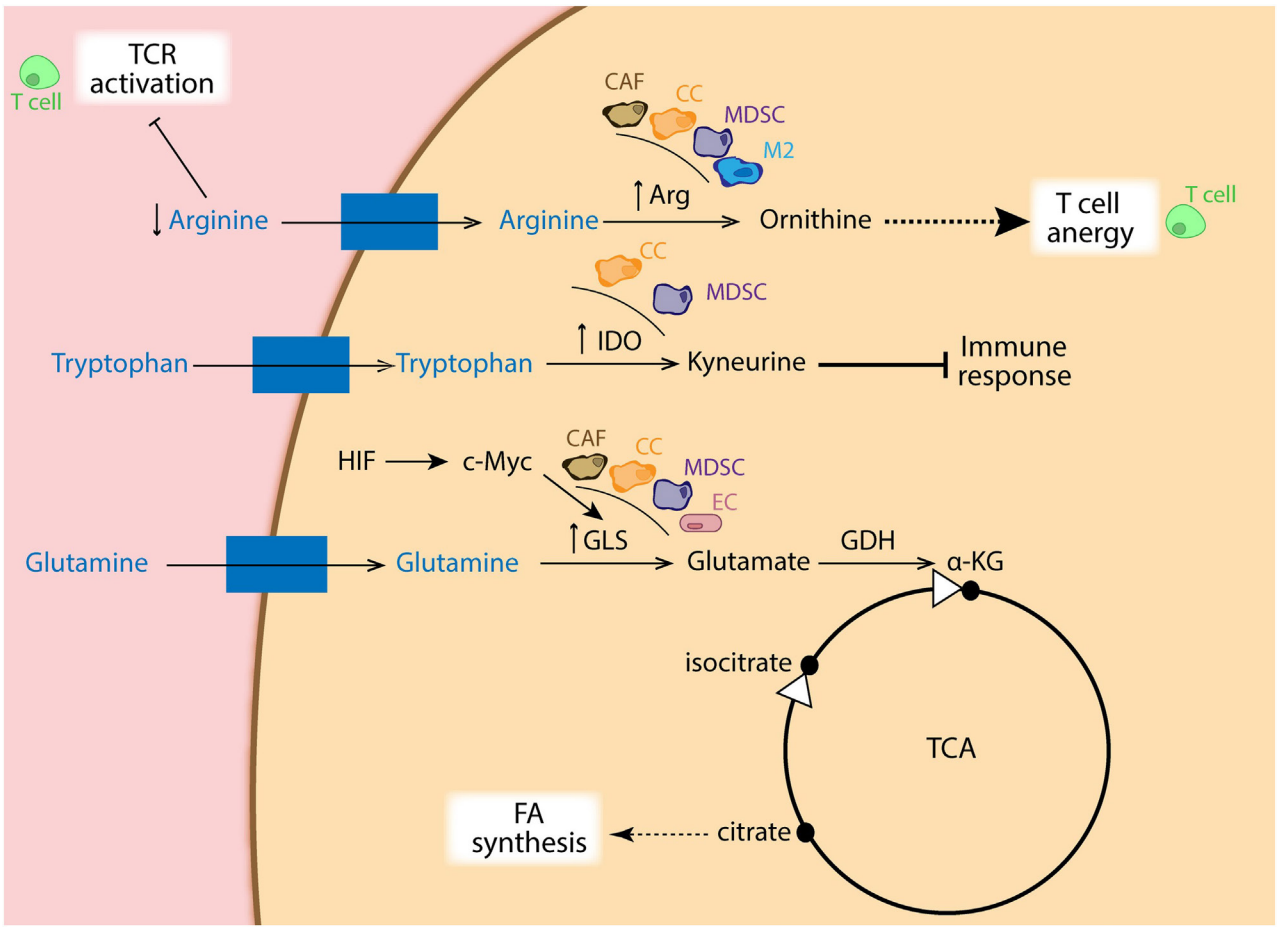

FIGURE 4 | Schematic representation of amino acid metabolism in different cell types. Cancer cells (CCs: orange), cancer-associated fibroblasts (CAFs: brown), myeloid-derived suppressor cells (MDSCs: purple), as well as M2 macrophages (blue) show increased expression of arginase, depleting the surrounding environment of arginine, which is essential for TCR activation in T cells (green). Ornithine coming from arginase metabolism also dampens T cell activation. Moreover, increased expression of indoleamine-2,3-dioxygenase (IDO) in CC and MDSCs favors catabolism of tryptophan into kyneurine, with immune suppressive effects. In addition, overexpression of GLS *via* HIF/c-Myc regulation in CA, CAFs, MDSCs, and endothelial cells (ECs: pink) generates glutamate, which is used to replenish intermediates of the TCA cycle, favoring FA synthesis from citrate.

Fibroblasts

Another important stromal cell of the TME is the CAF. The importance of CAFs relies not only on their ability to produce growth factors and chemokines regulating other stromal cells and cancer cells but also in their capacity to modify the extracellular matrix (ECM), facilitating tumor angiogenesis and invasiveness (105). CAFs is actually a mix of myofibroblast-like cells that ascend different types of cells, including fibroblasts, bone-marrow-derived stromal cells, ECs, and adipocytes (106–108). Despite their relevance in regulating tumor development, studies on the metabolism of CAFs have been limited. Interestingly, proliferating fibroblasts produce biomass for a next proliferation, while quiescent fibroblasts use biomass to replace oxidized lipids and degraded proteins, as well as synthesis of ECM proteins. Hypoxia pathway proteins are an important stimulus of this process, since they increase the expression of remodeling enzymes leading to increased tumor rigorousness and enhanced metastasis (109). In this respect, reduced PHD2 activity led to a diminished CAF-induced ECM remodeling and diminished metastasis (12, 110). Independent of their activation state, healthy fibroblasts incorporate glucose carbons in the TCA cycle at comparable rates (111). Fibroblasts have also been described to replenish intermediates of the TCA by a process known as anaplerosis. In particular, anaplerotic flux from pyruvate to oxaloacetate *via* pyruvate carboxylase in quiescent fibroblasts ensures continuity of the TCA cycle, whereas proliferating fibroblasts primarily use glutamine for anaplerosis. Like cancer cells, proliferating fibroblasts rely on PPP for biosynthesis. By contrast, quiescent fibroblasts generate NADPH *via* the PPP, which is essential for their survival (111). Moreover, CAFs also perform FA synthesis, essential for their proliferation (112). Production of ROS by cancer cells inhibits PHD2 (with subsequent gain of function of HIF-1α) and enhances NO production

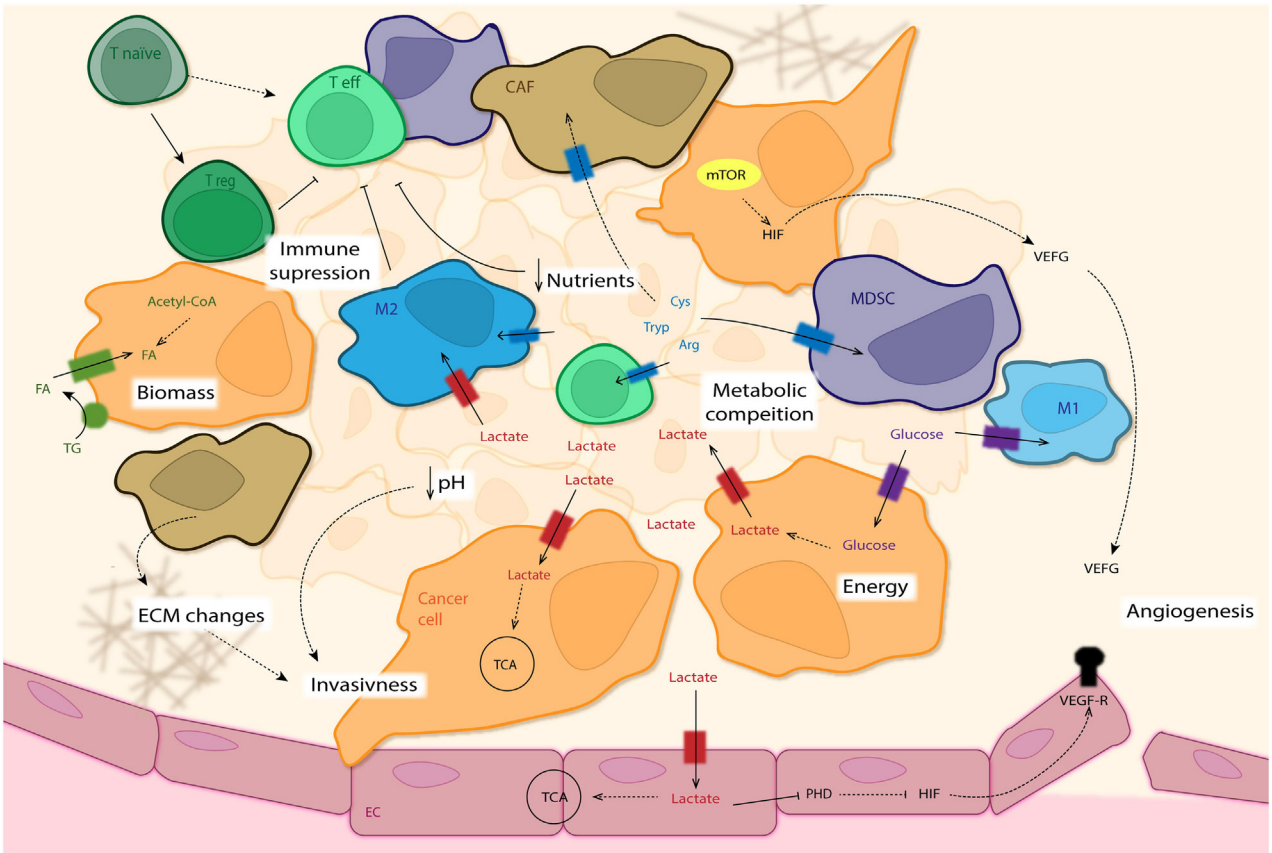

FIGURE 5 | The tumor microenvironment (TME). This overview summarizes the intricate interactions of the different cells types that constitute the tumor mass. Some cancer cells (CC: orange) use glycolysis as main source for energy production (independently of oxygen availability—Warburg effect), excreting lactate and leading to acidification of the surrounding area, which finally promotes invasiveness of CC. Other CCs as well as M2 macrophages (blue) and endothelial cells (ECs: pink) with access to adequate levels of oxygen use this lactate for oxidative phosphorylation (OxPhos). Lactate in ECs leads to inhibition of PHD and subsequent activation of HIF, enhancing expression of VEGF-Rs (among others) and promoting angiogenesis. Uptake of fatty acids (FA) as well as *de novo* synthesis is used for new biomass required for fast CC proliferation. Next to amino acid depletion in the tumor mass by M2 cells, cancer-associated fibroblasts (CAF: brown) and myeloid-derived suppressor cells (MDSCs: purple) establish a metabolic competition between pro- and anti-tumor forces present in the TME. The absence of nutrients needed for T effector cell activation (Teff: light green) leads to their anergy, with a differentiation shift toward T regulatory cells (Treg: dark green). The lack of nutrients leads to activation of the mTOR pathway in CCs and production of VEGF (among others) *via* HIF regulation, promoting angiogenesis.

by CAFs. This finally leads to dysfunctional mitochondria that are mitophaged, forcing CAFs to rely on glycolysis for ATP production, with accompanied increase in lactate production (113). High lactate production together with amino acids and keton bodies supply cancer cells with high-energy nutrients for oxidative metabolism in tumor oxygenated areas (114, 115). In addition, loss of HIF-1α in fibroblasts leads to vascular normalization, decreases hypoxia but increases breast cancer development (116). HIF1α-driven aerobic glycolysis in stromal cells supports cancer cell growth *via* the paracrine production of nutrients (such as lactate), which cancer cells can recycle (4, 102, 117, 118). As we will discuss in the following section, arginases (ArgI and ArgII) are important for immune suppression in the tumor, converting L-arginine to ornithine, resulting in T cell anergy and reduced anti-tumor response (119). CAFs that are localized to hypoxic regions in pancreatic tumors express high levels of ArgII, suggesting for CAF-mediated immunosuppression (120).

Immune Cells

Although numerous studies have indicated the involvement of almost every immune cells type, macrophages have been by far the most studied immune cell type during cancer development. As mentioned before, the importance of metabolism in regulating immune cell phenotype and function and its impact during tumor development is well known (121). Indeed, during the past years, a new field of study has emerged, focusing on the metabolism of the immune system also known as immunometabolism. This new area of research studies how changes in cell metabolism regulate the immune system during homeostasis as well as during inflammatory processes, including tumor-associated inflammation. Indeed, how metabolic changes in immune cells during tumor development regulate the contribution of these cells to disease progression has been the center of a great number of studies. As mentioned before, high levels of glycolysis but hampered angiogenesis inside hypoxic tumor areas can result in near glucose depletion and accumulation of waste products such as

lactate. Hence, anti-tumoral immune cells infiltrating TME face significant metabolic challenges to mount and sustain against the tumor. In this regard, T cells have been extensively studied as the main force fighting tumor growth, characterized by particular metabolic shifts according to their activation state (**Figure 5**).

T Cells

Resting naïve T cells require low amounts of glucose, amino acids, and FAs to sustain basic energetic and minimal replacement demands. More than 90% of their ATP production comes from FAO and OxPhos, whereas glutaminolysis and PPP contribute to biosynthesis purposes. Upon activation, T cells increase glucose and glutamine catabolism for nucleotide and lipid synthesis that are essential for cell growth, while OxPhos for ATP production is maintained (122–125) (**Figure 5**). Indeed, glycolysis has been described to be essential for T cell effector functions, since its impairment suppresses proliferation. TCR–CD28 co-stimulation triggers the shift from naïve to effector T (Teff) cells through PI3K/Akt/mTOR pathway, and activation of cMyc and HIF-1α transcription factors. This promotes glycolytic gene expression and post-translational modification essential to drive aerobic glycolysis and amino acid metabolism in Teff cells, while suppressing catabolic FAO for ATP (122, 126–128). On the other hand, T regulatory (Treg) cells orchestrate a pro-tumoral environment by inhibiting effector T cell responses in the tumor area. Contrary to Teff, Treg cells rely on both FAO and OxPhos for ATP upon activation (127, 129, 130). This metabolic state allows Treg cells to survive tumor conditions and exert their immunosuppressive effect, whereas anti-tumor effector T cells would face impair TCR signaling due to lack of glucose (122, 123, 129–131). Indeed, Treg expansion has been linked to activation of the nutrient stress sensor AMPK. Thus, when the AMP:ATP ratio increases due to the lack of nutrients, AMPK favors oxidative catabolic pathways (132). This metabolic shift implies that AMPK can immediately impact the balance of Teff and Treg cells *via* mTORC1 inhibition (133). Upon CD3/CD28 activation, T cells accumulate metabolites involved in anabolic pathways increasing FAS (122). In addition, it has been shown that mTORC impairment compromise *de novo* lipid synthesis in T cells through induction of the transcription factor SREBP (134). The importance of lipid metabolism in T cell biology has been also reported at the level of the FAS limiting rate enzyme ACC1, which deletion interferes with differentiation of naïve to effector T cells (135). However, ACC1 deletion did not affect the ability of naïve T cells to proliferate and differentiate into Treg (135, 136), suggesting FAS as an important metabolic checkpoint during activation-induced differentiation into Teff cells. Similar to cancer cells, PI3K–mTOR axis stimulates HIFα activity, downstream of the TCR activation (137, 138). Also, IL6 stimulation of T cells leads to JAK–STAT pathway increased transcription of HIF mRNA (126, 139). As mentioned before, metabolites themselves can also act as signaling molecules. In this regard, decreased flux through the TCA cycle may lower succinate levels. It has indeed been shown that succinate can stabilize HIF-1α, inducing transcription of several inflammatory cytokines (32). Although there are not many studies on the role of the HIF-pathway in T cells during tumorigenesis, previous studies have reported a role for this pathway during T cell-mediated inflammation and differentiation (Th17 and Treg balance). Dang et al. reported that HIF-1α enhances Th17 differentiation by direct transcriptional activation of RORγt, while inducing FoxP3 proteasomal degradation and dampening Treg differentiation (126). During Th17 cell development, glycolysis rate is increased through mTOR–HIF-1α signaling induction (127). The tumor protecting or promoting role of Th17 is still controversial due to its differently described phenotypes [for review, see Ref. (140)].

Moreover, it has been reported that T cell activation is blocked due to disruption of the electron transport chain leading to impaired mitochondrial ROS production (141). Apart from this, the hypoxic environment within the tumor area may protect tumor cells from anti-tumor immunity by HIF-1α-dependent upregulation of PD-L1 on cancer cells, which inhibits PD-1 expressing T effector cells (142). Moreover, high lactate levels in the tumor area have been shown to suppress the PI3K/Akt/mTOR pathway inhibiting glycolysis, finally leading to impaired T cells (128, 143, 144). Glycolysis inhibition can lead to increase expression of PD-1, which is associated with T cell exhaustion and non-responsiveness through inhibition of TCR and CD28-mediated co-stimulation, helping the tumor to escape immune surveillance (145). Also tryptophan has gotten attention as a limiting amino acid in T cell activation. Tryptophan metabolism is mainly regulated by IDO, highly expressed by cancers, and in fact correlated with poor prognosis (146) (**Figure 4**). Using a mouse sarcoma model, Chang et al. showed that glucose restricts T cells, leading to hampered mTOR activity, glycolytic capacity, and INFγ production. The result is enhanced tumor progression. Checkpoint blockade using antibodies against CTLA4 PD1 and PDL1 restore all previous changes (88). Recent work supports the hypothesis that lactic acid blunts the immune response mediated by T and NK cells (147).

Macrophages

Historically, macrophages have been classified as M1 (classically activated) and M2 (alternatively activated) according to their pro- or anti-inflammatory state, respectively. However, more recently, the idea of a multidimensional spectrum rather than dual macrophage activated states has emerged (148, 149). Since specific stimuli induce specific functional outcomes, it is expected that different states of polarization present a particular metabolism. M1-phenotyped macrophages are highly glycolytic and characterized by an increased induction of the strong glycolytic enzyme 6-phosphofructo-2-kinase/fructose-2,6-biphosphatase 3 isoform (PFKFB3) (150), conferring them an energetic advantage in hypoxic regions (151). This glycolytic state is mediated by the Akt/mTOR/HIF-1α pathway (32) and has been shown to induce TNF expression (152). The latter suggests a direct regulation of the inflammatory phenotype of macrophages depending on the glycolytic pathway. In relation to their anti-inflammatory role, M1 macrophages also use PPP and malic enzyme in order to produce high amounts of NO and ROS for killing pathogens, as well as NADPH to protect themselves from this high oxidative burst (153). On the other hand, M2 macrophages present high rates of FAO and OxPhos, with low glycolytic activity due to the expression of the weak glycolytic activator PFKFB1 isoform

(154, 155) (**Figure 2**). O'Neill et al. showed that M2 polarization upon IL-4 signaling stimulates mitobiogenesis by upregulating PGCβ, enhancing the metabolic switch to FAO (155). Also, M2 macrophages reduce PPP flux and GSH *via* induction of carbohydrate kinase-like protein (CARKL) (156). Traditionally, TAMs have been related to a more M2 anti-inflammatory and pro-tumor phenotype. However, the fluctuating levels of lactate and oxygen in the heterogeneous tumor mass induce differential macrophage responses depending on their functional plasticity in tumor. In general, a lactic acid-induced polarization to M2 has been reported, inducing an immunosuppressive and tissue remodeling phenotype. This is characterized by the production of VEGF and arginase in a HIF-1α dependent manner (43, 121). Also, TAMs respond and adapt to different oxygen levels through activation of the HIF pathway. In this regard, it has been shown that expression of HIF-1α has a protective role in hypoxic areas, since loss of this transcription factor leads to decrease expression of IL-6, TNF, and iNOS, as well as increased CD206, all of them characteristic markers of the M2 phenotype (15, 17). TAMs are also characterized by high expression of arginase enzyme (induced by the high lactate levels in the TME). This has been shown to impair anti-tumor T cell function due to depletion of the arginine pool required for NO and protein synthesis leading to TCR function impairment (19, 43, 157, 158). Apart from this, recent studies have described the importance of iron metabolism in macrophage polarization. M1 macrophages express reduced levels of ferroportin, the iron transporter, but high levels of H-ferritin involved in iron storage, whereas M2 macrophages present the opposite profile. Therefore, iron sequestration in M1 macrophages is believed to restrict both bacterial and tumor growth, while M2 macrophages release iron, which promotes tissue repair and tumor cell proliferation (159, 160). In addition, iron constitutes a cofactor of the PHD enzymes in the hypoxia pathway. Therefore, intracellular iron levels directly regulate HIF-1α stability crucial for the survival and pro-tumor function of TAMs (161). In a tumor setting, TAMs also undergo changes in their lipid profile. It has been shown that M-CSF secreted from tumor cells leads to enhanced expression of FASN in macrophages, which polarize to an IL-10 expressing pro-tumoral phenotype (162).

Myeloid-Derived Suppressor Cells (MDSCs)

Another important immune cell type that has recently gained great attention is the MDSCs that, as its name indicates, is functionally defined by its potent immunosuppressive activity in both innate and adaptive immunity. This cell population comprises two major subsets: monocytic MDSCs (M-MDSCs) and polymorphonuclear MDSCs (PMN-MDSCs) (163). G-CSF has been described to play a critical role in differentiating and mobilizing bone marrow granulocytic precursors within tumors (164); whereas depending on the magnitude and context of the stimulus, GM-CSF can induce accumulation of these suppressor subsets thereby inhibiting proliferation as well as anti-tumor ability of neu-specific T cells (165, 166). In addition, it has been shown that IL-4Rα activation through IL-4 and IL-13 exposure evokes MDSCs suppressive mechanism in a STAT6-dependent manner (167, 168). MDSCs promote immune dysfunction by using different mechanisms, either directly *via* impairment of T cell amino acid metabolism or through regulation of oxidative stress, which finally interferes with T cell viability, migration, and activation. Also, MDSC are able to indirectly induce other immune regulatory cells, such as Treg cells and TAMs (169–171). The same as macrophages, MDSCs show high expression of arginase, depleting arginine from the TME essential for TCR activation and T cell proliferation (172, 173). MDSCs also sequester the amino acid cysteine, which is indispensable for T-cell activation (174) and expresses IDO enzyme for tryptophan catabolism (175, 176). Deprivation of the later has been shown to induce expansion of the Treg cell population (177). Combined expression of nitric oxide synthase, arginase, and NADPH oxidase confers MDSC important regulators of oxidative stress in TME (178–181). Therefore, presence of RNI (mainly derived from M-MDSC), and ROI (mainly from PMN-MDSC) downregulates TCR and IL2 receptor signaling, inhibiting T cell activation and proliferation (170). In addition to this, MDSCs show enhanced FA uptake and high expression of FAO enzymes, accompanied by an increased mitochondrial mass and oxygen consumption rate (182). Corzo et al. described the role of the hypoxia pathway in MDSCs, with HIF-1α as main responsible for MDSC differentiation and function in TME (183). In addition, HIF-1α-mediated expression of PD-L1 is essential for mediating MDSC immune suppression (as discussed in the previous sections) (184). Also hypoxia can enhance MDSC migration to the tumor site *via* HIF-1α-mediated production of chemokines (185, 186). Hypoxia also influences seeding of MDSCs in the pre-metastatic niche by stimulating increased secretion of lysyl oxidase (187–189). This process drives ECM remodeling in the metastatic niche and suppresses NK anti-tumor response (188).

Neutrophils

During the past few years, the presence of tumor-associated neutrophils (TANs) has gained attention due to their pivotal role in tumor development. Indeed, a dual effect has been proposed for TANs during onset (190). In this regard, Fridlender et al. showed that in the absence of TGFβ, TANs encourage Teff response and anti-tumor activity, whereas in the presence of TGFβ they exhibit tumor promoting activity (191). Neutrophils comprise a significant proportion of the inflammatory infiltrate in cancerous lesions and high levels of blood neutrophils were observed in patients suffering from advanced stage tumors (192). In many cancer types, such as bronchoalveolar carcinoma (193), metastatic melanoma (192), and andrenal carcinoma (194), neutrophil accumulation was associated with increased aggressiveness and poor prognosis (195). By contrast, high neutrophil counts in gastric tumors correlate with favorable prognosis (196). Since neutrophils constitute an already mature population that does not proliferate, possible changes in their metabolism have not been studied in depth. Regarding their metabolism, neutrophils are strongly committed to glycolysis and PPP, whereas their few mitochondria are used for maintenance of the redox balance. It has been shown that their high rates of glycolysis are necessary for the generation of ATP, in which HIF-1α is critically involved by regulating the expression of key glycolytic

enzymes (197). Apart from its main role in energy metabolism, glycolysis has been shown to be essential for some neutrophil functions such as oxidative burst and chemotaxis (198). Indeed, Thompson et al. reported that murine HIF-2α deficient inflammatory neutrophils displayed no impairment of chemotaxis, phagocytosis, or respiratory burst but elevated sensitivity to apoptosis leading to reduced neutrophilic inflammation (199). It has also been shown that hypoxia can promote neutrophil recruitment by modifying the adherence properties of ECs to neutrophils (200). In addition, FAS has been reported to have some relevance in neutrophil biology. In this regard, Lodhi et al. showed that peroxisomal lipid synthesis drives inflammation by supporting neutrophil membrane phospholipid composition as well as viability (201). Another important feature of neutrophils is the formation of neutrophil extracellular traps (NETs). Brinkmann et al. described for the first time that activated neutrophils are able to release their chromatin (DNA and histones) loaded with granule enzymes forming an extracellular mesh-like structure that can both trap and kill extracellular organisms (202). Glucose uptake, glycolysis, and a shift toward PPP have been shown to be essential for NETs formation (203, 204). Beyond their bactericidal role, NETs has been described to sequester tumor cells and promote metastasis (205). Also, association of adhesion molecule and cytokines to NETs has been related to cancer-induced organ failure (206).

Therapy Perspectives

Regarding tumor therapy, there are different approaches that can be used, including targeting of the cancer cells, or switching the nature of the immune cell to a more anti-tumoral state. During the past decade, a lot of attention has been given to try and selectively kill the tumor based on their metabolic alterations (207, 208). Indeed, increasing evidence supports the idea that dysregulated cellular metabolism is connected to drug resistance during cancer therapy. Therefore, combining cellular metabolism inhibitors with chemotherapeutic drugs constitutes a promising strategy to overcome this.

It has also become clear that there is much more the only Warburg effect when it comes to the metabolic rearrangements associated with malignant transformations. Indeed, there is also an increased flux through the PPP, higher rates of glutamine consumption and lipid biosynthesis, maintenance of redox homeostasis and limited levels of autophagy, at least in the first steps of oncogenesis (209–211).

Glycose Metabolism

Due to the high glycolic rate in developing tumors, regulation of this pathway in cancer cells has been considerately studied. Starting by targeting glucose intake, the GLUT1 inhibitor WZb117 has been shown to reduce ATP production and ER stress induction in cancer cells, with a synergistic anticancer effect in combination with cisplatin or paclitaxel (212). Also, under hypoxia conditions, Cao et al. reported that the GLUT1 inhibitor phloretin significantly enhances anticancer effects of the antibiotic daunorubicin, overcoming hypoxia-conferred drug resistance (213). In the same line, inhibition of glycolysis with 2-DG (glucose analog for hexokinase) in combination with radiation or chemotherapy treatments, enhance clinical efficacy of the latter (214). A great number of studies have focused on the importance that the highly expressed PKM2 enzyme in cancer cells has in conferring resistance to therapy (48, 215–217) (**Figure 2**). Inhibition of this last rate-limiting enzyme in the glycolytic pathway, increases apoptosis and inhibits proliferation during cisplatin (218), and docetaxel (219) treatment. In the last step of the glycolytic pathway, LDHA expression and activity has been reported to be higher in Taxol-resistant breast cancer cells. Inhibition of LDHA by oxamate (a pyruvate analog) in combination with paclitaxel treatment has shown synergistic effect on taxol-resistant cells by promoting apoptosis (220). Regulating the shift between glycolysis and TCA, PDK inhibits PDH conversion of pyruvate to acetyl-CoA. Inhibition of PDK3 (functional of pyruvate isoform even in high concentration) has shown to diminish hypoxia-induced resistance in cervical and colon cancer (221, 222).

Recent evidence also indicates that modulation of immunometabolism plays an important role in controlling immune responses against cancer progression. Indeed, several studies have focused on targeting metabolic pathways to enhance T cell function and persistence. One of the most promising is the use of PD-1 blocking antibodies to rescue T cell glycolysis and enhance Teff functions (223). By contrast, inhibition of mTOR (224, 225) or AMPK (226, 227) has been shown to lead to controversial results.

Lipid Metabolism

Since proliferation of cells requires the generation of novel phospholipid membranes, targeting *de novo* lipogenesis or steroidogenesis would also be a potential anticancer therapy approach (68). Several enzymes involved in these synthesis pathways, including FASN (228, 229), ACLY (230), ACCs (231), choline kinase (232, 233), monoglyceride lipase (75), and HMGCR (234) have been ascribed critical roles in oncogenesis or tumor progression *in vivo*, yet have not been tested in clinical settings. Indeed, FAS is significantly upregulated and correlates with poor prognosis in many types of cancer. Therefore, it is not surprising that several FAS inhibitors, such as cerulenin, C75, orlistat, C93, or GSL 837149a, have shown anti-tumor activity (**Figure 3**). In addition, the combination of FAS inhibition with docetaxel (235), trastuzumab (236), or adriamycin (237) treatment increases therapy sensitivity in breast cancer. Cancer cell metabolism is also highly dependent on glutaminolysis. It has been shown that glutamine in combination with leucine activates mTORC1 by enhancing glutaminolysis and α-ketoglutarate production (238). Targeting glutaminolysis by using the mTORC1 inhibitor rapamycin has been reported to enhance cisplatin treatment in gastric cancer (239). Pharmacological inhibition of FAO functions in MDSC, averts immune inhibitory pathways and decreases the production of inhibitory cytokines. Consequently, blocking FAO postponed tumor growth in a T-cell-dependent way, and increased the anti-tumor effect after adoptive T cell treatment (182). COX-2 inhibitors or reducing COX-2 expression in 3LL cells, obstructed their capacity to induce arginase I in MDSC (240).

Amino Acid Metabolism

Inhibitors of folate metabolism, thymidine and deoxynucleotide synthesis and elongation of nucleic acid are the so-called anti-metabolites and serve as standard chemotherapeutic regimens against many human neoplasms (241). Unfortunately, these agents are linked to toxicity in bone marrow and intestinal epithelium, as these are highly proliferating tissues. Compound 968 (242) and bis-2-(5-phenylacetamido-1,2,4-thiadiazol-2-yl) ethyl sulfide, two specific GLS inhibitors (243), diminish glutamine catabolism and delay tumor growth in models of cancer. Targeting glutamate conversion to α-ketoglutarate by aminotransferases also diminishes tumor growth (244, 245). Replenishing the TCA cycle intermediates by providing substrates, such as glutamine, sustains mitochondrial metabolism in tumor cells (63) (**Figure 4**).

Mitochondria Respiration

Many types of tumor are highly dependent on OxPhos for their ATP (246–248). Therefore, these cells are probably sensitive to treatments that reduce mitochondrial ATP production. Moreover, inhibiting this mitochondrial ATP production would synergize together with approaches that diminish glycolysis, including inhibitors of the PI3K signaling pathway (249). It has been shown that phenformin (biguanide) inhibits mitochondrial complex I and in that way exerts its anti-tumor effects in experimental cancer models (250). Metformin has also antineoplastic activity (251). This appears to be independent of glycemia (252) and might reflect the ability to preferentially kill cancer stem cells, block mitochondrial respiration, intensify glutamine addiction, or limit inflammatory responses driving tumor growth (253–256). Indeed, its action is to specifically inhibit Mitochondrial complex 1, which in turn activates AMPK as a consequence of ATP decrease (257).

CONCLUSION

Our understanding of metabolic changes in cancer development has improved significantly over the past years. However, the influence of the hypoxia pathway proteins on the metabolic pathways in tumor cells and the TME is still not entirely known. In a vast amount of physiological as well as pathological situations, hypoxia-induced rewiring permits survival during metabolic stress. Conversely, this drives cancer progression, causing enhanced lethality due to resistance to therapy and greater metastatic potential. Therefore, more research is necessary to better understand hypoxia-induced alterations in cellular metabolism and eventually target these pathways, thereby eliminating malignant cells.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

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REFERENCES

1. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. *Cell* (2011) 144:646–74. doi:10.1016/j.cell.2011.02.013
2. Ward PS, Thompson CB. Metabolic reprogramming: a cancer hallmark even Warburg did not anticipate. *Cancer Cell* (2012) 21:297–308. doi:10.1016/j.ccr.2012.02.014
3. Quail DF, Joyce JA. Microenvironmental regulation of tumor progression and metastasis. *Nat Med* (2013) 19:1423–37. doi:10.1038/nm.3394
4. Sonveaux P, Végran F, Schroeder T, Wergin MC, Verrax J, Rabbani ZN, et al. Targeting lactate-fueled respiration selectively kills hypoxic tumor cells in mice. *J Clin Invest* (2008) 118:3930. doi:10.1172/JCI36843
5. Vaupel P, Mayer A. Hypoxia in cancer: significance and impact on clinical outcome. *Cancer Metastasis Rev* (2007) 26:225–39. doi:10.1007/s10555-007-9055-1
6. Meneses AM, Wielockx B. PHD2: from hypoxia regulation to disease progression. *Hypoxia (Auckl)* (2016) 4:53–67. doi:10.2147/HP.S53576
7. Keith B, Johnson RS, Simon MC. HIF1α and HIF2α: sibling rivalry in hypoxic tumour growth and progression. *Nat Rev Cancer* (2012) 12:9–22. doi:10.1038/nrc3183
8. Sormendi S, Wielockx B. HIF-pathway proteins: central regulators of tumor immunology. *Transl Cancer Res* (2016) 5:S1503–8. doi:10.21037/tcr.2016.12.30
9. Berra E, Benizri E, Ginouvès A, Volmat V, Roux D, Pouysségur J. HIF prolyl-hydroxylase 2 is the key oxygen sensor setting low steady-state levels of HIF-1alpha in normoxia. *EMBO J* (2003) 22:4082–90. doi:10.1093/emboj/cdg392
10. Klotzsche-von Ameln A, Muschter A, Mamlouk S, Kalucka J, Prade I, Franke K, et al. Inhibition of HIF prolyl hydroxylase-2 blocks tumor growth in mice through the anti-proliferative activity of transforming growth factorβ. *Cancer Res* (2011) 71(9):3306–16. doi:10.1158/0008-5472.CAN-10-3838
11. Klotzsche-von Ameln A, Muschter A, Heimesaat MM, Breier G, Wielockx B. HIF prolyl hydroxylase-2 inhibition diminishes tumor growth through matrix metalloproteinase-induced TGFβ activation. *Cancer Biol Ther* (2012) 13:216–23. doi:10.4161/cbt.13.4.18830
12. Kuchnio A, Moens S, Bruning U, Kuchnio K, Cruys B, Thienpont B, et al. The cancer cell oxygen sensor PHD2 promotes metastasis via activation of cancer-associated fibroblasts. *Cell Rep* (2015) 12:992–1005. doi:10.1016/j.celrep.2015.07.010
13. Mazzone M, Dettori D, De Oliveira RL, Loges S, Schmidt T, Jonckx B, et al. Heterozygous deficiency of PHD2 restores tumor oxygenation and inhibits metastasis via endothelial normalization. *Cell* (2009) 136:839–51. doi:10.1016/j.cell.2009.01.020
14. Branco-Price C, Zhang N, Schnelle M, Evans C, Katschinski DM, Liao D, et al. Endothelial cell HIF-1α and HIF-2α differentially regulate metastatic success. *Cancer Cell* (2012) 21:52–65. doi:10.1016/j.ccr.2011.11.017
15. Mamlouk S, Wielockx B. Hypoxia-inducible factors as key regulators of tumor inflammation. *Int J Cancer* (2013) 132:2721–9. doi:10.1002/ijc.27901
16. LaGory EL, Giaccia AJ. The ever-expanding role of HIF in tumour and stromal biology. *Nat Cell Biol* (2016) 18:356–65. doi:10.1038/ncb3330
17. Werno C, Menrad H, Weigert A, Dehne N, Goerdt S, Schledzewski K, et al. Knockout of HIF-1α in tumor-associated macrophages enhances M2

polarization and attenuates their pro-angiogenic responses. *Carcinogenesis* (2010) 31:1863–72. doi:10.1093/carcin/bgq088
18. Imtiyaz HZ, Williams EP, Hickey MM, Patel SA, Durham AC, Yuan L-J, et al. Hypoxia-inducible factor 2α regulates macrophage function in mouse models of acute and tumor inflammation. *J Clin Invest* (2010) 120:2699. doi:10.1172/JCI39506
19. Doedens AL, Stockmann C, Rubinstein MP, Liao D, Zhang N, Denardo DG, et al. Macrophage expression of hypoxia-inducible factor-1α suppresses T-cell function and promotes tumor progression. *Cancer Res* (2010) 70:7465–75. doi:10.1158/0008-5472.CAN-10-1439
20. Mamlouk S, Kalucka J, Singh RP, Franke K, Muschter A, Langer A, et al. Loss of prolyl hydroxylase-2 in myeloid cells and T-lymphocytes impairs tumor development. *Int J Cancer* (2014) 134:849–58. doi:10.1002/ijc.28409
21. Clever D, Roychoudhuri R, Constantinides MG, Askenase MH, Sukumar M, Klebanoff CA, et al. Oxygen sensing by T cells establishes an immunologically tolerant metastatic niche. *Cell* (2016) 166:1117–31.e1114. doi:10.1016/j.cell.2016.07.032
22. Kumar V, Gabrilovich DI. Hypoxia-inducible factors in regulation of immune responses in tumour microenvironment. *Immunology* (2014) 143:512–9. doi:10.1111/imm.12380
23. Zeng W, Liu P, Pan W, Singh SR, Wei Y. Hypoxia and hypoxia inducible factors in tumor metabolism. *Cancer Lett* (2015) 356:263–7. doi:10.1016/j.canlet.2014.01.032
24. Corcoran SE, O'Neill LA. HIF1α and metabolic reprogramming in inflammation. *J Clin Invest* (2016) 126:3699–707. doi:10.1172/JCI84431
25. Eales K, Hollinshead K, Tennant D. Hypoxia and metabolic adaptation of cancer cells. *Oncogenesis* (2016) 5:e190. doi:10.1038/oncsis.2015.50
26. Selfridge AC, Cavadas MA, Scholz CC, Campbell EL, Welch LC, Lecuona E, et al. Hypercapnia suppresses the HIF-dependent adaptive response to hypoxia. *J Biol Chem* (2016) 291:11800–8. doi:10.1074/jbc.M116.713941
27. Chandel NS, Mcclintock DS, Feliciano CE, Wood TM, Melendez JA, Rodriguez AM, et al. Reactive oxygen species generated at mitochondrial complex III stabilize hypoxia-inducible factor-1α during hypoxia a mechanism of O_2 sensing. *J Biol Chem* (2000) 275:25130–8. doi:10.1074/jbc.M001914200
28. Masson N, Singleton RS, Sekirnik R, Trudgian DC, Ambrose LJ, Miranda MX, et al. The FIH hydroxylase is a cellular peroxide sensor that modulates HIF transcriptional activity. *EMBO Rep* (2012) 13:251–7. doi:10.1038/embor.2012.9
29. Boulahbel H, Durán RV, Gottlieb E. *Prolyl Hydroxylases as Regulators of Cell Metabolism*. Biochem Soc Trans, (2009) 37(1):291–4.
30. Yang M, Soga T, Pollard PJ, Adam J. The emerging role of fumarate as an oncometabolite. *Front Oncol* (2012) 2:85. doi:10.3389/fonc.2012.00085
31. Ratcliffe PJ. Oxygen sensing and hypoxia signalling pathways in animals: the implications of physiology for cancer. *J Physiol* (2013) 591:2027–42. doi:10.1113/jphysiol.2013.251470
32. Tannahill G, Curtis A, Adamik J, Palsson-Mcdermott E, Mcgettrick A, Goel G, et al. Succinate is a danger signal that induces IL-1β via HIF-1α. *Nature* (2013) 496:238. doi:10.1038/nature11986
33. Warburg O. The metabolism of carcinoma cells. *J Cancer Res* (1925) 9:148–63. doi:10.1158/jcr.1925.148
34. Shestov AA, Liu X, Ser Z, Cluntun AA, Hung YP, Huang L, et al. Quantitative determinants of aerobic glycolysis identify flux through the enzyme GAPDH as a limiting step. *Elife* (2014) 3:e03342. doi:10.7554/eLife.03342
35. DeBerardinis RJ, Lum JJ, Hatzivassiliou G, Thompson CB. The biology of cancer: metabolic reprogramming fuels cell growth and proliferation. *Cell Metab* (2008) 7:11–20. doi:10.1016/j.cmet.2007.10.002
36. Levine AJ, Puzio-Kuter AM. The control of the metabolic switch in cancers by oncogenes and tumor suppressor genes. *Science* (2010) 330:1340–4. doi:10.1126/science.1193494
37. Cairns RA, Harris IS, Mak TW. Regulation of cancer cell metabolism. *Nat Rev Cancer* (2011) 11:85–95. doi:10.1038/nrc2981
38. Koppenol WH, Bounds PL, Dang CV. Otto Warburg's contributions to current concepts of cancer metabolism. *Nat Rev Cancer* (2011) 11:325–37. doi:10.1038/nrc3038
39. Dang CV. Links between metabolism and cancer. *Genes Dev* (2012) 26:877–90. doi:10.1101/gad.189365.112
40. Patra KC, Wang Q, Bhaskar PT, Miller L, Wang Z, Wheaton W, et al. Hexokinase 2 is required for tumor initiation and maintenance and its systemic deletion is therapeutic in mouse models of cancer. *Cancer Cell* (2013) 24:213–28. doi:10.1016/j.ccr.2013.06.014
41. Boroughs LK, DeBerardinis RJ. Metabolic pathways promoting cancer cell survival and growth. *Nat Cell Biol* (2015) 17:351. doi:10.1038/ncb3124
42. Gatenby RA, Gawlinski ET. A reaction-diffusion model of cancer invasion. *Cancer Res* (1996) 56:5745–53.
43. Colegio OR, Chu N-Q, Szabo AL, Chu T, Rhebergen AM, Jairam V, et al. Functional polarization of tumour-associated macrophages by tumour-derived lactic acid. *Nature* (2014) 513:559. doi:10.1038/nature13490
44. Bonuccelli G, Tsirigos A, Whitaker-Menezes D, Pavlides S, Pestell RG, Chiavarina B, et al. Ketones and lactate "fuel" tumor growth and metastasis: evidence that epithelial cancer cells use oxidative mitochondrial metabolism. *Cell Cycle* (2010) 9:3506–14. doi:10.4161/cc.9.17.12731
45. Migneco G, Whitaker-Menezes D, Chiavarina B, Castello-Cros R, Pavlides S, Pestell RG, et al. Glycolytic cancer associated fibroblasts promote breast cancer tumor growth, without a measurable increase in angiogenesis: evidence for stromal-epithelial metabolic coupling. *Cell Cycle* (2010) 9:2412–22. doi:10.4161/cc.9.12.11989
46. Lehninger A, Nelson D, Cox M. *Principles of Biochemistry*. 2nd ed. New York: Worth (1993).
47. Wong N, Ojo D, Yan J, Tang D. PKM2 contributes to cancer metabolism. *Cancer Lett* (2015) 356:184–91. doi:10.1016/j.canlet.2014.01.031
48. Christofk HR, Vander Heiden MG, Harris MH, Ramanathan A, Gerszten RE, Wei R, et al. The M2 splice isoform of pyruvate kinase is important for cancer metabolism and tumour growth. *Nature* (2008) 452:230. doi:10.1038/nature06734
49. Vander Heiden MG, Christofk HR, Schuman E, Subtelny AO, Sharfi H, Harlow EE, et al. Identification of small molecule inhibitors of pyruvate kinase M2. *Biochem Pharmacol* (2010) 79:1118–24. doi:10.1016/j.bcp.2009.12.003
50. Anastasiou D, Yu Y, Israelsen WJ, Jiang J-K, Boxer MB, Hong BS, et al. Pyruvate kinase M2 activators promote tetramer formation and suppress tumorigenesis. *Nat Chem Biol* (2012) 8:839–47. doi:10.1038/nchembio1212-1008b
51. Luo W, Hu H, Chang R, Zhong J, Knabel M, O'Meally R, et al. Pyruvate kinase M2 is a PHD3-stimulated coactivator for hypoxia-inducible factor 1. *Cell* (2011) 145:732–44. doi:10.1016/j.cell.2011.03.054
52. Kim J-W, Tchernyshyov I, Semenza GL, Dang CV. HIF-1-mediated expression of pyruvate dehydrogenase kinase: a metabolic switch required for cellular adaptation to hypoxia. *Cell Metab* (2006) 3:177–85. doi:10.1016/j.cmet.2006.02.002
53. Chae YC, Vaira V, Caino MC, Tang HY, Seo JH, Kossenkov AV, et al. Mitochondrial Akt regulation of hypoxic tumor reprogramming. *Cancer Cell* (2016) 30:257–72. doi:10.1016/j.ccell.2016.07.004
54. Brugarolas JB, Vazquez F, Reddy A, Sellers WR, Kaelin WG. TSC2 regulates VEGF through mTOR-dependent and-independent pathways. *Cancer Cell* (2003) 4:147–58. doi:10.1016/S1535-6108(03)00187-9
55. Mayerhofer M, Valent P, Sperr WR, Griffin JD, Sillaber C. BCR/ABL induces expression of vascular endothelial growth factor and its transcriptional activator, hypoxia inducible factor-1α, through a pathway involving phosphoinositide 3-kinase and the mammalian target of rapamycin. *Blood* (2002) 100:3767–75. doi:10.1182/blood-2002-01-0109
56. Pollard P, Briere J, Alam N, Barwell J, Barclay E, Wortham N, et al. Accumulation of Krebs cycle intermediates and over-expression of HIF1α in tumours which result from germline FH and SDH mutations. *Hum Mol Genet* (2005) 14:2231–9. doi:10.1093/hmg/ddi227
57. Lu H, Dalgard CL, Mohyeldin A, Mcfate T, Tait AS, Verma A. Reversible inactivation of HIF-1 prolyl hydroxylases allows cell metabolism to control basal HIF-1. *J Biol Chem* (2005) 280:41928–39. doi:10.1074/jbc.M508718200
58. Reitman ZJ, Yan H. Isocitrate dehydrogenase 1 and 2 mutations in cancer: alterations at a crossroads of cellular metabolism. *J Natl Cancer Inst* (2010) 102:932–41. doi:10.1093/jnci/djq187
59. Ye D, Ma S, Xiong Y, Guan K-L. R-2-hydroxyglutarate as the key effector of IDH mutations promoting oncogenesis. *Cancer Cell* (2013) 23:274–6. doi:10.1016/j.ccr.2013.03.005
60. Wong BW, Kuchnio A, Bruning U, Carmeliet P. Emerging novel functions of the oxygen-sensing prolyl hydroxylase domain enzymes. *Trends Biochem Sci* (2013) 38:3–11. doi:10.1016/j.tibs.2012.10.004

61. Fogal V, Richardson AD, Karmali PP, Scheffler IE, Smith JW, Ruoslahti E. Mitochondrial p32 protein is a critical regulator of tumor metabolism via maintenance of oxidative phosphorylation. *Mol Cell Biol* (2010) 30:1303–18. doi:10.1128/MCB.01101-09
62. Weinberg F, Hamanaka R, Wheaton WW, Weinberg S, Joseph J, Lopez M, et al. Mitochondrial metabolism and ROS generation are essential for Kras-mediated tumorigenicity. *Proc Natl Acad Sci U S A* (2010) 107:8788–93. doi:10.1073/pnas.1003428107
63. Guo JY, Chen H-Y, Mathew R, Fan J, Strohecker AM, Karsli-Uzunbas G, et al. Activated Ras requires autophagy to maintain oxidative metabolism and tumorigenesis. *Genes Dev* (2011) 25:460–70. doi:10.1101/gad.2016311
64. Fan J, Ye J, Kamphorst JJ, Shlomi T, Thompson CB, Rabinowitz JD. Quantitative flux analysis reveals folate-dependent NADPH production. *Nature* (2014) 510:298. doi:10.1038/nature13236
65. Lewis CA, Parker SJ, Fiske BP, Mccloskey D, Gui DY, Green CR, et al. Tracing compartmentalized NADPH metabolism in the cytosol and mitochondria of mammalian cells. *Mol Cell* (2014) 55:253–63. doi:10.1016/j.molcel.2014.05.008
66. Ackerman D, Simon MC. Hypoxia, lipids, and cancer: surviving the harsh tumor microenvironment. *Trends Cell Biol* (2014) 24:472–8. doi:10.1016/j.tcb.2014.06.001
67. de Padua MC, Delodi G, Vucetic M, Durivault J, Vial V, Bayer P, et al. Disrupting glucose-6-phosphate isomerase fully suppresses the "Warburg effect" and activates OXPHOS with minimal impact on tumor growth except in hypoxia. *Oncotarget* (2017) 8:87623–37. doi:10.18632/oncotarget.21007
68. Menendez JA, Lupu R. Fatty acid synthase and the lipogenic phenotype in cancer pathogenesis. *Nat Rev Cancer* (2007) 7:763. doi:10.1038/nrc2222
69. Zaidi N, Lupien L, Kuemmerle NB, Kinlaw WB, Swinnen JV, Smans K. Lipogenesis and lipolysis: the pathways exploited by the cancer cells to acquire fatty acids. *Prog Lipid Res* (2013) 52:585–9. doi:10.1016/j.plipres.2013.08.005
70. Kuemmerle NB, Rysman E, Lombardo PS, Flanagan AJ, Lipe BC, Wells WA, et al. Lipoprotein lipase links dietary fat to solid tumor cell proliferation. *Mol Cancer Ther* (2011) 10:427–36. doi:10.1158/1535-7163.MCT-10-0802
71. Griffiths B, Lewis CA, Bensaad K, Ros S, Zhang Q, Ferber EC, et al. Sterol regulatory element binding protein-dependent regulation of lipid synthesis supports cell survival and tumor growth. *Cancer Metab* (2013) 1:3. doi:10.1186/2049-3002-1-3
72. Ozcan U, Ozcan L, Yilmaz E, Düvel K, Sahin M, Manning BD, et al. Loss of the tuberous sclerosis complex tumor suppressors triggers the unfolded protein response to regulate insulin signaling and apoptosis. *Mol Cell* (2008) 29:541–51. doi:10.1016/j.molcel.2007.12.023
73. Young RM, Ackerman D, Quinn ZL, Mancuso A, Gruber M, Liu L, et al. Dysregulated mTORC1 renders cells critically dependent on desaturated lipids for survival under tumor-like stress. *Genes Dev* (2013) 27:1115–31. doi:10.1101/gad.198630.112
74. Schug ZT, Peck B, Jones DT, Zhang Q, Grosskurth S, Alam IS, et al. Acetyl-CoA synthetase 2 promotes acetate utilization and maintains cancer cell growth under metabolic stress. *Cancer Cell* (2015) 27:57–71. doi:10.1016/j.ccell.2014.12.002
75. Nomura DK, Long JZ, Niessen S, Hoover HS, Ng S-W, Cravatt BF. Monoacylglycerol lipase regulates a fatty acid network that promotes cancer pathogenesis. *Cell* (2010) 140:49–61. doi:10.1016/j.cell.2009.11.027
76. Metallo CM, Gameiro PA, Bell EL, Mattaini KR, Yang J, Hiller K, et al. Reductive glutamine metabolism by IDH1 mediates lipogenesis under hypoxia. *Nature* (2012) 481:380. doi:10.1038/nature10602
77. Son J, Lyssiotis CA, Ying H, Wang X, Hua S, Ligorio M, et al. Glutamine supports pancreatic cancer growth through a KRAS-regulated metabolic pathway. *Nature* (2013) 496:101. doi:10.1038/nature12040
78. Hao Y, Samuels Y, Li Q, Krokowski D, Guan B-J, Wang C, et al. Oncogenic PIK3CA mutations reprogram glutamine metabolism in colorectal cancer. *Nat Commun* (2016) 7:11971. doi:10.1038/ncomms11971
79. Corn PG, Ricci MS, Scata KA, Arsham AM, Simon MC, Dicker DT, et al. Mxi1 is induced by hypoxia in a HIF-1-dependent manner and protects cells from c-Myc-induced apoptosis. *Cancer Biol Ther* (2005) 4:1285–94. doi:10.4161/cbt.4.11.2299
80. Gordan JD, Bertout JA, Hu C-J, Diehl JA, Simon MC. HIF-2α promotes hypoxic cell proliferation by enhancing c-myc transcriptional activity. *Cancer Cell* (2007) 11:335–47. doi:10.1016/j.ccr.2007.02.006
81. Mullen AR, Wheaton WW, Jin ES, Chen P-H, Sullivan LB, Cheng T, et al. Reductive carboxylation supports growth in tumor cells with defective mitochondria. *Nature* (2012) 481:385. doi:10.1038/nature10642
82. Kim WY, Safran M, Buckley MRM, Ebert BL, Glickman J, Bosenberg M, et al. Failure to prolyl hydroxylate hypoxia-inducible factor alpha phenocopies VHL inactivation in vivo. *EMBO J* (2006) 25:4650–62. doi:10.1038/sj.emboj.7601300
83. Gameiro PA, Yang J, Metelo AM, Pérez-Carro R, Baker R, Wang Z, et al. In vivo HIF-mediated reductive carboxylation is regulated by citrate levels and sensitizes VHL-deficient cells to glutamine deprivation. *Cell Metab* (2013) 17:372–85. doi:10.1016/j.cmet.2013.02.002
84. Fendt S-M, Bell EL, Keibler MA, Olenchock BA, Mayers JR, Wasylenko TM, et al. Reductive glutamine metabolism is a function of the α-ketoglutarate to citrate ratio in cells. *Nat Commun* (2013) 4:2236. doi:10.1038/ncomms3236
85. Opitz CA, Litzenburger UM, Sahm F, Ott M, Tritschler I, Trump S, et al. Anendogenous tumour-promoting ligand of the human aryl hydrocarbon receptor. *Nature* (2011) 478:197. doi:10.1038/nature10491
86. Uyttenhove C, Pilotte L, Théate I, Stroobant V, Colau D, Parmentier N, et al. Evidence for a tumoral immune resistance mechanism based on tryptophan degradation by indoleamine 2, 3-dioxygenase. *Nat Med* (2003) 9:1269. doi:10.1038/nm934
87. Wang T, Liu G, Wang R. The intercellular metabolic interplay between tumor and immune cells. *Front Immunol* (2014) 5:358. doi:10.3389/fimmu.2014.00358
88. Chang C-H, Qiu J, O'Sullivan D, Buck MD, Noguchi T, Curtis JD, et al. Metabolic competition in the tumor microenvironment is a driver of cancer progression. *Cell* (2015) 162:1229–41. doi:10.1016/j.cell.2015.08.016
89. Ho P-C, Bihuniak JD, Macintyre AN, Staron M, Liu X, Amezquita R, et al. Phosphoenolpyruvate is a metabolic checkpoint of anti-tumor T cell responses. *Cell* (2015) 162:1217–28. doi:10.1016/j.cell.2015.08.012
90. De Bock K, Georgiadou M, Schoors S, Kuchnio A, Wong BW, Cantelmo AR, et al. Role of PFKFB3-driven glycolysis in vessel sprouting. *Cell* (2013) 154:651–63. doi:10.1016/j.cell.2013.06.037
91. Quintero M, Colombo SL, Godfrey A, Moncada S. Mitochondria as signaling organelles in the vascular endothelium. *Proc Natl Acad Sci U S A* (2006) 103:5379–84. doi:10.1073/pnas.0601026103
92. Leopold JA, Zhang Y-Y, Scribner AW, Stanton RC, Loscalzo J. Glucose-6-phosphate dehydrogenase overexpression decreases endothelial cell oxidant stress and increases bioavailable nitric oxide. *Arterioscler Thromb Vasc Biol* (2003) 23:411–7. doi:10.1161/01.ATV.0000056744.26901.BA
93. Unterluggauer H, Mazurek S, Lener B, Hütter E, Eigenbrodt E, Zwerschke W, et al. Premature senescence of human endothelial cells induced by inhibition of glutaminase. *Biogerontology* (2008) 9:247–59. doi:10.1007/s10522-008-9134-x
94. Polet F, Feron O. Endothelial cell metabolism and tumour angiogenesis: glucose and glutamine as essential fuels and lactate as the driving force. *J Intern Med* (2013) 273:156–65. doi:10.1111/joim.12016
95. Avraham-Davidi I, Ely Y, Pham VN, Castranova D, Grunspan M, Malkinson G, et al. ApoB-containing lipoproteins regulate angiogenesis by modulating expression of VEGF receptor 1. *Nat Med* (2012) 18:967–73. doi:10.1038/nm.2759
96. Schoors S, Bruning U, Missiaen R, Queiroz KC, Borgers G, Elia I, et al. Fatty acid carbon is essential for dNTP synthesis in endothelial cells. *Nature* (2015) 520:192. doi:10.1038/nature14362
97. Coulon C, Georgiadou M, Roncal C, De Bock K, Langenberg T, Carmeliet P. From vessel sprouting to normalization. *Arterioscler Thromb Vasc Biol* (2010) 30:2331–6. doi:10.1161/ATVBAHA.110.214106
98. Skuli N, Liu L, Runge A, Wang T, Yuan L, Patel S, et al. Endothelial deletion of hypoxia-inducible factor-2α (HIF-2α) alters vascular function and tumor angiogenesis. *Blood* (2009) 114:469–77. doi:10.1182/blood-2008-12-193581
99. Skuli N, Majmundar AJ, Krock BL, Mesquita RC, Mathew LK, Quinn ZL, et al. Endothelial HIF-2α regulates murine pathological angiogenesis and revascularization processes. *J Clin Invest* (2012) 122:1427. doi:10.1172/JCI57322
100. Boidot R, Végran F, Meulle A, Lebreton A, Dessy C, Sonveaux P, et al. Regulation of monocarboxylate transporter MCT1 expression by p53 mediates inward and outward lactate fluxes in tumors. *Cancer Res* (2011) 72:939–48. doi:10.1158/0008-5472.CAN-11-2474

101. De Saedeleer CJ, Copetti T, Porporato PE, Verrax J, Feron O, Sonveaux P. Lactate activates HIF-1 in oxidative but not in Warburg-phenotype human tumor cells. *PLoS One* (2012) 7:e46571. doi:10.1371/journal.pone.0046571
102. Sonveaux P, Copetti T, De Saedeleer CJ, Végran F, Verrax J, Kennedy KM, et al. Targeting the lactate transporter MCT1 in endothelial cells inhibits lactate-induced HIF-1 activation and tumor angiogenesis. *PLoS One* (2012) 7:e33418. doi:10.1371/journal.pone.0033418
103. Végran F, Boidot R, Michiels C, Sonveaux P, Feron O. Lactate influx through the endothelial cell monocarboxylate transporter MCT1 supports an NF-κB/IL-8 pathway that drives tumor angiogenesis. *Cancer Res* (2011) 71:2550–60. doi:10.1158/0008-5472.CAN-10-2828
104. Ruan G-X, Kazlauskas A. Lactate engages receptor tyrosine kinases Axl, Tie2 and VEGFR-2 to activate PI3K/Akt and promote angiogenesis. *J Biol Chem* (2013) 288(29):21161–72. doi:10.1074/jbc.M113.474619
105. Kalluri R, Zeisberg M. Fibroblasts in cancer. *Nat Rev Cancer* (2006) 6:392. doi:10.1038/nrc1877
106. Direkze NC, Hodivala-Dilke K, Jeffery R, Hunt T, Poulsom R, Oukrif D, et al. Bone marrow contribution to tumor-associated myofibroblasts and fibroblasts. *Cancer Res* (2004) 64:8492–5. doi:10.1158/0008-5472.CAN-04-1708
107. Hinz B, Phan SH, Thannickal VJ, Galli A, Bochaton-Piallat M-L, Gabbiani G. The myofibroblast: one function, multiple origins. *Am J Pathol* (2007) 170:1807–16. doi:10.2353/ajpath.2007.070112
108. Bochet L, Lehuédé C, Dauvillier S, Wang YY, Dirat B, Laurent V, et al. Adipocyte-derived fibroblasts promote tumor progression and contribute to the desmoplastic reaction in breast cancer. *Cancer Res* (2013) 73:5657–68. doi:10.1158/0008-5472.CAN-13-0530
109. Gilkes DM, Semenza GL, Wirtz D. Hypoxia and the extracellular matrix: drivers of tumour metastasis. *Nat Rev Cancer* (2014) 14:430–9. doi:10.1038/nrc3726
110. Madsen CD, Pedersen JT, Venning FA, Singh LB, Moeendarbary E, Charras G, et al. Hypoxia and loss of PHD2 inactivate stromal fibroblasts to decrease tumour stiffness and metastasis. *EMBO Rep* (2015) 16:1394–408. doi:10.15252/embr.201540107
111. Lemons JM, Feng X-J, Bennett BD, Legesse-Miller A, Johnson EL, Raitman I, et al. Quiescent fibroblasts exhibit high metabolic activity. *PLoS Biol* (2010) 8:e1000514. doi:10.1371/journal.pbio.1000514
112. Santolla MF, Lappano R, De Marco P, Pupo M, Vivacqua A, Sisci D, et al. G protein-coupled estrogen receptor mediates the up-regulation of fatty acid synthase induced by 17β-estradiol in cancer cells and cancer-associated fibroblasts. *J Biol Chem* (2012) 287:43234–45. doi:10.1074/jbc.M112.417303
113. Pavlides S, Vera I, Gandara R, Sneddon S, Pestell RG, Mercier I, et al. Warburg meets autophagy: cancer-associated fibroblasts accelerate tumor growth and metastasis via oxidative stress, mitophagy, and aerobic glycolysis. *Antioxid Redox Signal* (2012) 16:1264–84. doi:10.1089/ars.2011.4243
114. Lisanti MP, Martinez-Outschoorn UE, Chiavarina B, Pavlides S, Whitaker-Menezes D, Tsirigos A, et al. Understanding the "lethal" drivers of tumor-stroma co-evolution: emerging role (s) for hypoxia, oxidative stress and autophagy/mitophagy in the tumor microenvironment. *Cancer Biol Ther* (2010) 10:537–42. doi:10.4161/cbt.10.6.13370
115. Chaudhri VK, Salzler GG, Dick SA, Buckman MS, Sordella R, Karoly ED, et al. Metabolic alterations in lung cancer-associated fibroblasts correlated with increased glycolytic metabolism of the tumor. *Mol Cancer Res* (2013) 11:579–92. doi:10.1158/1541-7786.MCR-12-0437-T
116. Kim J-W, Evans C, Weidemann A, Takeda N, Lee YS, Stockmann C, et al. Loss of fibroblast HIF-1α accelerates tumorigenesis. *Cancer Res* (2012) 72:3187–95. doi:10.1158/0008-5472.CAN-12-0534
117. Chiavarina B, Martinez-Outschoorn UE, Whitaker-Menezes D, Howell A, Tanowitz HB, Pestell RG, et al. Metabolic reprogramming and two-compartment tumor metabolism: opposing role (s) of HIF1α and HIF2α in tumor-associated fibroblasts and human breast cancer cells. *Cell Cycle* (2012) 11:3280–9. doi:10.4161/cc.21643
118. Fiaschi T, Marini A, Giannoni E, Taddei ML, Gandellini P, De Donatis A, et al. Reciprocal metabolic reprogramming through lactate shuttle coordinately influences tumor-stroma interplay. *Cancer Res* (2012) 72:5130–40. doi:10.1158/0008-5472.CAN-12-1949
119. Bronte V, Zanovello P. Regulation of immune responses by L-arginine metabolism. *Nat Rev Immunol* (2005) 5:641. doi:10.1038/nri1668
120. Ino Y, Yamazaki-Itoh R, Oguro S, Shimada K, Kosuge T, Zavada J, et al. Arginase II expressed in cancer-associated fibroblasts indicates tissue hypoxia and predicts poor outcome in patients with pancreatic cancer. *PLoS One* (2013) 8:e55146. doi:10.1371/journal.pone.0055146
121. Biswas SK. Metabolic reprogramming of immune cells in cancer progression. *Immunity* (2015) 43:435–49. doi:10.1016/j.immuni.2015.09.001
122. Wang R, Dillon CP, Shi LZ, Milasta S, Carter R, Finkelstein D, et al. The transcription factor Myc controls metabolic reprogramming upon T lymphocyte activation. *Immunity* (2011) 35:871–82. doi:10.1016/j.immuni.2011.09.021
123. Wang R, Green DR. Metabolic reprogramming and metabolic dependency in T cells. *Immunol Rev* (2012) 249:14–26. doi:10.1111/j.1600-065X.2012.01155.x
124. Maclver NJ, Michalek RD, Rathmell JC. Metabolic regulation of T lymphocytes. *Annu Rev Immunol* (2013) 31:259–83. doi:10.1146/annurev-immunol-032712-095956
125. Gerriets VA, Kishton RJ, Nichols AG, Macintyre AN, Inoue M, Ilkayeva O, et al. Metabolic programming and PDHK1 control CD4+ T cell subsets and inflammation. *J Clin Invest* (2015) 125:194. doi:10.1172/JCI76012
126. Dang EV, Barbi J, Yang H-Y, Jinasena D, Yu H, Zheng Y, et al. Control of TH 17/Treg balance by hypoxia-inducible factor 1. *Cell* (2011) 146:772–84. doi:10.1016/j.cell.2011.07.033
127. Shi LZ, Wang R, Huang G, Vogel P, Neale G, Green DR, et al. HIF1α-dependent glycolytic pathway orchestrates a metabolic checkpoint for the differentiation of TH17 and Treg cells. *J Exp Med* (2011) 208:1367–76. doi:10.1084/jem.20110278
128. Waickman AT, Powell JD. mTOR, metabolism, and the regulation of T-cell differentiation and function. *Immunol Rev* (2012) 249:43–58. doi:10.1111/j.1600-065X.2012.01152.x
129. Michalek RD, Gerriets VA, Jacobs SR, Macintyre AN, Maciver NJ, Mason EF, et al. Cutting edge: distinct glycolytic and lipid oxidative metabolic programs are essential for effector and regulatory CD4+ T cell subsets. *J Immunol* (2011) 186:3299–303. doi:10.4049/jimmunol.1003613
130. Macintyre AN, Gerriets VA, Nichols AG, Michalek RD, Rudolph MC, Deoliveira D, et al. The glucose transporter Glut1 is selectively essential for CD4 T cell activation and effector function. *Cell Metab* (2014) 20:61–72. doi:10.1016/j.cmet.2014.05.004
131. Cham CM, Driessens G, O'Keefe JP, Gajewski TF. Glucose deprivation inhibits multiple key gene expression events and effector functions in CD8+ T cells. *Eur J Immunol* (2008) 38:2438–50. doi:10.1002/eji.200838289
132. Shackelford DB, Shaw RJ. The LKB1-AMPK pathway: metabolism and growth control in tumor suppression. *Nat Rev Cancer* (2009) 9:563. doi:10.1038/nrc2676
133. Delgoffe GM, Kole TP, Zheng Y, Zarek PE, Matthews KL, Xiao B, et al. The mTOR kinase differentially regulates effector and regulatory T cell lineage commitment. *Immunity* (2009) 30:832–44. doi:10.1016/j.immuni.2009.04.014
134. Yang K, Shrestha S, Zeng H, Karmaus PW, Neale G, Vogel P, et al. T cell exit from quiescence and differentiation into Th2 cells depend on raptor-mTORC1-mediated metabolic reprogramming. *Immunity* (2013) 39:1043–56. doi:10.1016/j.immuni.2013.09.015
135. Berod L, Friedrich C, Nandan A, Freitag J, Hagemann S, Harmrolfs K, et al. De novo fatty acid synthesis controls the fate between regulatory T and T helper 17 cells. *Nat Med* (2014) 20:1327–33. doi:10.1038/nm.3704
136. Lee J, Walsh MC, Hoehn KL, James DE, Wherry EJ, Choi Y. Regulator of fatty acid metabolism, acetyl coenzyme a carboxylase 1, controls T cell immunity. *J Immunol* (2014) 192:3190–9. doi:10.4049/jimmunol.1302985
137. Nakamura H, Makino Y, Okamoto K, Poellinger L, Ohnuma K, Morimoto C, et al. TCR engagement increases hypoxia-inducible factor-1α protein synthesis via rapamycin-sensitive pathway under hypoxic conditions in human peripheral T cells. *J Immunol* (2005) 174:7592–9. doi:10.4049/jimmunol.174.12.7592
138. Chisolm DA, Weinmann AS. TCR-signaling events in cellular metabolism and specialization. *Front Immunol* (2015) 6:292. doi:10.3389/fimmu.2015.00292
139. Noman MZ, Buart S, Van Pelt J, Richon C, Hasmim M, Leleu N, et al. The cooperative induction of hypoxia-inducible factor-1α and STAT3 during hypoxia induced an impairment of tumor susceptibility to CTL-mediated cell lysis. *J Immunol* (2009) 182:3510–21. doi:10.4049/jimmunol.0800854

140. Bailey SR, Nelson MH, Himes RA, Li Z, Mehrotra S, Paulos CM. Th17 cells in cancer: the ultimate identity crisis. *Front Immunol* (2014) 5:276. doi:10.3389/fimmu.2014.00276
141. Sena LA, Li S, Jairaman A, Prakriya M, Ezponda T, Hildeman DA, et al. Mitochondria are required for antigen-specific T cell activation through reactive oxygen species signaling. *Immunity* (2013) 38:225–36. doi:10.1016/j.immuni.2012.10.020
142. Barsoum IB, Koti M, Siemens DR, Graham CH. Mechanisms of hypoxia-mediated immune escape in cancer. *Cancer Res* (2014) 74:7185–90. doi:10.1158/0008-5472.CAN-14-2598
143. Chen JL-Y, Lucas JE, Schroeder T, Mori S, Wu J, Nevins J, et al. The genomic analysis of lactic acidosis and acidosis response in human cancers. *PLoS Genet* (2008) 4:e1000293. doi:10.1371/journal.pgen.1000293
144. Düvel K, Yecies JL, Menon S, Raman P, Lipovsky AI, Souza AL, et al. Activation of a metabolic gene regulatory network downstream of mTOR complex 1. *Mol Cell* (2010) 39:171–83. doi:10.1016/j.molcel.2010.06.022
145. Pauken KE, Wherry EJ. Overcoming T cell exhaustion in infection and cancer. *Trends Immunol* (2015) 36:265–76. doi:10.1016/j.it.2015.02.008
146. Platten M, Von Knebel Doeberitz N, Oezen I, Wick W, Ochs K. Cancer immunotherapy by targeting IDO1/TDO and their downstream effectors. *Front Immunol* (2014) 5:673. doi:10.3389/fimmu.2014.00673
147. Brand A, Singer K, Koehl GE, Kolitzus M, Schoenhammer G, Thiel A, et al. LDHA-associated lactic acid production blunts tumor immunosurveillance by T and NK cells. *Cell Metab* (2016) 24:657–71. doi:10.1016/j.cmet.2016.08.011
148. Murray PJ, Allen JE, Biswas SK, Fisher EA, Gilroy DW, Goerdt S, et al. Macrophage activation and polarization: nomenclature and experimental guidelines. *Immunity* (2014) 41:14–20. doi:10.1016/j.immuni.2014.06.008
149. Xue J, Schmidt SV, Sander J, Draffehn A, Krebs W, Quester I, et al. Transcriptome-based network analysis reveals a spectrum model of human macrophage activation. *Immunity* (2014) 40:274–88. doi:10.1016/j.immuni.2014.01.006
150. Rodríguez-Prados J-C, Través PG, Cuenca J, Rico D, Aragonés J, Martín-Sanz P, et al. Substrate fate in activated macrophages: a comparison between innate, classic, and alternative activation. *J Immunol* (2010) 185:605–14. doi:10.4049/jimmunol.0901698
151. Nizet V, Johnson RS. Interdependence of hypoxic and innate immune responses. *Nat Rev Immunol* (2009) 9:609. doi:10.1038/nri2607
152. Dietl K, Renner K, Dettmer K, Timischl B, Eberhart K, Dorn C, et al. Lactic acid and acidification inhibit TNF secretion and glycolysis of human monocytes. *J Immunol* (2010) 184:1200–9. doi:10.4049/jimmunol.0902584
153. O'Neill LA. A critical role for citrate metabolism in LPS signalling. *Biochem J* (2011) 438:e5–6. doi:10.1042/BJ20111386
154. Vats D, Mukundan L, Odegaard JI, Zhang L, Smith KL, Morel CR, et al. Oxidative metabolism and PGC-1β attenuate macrophage-mediated inflammation. *Cell Metab* (2006) 4:13–24. doi:10.1016/j.cmet.2006.08.006
155. O'Neill LA, Hardie DG. Metabolism of inflammation limited by AMPK and pseudo-starvation. *Nature* (2013) 493:346. doi:10.1038/nature11862
156. Haschemi A, Kosma P, Gille L, Evans CR, Burant CF, Starkl P, et al. The sedoheptulose kinase CARKL directs macrophage polarization through control of glucose metabolism. *Cell Metab* (2012) 15:813–26. doi:10.1016/j.cmet.2012.04.023
157. Popovic PJ, Zeh HJ, Ochoa JB. Arginine and immunity. *J Nutr* (2007) 137:1681S–6S.
158. Mills C. M1 and M2 macrophages: oracles of health and disease. *Crit Rev Immunol* (2012) 32(6):463–88. doi:10.1615/CritRevImmunol.v32.i6.10
159. Recalcati S, Locati M, Marini A, Santambrogio P, Zaninotto F, De Pizzol M, et al. Differential regulation of iron homeostasis during human macrophage polarized activation. *Eur J Immunol* (2010) 40:824–35. doi:10.1002/eji.200939889
160. Cairo G, Recalcati S, Mantovani A, Locati M. Iron trafficking and metabolism in macrophages: contribution to the polarized phenotype. *Trends Immunol* (2011) 32:241–7. doi:10.1016/j.it.2011.03.007
161. Maxwell PH, Wiesener MS, Chang GW, Clifford SC, Vaux EC, Cockman ME, et al. The tumour suppressor protein VHL targets hypoxia-inducible factors for oxygen-dependent proteolysis. *Nature* (1999) 399:271–5. doi:10.1038/20459
162. Park J, Lee SE, Hur J, Hong EB, Choi J-I, Yang J-M, et al. M-CSF from cancer cells induces fatty acid synthase and PPARβ/δ activation in tumor myeloid cells, leading to tumor progression. *Cell Rep* (2015) 10:1614–25. doi:10.1016/j.celrep.2015.02.024
163. Bronte V, Brandau S, Chen S-H, Colombo MP, Frey AB, Greten TF, et al. Recommendations for myeloid-derived suppressor cell nomenclature and characterization standards. *Nat Commun* (2016) 7:12150. doi:10.1038/ncomms12150
164. Kowanetz M, Wu X, Lee J, Tan M, Hagenbeek T, Qu X, et al. Granulocyte-colony stimulating factor promotes lung metastasis through mobilization of Ly6G+ Ly6C+ granulocytes. *Proc Natl Acad Sci U S A* (2010) 107:21248–55. doi:10.1073/pnas.1015855107
165. Bronte V, Apolloni E, Cabrelle A, Ronca R, Serafini P, Zamboni P, et al. Identification of a CD11b+/Gr-1+/CD31+ myeloid progenitor capable of activating or suppressing CD8+ T cells. *Blood* (2000) 96:3838–46.
166. Morales JK, Kmieciak M, Knutson KL, Bear HD, Manjili MH. GM-CSF is one of the main breast tumor-derived soluble factors involved in the differentiation of CD11b-Gr1-bone marrow progenitor cells into myeloid-derived suppressor cells. *Breast Cancer Res Treat* (2010) 123:39–49. doi:10.1007/s10549-009-0622-8
167. Bronte V, Serafini P, De Santo C, Marigo I, Tosello V, Mazzoni A, et al. IL-4-induced arginase 1 suppresses alloreactive T cells in tumor-bearing mice. *J Immunol* (2003) 170:270–8. doi:10.4049/jimmunol.170.1.270
168. Gallina G, Dolcetti L, Serafini P, De Santo C, Marigo I, Colombo MP, et al. Tumors induce a subset of inflammatory monocytes with immunosuppressive activity on CD8+ T cells. *J Clin Invest* (2006) 116:2777. doi:10.1172/JCI28828
169. Rodríguez PC, Ochoa AC. Arginine regulation by myeloid derived suppressor cells and tolerance in cancer: mechanisms and therapeutic perspectives. *Immunol Rev* (2008) 222:180–91. doi:10.1111/j.1600-065X.2008.00608.x
170. Gabrilovich DI, Ostrand-Rosenberg S, Bronte V. Coordinated regulation of myeloid cells by tumours. *Nat Rev Immunol* (2012) 12:253. doi:10.1038/nri3175
171. Ugel S, De Sanctis F, Mandruzzato S, Bronte V. Tumor-induced myeloid deviation: when myeloid-derived suppressor cells meet tumor-associated macrophages. *J Clin Invest* (2015) 125:3365–76. doi:10.1172/JCI80006
172. Rodriguez PC, Quiceno DG, Zabaleta J, Ortiz B, Zea AH, Piazuelo MB, et al. Arginase I production in the tumor microenvironment by mature myeloid cells inhibits T-cell receptor expression and antigen-specific T-cell responses. *Cancer Res* (2004) 64:5839–49. doi:10.1158/0008-5472.CAN-04-0465
173. Rodriguez PC, Ernstoff MS, Hernandez C, Atkins M, Zabaleta J, Sierra R, et al. Arginase I-producing myeloid-derived suppressor cells in renal cell carcinoma are a subpopulation of activated granulocytes. *Cancer Res* (2009) 69:1553–60. doi:10.1158/0008-5472.CAN-08-1921
174. Srivastava MK, Sinha P, Clements VK, Rodriguez P, Ostrand-Rosenberg S. Myeloid-derived suppressor cells inhibit T-cell activation by depleting cystine and cysteine. *Cancer Res* (2010) 70:68–77. doi:10.1158/0008-5472.CAN-09-2587
175. Munn DH, Shafizadeh E, Attwood JT, Bondarev I, Pashine A, Mellor AL. Inhibition of T cell proliferation by macrophage tryptophan catabolism. *J Exp Med* (1999) 189:1363–72. doi:10.1084/jem.189.9.1363
176. Martinez FO, Gordon S, Locati M, Mantovani A. Transcriptional profiling of the human monocyte-to-macrophage differentiation and polarization: new molecules and patterns of gene expression. *J Immunol* (2006) 177:7303–11. doi:10.4049/jimmunol.177.10.7303
177. Grohmann U, Bronte V. Control of immune response by amino acid metabolism. *Immunol Rev* (2010) 236:243–64. doi:10.1111/j.1600-065X.2010.00915.x
178. Nagaraj S, Gupta K, Pisarev V, Kinarsky L, Sherman S, Kang L, et al. Altered recognition of antigen is a novel mechanism of CD8+ T cell tolerance in cancer. *Nat Med* (2007) 13:828. doi:10.1038/nm1609
179. Corzo CA, Cotter MJ, Cheng P, Cheng F, Kusmartsev S, Sotomayor E, et al. Mechanism regulating reactive oxygen species in tumor-induced myeloid-derived suppressor cells. *J Immunol* (2009) 182:5693–701. doi:10.4049/jimmunol.0900092
180. Highfill SL, Rodriguez PC, Zhou Q, Goetz CA, Koehn BH, Veenstra R, et al. Bone marrow myeloid-derived suppressor cells (MDSCs) inhibit graft-versus-host disease (GVHD) via an arginase-1-dependent mechanism that is up-regulated by interleukin-13. *Blood* (2010) 116:5738–47. doi:10.1182/blood-2010-06-287839

181. Arina A, Bronte V. Myeloid-derived suppressor cell impact on endogenous and adoptively transferred T cells. *Curr Opin Immunol* (2015) 33:120–5. doi:10.1016/j.coi.2015.02.006
182. Hossain F, Al-Khami AA, Wyczechowska D, Hernandez C, Zheng L, Reiss K, et al. Inhibition of fatty acid oxidation modulates immunosuppressive functions of myeloid-derived suppressor cells and enhances cancer therapies. *Cancer Immunol Res* (2015) 3:1236–47. doi:10.1158/2326-6066.CIR-15-0036
183. Corzo CA, Condamine T, Lu L, Cotter MJ, Youn J-I, Cheng P, et al. HIF-1α regulates function and differentiation of myeloid-derived suppressor cells in the tumor microenvironment. *J Exp Med* (2010) 207(11):2439–53. doi:10.1084/jem.20100587
184. Noman MZ, Desantis G, Janji B, Hasmim M, Karray S, Dessen P, et al. PD-L1 is a novel direct target of HIF-1α, and its blockade under hypoxia enhanced MDSC-mediated T cell activation. *J Exp Med* (2014) 211(5):781–90. doi:10.1084/jem.20131916
185. Ye X-Z, Yu S-C, Bian X-W. Contribution of myeloid-derived suppressor cells to tumor-induced immune suppression, angiogenesis, invasion and metastasis. *J Genet Genomics* (2010) 37:423–30. doi:10.1016/S1673-8527(09)60061-8
186. Acharyya S, Oskarsson T, Vanharanta S, Malladi S, Kim J, Morris PG, et al. A CXCL1 paracrine network links cancer chemoresistance and metastasis. *Cell* (2012) 150:165–78. doi:10.1016/j.cell.2012.04.042
187. Erler JT, Bennewith KL, Nicolau M, Dornhöfer N, Kong C, Le Q-T, et al. Lysyl oxidase is essential for hypoxia-induced metastasis. *Nature* (2006) 440:1222. doi:10.1038/nature04695
188. Sceneay J, Chow MT, Chen A, Halse HM, Wong CS, Andrews DM, et al. Primary tumor hypoxia recruits CD11b+/Ly6Cmed/Ly6G+ immune suppressor cells and compromises NK cell cytotoxicity in the premetastatic niche. *Cancer Res* (2012) 72:3906–11. doi:10.1158/0008-5472.CAN-11-3873
189. Cox TR, Rumney RM, Schoof EM, Perryman L, Høye AM, Agrawal A, et al. The hypoxic cancer secretome induces pre-metastatic bone lesions through lysyl oxidase. *Nature* (2015) 522:106–10. doi:10.1038/nature14492
190. Mantovani A, Cassatella MA, Costantini C, Jaillon S. Neutrophils in the activation and regulation of innate and adaptive immunity. *Nat Rev Immunol* (2011) 11:519. doi:10.1038/nri3024
191. Fridlender ZG, Sun J, Kim S, Kapoor V, Cheng G, Ling L, et al. Polarization of tumor-associated neutrophil phenotype by TGF-β: "N1" versus "N2" TAN. *Cancer Cell* (2009) 16:183–94. doi:10.1016/j.ccr.2009.06.017
192. Schmidt H, Bastholt L, Geertsen P, Christensen IJ, Larsen S, Gehl J, et al. Elevated neutrophil and monocyte counts in peripheral blood are associated with poor survival in patients with metastatic melanoma: a prognostic model. *Br J Cancer* (2005) 93:273. doi:10.1038/sj.bjc.6602702
193. Bellocq A, Antoine M, Flahault A, Philippe C, Crestani B, Bernaudin J-F, et al. Neutrophil alveolitis in bronchioloalveolar carcinoma: induction by tumor-derived interleukin-8 and relation to clinical outcome. *Am J Pathol* (1998) 152:83.
194. Atzpodien J, Reitz M. Peripheral blood neutrophils as independent immunologic predictor of response and long-term survival upon immunotherapy in metastatic renal-cell carcinoma. *Cancer Biother Radiopharm* (2008) 23:129–34. doi:10.1089/cbr.2007.0429
195. Reid MD, Basturk O, Thirabanjasak D, Hruban RH, Klimstra DS, Bagci P, et al. Tumor-infiltrating neutrophils in pancreatic neoplasia. *Mod Pathol* (2011) 24:1612. doi:10.1038/modpathol.2011.113
196. Caruso RA, Bellocco R, Pagano M, Bertoli G, Rigoli L, Inferrera C. Prognostic value of intratumoral neutrophils in advanced gastric carcinoma in a high-risk area in northern Italy. *Mod Pathol* (2002) 15:831. doi:10.1097/01.MP.0000020391.98998.6B
197. Cramer T, Yamanishi Y, Clausen BE, Förster I, Pawlinski R, Mackman N, et al. HIF-1α is essential for myeloid cell-mediated inflammation. *Cell* (2003) 112:645–57. doi:10.1016/S0092-8674(03)00154-5
198. Jun HS, Weinstein DA, Lee YM, Mansfield BC, Chou JY. Molecular mechanisms of neutrophil dysfunction in glycogen storage disease type Ib. *Blood* (2014) 123:2843–53. doi:10.1182/blood-2013-05-502435
199. Thompson AR, Elks PM, Marriott HM, Eamsamarng S, Higgins KR, Lewis A, et al. Hypoxia-inducible factor 2α regulates key neutrophil functions in humans, mice, and zebrafish. *Blood* (2014) 123:366–76. doi:10.1182/blood-2013-05-500207
200. Yoshida N, Granger DN, Anderson DC, Rothlein R, Lane C, Kvietys PR. Anoxia/reoxygenation-induced neutrophil adherence to cultured endothelial cells. *Am J Physiol* (1992) 262:H1891–8. doi:10.1152/ajpheart.1992.262.6.H1891
201. Lodhi IJ, Wei X, Yin L, Feng C, Adak S, Abou-Ezzi G, et al. Peroxisomal lipid synthesis regulates inflammation by sustaining neutrophil membrane phospholipid composition and viability. *Cell Metab* (2015) 21:51–64. doi:10.1016/j.cmet.2014.12.002
202. Brinkmann V, Reichard U, Goosmann C, Fauler B, Uhlemann Y, Weiss DS, et al. Neutrophil extracellular traps kill bacteria. *Science* (2004) 303:1532–5. doi:10.1126/science.1092385
203. Azevedo EP, Rochael NC, Guimarães-Costa AB, De Souza-Vieira TS, Ganilho J, Saraiva EM, et al. A metabolic shift toward pentose phosphate pathway is necessary for amyloid fibril-and phorbol 12-myristate 13-acetate-induced neutrophil extracellular trap (NET) formation. *J Biol Chem* (2015) 290:22174–83. doi:10.1074/jbc.M115.640094
204. Rodríguez-Espinosa O, Rojas-Espinosa O, Moreno-Altamirano MMB, López-Villegas EO, Sánchez-García FJ. Metabolic requirements for neutrophil extracellular traps formation. *Immunology* (2015) 145:213–24. doi:10.1111/imm.12437
205. Cools-Lartigue J, Spicer J, Mcdonald B, Gowing S, Chow S, Giannias B, et al. Neutrophil extracellular traps sequester circulating tumor cells and promote metastasis. *J Clin Invest* (2013) 123:3446. doi:10.1172/JCI67484
206. Cedervall J, Zhang Y, Huang H, Zhang L, Femel J, Dimberg A, et al. Neutrophil extracellular traps accumulate in peripheral blood vessels and compromise organ function in tumor-bearing animals. *Cancer Res* (2015) 75:2653–62. doi:10.1158/0008-5472.CAN-14-3299
207. Tennant DA, Durán RV, Gottlieb E. Targeting metabolic transformation for cancer therapy. *Nat Rev Cancer* (2010) 10:267. doi:10.1038/nrc2817
208. Vander Heiden MG. Targeting cancer metabolism: a therapeutic window opens. *Nat Rev Drug Discov* (2011) 10:671. doi:10.1038/nrd3504
209. Vander Heiden MG, Cantley LC, Thompson CB. Understanding the Warburg effect: the metabolic requirements of cell proliferation. *Science* (2009) 324:1029–33. doi:10.1126/science.1160809
210. Schulze A, Harris AL. How cancer metabolism is tuned for proliferation and vulnerable to disruption. *Nature* (2012) 491:364. doi:10.1038/nature11706
211. White E. Deconvoluting the context-dependent role for autophagy in cancer. *Nat Rev Cancer* (2012) 12:401. doi:10.1038/nrc3262
212. Liu Y, Cao Y, Zhang W, Bergmeier S, Qian Y, Akbar H, et al. A small-molecule inhibitor of glucose transporter 1 downregulates glycolysis, induces cell-cycle arrest, and inhibits cancer cell growth in vitro and in vivo. *Mol Cancer Ther* (2012) 11:1672–82. doi:10.1158/1535-7163.MCT-12-0131
213. Cao X, Fang L, Gibbs S, Huang Y, Dai Z, Wen P, et al. Glucose uptake inhibitor sensitizes cancer cells to daunorubicin and overcomes drug resistance in hypoxia. *Cancer Chemother Pharmacol* (2007) 59:495–505. doi:10.1007/s00280-006-0291-9
214. Dwarakanath B, Jain V. Targeting glucose metabolism with 2-deoxy-D-glucose for improving cancer therapy. *Future Oncol* (2009) 5:581–5. doi:10.2217/fon.09.44
215. Yoo BC, Ku JL, Hong SH, Shin YK, Park SY, Kim HK, et al. Decreased pyruvate kinase M2 activity linked to cisplatin resistance in human gastric carcinoma cell lines. *Int J Cancer* (2004) 108:532–9. doi:10.1002/ijc.11604
216. Martinez-Balibrea E, Plasencia C, Ginés A, Martinez-Cardús A, Musulén E, Aguilera R, et al. A proteomic approach links decreased pyruvate kinase M2 expression to oxaliplatin resistance in patients with colorectal cancer and in human cell lines. *Mol Cancer Ther* (2009) 8:771–8. doi:10.1158/1535-7163.MCT-08-0882
217. Li SL, Ye F, Cai WJ, Hu HD, Hu P, Ren H, et al. Quantitative proteome analysis of multidrug resistance in human ovarian cancer cell line. *J Cell Biochem* (2010) 109:625–33. doi:10.1002/jcb.22413
218. Guo W, Zhang Y, Chen T, Wang Y, Xue J, Zhang Y, et al. Efficacy of RNAi targeting of pyruvate kinase M2 combined with cisplatin in a lung cancer model. *J Cancer Res Clin Oncol* (2011) 137:65–72. doi:10.1007/s00432-010-0860-5
219. Shi HS, Li D, Zhang J, Wang YS, Yang L, Zhang HL, et al. Silencing of pkm2 increases the efficacy of docetaxel in human lung cancer xenografts in mice. *Cancer Sci* (2010) 101:1447–53. doi:10.1111/j.1349-7006.2010.01562.x

220. Zhou M, Zhao Y, Ding Y, Liu H, Liu Z, Fodstad O, et al. Warburg effect in chemosensitivity: targeting lactate dehydrogenase-A re-sensitizes taxol-resistant cancer cells to taxol. *Mol Cancer* (2010) 9:33. doi:10.1186/1476-4598-9-33
221. Lu C-W, Lin S-C, Chen K-F, Lai Y-Y, Tsai S-J. Induction of pyruvate dehydrogenase kinase-3 by hypoxia-inducible factor-1 promotes metabolic switch and drug resistance. *J Biol Chem* (2008) 283:28106–14. doi:10.1074/jbc.M803508200
222. Lu C-W, Lin S-C, Chien C-W, Lin S-C, Lee C-T, Lin B-W, et al. Overexpression of pyruvate dehydrogenase kinase 3 increases drug resistance and early recurrence in colon cancer. *Am J Pathol* (2011) 179:1405–14. doi:10.1016/j.ajpath.2011.05.050
223. Patsoukis N, Bardhan K, Chatterjee P, Sari D, Liu B, Bell LN, et al. PD-1 alters T-cell metabolic reprogramming by inhibiting glycolysis and promoting lipolysis and fatty acid oxidation. *Nat Commun* (2015) 6:6692. doi:10.1038/ncomms7692
224. Gomez-Pinillos A, Ferrari AC. mTOR signaling pathway and mTOR inhibitors in cancer therapy. *Hematol Oncol Clin North Am* (2012) 26:483–505. doi:10.1016/j.hoc.2012.02.014
225. Zaytseva YY, Valentino JD, Gulhati P, Evers BM. mTOR inhibitors in cancer therapy. *Cancer Lett* (2012) 319:1–7. doi:10.1016/j.canlet.2012.01.005
226. Yue W, Yang CS, Dipaola RS, Tan X-L. Repurposing of metformin and aspirin by targeting AMPK-mTOR and inflammation for pancreatic cancer prevention and treatment. *Cancer Prev Res* (2014) 7:388–97. doi:10.1158/1940-6207.CAPR-13-0337
227. Li W, Saud SM, Young MR, Chen G, Hua B. Targeting AMPK for cancer prevention and treatment. *Oncotarget* (2015) 6:7365. doi:10.18632/oncotarget.3629
228. Alli PM, Pinn ML, Jaffee EM, Mcfadden JM, Kuhajda FP. Fatty acid synthase inhibitors are chemopreventive for mammary cancer in neu-N transgenic mice. *Oncogene* (2005) 24:39. doi:10.1038/sj.onc.1208174
229. Seguin F, Carvalho M, Bastos D, Agostini M, Zecchin K, Alvarez-Flores M, et al. The fatty acid synthase inhibitor orlistat reduces experimental metastases and angiogenesis in B16-F10 melanomas. *Br J Cancer* (2012) 107:977. doi:10.1038/bjc.2012.355
230. Hatzivassiliou G, Zhao F, Bauer DE, Andreadis C, Shaw AN, Dhanak D, et al. ATP citrate lyase inhibition can suppress tumor cell growth. *Cancer Cell* (2005) 8:311–21. doi:10.1016/j.ccr.2005.09.008
231. Beckers A, Organe S, Timmermans L, Scheys K, Peeters A, Brusselmans K, et al. Chemical inhibition of acetyl-CoA carboxylase induces growth arrest and cytotoxicity selectively in cancer cells. *Cancer Res* (2007) 67:8180–7. doi:10.1158/0008-5472.CAN-07-0389
232. Yalcin A, Clem B, Makoni S, Clem A, Nelson K, Thornburg J, et al. Selective inhibition of choline kinase simultaneously attenuates MAPK and PI3K/AKT signaling. *Oncogene* (2010) 29:139. doi:10.1038/onc.2009.317
233. Clem BF, Clem AL, Yalcin A, Goswami U, Arumugam S, Telang S, et al. A novel small molecule antagonist of choline kinase-α that simultaneously suppresses MAPK and PI3K/AKT signaling. *Oncogene* (2011) 30:3370. doi:10.1038/onc.2011.51
234. Cao Z, Fan-Minogue H, Bellovin DI, Yevtodiyenko A, Arzeno J, Yang Q, et al. MYC phosphorylation, activation, and tumorigenic potential in hepatocellular carcinoma are regulated by HMG-CoA reductase. *Cancer Res* (2011) 71:2286–97. doi:10.1158/0008-5472.CAN-10-3367
235. Menendez JA, Lupu R, Colomer R. Inhibition of tumor-associated fatty acid synthase hyperactivity induces synergistic chemosensitization of HER-2/neu-overexpressing human breast cancer cells to docetaxel (taxotere). *Breast Cancer Res Treat* (2004) 84:183–95. doi:10.1023/B:BREA.0000018409.59448.60
236. Vazquez-Martin A, Colomer R, Brunet J, Menendez JA. Pharmacological blockade of fatty acid synthase (FASN) reverses acquired autoresistance to trastuzumab (Herceptin™) by transcriptionally inhibiting 'HER2 super-expression' occurring in high-dose trastuzumab-conditioned SKBR3/Tzb100 breast cancer cells. *Int J Oncol* (2007) 31:769–76. doi:10.3892/ijo.31.4.769
237. Liu H, Liu Y, Zhang J-T. A new mechanism of drug resistance in breast cancer cells: fatty acid synthase overexpression-mediated palmitate overproduction. *Mol Cancer Ther* (2008) 7:263–70. doi:10.1158/1535-7163.MCT-07-0445
238. Durán RV, Oppliger W, Robitaille AM, Heiserich L, Skendaj R, Gottlieb E, et al. Glutaminolysis activates Rag-mTORC1 signaling. *Mol Cell* (2012) 47:349–58. doi:10.1016/j.molcel.2012.05.043
239. Kamata S, Kishimoto T, Kobayashi S, Miyazaki M, Ishikura H. Possible involvement of persistent activity of the mammalian target of rapamycin pathway in the cisplatin resistance of AFP-producing gastric cancer cells. *Cancer Biol Ther* (2007) 6:1036–43. doi:10.4161/cbt.6.7.4253
240. Rodriguez PC, Hernandez CP, Quiceno D, Dubinett SM, Zabaleta J, Ochoa JB, et al. Arginase I in myeloid suppressor cells is induced by COX-2 in lung carcinoma. *J Exp Med* (2005) 202:931–9. doi:10.1084/jem.20050715
241. Chabner BA, Roberts TG Jr. Chemotherapy and the war on cancer. *Nat Rev Cancer* (2005) 5:65. doi:10.1038/nrc1529
242. Wang J-B, Erickson JW, Fuji R, Ramachandran S, Gao P, Dinavahi R, et al. Targeting mitochondrial glutaminase activity inhibits oncogenic transformation. *Cancer Cell* (2010) 18:207–19. doi:10.1016/j.ccr.2010.08.009
243. Le A, Lane AN, Hamaker M, Bose S, Gouw A, Barbi J, et al. Glucose-independent glutamine metabolism via TCA cycling for proliferation and survival in B cells. *Cell Metab* (2012) 15:110–21. doi:10.1016/j.cmet.2011.12.009
244. Thornburg JM, Nelson KK, Clem BF, Lane AN, Arumugam S, Simmons A, et al. Targeting aspartate aminotransferase in breast cancer. *Breast Cancer Res* (2008) 10:R84. doi:10.1186/bcr2154
245. Qing G, Li B, Vu A, Skuli N, Walton ZE, Liu X, et al. ATF4 regulates MYC-mediated neuroblastoma cell death upon glutamine deprivation. *Cancer Cell* (2012) 22:631–44. doi:10.1016/j.ccr.2012.09.021
246. Caro P, Kishan AU, Norberg E, Stanley IA, Chapuy B, Ficarro SB, et al. Metabolic signatures uncover distinct targets in molecular subsets of diffuse large B cell lymphoma. *Cancer Cell* (2012) 22:547–60. doi:10.1016/j.ccr.2012.08.014
247. Haq R, Shoag J, Andreu-Perez P, Yokoyama S, Edelman H, Rowe GC, et al. Oncogenic BRAF regulates oxidative metabolism via PGC1α and MITF. *Cancer Cell* (2013) 23:302–15. doi:10.1016/j.ccr.2013.02.003
248. Vazquez F, Lim J-H, Chim H, Bhalla K, Girnun G, Pierce K, et al. PGC1α expression defines a subset of human melanoma tumors with increased mitochondrial capacity and resistance to oxidative stress. *Cancer Cell* (2013) 23:287–301. doi:10.1016/j.ccr.2012.11.020
249. Engelman JA, Chen L, Tan X, Crosby K, Guimaraes AR, Upadhyay R, et al. Effective use of PI3K and MEK inhibitors to treat mutant Kras G12D and PIK3CA H1047R murine lung cancers. *Nat Med* (2008) 14:1351–6. doi:10.1038/nm.1890
250. Birsoy K, Possemato R, Lorbeer FK, Bayraktar EC, Thiru P, Yucel B, et al. Metabolic determinants of cancer cell sensitivity to glucose limitation and biguanides. *Nature* (2014) 508:108. doi:10.1038/nature13110
251. Foretz M, Hébrard S, Leclerc J, Zarrinpashneh E, Soty M, Mithieux G, et al. Metformin inhibits hepatic gluconeogenesis in mice independently of the LKB1/AMPK pathway via a decrease in hepatic energy state. *J Clin Invest* (2010) 120:2355. doi:10.1172/JCI40671
252. Bowker SL, Majumdar SR, Veugelers P, Johnson JA. Increased cancer-related mortality for patients with type 2 diabetes who use sulfonylureas or insulin. *Diabetes Care* (2006) 29:254–8. doi:10.2337/dc06-0997
253. Buzzai M, Jones RG, Amaravadi RK, Lum JJ, Deberardinis RJ, Zhao F, et al. Systemic treatment with the antidiabetic drug metformin selectively impairs p53-deficient tumor cell growth. *Cancer Res* (2007) 67:6745–52. doi:10.1158/0008-5472.CAN-06-4447
254. Hirsch HA, Iliopoulos D, Tsichlis PN, Struhl K. Metformin selectively targets cancer stem cells, and acts together with chemotherapy to block tumor growth and prolong remission. *Cancer Res* (2009) 69:7507–11. doi:10.1158/0008-5472.CAN-09-2994
255. Fendt S-M, Bell EL, Keibler MA, Davidson SM, Wirth GJ, Fiske B, et al. Metformin decreases glucose oxidation and increases the dependency of prostate cancer cells on reductive glutamine metabolism. *Cancer Res* (2013) 73:4429–38. doi:10.1158/0008-5472.CAN-13-0080
256. Hirsch HA, Iliopoulos D, Struhl K. Metformin inhibits the inflammatory response associated with cellular transformation and cancer stem cell growth. *Proc Natl Acad Sci U S A* (2013) 110:972–7. doi:10.1073/pnas.1221055110
257. Pelletier J, Roux D, Viollet B, Mazure NM, Pouyssegur J. AMP-activated protein kinase is dispensable for maintaining ATP levels and for survival

following inhibition of glycolysis, but promotes tumour engraftment of Ras-transformed fibroblasts. *Oncotarget* (2015) 6:11833–47. doi:10.18632/oncotarget.3738

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How Endothelial Cells Adapt Their Metabolism to Form Vessels in Tumors

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Endothelial cells (ECs) line blood vessels, i.e., vital conduits for oxygen and nutrient delivery to distant tissues. While mostly present as quiescent "phalanx" cells throughout adult life, ECs can rapidly switch to a migratory "tip" cell and a proliferative "stalk" cell, and sprout into avascular tissue to form new blood vessels. The angiogenic switch has long been considered to be primarily orchestrated by the activity of angiogenic molecules. However, recent evidence illustrates an instrumental role of cellular metabolism in vessel sprouting, whereby ECs require specific metabolic adaptations to grow. Here, we overview the emerging picture that tip, stalk, and phalanx cells have distinct metabolic signatures and discuss how these signatures can become deregulated in pathological conditions, such as in cancer.

Keywords: angiogenesis, metabolism, sprouting, tip and stalk cells, tumor angiogenesis

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THE BASICS OF ENDOTHELIAL CELL (EC) BIOLOGY

Endothelial cells line blood vessels, an intricate network of functional conduits throughout the body, which is vital for the maintenance of tissue homeostasis. In healthy adults, ECs remain in a quiescent state for protracted periods. Nevertheless, quiescent blood vessels retain the ability to promptly respond to pro-angiogenic cues in the tightly coordinated process of angiogenesis. The angiogenic switch is characterized by active proliferation and migration of ECs to form new sprouts. A key player that orchestrates vascularization of oxygen- and nutrient-deprived tissues is vascular endothelial growth factor A (VEGF-A, hereafter referred to as VEGF), which signals primarily through VEGF receptor 2 (VEGFR2) (1). For vessel branching to occur, activated ECs must differentiate into three different subtypes, namely (i) migratory tip cells, which lead the sprout, (ii) proliferating stalk cells, responsible for sprout elongation, and (iii) quiescent phalanx cells, which line the newly established perfused vessel (1) (**Figure 1A**). The distinct morphological features and functional properties that distinguish these EC subtypes will be summarized in the next section. The angiogenic switch is metabolically taxing; recent evidence suggests that ECs adapt their metabolism during vessel sprouting to fulfill their needs of biomass production and to sustain high-energy requirements (2, 3). Furthermore, EC metabolism has been proven to co-determine vessel sprouting; thus, challenging the broadly accepted belief that angiogenesis is mainly (only) governed by angiogenic (growth factor) signals (2).

Here, we will briefly overview some key angiogenic determinants of vessel sprouting and EC quiescence [for further details, the reader is referred to more comprehensive reviews (1, 4, 5)]. We will then discuss metabolic pathways that are activated in angiogenic versus quiescent ECs, with particular emphasis to the metabolic reprogramming underlying the angiogenic switch.

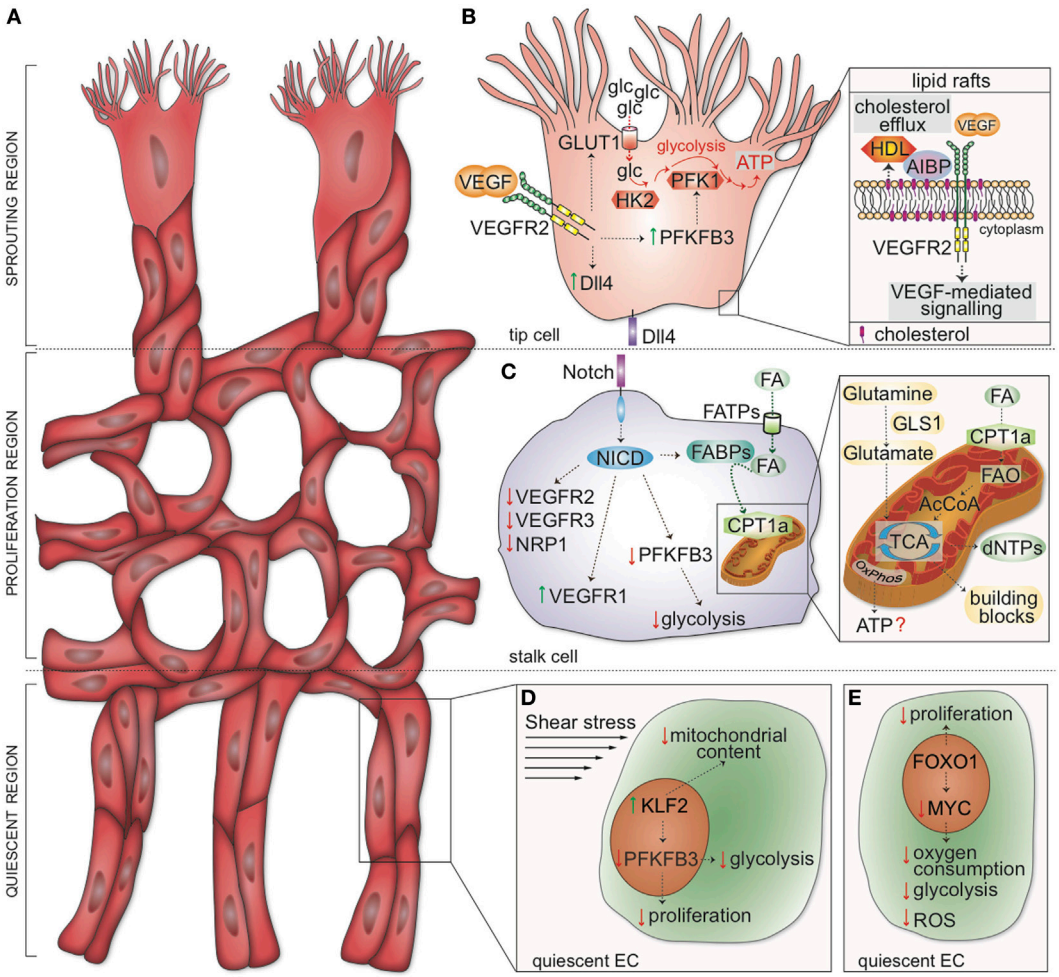

FIGURE 1 | Metabolic and genetic determinants regulating tip, stalk, and phalanx cell function. **(A)** Schematic representation of the vascular front. Three distinct zones are identified: the sprouting region (consisting of migrating tip cells), the proliferation region (containing stalk cells elongating the sprouts), and the quiescent region (where phalanx cells mature into quiescent cells). **(B)** In tip cells, VEGF-mediated activation of VEGFR2 induces transcription of Dll4. Concomitantly, VEGFR2 signaling upregulates GLUT1 and PFKFB3, thus increasing glucose uptake and glycolysis to induce localized adenosine triphosphate (ATP) generation at lamellipodia and filopodia. HK2 is also required for tip cell functions. Inset: cholesterol transfer to HDL is facilitated by AIBP; the rate of cholesterol efflux from the plasma membrane determines the formation of lipid rafts, positively affecting VEGFR2 dimerization and activation. **(C)** In stalk cells, Dll4 induces the cleavage of the NICD. In turn, NICD drives the expression of VEGFR1 while downregulating VEGFR2, VEGFR3, and NRP1. FAs are taken up into the cells by FATPs and, in the cytoplasm, bound by FABP. NICD represses PFKFB3 transcription and enhances FABP expression. Inset: CPT1a shuttles FAs into the mitochondria. Mitochondrial FAO generates acetyl-CoA, which enters the TCA cycle and supports dNTP synthesis; OxPhos is seemingly used for minimal ATP generation in endothelial cells (ECs). The conversion of glutamine to glutamate by GLS1 sustains the TCA cycle (anaplerosis) and contributes to building block production. **(D)** Shear stress induces the expression of KLF2, which in turn inhibits proliferation, reduces the mitochondrial content, and lowers glycolysis (by downregulating PFKFB3). **(E)** FOXO1 suppresses EC proliferation and inhibits the transcription factor MYC. As a consequence, oxygen consumption, glycolysis, and reactive oxygen species (ROS) production are lowered in quiescent ECs. VEGF, vascular endothelial growth factor; VEGFR2, vascular endothelial growth factor receptor 2; glc, glucose; GLUT1, glucose transporter-1; PFK-1, phosphofructokinase; PFKFB3, 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase 3; ATP, adenosine triphosphate; HK2, hexokinase 2; AIBP, apoA-I binding protein; Dll4, Delta-like ligand 4; HDL, high-density lipoprotein particles; NICD, Notch intracellular domain; FA, fatty acids; FATP, FA transporter protein; FABP, FA binding proteins; CPT1a, carnitine palmitoyltransferase 1a; FAO, FA β-oxidation; AcCoA, acetyl coenzyme A; TCA tricarboxylic acid cycle; OxPhos, oxidative phosphorylation; dNTPs, deoxynucleotides; GLS1, glutaminase 1; FOXO1, forkhead box O transcription factor 1; KLF2, Krüppel-like Factor 2.

GENETIC SIGNALS IN THE REGULATION OF VESSEL SPROUTING

The primary function of tip cells is to lead the developing sprout in response to pro-angiogenic cues. Tip cells have a minimal proliferation rate and are characterized by a particular morphology: they are highly polarized and extend numerous filopodia and lamellipodia to allow cellular movements (6). Moreover, tip cells are rich in cell-surface receptors and molecules involved in extracellular matrix degradation and basement membrane deposition (7). On the contrary, stalk cells generate fewer filopodia and proliferate behind the tip cells to ensure sprout elongation

and lumen formation (8). The specification of ECs into tip and stalk cells is tightly orchestrated by the VEGF and Notch signaling pathways (5). At the vascular front, the ECs exposed to the highest concentration of VEGF are selected to become tip cells. Activation of the cognate receptor VEGFR2 initiates an intracellular cascade in tip cells that induces the expression of the Notch ligand delta-like ligand 4 (Dll4) (5) (**Figure 1B**). Subsequent activation of Notch signaling in neighboring ECs *via* Dll4 binding results in the cleavage of the Notch intracellular domain (NICD); in turn, the NICD activates a transcriptional program that results in reduced expression of VEGFR2, VEGFR3, and the VEGF coreceptor neuropilin 1 (NRP1), with concomitant upregulation of the VEGF trap VEGFR1 (5) (**Figure 1C**). Thus, Notch signaling out-competes the ability of an EC to become a tip cell and instead promotes a stalk cell phenotype. Moreover, during angiogenic sprouting, the high turnover of VE-cadherin, a key junctional adhesion molecule in ECs, facilitates cell migration (9). Notch signaling also reduces the adhesiveness of VE-cadherin junctions and consequently compromises the ability of these cells to reshuffle positions within the sprout (10). However, the phenotype of tip and stalk cells is not fixed: dynamic rearrangements occur as the vessel network expands; with tip cells frequently being overtaken by stalk cells that move to the front and become new tips (11, 12). Notably, a novel EC topology was recently described in growing vessels, according to which at least two filopodia-extending ECs are present at the tip of the sprout (13). The polarization of tip ECs along the longitudinal border seemingly allows for apical polarization and lumen formation (13).

The process of anastomosis, the contact between tip cells of neighboring sprouts, establishes new vessel connections (1). Once tissue vascularization is restored, the levels of pro-angiogenic factors are reduced and ECs establish a quiescent phenotype. Quiescent ECs, also termed phalanx cells, are highly interconnected by junctional molecules such as VE-cadherin and tight junction proteins that mechanically strengthen the vessel's wall and create a barrier (1). Perfusion induces vascular maturation by re-establishing pericyte recruitment and maturation and basal membrane deposition to promote vessel stabilization (1).

METABOLISM AT THE TIP: GLYCOLYSIS AS A FUEL

Endothelial cells are exposed to high concentrations of oxygen in the blood. Oxidative phosphorylation would, therefore, be expected to be the favored energy-generating pathway. On the contrary, only <15% of the total amount of ATP is produced through oxidation of glucose, glutamine, and fatty acids (FA), and sprouting ECs have a lower oxygen consumption rate than other cell types (2). In turn, glycolysis yields 85% of the total cellular ATP content (2). Even if it seems counterintuitive at first sight, as the yield of ATP per mole glucose from mitochondrial respiration (36 mol ATP) is much higher than from anaerobic metabolism (2 mol ATP), the preference for glycolysis might have several advantages. First, in conditions of unlimited glucose, high glycolytic flux can produce more ATP in a shorter time with respect to oxidative metabolism (14), which represents an advantage for cells that have to rapidly migrate into avascular tissues in order to restore the physiological oxygen levels. Also, by relying primarily on anaerobic metabolism for ATP production, ECs spare oxygen for transfer to perivascular cells. In addition, glucose can also be shunted into glycolytic side pathways such as the pentose-phosphate pathway and the serine biosynthesis pathway utilized for biomass production (15). Last, a reduction in mitochondrial respiration reduces the amount of reactive oxygen species (ROS) generated (16).

Upon VEGF stimulation, ECs rewire their metabolism to meet the elevated energetic and biosynthetic needs of the angiogenic state. The VEGF-dependent upregulation of the glycolysis regulator 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase 3 (PFKFB3), an activator of phosphofructokinase 1, is paralleled by an increase in glycolytic flux (2) (**Figure 1B**). Notably, activation of the pro-stalk signaling Notch receptor *via* Dll4 reduces PFKFB3-driven glycolysis (**Figure 1C**), arguing for a high glycolytic activity as metabolic feature of tip cells (2). Consistently, genetic loss or pharmacologic inhibition of PFKFB3 diminishes tip cell behavior and reduces competitiveness for the tip position, consequently impairing EC sprouting *in vitro* and vessel outgrowth *in vivo* (2). On the contrary, overexpression of PFKFB3 reverts Notch-instructed stalk cells into tip cells, suggesting that glycolysis can even override genetic signals modulating EC specification (2). Besides PFKFB3, the glycolytic enzyme hexokinase 2 (HK2) has been recently implicated in the processes of angiogenesis and lymphangiogenesis. Pan-endothelial *Hk2* deletion during embryonic development induces defects in angiogenesis, and *Hk2* excision soon after birth (P0) impairs the development of the retinal vasculature by reducing the number of both tip cells and branch points (17).

In motile ECs, bulky mitochondria are positioned in the perinuclear cytosol; glycolytic enzymes, instead, associate with F-actin and compartmentalize in lamellipodia and filopodia (2) to generate high and localized amounts of ATP. Thus, glycolysis fuels actin–myosin contraction and enables cytoskeleton remodeling during EC migration by locally producing high amounts of ATP (2). Dynamic rearrangements at the tip of the sprout allow for a continuous selection and repositioning of the more competitive tip cell to ensure maximal fitness of the growing sprout (11, 12). Remarkably, computational simulations, validated by experimentations, predicted that glycolytic ATP generation, required for cellular rearrangements, modulates intercellular adhesion by affecting VE-cadherin turnover (18). Of note, these computational simulations predicted that PFKFB3 blockade in combination with anti-VEGF treatment might normalize vessel sprouting in models of deregulated EC rearrangement due to excessive VEGF levels (as observed in conditions of pathological angiogenesis) (18). Lowering glycolysis in ECs by genetic and/or pharmacological means indeed promotes tumor vessel normalization (19).

Glycolysis is not the only metabolic determinant of tip cells. Indeed, cholesterol turnover and efflux are indispensable to maintain normal cellular functions. Generally, cholesterol is loaded onto ApoA-1-containing high-density lipoprotein particles (HDL) in an ATP-binding cassette transporters-dependent fashion (20). The apoA-I binding protein (AIBP)

favors cholesterol transfer from ECs to HDL (**Figure 1B**). In the presence of AIBP and HDL, the rate of cholesterol efflux is accelerated, thereby impairing formation of plasma membrane lipid rafts, essential for VEGFR2 dimerization and endocytosis, overall thus inhibiting VEGF-mediated signaling (20). In zebrafish embryos, tip cells have a higher content of lipid rafts than stalk cells, suggesting a cholesterol-dependent positive regulation of VEGFR2 signaling at the tip of the sprout (20). Interfering with cholesterol efflux abrogates these morphological differences and results in dysregulated angiogenesis (20).

Furthermore, glutamine deprivation or inhibition of glutaminase 1 (GLS1), an enzyme of amino acid metabolism that converts glutamine to glutamate, negatively affects tricarboxylic acid (TCA) cycle anaplerosis, macromolecule production, and redox homeostasis in ECs (21, 22). The inhibition of glutamine metabolism impairs EC proliferation and migration, causes severe vessel sprouting defects *in vivo,* and decreases pathological ocular angiogenesis (21, 22). In particular, the silencing of GLS1 in ECs reduces their competitiveness to obtain the tip position in spheroid sprouts *in vitro* (21). In a separate study, microarray analysis of ECs dissected from the postnatal retinal vasculature showed increased expression of glutaminase 2 (GLS2) (23). Yet, the exact role of GLS2 in angiogenesis is still unclear.

METABOLIC DETERMINANTS OF STALK CELL PROLIFERATION

Despite the pivotal role of glycolysis in the specification and functioning of tip cells, this metabolic pathway is also associated with stalk cell functions. Indeed, these actively proliferating cells need to generate high amounts of biomass (macromolecules) that are in part derived from the non-oxidative pentose-phosphate pathway and the serine biosynthesis pathway, side branches of glycolysis (24). Rapidly proliferating ECs, such as tumor ECs, increase glucose uptake, diversion of glycolytic intermediates into these anabolic pathways, and incorporation of glucose carbons into nucleotides (19). Consistently, treatment of ECs with Dll4 (a Notch signaling activator) suppresses progression through the cell cycle while concomitantly reducing glycolysis at the late G_1 cell cycle phase (25). In addition, PFKFB3 inhibition *in vitro* impairs EC sprouting induced by Notch blockade (2), thus underscoring the ability of the glycolytic activator PFKFB3 to overcome the metabolic break induced by Notch.

Fatty acid metabolism is also essential for EC proliferation (**Figure 1C**). The uptake of FAs across the plasma membrane can occur *via* a "flip-flop" mechanism or *via* specific transport proteins, such as CD36 and FA transport proteins (26). Once shuttled to the inner side of the plasma membrane, FAs are recruited by FA binding proteins (FABPs), which are responsible for their intracellular trafficking (26). Of note, FABP4 regulates proliferation and sprouting of ECs (27, 28). The shuttling of activated FA-CoA to mitochondria is dependent on the activity of the carnitine palmitoyltransferases (CPTs), a rate-controlling step of FA β-oxidation (FAO) (29). Genetic or pharmacological inhibition of CPT1a, the most abundant isoform in ECs, reduces EC proliferation without affecting the migratory capability of ECs (30). Endothelial CPT1a loss in mice impairs postnatal vascular development of the retina and reduces branching angiogenesis without altering filopodia formation or vessel maturation, thus suggesting that FAO exquisitely modulates stalk cell behavior (30). In addition, pharmacological inhibition of FAO affects the barrier function by inducing EC hyper-permeability (31).

A role for oxidative phosphorylation in ATP generation was documented in proliferating ECs (32) (**Figure 1C**). However, the magnitude and relevance of these findings remain debated, since oxidative metabolism in ECs accounts for the generation of only less than 15% of ATP (2) and, consistently, the defect observed upon CPT1a silencing is not due to insufficient ATP production or defective oxygen consumption (30). Instead, ^{13}C-palmitate tracing experiments uncovered that FAO generates acetyl coenzyme A (acetyl-CoA), which helps to sustain, in conjunction with an anaplerotic substrate, the TCA cycle and deoxynucleotide (dNTP) synthesis for proliferation (30) (**Figure 1C**). In line with these observations, silencing of CPT1a depletes the cellular pool of dNTPs, which are restored upon supplementation with acetate, a precursor of acetyl-CoA (product of FAO) (30). Interestingly, acetate- or dNTP supplementation recovers the sprouting defect observed upon CPT1a silencing *in vitro* (30). Thus, the selective role of FAO in stalk but not tip cells implies distinct metabolic signatures for these different EC subphenotypes.

METABOLISM OF QUIESCENT PHALANX ECs

When cells are exposed to unfavorable conditions to sustain cell proliferation, many healthy (non-transformed) cells enter a non-dividing state while still keeping the ability to become proliferative. This state of reversible cell cycle arrest in the G_0/G_1 phase, also known as quiescence, is common in many non-transformed cell types in different species (e.g., bacterial, yeast, or mammalian cells). The switch from quiescence to proliferation (and *vice versa*) is accompanied with substantial changes in metabolism. As an example, quiescent T cells derive most of their ATP from oxidative phosphorylation (33, 34). By contrast, activated T cells rely on glycolysis, facilitated by increased glucose transport (35). Similarly, B-cell activation in response to interleukin-3 induces a 8-fold increase in glycolytic flux (36).

As mentioned above, ECs build new blood vessels when oxygen and nutrients are low and become quiescent once functional vessels have been perfused, and levels of oxygen and nutrients are restored (1, 4). Although the metabolism of quiescent ECs remains poorly characterized, recent data showed that ECs rewire their metabolism when exiting the cell cycle. Upon (genetic or chemical) inhibition of PFKFB3, proliferating ECs lower their glycolytic flux by 35–40% and display a quiescent phenotype (2, 37, 38). Similar results have been obtained upon Kruppel-like factor 2 (KLF2) overexpression, which mimics the anti-proliferative effect induced by laminar shear stress. KLF2 overexpression, as well as shear stress itself, reduces mitochondrial content and activity and causes a decrease in glycolytic flux to the same levels

observed upon PFKFB3 inhibition (39). Mechanistically, KLF2 represses the transcription of PFKFB3 (39) (**Figure 1D**). Of note, overexpression of PFKFB3 in ECs restores glycolytic flux and overcomes KLF2-mediated inhibition of proliferation (39).

Another recent study highlighted a role for forkhead box O (FOXO) transcription factor 1 (FOXO1) in coupling cellular growth/quiescence to metabolism in ECs (**Figure 1E**). Expression of constitutively active FOXO1 suppresses EC proliferation and lowers endothelial metabolic activity by decreasing glycolysis, lactate production, oxygen consumption, and the production of ROS (38). These changes in metabolic activity are promoted by FOXO1's ability to antagonize MYC signaling (38).

In a healthy adult mammalian organism, the quiescent endothelium has a long half-life of several years, and as long as it remains healthy, it secures vascular homeostasis through vasculoprotective mechanisms, including vasodilation, thromboresistance, anti-inflammation, etc. (40). However, upon aging, quiescent ECs risk becoming dysfunctional, thereby contributing to the progression of various cardiovascular diseases (CVD). The metabolic features of dysfunctional ECs during aging have not been fully elucidated yet and further studies are warranted to shed light on the mechanisms of metabolic (mal)-adaptation, but because of the importance of EC dysfunction in CVD, we will briefly discuss the current knowledge on this topic.

AGING AND METABOLISM IN ECs

Advanced age is an independent risk factor for life-threatening diseases, including coronary artery disease, stroke, and hypertension, which are directly related to aging-associated EC dysfunction (41). With time, aging blood vessels become stiffer and thicker, which results in decreased flexibility in adjusting vessel shape and function in response to tissue demands. The age-associated remodeling of the vasculature involves an impairment of vasorelaxation, an increase in vascular permeability, inflammation, and fibrosis, and an impairment of angiogenesis (42–44).

Emerging evidence arises about the underlying (metabolic) mechanisms of the aging-associated EC dysfunction. A common mechanism seems to be mitochondrial DNA damage and mitochondrial dysfunction, with decreased production of ATP and upregulation of ROS production (45, 46). The mitochondrial respiration chain and NAD(P)H oxidases (Nox) are important sources of ROS production in ECs (47). In aged animals, inflammatory markers such as the intercellular adhesion molecule 1 (ICAM1), a leukocyte adhesion molecule that promotes vessel wall inflammation, are upregulated with respect to young animals (48). This increased expression appears to be partially dependent on ROS, as long-term treatment with mitochondria-targeted antioxidant treatment abrogates the increased expression of ICAM1 (48). Also, the NADPH Oxidase 4 (Nox4), which is vasculoprotective at a young age (49), is upregulated in pulmonary arteries of aged rats and has been implicated in oxidative stress (50). In agreement, upon knockdown of Nox4, the replicative lifespan of ECs is extended and oxidative DNA damage is reduced (51). In addition, a reduction in Sirtuin 1 activity in ECs has been proposed to contribute to vascular dysfunction in aging mice by reducing FA uptake and oxidation, and by inducing mitochondrial dysfunction, oxidative stress, and DNA damage (52). Not only the production of ROS is increased, but also ROS scavenging is impaired in the aged endothelium (50).

Endothelial cell dysfunction during vascular aging is also associated with reduced endothelial nitric oxide (NO) bioavailability. NO is an important cellular signaling molecule that mediates vasodilatation and stimulates angiogenesis (53). However, aging-induced oxidative stress can change the fate of endothelial NO synthase (eNOS) from a NO-producing to a superoxide anion-producing enzyme (in a process known as eNOS uncoupling), resulting in impaired NO generation (54).

Aging dysfunctional ECs may, thus, create a milieu in which vascular disease can flourish. It is, therefore, of great clinical relevance to deepen our knowledge on the mechanisms underlying age-related EC dysfunction and to assess benefits of interventions that may restore EC function.

METABOLISM OF ECs IN THE TUMOR MICROENVIRONMENT

The tumor microenvironment is characterized by a highly disorganized vessel network, which is morphologically and functionally abnormal. The vasculature appears irregular, tortuous, and non-homogeneous, and the ECs form weak junctions that result in increased leakiness and facilitate intravasation of cancer cells (4). A recent study demonstrated that the metabolic profile of ECs lining tumor blood vessels differs from healthy ECs (19). In particular, most glycolytic genes, including PFKFB3, are upregulated at the transcriptional level with respect to other pathways in central metabolism, thus supporting a higher dependence of tumor ECs on glucose metabolism (19). In agreement with these findings, competition for the availability of glucose between tumor-associated macrophages (TAMs) and tumor ECs influence the angiogenic response. Indeed, enhancing glycolysis in TAMs reduces glucose availability for ECs in the tumor microenvironment and favors normalization of the tumor vasculature (55) (**Figure 2**). In mice, endothelial deletion of both PFKFB3 alleles results in reduced vessel perfusion in tumors (56). Consistently, treatment with a high dose of the PFKFB3 blocker 3-(3-pyridinyl)-1-(4-pyridinyl)-2-propen-1-one (3PO) suppresses tumor EC proliferation and promotes EC death, ultimately causing tumor vessel disintegration (57). Surprisingly, however, PFKFB3 haplodeficiency or the treatment with a low dose of 3PO, which target the hyperglycolytic tumor ECs (but not the cancer cells), do not affect tumor growth but instead exert a favorable effect on tumor vessel normalization, characterized by remodeling of the tumor vasculature into a more normal one, with a tighter vascular barrier, more pericyte coverage (vessel maturation), and improved vessel perfusion and oxygenation. This results in reduced metastasis and amelioration of the delivery and response to chemotherapeutic agents (19) (**Figure 2**). A growing body of evidence in the past years has highlighted the efficacy of tumor vessel normalization in improving tumor perfusion and oxygenation as well as anti-cancer drug delivery (58). The finding that partial inhibition of glycolysis induces tumor vessel normalization paves the way for further investigations on the modulation of EC cellular metabolism as a therapeutic strategy.

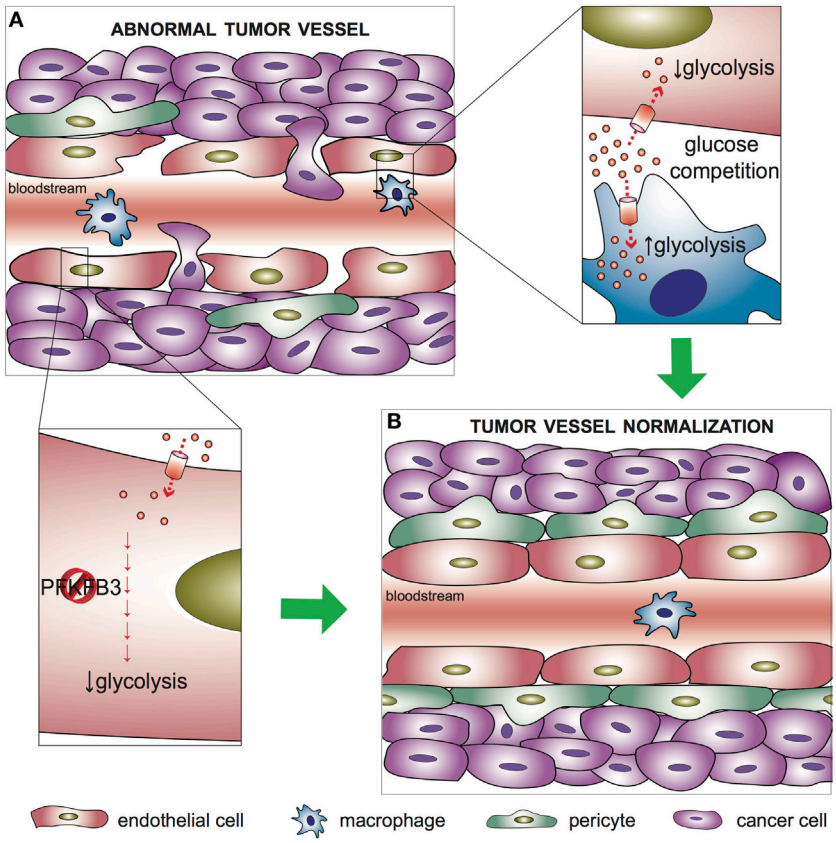

FIGURE 2 | Metabolic regulation of tumor vessel normalization. **(A)** In abnormal tumor vessels, endothelial cells (ECs) form weak junctions that, in combination with reduced pericyte coverage, result in increased leakiness and facilitate intravasation of cancer cells. **(B)** Targeting glycolysis in tumor ECs, which are hyperglycolytic with respect to healthy ECs, promotes normalization of the tumor vasculature. Upper left inset: tumor-associated macrophages (TAMs) compete with ECs for glucose availability in the tumor microenvironment; an increased glycolytic flux in TAMs favor tumor vessel normalization. Lower left inset: targeting PFKFB3 to partially reduce glycolysis in ECs improves vessel maturation, favors the tightening of the vascular barrier, and reduces cancer cell intravasation. PFKFB3, 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase 3.

CONCLUSION AND FUTURE PERSPECTIVES

Over the past few years, the pivotal role of metabolism in the regulation of EC behavior is becoming increasingly recognized. However, more efforts are needed to fully characterize the metabolic roadmap of ECs in health and disease, requiring global untargeted metabolomics analysis of patient samples in combination with the generation of EC-specific metabolic knockout animals. To date, therapeutic strategies to combat pathological angiogenesis primarily rely on VEGF signaling blockade (59, 60). However, efficacy of these treatments and improvements in survival are limited by acquired refractoriness and drug resistance (59, 60). There is, thus, an unmet need to develop additional anti-angiogenic therapies that operate *via* fundamentally different, complementary mechanisms. The concept of targeting metabolic pathways for improving current therapies is still in its infancy. Initial proof of concept has already been provided in preclinical animal studies, whereby blockade of PFKFB3 proved to be efficacious in reducing pathological angiogenesis of skin and bowel inflammation, and enhancing the anti-angiogenic effects of VEGF inhibitors (24, 56), and promoting tumor vessel normalization with reduced metastasis and improved responses to chemotherapy (19). Of note, in a preclinical mouse model of wet age-related macular degeneration, systemic administration of the PFKFB3 blocker 3PO inhibited choroidal neovascularization (24). Similarly, pathological ocular neovascularization is reduced by systemic administration of etomoxir, a CPT1 blocker (30).

A growing body of evidence suggests that EC functions can be modulated by metabolites in the blood or released by surrounding tissues. An evident question is whether dietary supplementation of metabolites might act in concert with current therapeutic strategies to ease vascular disease symptoms? In support of this concept, supplementation of acetate (a precursor of acetyl-CoA) promotes (lymph)-angiogenesis, *in vitro* as well as *in vivo* (3, 61).

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AZ, JK, CD, and PC wrote the paper and commented on the manuscript.

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REFERENCES

1. Potente M, Gerhardt H, Carmeliet P. Basic and therapeutic aspects of angiogenesis. *Cell* (2011) 146:873–87. doi:10.1016/j.cell.2011.08.039
2. De Bock K, Georgiadou M, Schoors S, Kuchnio A, Wong BW, Cantelmo AR, et al. Role of PFKFB3-driven glycolysis in vessel sprouting. *Cell* (2013) 154:651–63. doi:10.1016/j.cell.2013.06.037
3. Schoors S, Bruning U, Missiaen R, Queiroz KC, Borgers G, Elia I, et al. Fatty acid carbon is essential for dNTP synthesis in endothelial cells. *Nature* (2015) 520:192–7. doi:10.1038/nature14362
4. Carmeliet P, Jain RK. Molecular mechanisms and clinical applications of angiogenesis. *Nature* (2011) 473:298–307. doi:10.1038/nature10144
5. Blanco R, Gerhardt H. VEGF and Notch in tip and stalk cell selection. *Cold Spring Harb Perspect Med* (2013) 3:a006569. doi:10.1101/cshperspect.a006569
6. Isogai S, Lawson ND, Torrealday S, Horiguchi M, Weinstein BM. Angiogenic network formation in the developing vertebrate trunk. *Development* (2003) 130:5281–90. doi:10.1242/dev.00733
7. del Toro R, Prahst C, Mathivet T, Siegfried G, Kaminker JS, Larrivee B, et al. Identification and functional analysis of endothelial tip cell-enriched genes. *Blood* (2010) 116:4025–33. doi:10.1182/blood-2010-02-270819
8. Thurston G, Kitajewski J. VEGF and Delta-Notch: interacting signalling pathways in tumour angiogenesis. *Br J Cancer* (2008) 99:1204–9. doi:10.1038/sj.bjc.6604484
9. Perryn ED, Czirok A, Little CD. Vascular sprout formation entails tissue deformations and VE-cadherin-dependent cell-autonomous motility. *Dev Biol* (2008) 313:545–55. doi:10.1016/j.ydbio.2007.10.036
10. Bentley K, Franco CA, Philippides A, Blanco R, Dierkes M, Gebala V, et al. The role of differential VE-cadherin dynamics in cell rearrangement during angiogenesis. *Nat Cell Biol* (2014) 16:309–21. doi:10.1038/ncb2926
11. Jakobsson L, Franco CA, Bentley K, Collins RT, Ponsioen B, Aspalter IM, et al. Endothelial cells dynamically compete for the tip cell position during angiogenic sprouting. *Nat Cell Biol* (2010) 12:943–53. doi:10.1038/ncb2103
12. Arima S, Nishiyama K, Ko T, Arima Y, Hakozaki Y, Sugihara K, et al. Angiogenic morphogenesis driven by dynamic and heterogeneous collective endothelial cell movement. *Development* (2011) 138:4763–76. doi:10.1242/dev.068023
13. Pelton JC, Wright CE, Leitges M, Bautch VL. Multiple endothelial cells constitute the tip of developing blood vessels and polarize to promote lumen formation. *Development* (2014) 141:4121–6. doi:10.1242/dev.110296
14. Zheng J. Energy metabolism of cancer: glycolysis versus oxidative phosphorylation (review). *Oncol Lett* (2012) 4:1151–7. doi:10.3892/ol.2012.928
15. Verdegem D, Moens S, Stapor P, Carmeliet P. Endothelial cell metabolism: parallels and divergences with cancer cell metabolism. *Cancer Metab* (2014) 2:19. doi:10.1186/2049-3002-2-19
16. Zorov DB, Juhaszova M, Sollott SJ. Mitochondrial reactive oxygen species (ROS) and ROS-induced ROS release. *Physiol Rev* (2014) 94:909–50. doi:10.1152/physrev.00026.2013
17. Yu P, Wilhelm K, Dubrac A, Tung JK, Alves TC, Fang JS, et al. FGF-dependent metabolic control of vascular development. *Nature* (2017) 545:224–8. doi:10.1038/nature22322
18. Cruys B, Wong BW, Kuchnio A, Verdegem D, Cantelmo AR, Conradi LC, et al. Glycolytic regulation of cell rearrangement in angiogenesis. *Nat Commun* (2016) 7:12240. doi:10.1038/ncomms12240
19. Cantelmo AR, Conradi LC, Brajic A, Goveia J, Kalucka J, Pircher A, et al. Inhibition of the glycolytic activator PFKFB3 in endothelium induces tumor vessel normalization, impairs metastasis, and improves chemotherapy. *Cancer Cell* (2016) 30(6):968–85. doi:10.1016/j.ccell.2016.10.006
20. Fang L, Choi SH, Baek JS, Liu C, Almazan F, Ulrich F, et al. Control of angiogenesis by AIBP-mediated cholesterol efflux. *Nature* (2013) 498:118–22. doi:10.1038/nature12166
21. Huang H, Vandekeere S, Kalucka J, Bierhansl L, Zecchin A, Bruning U, et al. Role of glutamine and interlinked asparagine metabolism in vessel formation. *EMBO J* (2017) 36:2334–52. doi:10.15252/embj.201695518
22. Kim B, Li J, Jang C, Arany Z. Glutamine fuels proliferation but not migration of endothelial cells. *EMBO J* (2017) 36:2321–33. doi:10.15252/embj.201796436
23. Strasser GA, Kaminker JS, Tessier-Lavigne M. Microarray analysis of retinal endothelial tip cells identifies CXCR4 as a mediator of tip cell morphology and branching. *Blood* (2010) 115:5102–10. doi:10.1182/blood-2009-07-230284
24. Schoors S, De Bock K, Cantelmo AR, Georgiadou M, Ghesquiere B, Cauwenberghs S, et al. Partial and transient reduction of glycolysis by PFKFB3 blockade reduces pathological angiogenesis. *Cell Metab* (2014) 19:37–48. doi:10.1016/j.cmet.2013.11.008
25. Kalucka J, Missiaen R, Georgiadou M, Schoors S, Lange C, De Bock K, et al. Metabolic control of the cell cycle. *Cell Cycle* (2015) 14:3379–88. doi:10.1080/15384101.2015.1090068
26. Glatz JF, Luiken JJ, Bonen A. Membrane fatty acid transporters as regulators of lipid metabolism: implications for metabolic disease. *Physiol Rev* (2010) 90:367–417. doi:10.1152/physrev.00003.2009
27. Elmasri H, Karaaslan C, Teper Y, Ghelfi E, Weng M, Ince TA, et al. Fatty acid binding protein 4 is a target of VEGF and a regulator of cell proliferation in endothelial cells. *FASEB J* (2009) 23:3865–73. doi:10.1096/fj.09-134882
28. Elmasri H, Ghelfi E, Yu CW, Traphagen S, Cernadas M, Cao H, et al. Endothelial cell-fatty acid binding protein 4 promotes angiogenesis: role of stem cell factor/c-kit pathway. *Angiogenesis* (2012) 15:457–68. doi:10.1007/s10456-012-9274-0
29. McGarry JD, Brown NF. The mitochondrial carnitine palmitoyltransferase system. From concept to molecular analysis. *Eur J Biochem* (1997) 244:1–14. doi:10.1111/j.1432-1033.1997.00001.x
30. Schoors S, Bruning U, Missiaen R, Queiroz KC, Borgers G, Elia I, et al. Corrigendum: fatty acid carbon is essential for dNTP synthesis in endothelial cells. *Nature* (2015) 526:144. doi:10.1038/nature14624
31. Patella F, Schug ZT, Persi E, Neilson LJ, Erami Z, Avanzato D, et al. Proteomics-based metabolic modeling reveals that fatty acid oxidation (FAO) controls endothelial cell (EC) permeability. *Mol Cell Proteomics* (2015) 14:621–34. doi:10.1074/mcp.M114.045575
32. Coutelle O, Hornig-Do HT, Witt A, Andree M, Schiffmann LM, Piekarek M, et al. Embelin inhibits endothelial mitochondrial respiration and impairs neoangiogenesis during tumor growth and wound healing. *EMBO Mol Med* (2014) 6:624–39. doi:10.1002/emmm.201303016
33. Krauss S, Zhang CY, Lowell BB. A significant portion of mitochondrial proton leak in intact thymocytes depends on expression of UCP2. *Proc Natl Acad Sci U S A* (2002) 99:118–22. doi:10.1073/pnas.012410699
34. Fox CJ, Hammerman PS, Thompson CB. Fuel feeds function: energy metabolism and the T-cell response. *Nat Rev Immunol* (2005) 5:844–52. doi:10.1038/nri1710
35. Frauwirth KA, Riley JL, Harris MH, Parry RV, Rathmell JC, Plas DR, et al. The CD28 signaling pathway regulates glucose metabolism. *Immunity* (2002) 16:769–77. doi:10.1016/S1074-7613(02)00323-0
36. Bauer DE, Harris MH, Plas DR, Lum JJ, Hammerman PS, Rathmell JC, et al. Cytokine stimulation of aerobic glycolysis in hematopoietic cells exceeds proliferative demand. *FASEB J* (2004) 18:1303–5. doi:10.1096/fj.03-1001fje
37. Merchan JR, Kovacs K, Railsback JW, Kurtoglu M, Jing Y, Pina Y, et al. Antiangiogenic activity of 2-deoxy-D-glucose. *PLoS One* (2010) 5:e13699. doi:10.1371/journal.pone.0013699

38. Wilhelm K, Happel K, Eelen G, Schoors S, Oellerich MF, Lim R, et al. FOXO1 couples metabolic activity and growth state in the vascular endothelium. *Nature* (2016) 529:216–20. doi:10.1038/nature16498
39. Doddaballapur A, Michalik KM, Manavski Y, Lucas T, Houtkooper RH, You X, et al. Laminar shear stress inhibits endothelial cell metabolism via KLF2-mediated repression of PFKFB3. *Arterioscler Thromb Vasc Biol* (2015) 35:137–45. doi:10.1161/ATVBAHA.114.304277
40. Pober JS, Sessa WC. Evolving functions of endothelial cells in inflammation. *Nat Rev Immunol* (2007) 7:803–15. doi:10.1038/nri2171
41. Barodka VM, Joshi BL, Berkowitz DE, Hogue CW Jr, Nyhan D. Review article: implications of vascular aging. *Anesth Analg* (2011) 112:1048–60. doi:10.1213/ANE.0b013e3182147e3c
42. Cosentino F, Francia P, Camici GG, Pelicci PG, Luscher TF, Volpe M. Final common molecular pathways of aging and cardiovascular disease: role of the p66Shc protein. *Arterioscler Thromb Vasc Biol* (2008) 28:622–8. doi:10.1161/ATVBAHA.107.156059
43. Kovacic JC, Moreno P, Hachinski V, Nabel EG, Fuster V. Cellular senescence, vascular disease, and aging: part 1 of a 2-part review. *Circulation* (2011) 123(15):1650–60. doi:10.1161/CIRCULATIONAHA.110.007021
44. Kovacic JC, Moreno P, Nabel EG, Hachinski V, Fuster V. Cellular senescence, vascular disease, and aging: part 2 of a 2-part review: clinical vascular disease in the elderly. *Circulation* (2011) 123(17):1900–10. doi:10.1161/CIRCULATIONAHA.110.009118
45. Virdis A, Ghiadoni L, Giannarelli C, Taddei S. Endothelial dysfunction and vascular disease in later life. *Maturitas* (2010) 67:20–4. doi:10.1016/j.maturitas.2010.04.006
46. Lopez-Otin C, Blasco MA, Partridge L, Serrano M, Kroemer G. The hallmarks of aging. *Cell* (2013) 153:1194–217. doi:10.1016/j.cell.2013.05.039
47. Pendyala S, Usatyuk PV, Gorshkova IA, Garcia JG, Natarajan V. Regulation of NADPH oxidase in vascular endothelium: the role of phospholipases, protein kinases, and cytoskeletal proteins. *Antioxid Redox Signal* (2009) 11:841–60. doi:10.1089/ARS.2008.2231
48. Zinovkin RA, Romaschenko VP, Galkin II, Zakharova VV, Pletjushkina OY, Chernyak BV, et al. Role of mitochondrial reactive oxygen species in age-related inflammatory activation of endothelium. *Aging (Albany NY)* (2014) 6:661–74. doi:10.18632/aging.100685
49. Drummond GR, Sobey CG. Endothelial NADPH oxidases: which NOX to target in vascular disease? *Trends Endocrinol Metab* (2014) 25:452–63. doi:10.1016/j.tem.2014.06.012
50. Podlutsky A, Ballabh P, Csiszar A. Oxidative stress and endothelial dysfunction in pulmonary arteries of aged rats. *Am J Physiol Heart Circ Physiol* (2010) 298:H346–51. doi:10.1152/ajpheart.00972.2009
51. Lener B, Koziel R, Pircher H, Hutter E, Greussing R, Herndler-Brandstetter D, et al. The NADPH oxidase Nox4 restricts the replicative lifespan of human endothelial cells. *Biochem J* (2009) 423:363–74. doi:10.1042/BJ20090666
52. Kong D, Zhan Y, Liu Z, Ding T, Li M, Yu H, et al. SIRT1-mediated ERbeta suppression in the endothelium contributes to vascular aging. *Aging Cell* (2016) 15(6):1092–102. doi:10.1111/acel.12515
53. Forstermann U, Munzel T. Endothelial nitric oxide synthase in vascular disease: from marvel to menace. *Circulation* (2006) 113:1708–14. doi:10.1161/CIRCULATIONAHA.105.602532
54. Forstermann U, Sessa WC. Nitric oxide synthases: regulation and function. *Eur Heart J* (2012) 33:829–37, 837a–837d. doi:10.1093/eurheartj/ehr304
55. Wenes M, Shang M, Di Matteo M, Goveia J, Martìn-Pérez R, Serneels J, et al. Macrophage metabolism controls tumor blood vessel morphogenesis and metastasis. *Cell Metab* (2016) 24(5):701–15. doi:10.1016/j.cmet.2016.09.008
56. Xu Y, An X, Guo X, Habtetsion TG, Wang Y, Xu X, et al. Endothelial PFKFB3 plays a critical role in angiogenesis. *Arterioscler Thromb Vasc Biol* (2014) 34:1231–9. doi:10.1161/ATVBAHA.113.303041
57. Conradi LC, Brajic A, Cantelmo AR, Bouche A, Kalucka J, Pircher A, et al. Tumor vessel disintegration by maximum tolerable PFKFB3 blockade. *Angiogenesis* (2017) 20(4):599–613. doi:10.1007/s10456-017-9573-6
58. Carmeliet P, Jain RK. Principles and mechanisms of vessel normalization for cancer and other angiogenic diseases. *Nat Rev Drug Discov* (2011) 10:417–27. doi:10.1038/nrd3455
59. Singh M, Ferrara N. Modeling and predicting clinical efficacy for drugs targeting the tumor milieu. *Nat Biotechnol* (2012) 30:648–57. doi:10.1038/nbt.2286
60. Jayson GC, Kerbel R, Ellis LM, Harris AL. Antiangiogenic therapy in oncology: current status and future directions. *Lancet* (2016) 388:518–29. doi:10.1016/S0140-6736(15)01088-0
61. Wong BW, Wang X, Zecchin A, Thienpont B, Cornelissen I, Kalucka J, et al. The role of fatty acid beta-oxidation in lymphangiogenesis. *Nature* (2017) 542:49–54. doi:10.1038/nature21028

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REVIEW
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Diverse Roles of Mitochondria in Immune Responses: Novel Insights Into Immuno-Metabolism

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Lack of immune system cells or impairment in differentiation of immune cells is the basis for many chronic diseases. Metabolic changes could be the root cause for this immune cell impairment. These changes could be a result of altered transcription, cytokine production from surrounding cells, and changes in metabolic pathways. Immunity and mitochondria are interlinked with each other. An important feature of mitochondria is it can regulate activation, differentiation, and survival of immune cells. In addition, it can also release signals such as mitochondrial DNA (mtDNA) and mitochondrial ROS (mtROS) to regulate transcription of immune cells. From current literature, we found that mitochondria can regulate immunity in different ways. *First*, alterations in metabolic pathways (TCA cycle, oxidative phosphorylation, and FAO) and mitochondria induced transcriptional changes can lead to entirely different outcomes in immune cells. For example, M1 macrophages exhibit a broken TCA cycle and have a pro-inflammatory role. By contrast, M2 macrophages undergo β-oxidation to produce anti-inflammatory responses. In addition, amino acid metabolism, especially arginine, glutamine, serine, glycine, and tryptophan, is critical for T cell differentiation and macrophage polarization. *Second*, mitochondria can activate the inflammatory response. For instance, mitochondrial antiviral signaling and NLRP3 can be activated by mitochondria. *Third*, mitochondrial mass and mobility can be influenced by fission and fusion. Fission and fusion can influence immune functions. *Finally,* mitochondria are placed near the endoplasmic reticulum (ER) in immune cells. Therefore, mitochondria and ER junction signaling can also influence immune cell metabolism. Mitochondrial machinery such as metabolic pathways, amino acid metabolism, antioxidant systems, mitochondrial dynamics, mtDNA, mitophagy, and mtROS are crucial for immune functions. Here, we have demonstrated how mitochondria coordinate to alter immune responses and how changes in mitochondrial machinery contribute to alterations in immune responses. A better understanding of the molecular components of mitochondria is necessary. This can help in the development of safe and effective immune therapy or prevention of chronic diseases. In this review, we have presented an updated prospective of the mitochondrial machinery that drives various immune responses.

Keywords: oxidative phosphorylation, fatty acid oxidation, TCA cycle, regulatory T cell, memory T cell

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INTRODUCTION

Mitochondria have many fundamental functions such as energy production, providing metabolites for building macromolecules, and aiding in differentiation, apoptosis, and cell cycle. After reviewing recent literature, two mitochondrial functions appeared to be intriguing. *First*, mitochondria and the endoplasmic reticulum (ER) communicate with each other through signaling molecules (1). *Second*, mitochondria are associated with NLRP3 inflammasome activation (2). Mitochondria are localized near the ER to supply energy for protein and lipid synthesis. For example, Bantug et al. have highlighted that ER and mitochondria junction signaling is critical in CD8$^+$ memory T cells. Mechanistically, this happens in crosstalk between ER and mitochondria by mammalian target of rapamycin complex 2, AKT (protein kinase B), and glycogen synthase kinase 3β mediated signaling which promotes respiration (**Figure 1**.9) (3).

Immune response and metabolism are closely dependent on each other. During immune response, immune cells transition from metabolic quiescence to active phase. This transition is associated with a metabolic shift from catabolic to anabolic state. During the quiescence state, macromolecules undergo catabolic pathways to produce energy and support long-term survival. During the anabolic state, macromolecules are synthesized and support a balance between the need for ATP and required metabolites. Depending on the need, immune cells choose a specific pathway such as β-oxidation to generate more ATP than glycolysis.

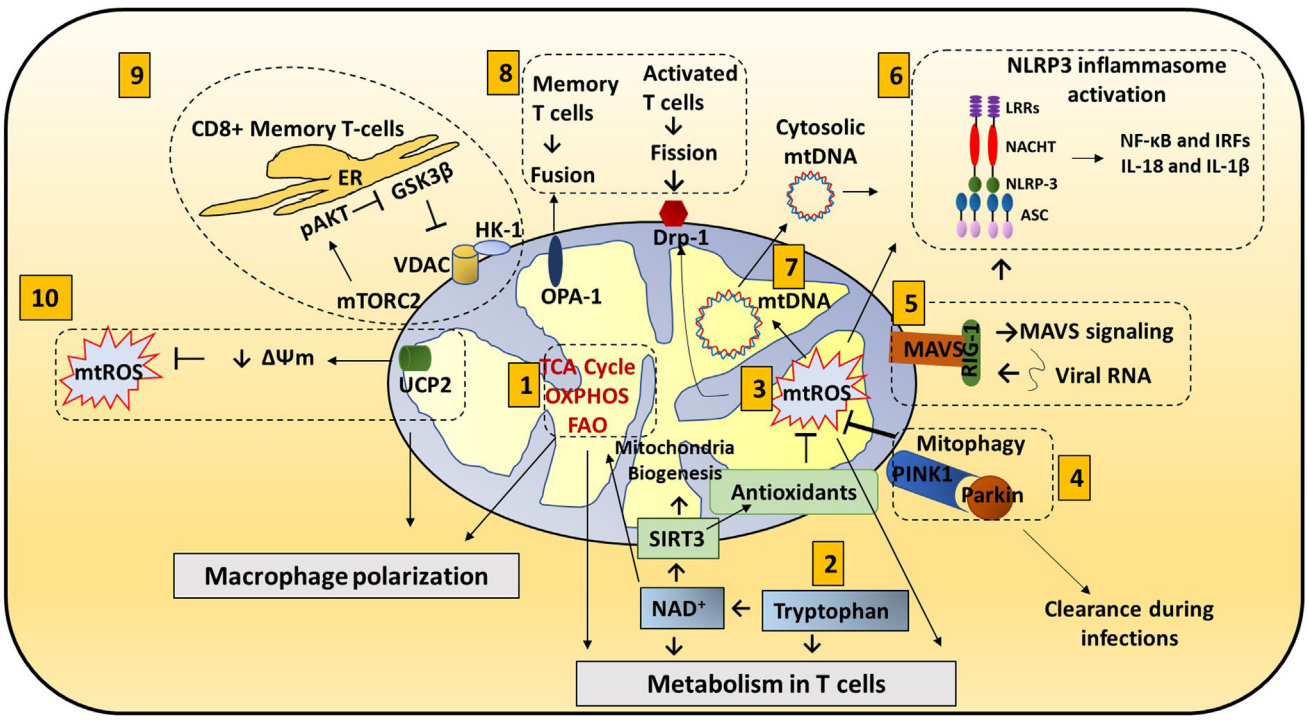

FIGURE 1 | Mitochondria roles in immune responses: mitochondrial components are involved in immune functions. (1) *Metabolic pathways* such as TCA cycle, oxidative phosphorylation (OXPHOS), and fatty acid oxidation (FAO) are important for macrophage polarization and T cell differentiation. (2) *Amino acid metabolism* (Tryptophan metabolism is shown here.) also contributes to mitochondrial immune functions. Tryptophan depletion can cause immune tolerance. NAD$^+$ is synthesized from tryptophan. NAD$^+$ contributes to SIRT3-mediated mitochondrial biogenesis. In addition, NAD$^+$ is required for metabolic pathways and CD4$^+$ T cell differentiation. (3) *Mitochondrial ROS* (*mtROS*) control immune cell transcription, metabolism, and NLRP3 mediated inflammation. Antioxidants such as glutathione balance mtROS. SIRT3 is involved in regulating many mitochondrial proteins such as IDH2 (TCA cycle), MnSOD [reactive oxygen species (ROS) balance], and FOXO3. SIRT3 inhibits mtROS. (4) *Mitophagy* is crucial for removing damaged mitochondria. Damaged mitochondria are a source of mtROS so mitophagy balances mtROS. Pink1, localized on the mitochondrial outer membrane, binds to parkin and initiates mitophagy. Parkin mutations can increase the susceptibility to the intracellular bacteria *Mycobacterium leprae* and *Salmonella enterica*. Decreased mitophagy results in increased ROS which further increases the susceptibility to infections. Hepatitis B and C viruses use mitophagy to their benefit. These viruses protect themselves from mitochondria induced apoptosis by activating mitophagy. (5) Mitochondrial antiviral signaling (*MAVS*) in the mitochondria membrane initiates the inflammatory response. Upon viral infections, viral RNA is sensed by RIG-I. RIG-I activates MAVS (located on the outer membrane of mitochondria). MAVS signaling can activate NLRP3 inflammasome and NF-κB/IRF transcription. (6) *NLRP3 inflammasome* contributes to the activation of caspase-1 and leads to NF-κB signaling and production of IL-1β and IL-18. (7) *Mitochondrial DNA* (*mtDNA*) can be released from mitochondria into the cytosol and activate the NLRP3 inflammasome and production of IL-1β and IL-18. Also, mtROS can induce mtDNA mutation. (8) *Mitochondrial dynamics*, fission (OPA-1) and fusion (Drp1), is associated with activated T cells and memory T cells, respectively. (9) *ER and mitochondria junction:* in CD8$^+$ memory T cells, mitochondria are placed near the ER. HK-1 (hexokinase-1, an important enzyme in glycolysis) in conjunction with pAKT, mammalian target of rapamycin complex 2 (mTORC2), and glycogen synthase kinase 3β (GSK-3β) mediated signaling cascades regulate CD8$^+$ memory T cell metabolism. (10) *Uncoupling protein 2* (*UCP2*), a mitochondrial membrane protein, allows protons to enter the mitochondrial matrix, thereby decreasing mitochondrial membrane potential which further decreases mtROS.

Thus, ATP and metabolic intermediates provide the signals to activate immune responses (4–6).

Hence, mitochondria, the chief organelle for metabolism, have emerged to play crucial roles in the maintenance and establishment of immune responses. Mitochondrial machinery such as metabolic pathways, amino acid metabolism, antioxidant systems, mitochondrial dynamics, mitochondrial DNA (mtDNA), mitophagy, and mitochondrial ROS (mtROS) are crucial for immune functions. In this review, we have presented an updated prospective of the mitochondrial machinery that drives various immune responses.

METABOLIC PATHWAYS ARE TIGHTLY CONTROLLED IN IMMUNE CELLS

Mitochondrial metabolic events can have tremendous impact on immune cell function. These metabolic events could be guided by the internal signals from the cell itself or influenced by other surrounding cells. Here, we have pointed out how oxidative phosphorylation (OXPHOS), fatty acid metabolism, and amino acid metabolism in mitochondria influence immune cell activity (**Figure 1**.1).

OXPHOS Affects Immune Cell Activity

M1 and M2 Polarization

There are two categories of macrophages—M1 (classically activated) and M2 (alternatively activated). Polarization of monocytes to M1 or M2 phenotypes is controlled by the cytokines produced by other immune cells. M1 macrophages are activated by IFN-γ produced by Th1 cells and lipopolysaccharide (LPS). Plus, M1 exhibits nitric oxide (NO) production (7) and a pro-inflammatory phenotype. Contrarily, M2 macrophages are activated by IL-4 or IL-13 to regulate anti-inflammation and promote Th2 response and tissue repair (**Figure 2A**) (8).

Uncoupling Protein 2 (UCP2) and Macrophage Polarization

Mitochondrial UCP2 is localized in the mitochondrial inner membrane and shuttle protons toward the matrix (**Figure 1**.10). There is increasing evidence supporting that UCP2 controls mitochondria derived reactive oxygen species (ROS). UCP2 can also influence polarization of macrophages. UCP2 expression is decreased in M1 macrophages. By blocking UCP2, there is a decrease in IL-4 induced M2 macrophage activation (9). However, how UCP2 is regulated in other immune cells is not well elucidated.

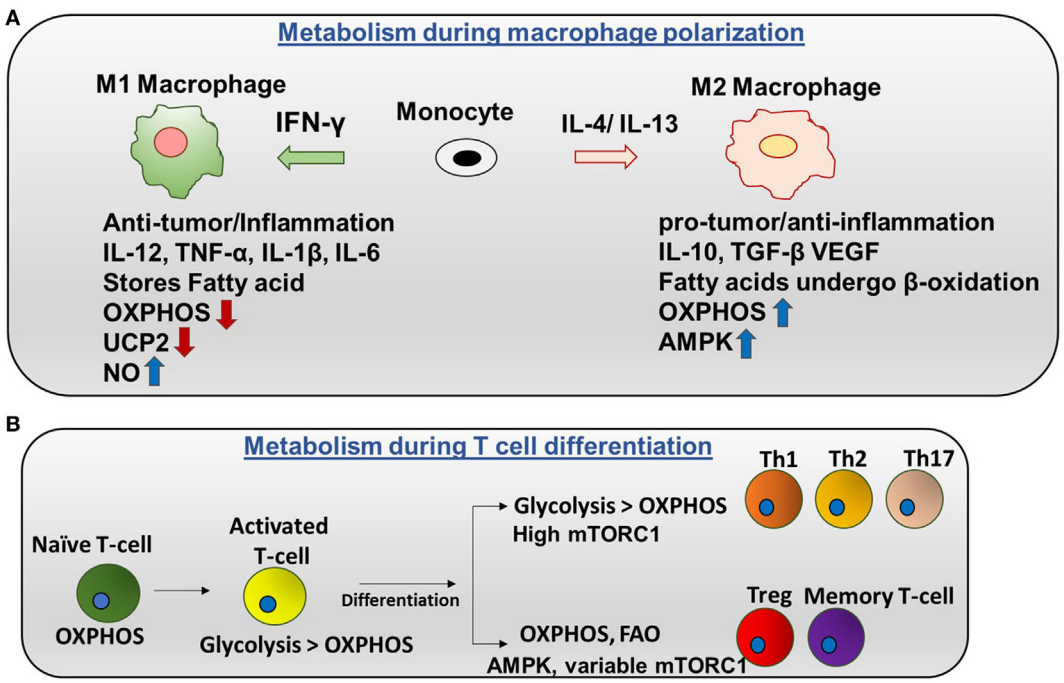

FIGURE 2 | Metabolic regulation in macrophages and T cells: **(A)** metabolism during macrophage polarization: polarization of monocytes to M1 or M2 phenotypes is controlled by the cytokines produced by other immune cells. *M1 macrophages* are activated by IFN-γ produced by Th1 cells. M1 macrophages tend to store surplus FA as triacylglycerols and cholesteryl esters in lipid droplets, and they exhibit higher aerobic glycolysis and lower oxidative phosphorylation (OXPHOS). Nitric oxide production is higher in M1. Uncoupling protein 2 (UCP2) expression is decreased in M1 macrophages. Contrarily, *M2 macrophages* are activated by IL-4 or IL-13 to regulate anti-inflammation and promote Th2 response and tissue repair. M2 macrophages adopt a metabolic program dominated by fatty acid-fueled OXPHOS and channel FA toward re-esterification and β-oxidation. Silencing UCP2 impairs M2 macrophage activation by IL-4. High adenosine monophosphate-activated protein kinase (AMPK) and low NO is the reason for high OXPHOS in M2 macrophages. **(B)** Metabolism during T cells differentiation: naïve T cells are dependent on OXPHOS as their primary metabolic pathway. By contrast, activated T cells exhibit higher glycolysis than OXPHOS. After differentiation, Th1, Th2, and Th17 have higher glycolysis than OXPHOS and high mTORC1 activity. Memory T cells and regulatory T cells undergo AMPK-dependent FAO and have variable mTORC1.

TCA Cycle in M1 Macrophages

Metabolic events are tightly controlled in M1 and M2 macrophages. Mechanistically in M1 macrophages, TCA (tricarboxylic acid) cycle exhibits two breaks (**Figure 3A**) (10, 11). *First break* occurs in the enzymatic step involving isocitrate dehydrogenase (IDH). This results in increased citrate and itaconic acid levels. Citrate is the precursor for fatty acid (FA) synthesis, prostaglandin (PG), and nitric oxide (NO) production. Itaconic acid has anti-bacterial properties which supports the notion that M1 macrophages have inflammatory function. Interestingly, IDH1 and IDH2 are the enzymes that catalyze decarboxylation of isocitrate to α-ketoglutarate outside and inside of the mitochondria, respectively (12). IDH2 plays a vital role in the formation of NADPH which is critical for ROS balance in the mitochondria (13). *Second break* occurs in the enzymatic step involving succinate dehydrogenase. This causes an increase in the expression of succinate. Succinate stabilizes HIF-1α. HIF-1α binds to the IL-1β promoter and promotes IL-1β production. Increased aspartate arginosuccinate shunt will further increase the flow of the TCA cycle. Therefore, this will increase citrate levels and the urea cycle that contribute to NO production. Inhibition of aspartate aminotransferase inhibits NO and IL-6 in M1 macrophages. Thus, the production of NO, IL-1β, and itaconic acid can promote inflammatory functions (14). Also, glutamine metabolism also impacts TCA cycle in M1 macrophages.

TCA Cycle in M2 Macrophages

M2 macrophages adopt a metabolic program fueled by fatty acids and OXPHOS. Low NO production and high adenosine monophosphate-activated protein kinase (AMPK) can be the reason for the high rate of OXPHOS in M2 macrophages (**Figure 4A**) (15). Knockdown of pyruvate dehydrogenase kinase 1 diminishes M1 activation whereas it enhances M2 activation of macrophages (16).

OXPHOS in Adaptive Immune Cells

Naïve and regulatory T cells exhibit OXPHOS as their primary method of respiration while activated T cells depend on glycolysis. Naïve T cells depend on OXPHOS as their primary metabolic pathway. By contrast, activated T cells exhibit higher glycolysis than OXPHOS. After differentiation, Th1, Th2, and Th17 have higher glycolysis than OXPHOS and high mTORC1 activity.

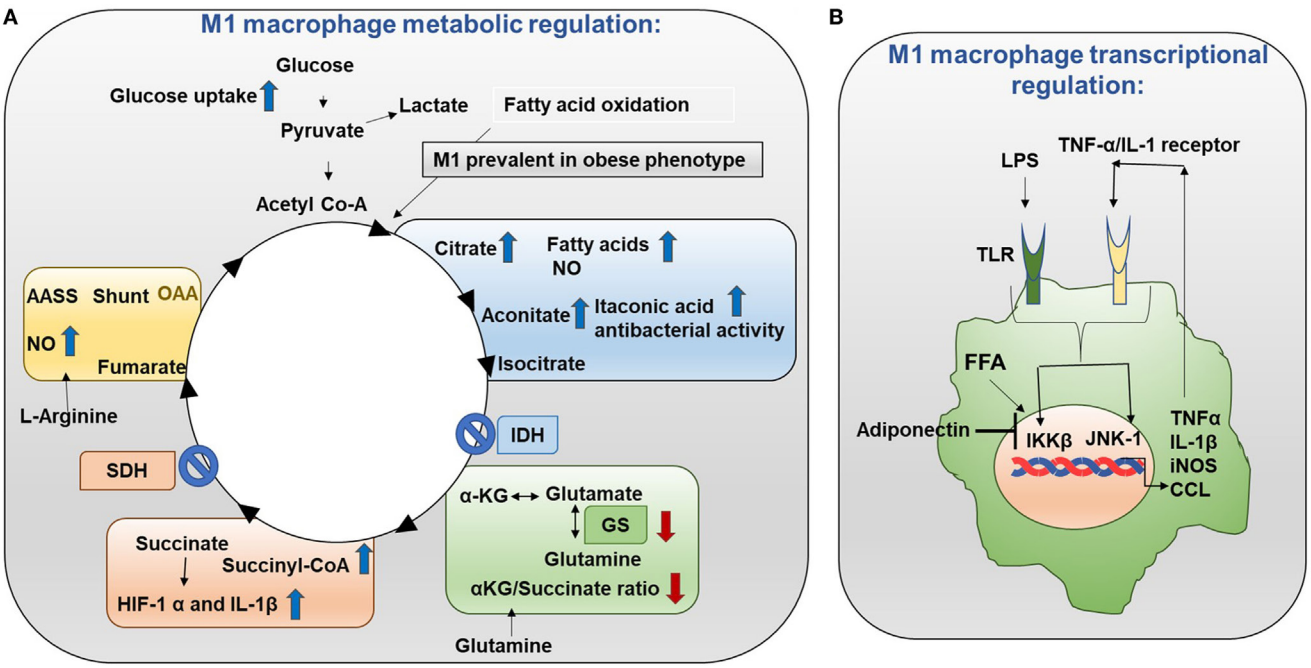

FIGURE 3 | Metabolism in M1 macrophages. **(A)** M1 macrophage metabolic regulation: M1 macrophages are prevalent in obese adipose tissue. Glucose uptake is increased in M1 macrophages. Importantly, the TCA cycle exhibits two breaks. The first break involves the enzyme isocitrate dehydrogenase (IDH) which results in increased levels of citrate and itaconic acid. Citrate feeds fatty acid (FA) synthesis for prostaglandin (PG) and nitric oxide (NO) production while itaconic acid has anti-bacterial properties. The second break happens with the enzyme succinate dehydrogenase (SDH) which causes increased succinate levels. Succinate stabilizes HIF-1α which binds to the interleukin (IL)-1β promoter boosting IL-1β production and inflammation. Increased flow through the aspartate arginosuccinate shunt (AASS) replenishes the TCA cycle which further increases citrate levels and feeds the urea cycle which contributes to NO production. Glutamine is converted into glutamate by glutamate synthase (GS). Glutamate can further be converted into α-ketoglutarate (αKG). Low αKG/succinate ratio strengthens M1 macrophage activation. Glutamine-synthetase inhibition skews M2-polarized macrophages toward the M1-like phenotype characterized by reduced intracellular glutamine and increased succinate with enhanced glucose flux through glycolysis which is partly related to HIF-1α activation. **(B)** M1 macrophage transcriptional regulation: in M1 macrophages, the TCA cycle is broken. As a result, citrate is converted to free fatty acids (FFAs). FFAs activate macrophage IKK and JNK1 signaling molecules to induce M1 polarization. Activation of IKK and JNK1 in macrophages produces IL-1β and TNFα which can initiate inflammatory response. Adiponectin is released from adipose tissue and is involved in the breakdown of fatty acids. Adiponectin signaling inhibits M1 programming.

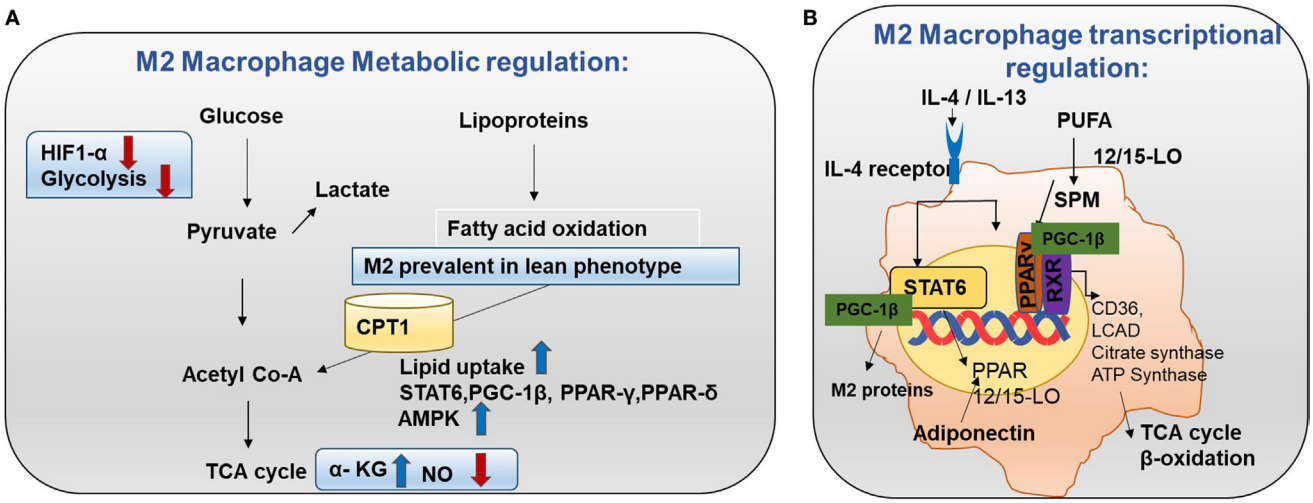

FIGURE 4 | Metabolism in M2 macrophages. **(A)** M2 macrophage metabolic regulation: M2 macrophages adopt a metabolic program fueled by fatty acids and oxidative phosphorylation (OXPHOS). M2 macrophages are seen in lean adipose tissue and lipid uptake is increased in M2 macrophages (8). Both OXPHOS and FAO are important for the anti-inflammatory function of M2 macrophages. In M2 macrophages, glycolysis is impaired due to low HIF-1α activity and low expression of the active 6-phosphofructo-2-kinase/fructose-2,6-biphosphatase 1 (PFKFB1). Also, high α-KG/succinate ratio promotes the M2 phenotype. This could be due to the high flow of the TCA cycle or glutamine metabolism. M2 macrophages will channel FA toward re-esterification and β-oxidation. β-oxidation is channeled through carinitine palmitoyl transferase 1 (CPT1) (9). The high rates of OXPHOS in M2 macrophages are caused by low nitric oxide (NO) production and high adenosine monophosphate-activated protein kinase (AMPK) activity. **(B)** M2 macrophage transcriptional regulation: omega-3 polyunsaturated fatty acids (PUFA) undergo enzymatic transformation by 12/15-LO (lipoxygenase) and other enzymes in the production of specialized pro-resolving lipid mediators (SPMs) and intermediate metabolites that are potent PPAR ligands. M2-inducing cytokines IL-4 and IL-13 promote STAT6 phosphorylation to induce transcription of PPARs (γ and δ) and their coactivator PPARγ-coactivator-1β (PGC-1β). M2 exhibit enhanced PPAR-γ activity. PPAR-γ forms a heterodimer with RXR to induce OXHOS regulating proteins. PGC-1β promotes the STAT6 transcription complex and expression of M2 proteins such as arginase-1 and the pattern-recognition/endocytic receptor CD206. The STAT6 transcription complex further increases transcription of TCA cycle enzymes such as citrate synthase, CD36 (fatty acid transporter), ATP synthase, 12/15 lipoxygenase (converts omega-3 PUFA to SPMs), and LCAD (long chain acyl dehydrogenase, an enzyme for β-oxidation). Adiponectin activates PPARs in M2 macrophages and consequently increases β-oxidation.

By contrast, memory T cells and regulatory T cells undergo AMPK-dependent FAO and have variable mTORC1 (**Figure 2B**) (17). Interestingly, glutamine is required for T cell proliferation. Thus, glutamine can provide the intermediate metabolites of the TCA cycle after being converted into α-ketoglutarate in a process called glutaminolysis. These metabolites are important for proliferation. In addition, $CD4^+$ T cells can grow in glucose depleted media supplemented with sodium pyruvate (18). This suggests that pyruvate can be utilized, and it is adequate for the survival of T cells in the absence of glucose. In addition, it has been reported that mitochondria undergo rapid changes during T cell activation including increasing mitochondrial mass, number, and mtDNA. This further suggests that mitochondrial metabolism may be crucial for T cell activation and proliferation.

Lactate Can Act as a Substrate for Mitochondrial Respiration

Lactate dehydrogenase controls a reversible reaction involving the conversion of lactate to pyruvate (19). Lactate is secreted from tumor cells to the tumor microenvironment (TME). This causes acidification of the TME known as acidosis. Acidosis can suppress the proliferation and cytokine production of cytotoxic T cells (20). Along with cytotoxic T cells, macrophages can be influenced by lactic acid through HIF-1α (21). Furthermore, T cell activation requires activation of nuclear factor of activated T cell (NFAT). High concentrations of lactate can inhibit production of the cytokine interferon gamma (IFN-γ) and transcription of NFAT in both NK cells and T cells. Moreover, genetically targeting LDHA in tumors helps to restore T cell infiltration and function (22). Similarly, umbilical cord derived mesenchymal stromal cells can produce a large amount of lactate. This leads to decreased OXPHOS and mitochondrial mass. Ultimately, this can alter the phenotype and function of dendritic cells (DCs) by inducing M2-type gene signature (23). This indicates that lactate concentration affects activation and survival of the immune cells.

In brief, metabolic changes in immune cells are primarily caused by alterations in metabolic pathways induced by transcriptional changes and TME. In M1 macrophages, the TCA cycle is broken with a decrease in OXPHOS. On the other hand, M2 TCA cycle is fueled by β-oxidation with an increase in OXPHOS. Furthermore, mitochondrial metabolism is important for activation of T cells. Acidification due to lactate production can suppress the T cell proliferation and cytokine production. Therefore, this evidence supports that remodeling OXPHOS in immune cells may be an essential component for developing more efficient therapy for autoimmune disease and cancer.

Fatty Acid Oxidation (FAO) in Immune Cells

FAO in Macrophages

M1 macrophages are prevalent in obese adipose tissue (24). Unsaturated fatty acids (nitrosylated fatty acid and omega-3

derived fatty acids) polarize macrophages toward the M1 phenotype (25). In addition, glucose uptake is increased in M1 macrophages (10). Hence due to their broken TCA cycle, M1 macrophages tend to store surplus fatty acid (FA) as triacylglycerol and cholesteryl esters in lipid droplets. By contrast, *M2 macrophages* are seen in lean adipose tissue (24), and their lipid uptake is increased (8). Both OXPHOS and FAO are important for the anti-inflammatory function of M2 macrophages. In M2 macrophages, glycolysis is impaired due to low HIF-1α activity and low expression of the active 6-phosphofructo-2-kinase/fructose-2,6-biphosphatase 1. Also, high α-KG/succinate ratio promotes the M2 phenotype. This could be due to high flow of the TCA cycle or glutamine metabolism. M2 macrophages will channel FA toward re-esterification and β-oxidation (**Figure 4A**) (11, 26–28). β-oxidation is channeled through carinitine palmitoyl transferase 1 (CPT1), but there are not enough studies supporting how CPT1 is regulated in different macrophages.

FAO Controls Transcription of M1 Proteins

In M1 macrophages, the TCA cycle is broken. As a result, citrate is converted to free fatty acids (FFAs). Th1 type cytokines and saturated FFAs activate macrophage IKK and JNK1 signaling molecules to induce M1 polarization. Activation of IKK and JNK1 in macrophages produce IL-1β and TNFα which can initiate inflammatory responses. In addition, IL-1β and TNFα can also activate adipocyte IKK and JNK to block insulin signaling resulting in insulin insensitivity (**Figure 3B**) (11). Adiponectin is released from adipose tissue and involved in glucose metabolism and break down of fatty acids (29). Adiponectin signaling inhibits M1 programming.

FAO Controls Transcription of M2 Proteins

Transcriptional changes in M2 can be the root cause for high FAO. Mechanistically, M2-inducing cytokines IL-4 and IL-13 promote STAT6 phosphorylation to induce transcription of PPARs (γ and δ) and their coactivator PPARγ-coactivator-1β (PGC-1β) (30). PGC-1β promotes the STAT6 transcription complex and expression of M2 proteins such as arginase-1 and the pattern recognition/endocytic receptor CD206 (**Figure 4B**). Subsequently, STAT6 transcription complex further increases transcription of TCA cycle enzymes such as citrate synthase, CD36 (fatty acid transporter), ATP synthase, 12/15 lipoxygenase (converts omega-3 polyunsaturated fatty acids to SPMs), and LCAD (long chain acyl dehydrogenase, an enzyme for β-oxidation) (11). Also, adiponectin activates PPARs in M2 macrophages and consequently increase β-oxidation. In this way, the M2 program is activated. It supports insulin sensitivity by lowering FFA and promoting lean phenotype. Most importantly, these transcriptional changes lead to β-oxidation in M2 macrophages (**Figure 4B**).

FAO in Memory T Cell Development

Memory T cells are critical for long-term immune response against re-infection by the same pathogen which mainly depends on FAO (31, 32). $CD8^+$ T cells play a crucial role in immune responses. Upon antigen stimulation, these cells differentiate into T effector and memory cells for long-term immunity. This differentiation process is closely associated with FAO through AMPK, lipolysis, and IL-7. AMPK is a key regulator of FAO. Metformin activates AMPK which enhances memory T cell development in tumor necrosis factor receptor-associated factor 6 (TRAF6)-deficient $CD8^+$ T cells (31). AMPK activation is an important factor for memory T cell development. In addition, lipolysis is the hydrolysis process through which stored lipids are converted into FA. Lysosomal acid lipase catalyzes this process. Memory T cells rely on the expression of lysosomal acid lipase to mobilize FA for FAO (32). IL-7 is required for memory T cell activation as well. Glycerol channel aquaporin 9 (AQP9) is utilized for glycerol transport and triglyceride synthesis, and IL-7 induces the expression of AQP9 in virus-specific memory $CD8^+$ T cells (33). AQP9-deficient memory T cells showed less survival because of decreased glycerol import, reduced triglyceride synthesis, and consequently limited FAO. This suggests that IL-7-mediated triglyceride synthesis is important for memory T cell development.

FAO in Regulatory T Cell Activation

Stimulated $CD4^+$ T lymphocytes can differentiate into effector T cells or inducible regulatory T cell. Michalek et al. found that FAO is required for regulatory T cells differentiation. Glucose metabolism is required for effector T cells and is selectively suppressed by FAO (34). Also, AMPK and mTOR-mediated FAO supports Treg differentiation (**Figure 2B**). Also, *de novo* synthesized fatty acids are important for FAO and OXPHOS in activated plasmocytoid DCs (35).

In brief, M1 macrophages prefer storing FA while β-oxidation is increased in M2 macrophages. In adipose tissue, M2 macrophages are associated with lean and insulin sensitive phenotype (through regulation of STAT6 and PPARs), whereas M1 macrophages are linked to obese and insulin insensitive phenotype (through regulation of IKK and JNK-1). Current evidence supports that adipose tissue activity is linked to M1 or M2 program activation and obesity. Activated memory T cells (through AMPK and AQP9) and pDCs also support FAO. Concisely, the activation of immune cells is closely related to FAO and the cytokines produced by immune cells which also affect transcriptional changes in the surrounding cells.

Amino Acid Metabolism in Immune Cells

Amino acid metabolism provides the metabolites needed for immune cells growth. L-Arginine supplementation in activated naïve T cells promotes OXPHOS, limits IFN-γ production, and most importantly helps to express memory T cell markers leading to longer cellular survival (36). Similarly, M1 macrophage polarization also requires amino acid metabolism. For example, arginosuccinate synthase is strongly upregulated in M1 macrophages (14). Inhibition of the aspartate–arginosuccinate shunt enzyme GOT1 (glutamic oxaloacetic transaminase) decreases NO production, and IL-6 production which is characteristic of M1 macrophages.

SHMT2 (serine hydroxymethyltransferase 2) catalyzes glycine synthesis from serine in mitochondria

SHMT2 is upregulated in activated $CD4^+$ T cells (37). SHMT2-deficient activated T cells have decreased purine levels compared with wild-type controls as well as increased DNA damage and

impaired survival. Furthermore, SHMT-2 is the primary source for glycine which is important for glutathione synthesis.

Tryptophan Metabolism Is Important for Immune Cell Response

L-tryptophan (Trp) cannot be produced by the human body and must be obtained from dietary sources. It is catabolized by indoleamine-2,3-dioxygenase-1 (IDO-1) to L-kynurenine (Kyn). Interferon-γ produced by Th1 cells stimulates IDO-1 production in antigen-presenting cells (APCs), and tumor cells are known to express high levels of IDO-1 (38, 39). Increased IDO-1 causes conversion of Trp to Kyn resulting in a decrease of Trp and an increase of Kyn in plasma. Kyn is a ligand for aryl hydrocarbon receptor (AhR). Increased Kyn causes immune tolerance by inhibiting proliferation of T cells and NK cells, inhibiting maturation of DCs, and increasing proliferation of immune suppresser cells (Treg and MDSC). Also, tryptophan depletion causes apoptosis of T cells due to nutrient deficiency (**Figure 5**) (40–43).

Tryptophan Also Affects Mitochondrial Function

Tryptophan metabolism partially occurs in mitochondria. Trp is one of the sources for NAD^+ biosynthesis (**Figure 5**). NAD^+ is an important metabolite for metabolic processes such as glycolysis, TCA cycle, and OXPHOS (44). During macrophage oxidative burst in the presence of NADPH oxidase, NADPH is oxidized to produce superoxide that is utilized to kill pathogens. Furthermore, NAD^+ and AhR are also linked to mitochondria. Mitochondrial AhR is localized in the inner membrane space of mitochondria which is also affected by Kyn (45). NAD^+ has recently gained much interest because $CD4^+$ T cell differentiation is controlled by NAD^+ (46). NAD-dependent deacetylase Sirtuin 3 (Sirt3) is localized in mitochondria. SIRT3 controls mitochondria biogenesis and

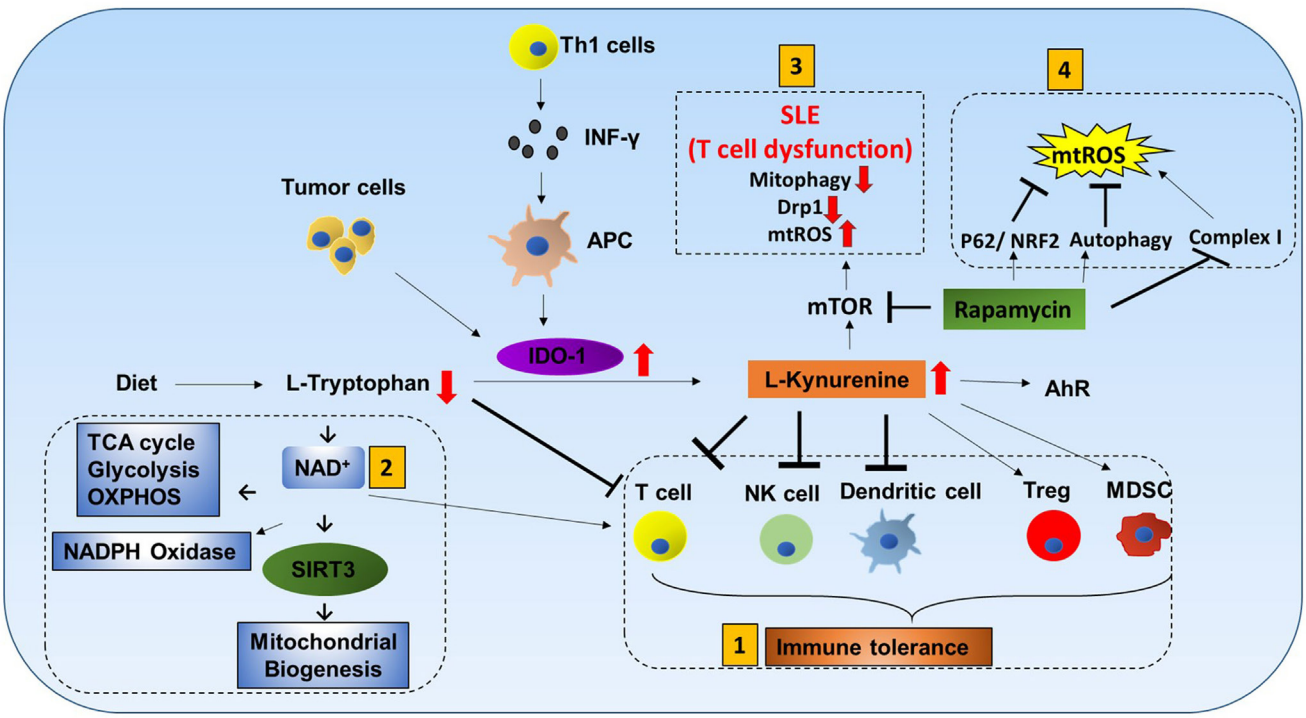

FIGURE 5 | Tryptophan metabolism mediated immune cell response. L-tryptophan (Trp) can only be obtained in the human body from dietary sources. It is catabolized by the enzyme indoleamine-2,3-dioxygenase-1 (IDO-1) to L-kynurenine (Kyn). Interferon-γ produced by Th1 cells stimulates IDO-1 production in antigen-presenting cells (APCs). Tumor cells are also known to have increased production of IDO-1. Increases in IDO-1 cause conversion of Trp to Kyn resulting in lower Trp and higher Kyn in plasma. Kyn is a ligand for aryl hydrocarbon receptor (AhR). Mitochondrial AhR (MitoAhR) is localized in the inner membrane space of mitochondria and is also affected by Kyn. (1) *Immune tolerance by kynurenine:* increased Kyn causes immune tolerance by inhibiting proliferation of both T cells and NK cells by inhibiting maturation of dendritic cells and increasing proliferation of immune suppresser cells (Treg and MDSC). Tryptophan depletion also causes apoptosis of T cells due to nutrient deficiency. (2) *NAD^+ mediated immune function:* in addition, Trp is one of the sources for NAD^+ biosynthesis. NAD^+ is an important metabolite for metabolic processes [glycolysis, TCA cycle, and oxidative phosphorylation (OXPHOS)] and production of NADPH. During oxidative burst in macrophages, NAPPH is oxidized in the presence of NADPH oxidase to produce superoxide which is utilized to kill pathogens. NAD^+ contributes to $CD4^+$ T cell differentiation and SIRT3-mediated mitochondrial biogenesis. (3) *mTOR inhibitor rapamycin can be used to treat systemic lupus erythematosus (SLE) and mitochondrial disease:* T cell dysfunction is associated with SLE. In SLE, an increase in kynurenine and kynurenine mediated mTOR stimulation was observed. In addition, accumulation of reactive oxygen species (ROS) producing mitochondria and decreased Drp1 are associated with SLE. Rapamycin can increase Drp1 by inhibiting mTORC1. (4) *Rapamycin inhibits mitochondrial ROS (mtROS):* rapamycin inhibits mtROS through several mechanisms. Complex I in mitochondria is a source of ROS generation. Rapamycin can inhibit mtROS production by inhibiting complex I formation. In addition to this, rapamycin can inhibit mtROS by inducing autophagy and activating p62 and the NRF2 pathway.

ATP production. Sirt3 can regulate IDH2 (TCA cycle), LCAD (β-oxidation), and MnSOD (mtROS balance) (**Figure 9**.1) (47). Furthermore, Sirt3 is dependent on NAD^+ (**Figure 1**.2) (48). This suggests that tryptophan metabolism is important for immune response, immune tolerance, and metabolism in mitochondria (**Figure 5**).

mTOR Inhibitor Rapamycin Can Be Used to Treat Systemic Lupus Erythematosus (SLE) and Mitochondrial Disease

T cell dysfunction is associated with SLE. In SLE, an increase in kynurenine and kynurenine-mediated mTOR stimulation was observed (49). When SLE patients were treated with rapamycin (mTORC1 inhibitor), progressive improvement was observed after 12 months in the clinical trial (50). Rapamycin treatment was also effective for mitochondrial diseases such as mitochondrial myopathy (51). In addition, accumulation of ROS producing mitochondria and decreased Drp1 are associated with SLE. Rapamycin can increase Drp1 by inhibiting mTORC1.

Rapamycin Inhibits mtROS

Rapamycin inhibits mtROS through several mechanisms. Complex I in mitochondria is a source of ROS generation. Proper assembly of various subunits of complex I is important. Rapamycin can inhibit matrix subunits (NDUFS3 and NDUFV2) as well as prohibitin (PhB) from binding to other membrane subunits (NDUFA9 and NDUFB9). Thus, rapamycin can inhibit mtROS production by inhibiting complex I formation (52, 53). In addition to this, rapamycin can inhibit mtROS by inducing autophagy and activating p62 and the NRF2 pathway (54).

Glutamine Metabolism Is Associated With Macrophage Polarization and T Cell Activation

α-Ketoglutarate is an intermediate product of the TCA cycle and is also produced by glutamine metabolism (**Figure 3A**). αKG is important for alternate M2 macrophage activation by FAO (**Figure 4A**). Low αKG/succinate ratio strengthens M1 macrophage activation and high αKG/succinate ratio promotes the M2 phenotype (55). In addition, glutamine-synthetase inhibition skews M2-polarized macrophages toward the M1-like phenotype characterized by reduced intracellular glutamine and increased succinate with enhanced glucose flux through glycolysis which is partly related to HIF-1α activation (56). Glutamine is also implicated in T cell functions. SNAT1 and SNAT2 (glutamine transporters) are increased in T cell activation (5). Lack of glutamine inhibits oxygen consumption in effector T cells and reduces ATP concentration. After LPS treatment of macrophages, glutamine metabolism was increased (15). Glutamine deficiency reduces lipid-induced lysosomal dysfunction, inflammasome activation, and cell death in macrophages but boosts autophagy (57).

Taken together, amino acid metabolism especially L-arginine, L-glutamine, L-tryptophan, and SHMT-2 (serine/glycine metabolism) plays a decisive role in the activation of T cells and macrophages.

MITOCHONDRIA ASSOCIATED CELL SIGNALING IN THE IMMUNITY

Mitochondria are a metabolic hub in the cell which functions to meet cellular needs. Obviously, this necessitates that mitochondria receive signals to alter their functions. However, accumulating evidence suggests that mitochondria not only receive signals but also actively provide signals. Mitochondria release proteins, lipids, metabolites, and ROS which can be used as signaling molecules. This crosstalk may coordinate cell-fate decisions and metabolic capacity depending on the cellular environment. Thus, we propose that mitochondria are an integral part of the decision-making process when cells receive immune cues.

Mitochondrial ROS

Mitochondria are hubs in cellular signaling and produce ROS that drive production of inflammatory cytokines and play a role in removing bacteria. Major sites of ROS production are complex I and III of electron transport chain (ETC) (**Figure 9**.1).

mtROS Contribute to Macrophage Polarization

H_2O_2 is released from mitochondria being the source for mtROS. He et al. reported that Cu-Zn SOD-mediated mtROS contribute to macrophage polarization (**Figure 9**.1). Cu-Zn SOD mediated H_2O_2 suppresses M1 phenotype and promotes M2 phenotype. This suggests that mtROS contribute to macrophage polarization (8, 15, 58).

mtROS Are Important for the Anti-Bacterial Activity of Immune Cells

Recent studies demonstrate that mtROS significantly contribute to the bactericidal activity and activation of M1 macrophages (59, 60). West et al. found that TRAF6 is recruited to mitochondria resulting in increased mitochondrial and cellular ROS generation. In addition, mitochondrial catalase functions as an antioxidant to control cellular ROS. Mitochondrial catalase transgenic macrophages are less effective at clearing bacteria. This evidence suggests that mtROS are important for the anti-bacterial functions of macrophages.

mtROS Contribute to NLRP3 Inflammasome Activation

The NLRP3 inflammasome is a cytosolic complex that plays a key role in innate immunity by participating in the production of pro-inflammatory cytokines interleukin-1β (IL-1β) and IL-18 (**Figure 6**) (61). The role of the NOD-like receptor (NLR) family of cytoplasmic pattern-recognition receptors in the initiation of inflammatory responses is gathering clear evidence. Structurally, NLRs typically consist of a variable N-terminal effector domain (PYD), a constant central nucleotide binding and oligomerization (NACHT) domain, and C-terminal leucine-rich repeats (**Figure 1**.6). Upon cellular stress, NLRP3 oligomerizes and exposes its effector domain for interaction with the adaptor protein ASC (apoptosis-associated speck-like protein containing a caspase recruitment domain) and then the complex recruits pro-caspase-1. Pro-caspase-1 clustering leads to its activation

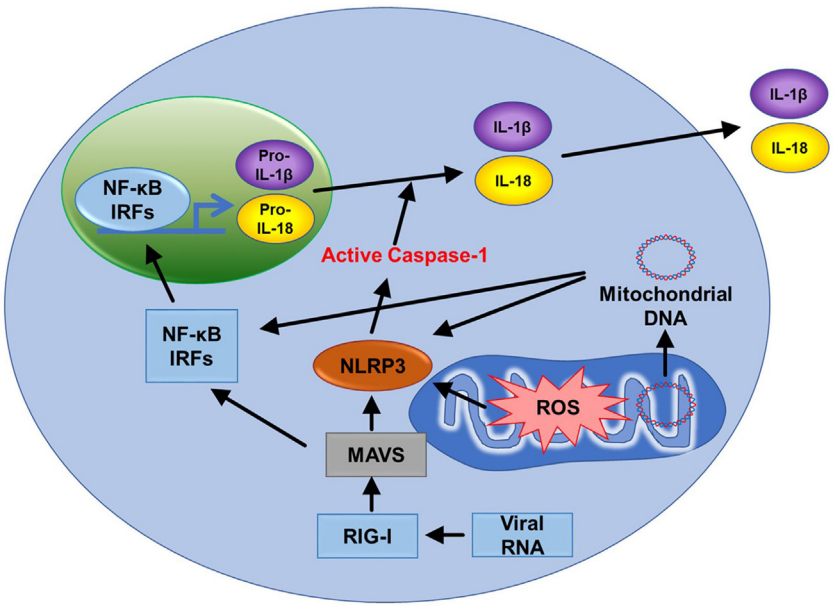

FIGURE 6 | Mitochondria related signal cascades to activate immunity. Mitochondrial antiviral signaling (MAVS) is activated by the viral RNA sensor RIG-I and promotes inflammatory and immune gene expression *via* transcription factor NF-κB and IRFs. MAVS receptors can be found in the mitochondrial outer membrane. Mitochondrial ROS (mtROS) are also able to activate MAVS independently of RNA. The NLRP3 inflammasome also signals from the outer mitochondrial membrane. Like MAVS, NLRP3 also responds to mtROS and can cause mitochondrial damage that promotes the generation of additional ROS. NLRP3 drives the production of IL-1β and IL-18. Mitochondrial DNA can also activate NLRP3. Finally, MAVS promotes oligomerization of NLRP3 at the mitochondria. Therefore, two major innate immune pathways (RIG-I/MAVS and NLRP3) are all dependent on mitochondria.

and then active caspase-1 cleaves a variety of cytoplasmic targets including IL-1β. In the last decade, several studies found that ROS supplied by mitochondria are essential in activating the inflammasome (2, 62). Nakahira et al. also found that damaged mitochondria in macrophages treated with NLRP3 activator produced high mtROS and then activated the NLRP3 inflammasome (62). In another study, inhibition of mitochondrial complex I- or III-mediated mtROS activated the NLRP3 inflammasome indicating mtROS can be a main source of NLRP3 inflammasome activation (**Figure 1**.3) (63). It suggests that mitochondria contribute to immune response *via* the NLRP3 inflammasome.

mtROS Are Important for T Cell Activation

Stimulation of the T cell receptor (TCR) drives T cells into rapid proliferation and differentiation. T cell activation induces a rapid increase in mtROS production (18). Sena et al. also found that mtROS from complex III are required for $CD4^+$ T cell activation and mitochondrial targeted antioxidant mitovitamin E attenuates IL-2 production. An alternative source of mtROS in mitochondria is mitochondrial glycerol-3-phosphate dehydrogenase 2 (GPD2). GPD2 oxidizes glycerol-3-phosphate to dihydroxyacetone phosphate leading to a hyper-reduced state of ubiquinone called ubiquinol in the inner mitochondrial membrane. Kamiński et al. found that GPD2 could directly produce ROS and accumulating ubiquinol could support ROS production at other ETC sites such as complex I (64). GPD2 depletion has been shown to inhibit mtROS production during T cell activation and decrease IL-2 expression. Mitochondrial and GPD2 induced ROS are important for T cell activation.

mtROS Also Function as a Signal in B Cell Activation and Antibody Production

Naïve B lymphocytes undergo diversification of their antigen receptor through somatic hyper-mutation, alteration of immunoglobulin function by class-switch recombination (CSR), and differentiation into antibody-secreting plasma cells (PCD) or memory B cells. Bach2 is a transcriptional factor which is required for antigen class switch in B cells. mtROS block heme synthesis in CSR, but it promotes heme synthesis in PCD. As a result, mtROS are promoted during CSR and suppressed during PCD. In support of this, mitochondrial mass and membrane potential are increased in CSR and decreased in PCD (65, 66). Hence, mtROS modulate B cell function by changing heme synthesis.

In short, mtROS are important for immune cell activities in T cells (mediated by GDP2), in macrophages (*via* TRAF6, ECSIT), and in B cells (*via* heme synthesis).

Mitophagy

Mitophagy is a form of autophagy that specifically eliminates damaged mitochondria for mitochondria quality control. Mitophagy is regulated by PTEN-induced putative kinase 1 (PINK1) and the ubiquitin ligase parkin (67). On the surface of mitochondria, PINK1 recruits parkin from the cytosol and activates parkin's E3 ligase activity (**Figure 1**.4). Parkin leads the ubiquitin conjugation of various outer membrane proteins to induce mitochondrial engulfment by an autophagosome followed by subsequent fusion with a lysosome for the clearance of damaged mitochondria.

Interruption in Mitophagy Leads to Increased Susceptibility to Pathogens

Roles for mitophagy during pathogen infection have been identified. Degradation of those mitochondria damaged by *Pseudomonas aeruginosa*-produced siderophores requires PINK1 in *C. elegans* (68). On the other hand, parkin mutation showed increased susceptibility to the intracellular pathogenic bacteria *Mycobacterium leprae* and *Salmonella enterica* which causes leprosy and typhoid fever, respectively (69–71). In addition, parkin-deficient mice and flies showed increased susceptibility to *Mycobacterium tuberculosis*, and bacteria proliferation was increased in macrophages (72).

Decreased Mitophagy in T Cell Increases ROS and Apoptosis. T cells utilize mitophagy to maintain their proper homeostasis. Autophagy-related protein 7 (Atg7) is required for the formation of autophagosomes.

Pua et al. found that Atg7-deficient T cells had enhanced mitochondrial content, increased ROS production, and expression of pro-apoptotic proteins like Bak, cytochrome *c*, and AIF (73).

In addition, vacuolar protein sorting 34 (Vps34) is a member of the class III PI3K family of lipid kinases. Vps34-deficient $CD4^+$ and $CD8^+$ T cells had increased amounts of ROS, mitochondrial mass, and impaired mitophagy (74).

Hepatitis B and C Viruses (HBV and HCV) Utilize Mitophagy to Their Benefit

Mitochondria also initiate apoptosis signals. HBV and HCV induce mitophagy to protect themselves from apoptotic signaling. HCV induces parkin and PINK1 leading to mitophagy and mitochondrial dysfunction (75). HBV was found to promote its replication in the cells by promoting PINK1/parkin mitophagy and fission to prevent pro-apoptotic stimuli (76).

Hence, proteins such as PINK1, parkin, ATG7, ATG5, and Vaps34 are important for mitophagy and are implicated in immune cell function. Absence of these proteins may increase the susceptibility to microbial infections and apoptosis of immune cells. Targeting these proteins can benefit HPV and HCV related infections.

Mitochondrial DNA

NLRP3 inflammasome facilitates activation of caspase-1, secretion of IL-1β and IL-18, and cell death (**Figure 6**). There are many factors that involve activation of NLRP3 inflammasome such as NF-κB, pathogen-associated molecular patterns, ATP, and potassium ion channels (77). Interestingly, mitochondrial apoptotic signaling can also activate the NLRP3 inflammasome. During mitochondrial dysfunction, mtDNA is released from mitochondria and into the cytosol. mtDNA can bind and activate the NLRP3 inflammasome (**Figure 6**) (62, 78). In addition, autophagic protein deficiency in macrophages promotes mitochondria dysfunction and consequent release of mtDNA into the cytosol and activation of the NLRP3 inflamasome. Collectively, this suggests that mtDNA mediated activation of NLRP3 is related to autophagy and immune responses. In contrast to this, Allam et al. found that mitochondrial apoptosis was not required for the NLRP3 inflammasome activation, but caspase-8-mediated apoptosis is required for its activation. Nevertheless, this report supports that dysfunctional mitochondria cause mtDNA release and activation of the NLRP3 inflammasome (**Figure 1**.7) (79). This gives the indication that mtDNA is necessary for NLRP3 inflammasome activation and mitochondrial apoptosis. In addition, high frequency of polymorphisms in the D loop region of mtDNA is observed in lymphocytes of immune-related pancytopenia patients (80). This suggests that mtDNA contribute to red and white blood cell development. How mtDNA is controlled in the case of leukemia or other blood cancers is still not clear.

Mitochondrial Antiviral Signaling

Mitochondrial antiviral signaling (MAVS) is a signaling protein located on the outer membrane of mitochondria. It is activated by a viral RNA sensor called retinoic acid-inducible gene I (RIG-I) (**Figure 6**). It activates pathways that regulate NF-κB and interferon regulatory transcription factors (IRFs) to promote gene expression (81). mtROS can drive MAVS oligomerization and production of type I interferon (82) highlighting that MAVS might be a key sensor of mtROS. Furthermore, MAVS associates with NLRP3 and promotes its oligomerization leading to caspase-1 activation (83). Recently, Hee and Cresswell found that MAVS directly interacts with the antiviral protein viperin acting as an immune defense mechanism against RNA viruses in macrophages (84). These studies support that MAVS is activated upon viral infections, induces immunogenic signaling/apoptosis, and mediates the effects of mtROS (**Figure 1**.5).

MITOCHONDRIAL DYNAMICS PLAYS A KEY ROLE IN IMMUNE CELL METABOLISM

Mitochondrial fission and fusion controls mitochondrial mass (85). Nutrient deprivation induces an increase in mitochondrial fusion and suppression of mitophagy. On the other hand, prolonged DNA damage leads to mitochondrial fission (86, 87). Mechanistically, fusion is controlled by two dynamin-like GTPases: mitofusin (Mfn1 and Mfn2) for the outer membrane and optic atrophy 1 (OPA1) for the inner membrane. Outer membrane fission is regulated by dynamin-related protein-1 (Drp1), mitochondria fission factor (MFF), Mid49, and mid52 (**Figure 7**) (88).

The mitochondrial fission and fusion process can have three cellular functions. *First,* it allows mixing of mtDNA content. Each individual mitochondrion has mtDNA that encodes for respiratory complexes, but DNA containing harmful mutations can be pathogenic. A high load of pathogenic DNA can attenuate respiratory function. Fission and fusion can compensate for this problem by mixing mtDNA in different compartments until the pathogenic content becomes too overwhelming. *Second,* smaller particles can move more efficiently so mitochondrial fission can promote mitochondrial mobility inside the cell, and fusion can promote tethering to other cellular structures such as the ER (89). *Third,* mitochondrial fusion can increase cristae formation and can provide more surface area for OXPHOS and FAO while

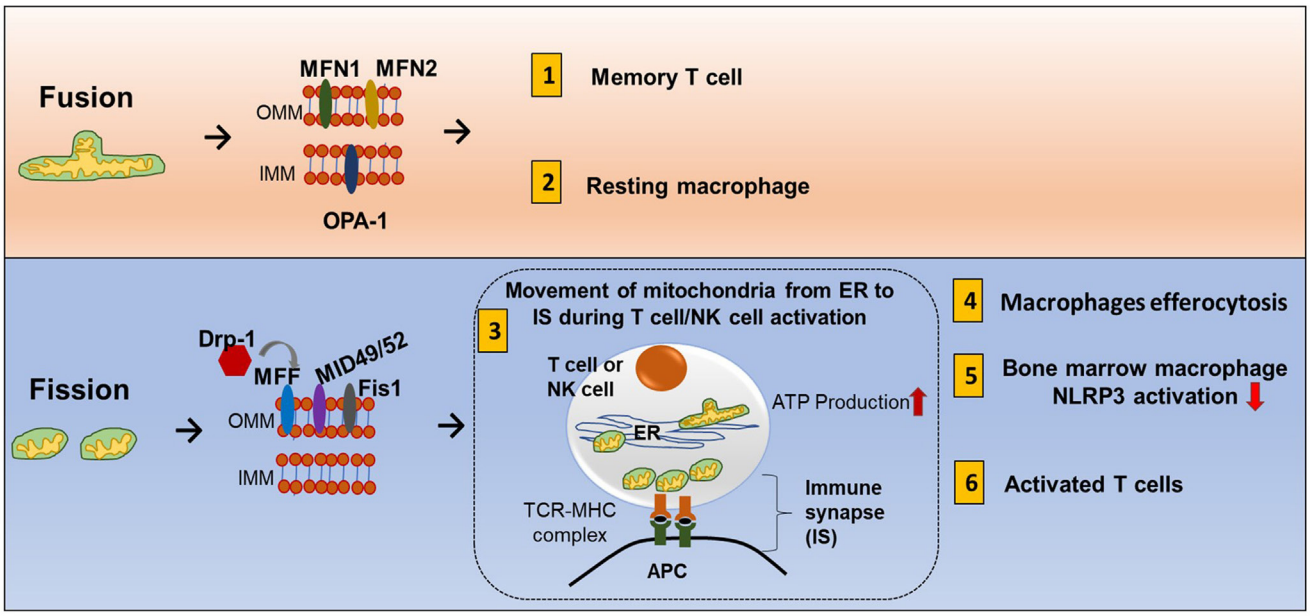

FIGURE 7 | Mitochondrial fission and fusion contribute to immune responses. Mitochondrial fission and fusion controls mitochondrial mass. Nutrient deprivation induces an increase in mitochondrial fusion and suppression of mitophagy. On the other hand, prolonged DNA damage leads to mitochondrial fission. *Fusion (tubular mitochondria)* is controlled by two dynamin-like GTPases for the outer membrane proteins (mitofusin MFN1 and MFN2) and inner membrane proteins (OPA1). *Fission (fragmented mitochondria)* is coordinated by four outer membrane proteins (Drp1, Mff, Mid49, and mid52). Fusion and fission of mitochondria contribute to immune response. (1) Memory T cells have increased fusion generating more oxidative phosphorylation (OXPHOS) and FAO. (2) Clearance of apoptotic cells by phagocytes is known as efferocytosis. Resting macrophages have displayed tubular mitochondria. (3) Fission contributes to mitochondria mobility. During antigen-specific T cell and NK cell activation, mitochondria fission increased, and mitochondria move toward the immune synapse (IS) and facilitate ATP production at the IS. (4) Uptake of apoptotic cells by macrophages during efferocytosis requires fission and increased Drp1. (5) In mouse-derived bone marrow macrophages, knocking down Drp1 initiates NLRP3 inflammasomal activation, and inducing mitochondrial fission attenuated NLRP3 inflammasomal assembly and activation. (6) Activated effector T cells have increased mitochondrial fission and exhibit higher rates of glycolysis.

fission eliminates dysfunctional mitochondria and is an adaptation for increased aerobic glycolysis.

During antigen-specific T cell and NK cell activation, mitochondria mobilize toward the immune synapse (IS) (**Figure 7**). Drp1, a fission factor, is required for translocation of the mitochondria (90, 91). Polarization of cytoskeletal structures, proteins, cell organelles, and transport of ions across membranes at the IS all require energy. This energy is provided by mitochondria. In addition, ATP is also released locally at the IS contributing to T cell function (92). Mitochondrial tethering is also associated with STIM-1 membrane trafficking from the ER to the plasma membrane in mast cells (93). Clearance of apoptotic cells by phagocytes is known as efferocytosis. Resting macrophages have displayed tubular mitochondria. Uptake of apoptotic cells by macrophages requires fission and increased Drp1 (**Figure 7**) (94). However, there is need of more research to know how fusion and fission regulate macrophage polarization.

Drp1 Influences ROS Production

Interestingly, Opa1 is deactivated upon ROS increase. Drp1 is activated and causes the fragmentation of mitochondria in neuronal cells (95). This is linked to iron overload, AMPK activation, MFF (96), and ubiquitination of A-kinase anchor protein 121 (97). Conversely, ROS induced mitophagy is suppressed by depleting Drp1 (98). In addition, accumulation of mitochondria and decreased Drp1 is associated with T cell dysfunction in SLE (SLE → T cell dysfunction → mitochondria accumulation → Drp1 depletion) (99). Rapamycin can increase Drp1 by inhibiting mTORC1 (100). This means that Drp1 can be targeted in ROS producing mitochondria and other immune diseases.

Fusion and Fission in Diverse Immune Cells

Activated effector T cells have an increase in mitochondrial fission and mitochondrial mass but less cristae formation to support aerobic glycolysis. Memory T cells have an increase in fusion which consequently causes OXPHOS and FAO to be increased (**Figure 1**.8) (101). The receptor-interacting protein kinase 3 (RIPK3) plays a crucial role in natural killer T cell (NKT cell)-mediated immune response *via* activation of the mitochondrial phosphatase phosphoglycerate mutase 5 (PGAM5). RIPK3-mediated PGAM5 promotes nuclear translocation of NFAT and dephosphorylating Drp1 in NKT cells (102). In addition, there is some evidence that mitochondrial dynamics is also related to inflammasomal activation. Obese rats have increased expression of Drp1 and NLRP3 and decreased fusion-relative protein optic

atropy-1 (OPA1) (103). In mouse bone marrow-derived macrophages, knocking down Drp1 initiates NLRP3 inflammasomal activation, and inducing mitochondrial fission attenuated NLRP3 inflammasomal assembly and activation (104).

In addition to mtDNA mixing, mobility, and metabolic regulation, mitochondrial dynamics also control the inflammasomal activation. However, how the genes for fusion and fission are controlled is still unknown.

MITOCHONDRIA ARE A NODE FOR ROS, AND ANTIOXIDANTS ARE IMPORTANT FOR THE NEUTRALIZATION OF ROS

Antioxidants Play an Important Role in Neutralizing mtROS

In mitochondria, superoxide $\left(O_2^-\right)$ is released from complex I (into the matrix) and III (into both the matrix and intermembrane space). This is converted to H_2O_2 in the presence of SOD. Catalase converts H_2O_2 to H_2O. H_2O_2 can also be converted to HOCl in the presence of myeloperoxidase. HOCl is produced by neutrophils to damage macromolecules of pathogens and ultimately kill the pathogens. H_2O_2 can also go through the fenton reaction to produce highly reactive OH^-. This can react with different components of the cell and cause damage. Glutathione converts OH^- to H_2O to reduce the cellular damage. Superoxide, OH^-, and H_2O_2 generated in mitochondria are the main sources of ROS. Hence, antioxidants such as catalase, Mn-SOD (manganese dismutase in mitochondria matrix), Cu/Zn-SOD (in the intermembrane space and cytosol), and glutathione play a crucial role by neutralizing the ROS (**Figure 9**.1) (105).

ROS Are Important to Maintain Cellular Homeostasis

Low or moderate concentrations of ROS help cells maintain healthy conditions. For example, oxidative burst in macrophages generated using NADPH oxidase releases H_2O_2 during phagocytosis to kill pathogens. H_2O_2 is converted into HOCl to kill pathogens by damaging macromolecules. H_2O_2 influences mtDNA and nuclear DNA transcription of antioxidant genes (NRF2, AP-1). However, it can lead to pathological conditions in the case of excess of ROS. Excess levels of ROS inhibit glucose transporter proteins and cause alterations in cellular signaling and oxidative damage to macromolecules. Antioxidants neutralize the ROS and maintain healthy conditions (**Figure 9**.2).

Glutathione Metabolism and Function

Glutathione can be considered "mother of the antioxidant defense system." Glutathione is synthesized from amino acids in a two-step reaction. In the presence of γ-glutamylcysteine synthetase, glutamic acid and cysteine are converted into γ-glutamylcysteine. Glycine is then added in the presence of glutathione synthetase to make glutathione. γ-glutamylcysteine synthetase regulates glutathione synthesis. Oxidative stress influences the function of γ-glutamylcysteine synthetase. Glutathione is oxidized and reduced to glutathione disulfide (GSSG) in a reversible reaction. NADPH from the pentose phosphate pathway reduces GSSG to form glutathione pathway (GSH). GSH is important for conversion of OH^- to H_2O. Mitochondrial GSH also contributes to detoxification of harmful lipids (lipids-OOH) and deglutathionylation of mitochondrial proteins (106).

Other Antioxidant Proteins Present in Mitochondria

In addition to glutathione, other antioxidants (Co-Q, SIRT3, FOXOs) also influence ROS. Coenzyme Q (Co-Q) is an essential antioxidant that carries electrons from complex I–II and III. Co-Q is synthesized in mitochondria requiring proteins coded in 12 genes. Mutations in Co-Q genes cause CoQ10 deficiency which leads to an increase in ROS and ATP depletion (107). Sirtuin 3 (SIRT 3) coordinates FAO and superoxide detoxification to control cellular ROS levels by deacetylation of acytyl co-A dehydrogenase, Mn-SOD, and FOXO3 (108).

Glutathione Depletion Leads to Impairment of Immune Cell Function

Glutathione and APCs

Antigen-presenting cells display MHC on their surface which interacts with TCRs on T cells to drive immune function and cytokine production. Glutathione levels within APCs and immune cells regulate their function. GSH depletion in APCs correlates with reduced secretion of Th1 cytokines. Increased intracellular GSH content stimulates IL-12 or IL-27 which in turn differentiates $CD4^+$ T cells to Th1 cells (109). In addition, activated macrophages and DCs secrete antioxidant precursors such as cysteine. Cysteine is taken up by T cells. This allows T cells to be protected from harm during antigen presentation.

Glutathione in Other Immune Cells

In support of this, glutathione depletion was observed in other immune cells. Decreased GSH/GSSG ratio in $CD8^+$ memory T cell leads to an increase in ROS and impairment of $CD8^+$ memory T cells (50). IL-17-producing γδ T cells (γδ17 T cells) have recently been found to promote tumor growth and metastasis. Low levels of glutathione are also observed in γδ17 T cells (110). These factors suggest that decreased GSH can disrupt immune cell function.

Glutathione During HIV Infection

It is well known that HIV suppresses the immune system. HIV infection is associated with elevated levels of ROS and decreased GSH. However, increasing GSH levels in NK cells reduces intracellular survival of pathogens in macrophages (111). HIV infection destroys $CD4^+$ T cells. Supplementation of L-GSH to HIV-positive patients with low $CD4^+$ T cell counts resulted in an increase in IL-12, IL-2, and IFN-γ and a decrease in IL-6, IL-10, and free radicals as well as stabilization of the levels of TGF-β, IL-1, and IL-17 (112). This implies that glutathione can impact cytokine production.

Glutathione and Post-Translational Modification of Proteins in Immune Cells

Glutathione can also reverse ROS-mediated post-translational modifications. Increased H_2O_2 production results in increased protein S-glutathionylation in both monocytes and differentiated macrophages. Protein S-glutathionylation can be prevented either by

the activity of antioxidant enzymes at the level of ROS scavenging or reversed by glutaredoxin-mediated deglutathionylation (113, 114).

In short, glutathione plays a vital role in APCs and T cell differentiation, the function of NK cells and macrophages, cytokine production in immune cells, and post-translational modifications of proteins in immune cells.

Antioxidants Regulate ROS-Mediated Cell Signaling Pathways in Immune Cells

Antioxidants neutralize the cellular ROS levels while also influencing transcription of antioxidant genes and post-translational modifications of proteins involved in antioxidant pathways. Transcription factors such as NRF2 and Keap1 control expression of antioxidant genes. Therefore, ROS levels are regulated by the GSH and NRF2–Keap1–Cul3 trimeric complex. T cell-mediated autoimmune disease is mediated by dysregulation of these pathways and elevated levels of ROS (**Figure 8**) (115).

T cell metabolism is highly dynamic. Proper T cell activation and differentiation is critical (116). It is reported that naïve T cells, CD4 Treg, and chronically stimulated T cells depends on OXPHOS as the primary metabolism method while CD8 effector and CD4 Th1 cells mainly depend on glycolysis (117). This metabolic switching may be controlled by ROS.

During activation, $CD4^+$ T cells must transition metabolically from OXPHOS to aerobic glycolysis to support proliferation and effector function. Low/moderate levels of ROS promote mTOR (**Figure 8**) (118), Myc, and NFAT activation. mTOR activation leads to increased glycolysis in T cells. Abrogation of GSH, which may cause high levels of ROS, impairs the inflammatory response of T cells. High AMPK levels inhibit mTOR and increase ROS (17, 119, 120). High ROS and diminished mTOR activation lead to decreased Myc and reduced transition to aerobic glycolysis in diabetogenic splenocytes. These results suggest that ROS are required for the metabolic transition (121). High levels of ROS and low GSH can lead to improper cellular signaling and post-translational modifications of proteins which can impact metabolic outcome.

Antioxidants Influence Mitochondrial Membrane Potential (MMP)

Mitochondrial membrane potential ($\Delta\Psi m$) is generated as protons are pumped outward from the matrix, a process that depends on substrate utilization and electron transport. This is a critical check point for cell death and ATP synthesis. Loss of membrane potential may result from any processes wherein protons move back toward the matrix. UCP2 acts like an antioxidant to reduce ROS by moving protons back to the matrix (122). Sirt3 and FOXO activation decrease mtROS. Similarly, mitophagy through activation of BNIP3 or NIX decreases mtROS by decreasing the number of damaged mitochondria that produce more ROS. $\Delta\Psi m$ plays a decisive role by driving ATP synthesis. $\Delta\Psi m$ is regulated by glutathione which depends on thioredoxin and NADPH generated in the pentose phosphate pathway (123). There is still a need for more research on potassium and calcium channels that also have an impact on MMP (124).

Antioxidants Impact on MMP, mtDNA, and ATP Synthesis

T cell activation and proliferation depend on the production of reactive oxygen intermediates (ROIs). Mitochondrial hyperpolarization (MHP) is associated with increased ROI, decreased GSH, and ATP depletion. This MHP is crucial for T cell activation (125–127). In cancer cells, mitochondrial H^+-ATP synthase is increased and ATPase inhibitory factor 1 is decreased when compared with normal tissues. This in turn promotes ROS production

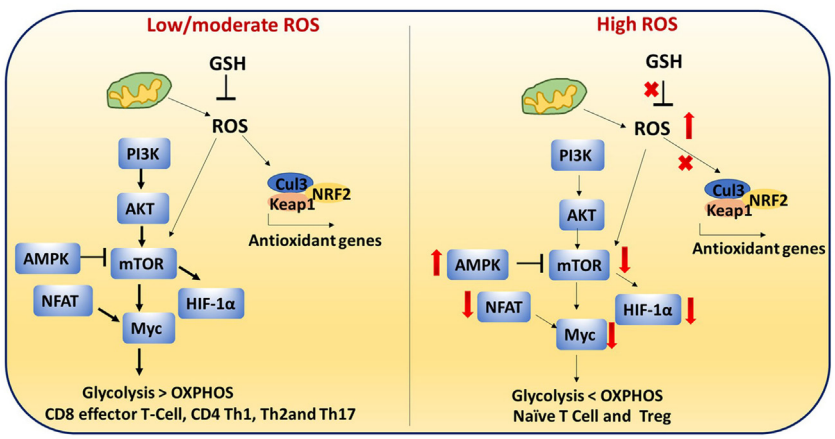

FIGURE 8 | Reactive oxygen species (ROS)-mediated cell signaling in T cells: low/moderate levels of ROS promote mTOR, Myc, and nuclear factor of activated T cell (NFAT) activation which is associated with increased glycolysis and inflammatory response in T cells. ROS levels are regulated by the glutathione pathway (GSH) and NRF2–Keap1–Cul3 trimeric complex. Abrogation of GSH and the trimeric complex, which may cause high levels of ROS, impairs the inflammatory response of T cells. In addition, high ROS and high adenosine monophosphate-activated protein kinase (AMPK) levels inhibit mTOR. It is reported that naïve T cells, $CD4^+$ regulatory T cells, and chronically stimulated T cells depend on OXPHOS as their primary metabolism method while $CD8^+$ effector and $CD4^+$ Th1 cells mainly depend on glycolysis. This metabolic switching is controlled by ROS.

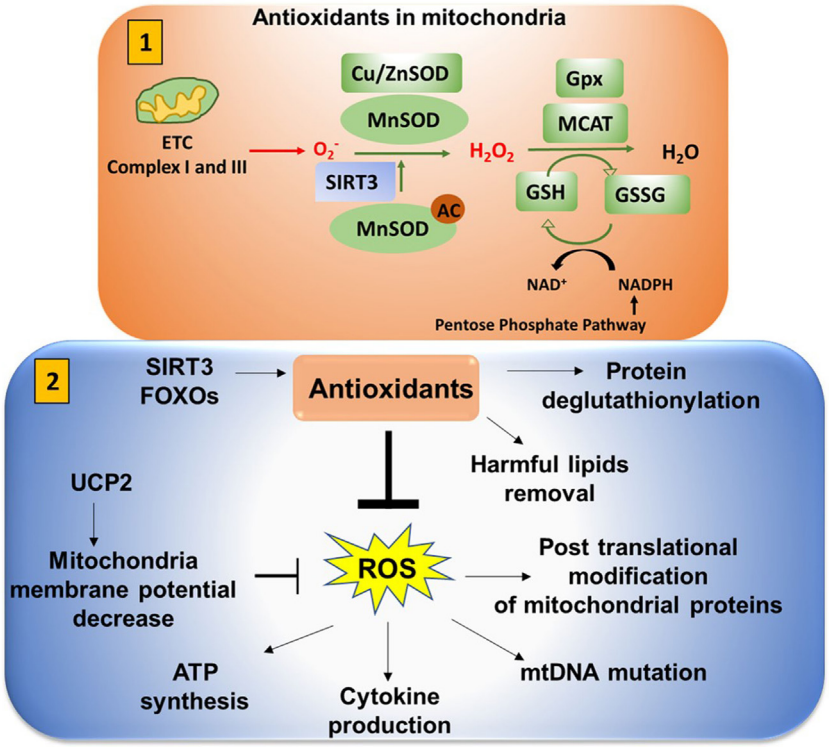

FIGURE 9 | Antioxidants neutralize mitochondrial ROS (mtROS). (1) *Antioxidants in mitochondria:* superoxide (O_2^-) is released from complex I (into the matrix) and III (into both the matrix and intermembrane space). This is converted to H_2O_2 in the presence of superoxide dismutase (SOD). Mn-SOD (manganese dismutase) is localized in mitochondria matrix and Cu/Zn-SOD (in the intermembrane space and cytosol). SIRT3 is involved in deacetylation of MnSOD. Catalase converts H_2O_2 to H_2O. Catalase is also localized in mitochondria (MCAT). Glutathione converts OH^- to H_2O to reduce the cellular damage. Glutathione (GSH) is oxidized and reduced to glutathione disulfide (GSSG) in a reversible reaction. NADPH from the pentose phosphate pathway reduces GSSG to form GSH in presence of glutathione peroxidase (Gpx). (2) *Antioxidants play an important role in neutralizing mtROS.* Uncoupling protein 2 (UCP2) (mitochondrial uncoupler protein), which helps to shuttle H^+ ions from the intermembrane space to the mitochondria matrix, decreases the membrane potential to inhibit excessive reactive oxygen species (ROS) production. Excessive ROS production can impact ATP synthesis, cytokine production, mitochondrial DNA (mtDNA) mutation, and post-translational modification of cellular proteins by S-nitosylation or glutathionylation.

and ATP depletion and further decreases the antioxidant defense system (128).

Excess ROS Can Lead to mtDNA Mutation

Reactive oxygen species can also cause mutations in mtDNA and further change MMP and ATP synthesis. mtDNA contains 37 genes. 12 proteins are encoded by mtDNA as structural proteins of mitochondrial enzyme complexes (I-IV). Nuclear genes encode about 1,500 mitochondrial proteins. Changes in cellular ROS levels can cause mutations in mtDNA which further disturbs the normal functions of mitochondrial proteins (105). Mutation load in the ATP6 gene leads to a defect in mitochondrial ATP synthase which can further increase MMP and ATP synthesis depletion (129).

DOES MITOCHONDRIAL IMMUNE REGULATION HAVE ANY THERAPEUTIC IMPORTANCE?

Based on previous evidence, mitochondria are important for autoimmune disease, mitochondrial disorders, and cancer. Selectively targeting OXPHOS can be effective for advanced melanoma (130). Also, altering mitochondrial machinery in sepsis can increase patient survival by 30% (131). From this, a new field has emerged called "mitochondrial medicine." Drugs can be developed selectively by targeting them to mitochondria causing a switch in immune programing (132, 133). Here, we have highlighted how antioxidants can be delivered into mitochondria and how it can be useful during ischemia reperfusion.

Role of Antioxidants in Mitochondria

Antioxidants can be targeted selectively to mitochondria to reduce oxidative stress (134, 135). Natural antioxidants (vitamin E, curcumin, ginko biloba, melatonin) in addition to targeted TPP-based antioxidants (MitoQ, Mito-VitE, Mito-α-lipoic acid, Mito-PBN), small peptide-based molecules (SS31, SS02, SS19, SS20), choline esters of glutathione, and *N*-acetyl-L-cysteine neutralize mtROS which further maintains normal MMP (136, 137). These molecules are preferentially taken up by mitochondria due to differential charge (negative charge in mitochondria and positive charge on the molecules). Antioxidant SS31 has been shown to inhibit fission proteins

(Drp1 and Fis1). Antioxidants can be used as targeted therapy for mitochondrial disorders (138). Also, TPP conjugated antioxidants have shown to potently inhibit cancer cell proliferation (139).

Mitochondrial Components Are Modified by Antioxidants During Ischemia/Reperfusion (IR)

Ischemia/reperfusion injury is the tissue damage caused by the return of blood supply after a period of ischemia leading to a state of hypoxia. ROS are known as the primary cause of ischemic tissue injury. In mitochondria, several protein complexes are modified by S-nitrosylation and S-glutathinylation (140). Complex I is modified by both processes. Complex I has two transitional states: active (A) and deactive (D). Complex I is S-nitrosylated during D state which can be reversed by thiol reductants. Reperfusion of ischemic tissue rapidly activates complex I and increases the generation of ROS which leads to cell death. The presence of MitoSNO (S-nitrosothiol) or S-nitrosylated agents during reperfusion selectively target the ND3 subunit of complex I at Cys39 to keep the complex in low activity and decrease ROS production. In this way, S-nitrosylation of ND3 at Cys39 in complex I protects against IR injury (141–143).

Other mitochondrial components have been shown to treat diseases. However, whether these can also be implicated in immune-related functions is still not known. For example, coenzyme Q10 in mitochondria can be utilized as a potential treatment for heart failure (144). Genipin is a UCP2 inhibitor that is useful for anticancer therapy (145). Circulating mtDNA can be used as potential biomarkers for chemotherapy induced cardiac damage (146). Whether circulating mtDNA and genipin can be useful for leukemia or other immune diseases is still unknown.

CONCLUDING REMARKS

The importance of mitochondria in immunity has become clearer. Besides controlling cell fate, mitochondria provide signaling platforms generated by MAVS, ROS, and mtDNA. Mitochondria balance redox status to fine-tune NLRP3 inflammasome activation. Maintenance of mitochondrial fidelity by mitophagy is important for cell fate and immunity. The protective role of mitophagy has the potential to treat inflammatory diseases with excess ROS and mitochondrial dysfunction. This idea is supported by the observation that mitochondrial antioxidants help ameliorate symptoms (147, 148). In addition, targeting this pathway can be a therapeutic strategy because defective mitophagy has been implicated in Parkinson's disease.

In addition, mutations in mitochondrial proteins have gained clinical importance. For instance, apoptosis inducing factor mitochondria associated-1 (AIFM-1) is related to mitochondria function and immune system regulation. Mutations in AIFM-1 are related to fatal encephalomyopathy in infants (149). Recurrent mutations in IDH2 are associated with angioimmunoblastic T cell lymphoma (150). Polymorphisms in MnSOD can lead to abortion during the first trimester of pregnancy (151). This evidence supports the conclusion that immunodeficiency is related to disrupted mitochondrial components. However, there is still need of more research to know what leads to these types of mutations/polymorphisms and how they affect immune cell functions.

For immunologists, mitochondria can be the powerhouse of immunity along with their roles as the powerhouse of the cell. After many decades of hard work, remarkable developments in immunology research have improved our understanding of the immune system, and immunologists are now better equipped with modern knowledge and techniques to cross over into other disciplines. Future work will continue to reveal the functions of mitochondria in immunity. For example, "What are the roles of mitochondrial fission and fusion as well as cristae remodeling in immunity?" or "Do mitochondria affect other innate immune cells such as innate lymphoid cells and granulocytes?" We hope this review will inspire research into many questions that remain to be explored. Coupling the unique benefits of studying mitochondria and immunity is beneficial for the enormous clinical relevance in human health and diseases.

AUTHOR CONTRIBUTIONS

This manuscript was conceived by MT, designed and written by SL, AA, CY, and MT, and revised by JP, J-HK, and ZY. MT supervised development of this paper as the principal investigator.

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REFERENCES

1. Grimm S. The ER-mitochondria interface: the social network of cell death. *Biochim Biophys Acta* (2012) 1823(2):327–34. doi:10.1016/j.bbamcr.2011.11.018
2. Zhou R, Yazdi AS, Menu P, Tschopp J. A role for mitochondria in NLRP3 inflammasome activation. *Nature* (2011) 469(7329):221–5. doi:10.1038/nature09663
3. Bantug GR, Fischer M, Grählert J, Balmer ML, Unterstab G, Develioglu L, et al. Mitochondria-endoplasmic reticulum contact sites function as immunometabolic hubs that orchestrate the rapid recall response of memory CD8(+) T cells. *Immunity* (2018) 48(3):542–555.e6. doi:10.1016/j.immuni.2018.02.012
4. Pearce EL, Pearce EJ. Metabolic pathways in immune cell activation and quiescence. *Immunity* (2013) 38(4):633–43. doi:10.1016/j.immuni.2013.04.005
5. Carr EL, Kelman A, Wu GS, Gopaul R, Senkevitch E, Aghvanyan A, et al. Glutamine uptake and metabolism are coordinately regulated by ERK/MAPK during T lymphocyte activation. *J Immunol* (2010) 185(2):1037–44. doi:10.4049/jimmunol.0903586
6. Le A, Lane AN, Hamaker M, Bose S, Gouw A, Barbi J, et al. Glucose-independent glutamine metabolism via TCA cycling for proliferation and survival in B cells. *Cell Metab* (2012) 15(1):110–21. doi:10.1016/j.cmet.2011.12.009
7. Wynn TA, Chawla A, Pollard JW. Macrophage biology in development, homeostasis and disease. *Nature* (2013) 496(7446):445–55. doi:10.1038/nature12034
8. He C, Carter AB. The metabolic prospective and redox regulation of macrophage polarization. *J Clin Cell Immunol* (2015) 6(6):371. doi:10.4172/2155-9899.1000371

9. De Simone R, Ajmone-Cat MA, Pandolfi M, Bernardo A, De Nuccio C, Minghetti L, et al. The mitochondrial uncoupling protein-2 is a master regulator of both M1 and M2 microglial responses. *J Neurochem* (2015) 135(1):147–56. doi:10.1111/jnc.13244
10. Mills EL, O'Neill LA. Reprogramming mitochondrial metabolism in macrophages as an anti-inflammatory signal. *Eur J Immunol* (2016) 46(1):13–21. doi:10.1002/eji.201445427
11. Shapiro H, Lutaty A, Ariel A. Macrophages, meta-inflammation, and immunometabolism. *ScientificWorldJournal* (2011) 11:2509–29. doi:10.1100/2011/397971
12. Phan K, Ng W, Lu VM, McDonald KL, Fairhall J, Reddy R, et al. Association between IDH1 and IDH2 mutations and preoperative seizures in patients with low-grade versus high-grade glioma: a systematic review and meta-analysis. *World Neurosurg* (2018) 111:e539–45. doi:10.1016/j.wneu.2017.12.112
13. Kong MJ, Han SJ, Kim JI, Park JW, Park KM. Mitochondrial NADP(+)-dependent isocitrate dehydrogenase deficiency increases cisplatin-induced oxidative damage in the kidney tubule cells. *Cell Death Dis* (2018) 9(5):488. doi:10.1038/s41419-018-0537-6
14. Jha AK, Huang SC, Sergushichev A, Lampropoulou V, Ivanova Y, Loginicheva E, et al. Network integration of parallel metabolic and transcriptional data reveals metabolic modules that regulate macrophage polarization. *Immunity* (2015) 42(3):419–30. doi:10.1016/j.immuni.2015.02.005
15. Tannahill GM, Curtis AM, Adamik J, Palsson-McDermott EM, McGettrick AF, Goel G, et al. Succinate is an inflammatory signal that induces IL-1beta through HIF-1alpha. *Nature* (2013) 496(7444):238–42. doi:10.1038/nature11986
16. Tan Z, Xie N, Cui H, Moellering DR, Abraham E, Thannickal VJ, et al. Pyruvate dehydrogenase kinase 1 participates in macrophage polarization via regulating glucose metabolism. *J Immunol* (2015) 194(12):6082–9. doi:10.4049/jimmunol.1402469
17. Coe DJ, Kishore M, Marelli-Berg F. Metabolic regulation of regulatory T cell development and function. *Front Immunol* (2014) 5:590. doi:10.3389/fimmu.2014.00590
18. Sena LA, Li S, Jairaman A, Prakriya M, Ezponda T, Hildeman DA, et al. Mitochondria are required for antigen-specific T cell activation through reactive oxygen species signaling. *Immunity* (2013) 38(2):225–36. doi:10.1016/j.immuni.2012.10.020
19. Arora R, Schmitt D, Karanam B, Tan M, Yates C, Dean-Colomb W. Inhibition of the Warburg effect with a natural compound reveals a novel measurement for determining the metastatic potential of breast cancers. *Oncotarget* (2015) 6(2):662–78. doi:10.18632/oncotarget.2689
20. Fischer K, Hoffmann P, Voelkl S, Meidenbauer N, Ammer J, Edinger M, et al. Inhibitory effect of tumor cell-derived lactic acid on human T cells. *Blood* (2007) 109(9):3812–9. doi:10.1182/blood-2006-07-035972
21. Colegio OR, Chu NQ, Szabo AL, Chu T, Rhebergen AM, Jairam V, et al. Functional polarization of tumour-associated macrophages by tumour-derived lactic acid. *Nature* (2014) 513(7519):559–63. doi:10.1038/nature13490
22. Buck MD, Sowell RT, Kaech SM, Pearce EL. Metabolic instruction of immunity. *Cell* (2017) 169(4):570–86. doi:10.1016/j.cell.2017.04.004
23. Selleri S, Bifsha P, Civini S, Pacelli C, Dieng MM, Lemieux W, et al. Human mesenchymal stromal cell-secreted lactate induces M2-macrophage differentiation by metabolic reprogramming. *Oncotarget* (2016) 7(21):30193–210. doi:10.18632/oncotarget.8623
24. Marino A, Menghini R, Fabrizi M, Casagrande V, Mavilio M, Stoehr R, et al. ITCH deficiency protects from diet-induced obesity. *Diabetes* (2014) 63(2):550–61. doi:10.2337/db13-0802
25. Adamson S, Leitinger N. Phenotypic modulation of macrophages in response to plaque lipids. *Curr Opin Lipidol* (2011) 22(5):335–42. doi:10.1097/MOL.0b013e32834a97e4
26. Rombaldova M, Janovska P, Kopecky J, Kuda O. Omega-3 fatty acids promote fatty acid utilization and production of pro-resolving lipid mediators in alternatively activated adipose tissue macrophages. *Biochem Biophys Res Commun* (2017) 490(3):1080–5. doi:10.1016/j.bbrc.2017.06.170
27. Ayers S, Switnicki MP, Angajala A, Lammel J, Arumanayagam AS, Webb P. Genome-wide binding patterns of thyroid hormone receptor beta. *PLoS One* (2014) 9(2):e81186. doi:10.1371/journal.pone.0081186
28. Lammel Lindemann JA, Angajala A, Engler DA, Webb P, Ayers SD. Thyroid hormone induction of human cholesterol 7 alpha-hydroxylase (Cyp7a1) in vitro. *Mol Cell Endocrinol* (2014) 388(1–2):32–40. doi:10.1016/j.mce.2014.02.003
29. Zubair H, Azim S, Srivastava SK, Ahmad A, Bhardwaj A, Aslam Khan M, et al. Glucose metabolism reprogrammed by overexpression of ikkepsilon promotes pancreatic tumor growth. *Cancer Res* (2016) 76(24):7254–64. doi:10.1158/0008-5472.CAN-16-1666
30. Vats D, Mukundan L, Odegaard JI, Zhang L, Smith KL, Morel CR, et al. Oxidative metabolism and PGC-1beta attenuate macrophage-mediated inflammation. *Cell Metab* (2006) 4(1):13–24. doi:10.1016/j.cmet.2006.08.006
31. Pearce EL, Walsh MC, Cejas PJ, Harms GM, Shen H, Wang LS, et al. Enhancing CD8 T-cell memory by modulating fatty acid metabolism. *Nature* (2009) 460(7251):103–7. doi:10.1038/nature08097
32. O'Sullivan D, van der Windt GJ, Huang SC, Curtis JD, Chang CH, Buck MD, et al. Memory CD8(+) T cells use cell-intrinsic lipolysis to support the metabolic programming necessary for development. *Immunity* (2014) 41(1):75–88. doi:10.1016/j.immuni.2014.06.005
33. Cui G, Staron MM, Gray SM, Ho PC, Amezquita RA, Wu J, et al. IL-7-induced glycerol transport and TAG synthesis promotes memory CD8+ T cell longevity. *Cell* (2015) 161(4):750–61. doi:10.1016/j.cell.2015.03.021
34. Michalek RD, Gerriets VA, Jacobs SR, Macintyre AN, MacIver NJ, Mason EF, 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. doi:10.4049/jimmunol.1003613
35. Wu D, Sanin DE, Everts B, Chen Q, Qiu J, Buck MD, et al. Type 1 interferons induce changes in core metabolism that are critical for immune function. *Immunity* (2016) 44(6):1325–36. doi:10.1016/j.immuni.2016.06.006
36. Geiger R, Rieckmann JC, Wolf T, Basso C, Feng Y, Fuhrer T, et al. L-arginine modulates T cell metabolism and enhances survival and anti-tumor activity. *Cell* (2016) 167(3):829–842.e13. doi:10.1016/j.cell.2016.09.031
37. Ron-Harel N, Santos D, Ghergurovich JM, Sage PT, Reddy A, Lovitch SB, et al. Mitochondrial biogenesis and proteome remodeling promote one-carbon metabolism for T cell activation. *Cell Metab* (2016) 24(1):104–17. doi:10.1016/j.cmet.2016.06.007
38. Gostner JM, Becker K, Kofler H, Strasser B, Fuchs D. Tryptophan metabolism in allergic disorders. *Int Arch Allergy Immunol* (2016) 169(4):203–15. doi:10.1159/000445500
39. Soliman H, Mediavilla-Varela M, Antonia S. Indoleamine 2,3-dioxygenase: is it an immune suppressor? *Cancer J* (2010) 16(4):354–9. doi:10.1097/PPO.0b013e3181eb3343
40. Strong MJ, Xu G, Coco J, Baribault C, Vinay DS, Lacey MR, et al. Differences in gastric carcinoma microenvironment stratify according to EBV infection intensity: implications for possible immune adjuvant therapy. *PLoS Pathog* (2013) 9(5):e1003341. doi:10.1371/journal.ppat.1003341
41. Ma N, Guo P, Zhang J, He T, Kim SW, Zhang G, et al. Nutrients mediate intestinal bacteria-mucosal immune crosstalk. *Front Immunol* (2018) 9:5. doi:10.3389/fimmu.2018.00005
42. Vera JF, Brenner MK, Dotti G. Immunotherapy of human cancers using gene modified T lymphocytes. *Curr Gene Ther* (2009) 9(5):396–408. doi:10.2174/156652309789753338
43. Jürgens B, Raberger J, Fuchs D, Heitger A. Indoleamine 2,3-dioxygenase in human hematopoietic stem cell transplantation. *Int J Tryptophan Res* (2010) 3:77–90. doi:10.4137/IJTR.S4076
44. Rodriguez Cetina Biefer H, Vasudevan A, Elkhal A. Aspects of tryptophan and nicotinamide adenine dinucleotide in immunity: a new twist in an old tale. *Int J Tryptophan Res* (2017) 10:1178646917713491. doi:10.1177/1178646917713491
45. Hwang HJ, Dornbos P, Steidemann M, Dunivin TK, Rizzo M, LaPres JJ. Mitochondrial-targeted aryl hydrocarbon receptor and the impact of 2,3,7,8-tetrachlorodibenzo-p-dioxin on cellular respiration and the mitochondrial proteome. *Toxicol Appl Pharmacol* (2016) 304:121–32. doi:10.1016/j.taap.2016.04.005
46. Tullius SG, Biefer HR, Li S, Trachtenberg AJ, Edtinger K, Quante M, et al. NAD+ protects against EAE by regulating CD4+ T-cell differentiation. *Nat Commun* (2014) 5:5101. doi:10.1038/ncomms6101
47. Duan W. Sirtuins: from metabolic regulation to brain aging. *Front Aging Neurosci* (2013) 5:36. doi:10.3389/fnagi.2013.00036
48. Wakino S, Hasegawa K, Itoh H. Sirtuin and metabolic kidney disease. *Kidney Int* (2015) 88(4):691–8. doi:10.1038/ki.2015.157

49. Perl A, Hanczko R, Lai ZW, Oaks Z, Kelly R, Borsuk R, et al. Comprehensive metabolome analyses reveal N-acetylcysteine-responsive accumulation of kynurenine in systemic lupus erythematosus: implications for activation of the mechanistic target of rapamycin. *Metabolomics* (2015) 11(5):1157–74. doi:10.1007/s11306-015-0772-0
50. Lai ZW, Kelly R, Winans T, Marchena I, Shadakshari A, Yu J, et al. Sirolimus in patients with clinically active systemic lupus erythematosus resistant to, or intolerant of, conventional medications: a single-arm, open-label, phase 1/2 trial. *Lancet* (2018) 391(10126):1186–96. doi:10.1016/S0140-6736(18)30485-9
51. Khan NA, Nikkanen J, Yatsuga S, Jackson C, Wang L, Pradhan S, et al. mTORC1 regulates mitochondrial integrated stress response and mitochondrial myopathy progression. *Cell Metab* (2017) 26(2):419–428.e5. doi:10.1016/j.cmet.2017.07.007
52. Miwa S, Jow H, Baty K, Johnson A, Czapiewski R, Saretzki G, et al. Low abundance of the matrix arm of complex I in mitochondria predicts longevity in mice. *Nat Commun* (2014) 5:3837. doi:10.1038/ncomms4837
53. Mimaki M, Wang X, McKenzie M, Thorburn DR, Ryan MT. Understanding mitochondrial complex I assembly in health and disease. *Biochim Biophys Acta* (2012) 1817(6):851–62. doi:10.1016/j.bbabio.2011.08.010
54. Ko JH, Yoon SO, Lee HJ, Oh JY. Rapamycin regulates macrophage activation by inhibiting NLRP3 inflammasome-p38 MAPK-NFkappaB pathways in autophagy- and p62-dependent manners. *Oncotarget* (2017) 8(25):40817–31. doi:10.18632/oncotarget.17256
55. Liu PS, Wang H, Li X, Chao T, Teav T, Christen S, et al. alpha-ketoglutarate orchestrates macrophage activation through metabolic and epigenetic reprogramming. *Nat Immunol* (2017) 18(9):985–94. doi:10.1038/ni.3796
56. Palmieri EM, Menga A, Martín-Pérez R, Quinto A, Riera-Domingo C, De Tullio G, et al. Pharmacologic or genetic targeting of glutamine synthetase skews macrophages toward an M1-like phenotype and inhibits tumor metastasis. *Cell Rep* (2017) 20(7):1654–66. doi:10.1016/j.celrep.2017.07.054
57. He L, Weber KJ, Schilling JD. Glutamine modulates macrophage lipotoxicity. *Nutrients* (2016) 8(4):215. doi:10.3390/nu8040215
58. He C, Ryan AJ, Murthy S, Carter AB. Accelerated development of pulmonary fibrosis via Cu,Zn-superoxide dismutase-induced alternative activation of macrophages. *J Biol Chem* (2013) 288(28):20745–57. doi:10.1074/jbc.M112.410720
59. West AP, Brodsky IE, Rahner C, Woo DK, Erdjument-Bromage H, Tempst P, et al. TLR signalling augments macrophage bactericidal activity through mitochondrial ROS. *Nature* (2011) 472(7344):476–80. doi:10.1038/nature09973
60. Rousset S, Emre Y, Join-Lambert O, Hurtaud C, Ricquier D, Cassard-Doulcier AM. The uncoupling protein 2 modulates the cytokine balance in innate immunity. *Cytokine* (2006) 35(3–4):135–42. doi:10.1016/j.cyto.2006.07.012
61. Dostert C, Pétrilli V, Van Bruggen R, Steele C, Mossman BT, Tschopp J. Innate immune activation through Nalp3 inflammasome sensing of asbestos and silica. *Science* (2008) 320(5876):674–7. doi:10.1126/science.1156995
62. Nakahira K, Haspel JA, Rathinam VA, Lee SJ, Dolinay T, Lam HC, et al. Autophagy proteins regulate innate immune responses by inhibiting the release of mitochondrial DNA mediated by the NALP3 inflammasome. *Nat Immunol* (2011) 12(3):222–30. doi:10.1038/ni.1980
63. Li N, Ragheb K, Lawler G, Sturgis J, Rajwa B, Melendez JA, et al. Mitochondrial complex I inhibitor rotenone induces apoptosis through enhancing mitochondrial reactive oxygen species production. *J Biol Chem* (2003) 278(10):8516–25. doi:10.1074/jbc.M210432200
64. Kamiński MM, Sauer SW, Kamiński M, Opp S, Ruppert T, Grigaravičius P, et al. T cell activation is driven by an ADP-dependent glucokinase linking enhanced glycolysis with mitochondrial reactive oxygen species generation. *Cell Rep* (2012) 2(5):1300–15. doi:10.1016/j.celrep.2012.10.009
65. Watanabe-Matsui M, Muto A, Matsui T, Itoh-Nakadai A, Nakajima O, Murayama K, et al. Heme regulates B-cell differentiation, antibody class switch, and heme oxygenase-1 expression in B cells as a ligand of Bach2. *Blood* (2011) 117(20):5438–48. doi:10.1182/blood-2010-07-296483
66. Jang KJ, Mano H, Aoki K, Hayashi T, Muto A, Nambu Y, et al. Mitochondrial function provides instructive signals for activation-induced B-cell fates. *Nat Commun* (2015) 6:6750. doi:10.1038/ncomms7750
67. Narendra DP, Jin SM, Tanaka A, Suen DF, Gautier CA, Shen J, et al. PINK1 is selectively stabilized on impaired mitochondria to activate Parkin. *PLoS Biol* (2010) 8(1):e1000298. doi:10.1371/journal.pbio.1000298
68. Kirienko NV, Ausubel FM, Ruvkun G. Mitophagy confers resistance to siderophore-mediated killing by *Pseudomonas aeruginosa*. *Proc Natl Acad Sci U S A* (2015) 112(6):1821–6. doi:10.1073/pnas.1424954112
69. Mira MT, Alcaïs A, Nguyen VT, Moraes MO, Di Flumeri C, Vu HT, et al. Susceptibility to leprosy is associated with PARK2 and PACRG. *Nature* (2004) 427(6975):636–40. doi:10.1038/nature02326
70. Malhotra D, Darvishi K, Lohra M, Kumar H, Grover C, Sood S, et al. Association study of major risk single nucleotide polymorphisms in the common regulatory region of PARK2 and PACRG genes with leprosy in an Indian population. *Eur J Hum Genet* (2006) 14(4):438–42. doi:10.1038/sj.ejhg.5201563
71. Ali S, Vollaard AM, Widjaja S, Surjadi C, van de Vosse E, van Dissel JT. PARK2/PACRG polymorphisms and susceptibility to typhoid and paratyphoid fever. *Clin Exp Immunol* (2006) 144(3):425–31. doi:10.1111/j.1365-2249.2006.03087.x
72. Manzanillo PS, Ayres JS, Watson RO, Collins AC, Souza G, Rae CS, et al. The ubiquitin ligase parkin mediates resistance to intracellular pathogens. *Nature* (2013) 501(7468):512–6. doi:10.1038/nature12566
73. Pua HH, Guo J, Komatsu M, He YW. Autophagy is essential for mitochondrial clearance in mature T lymphocytes. *J Immunol* (2009) 182(7):4046–55. doi:10.4049/jimmunol.0801143
74. Willinger T, Flavell RA. Canonical autophagy dependent on the class III phosphoinositide-3 kinase Vps34 is required for naive T-cell homeostasis. *Proc Natl Acad Sci U S A* (2012) 109(22):8670–5. doi:10.1073/pnas.1205305109
75. Kim SJ, Syed GH, Siddiqui A. Hepatitis C virus induces the mitochondrial translocation of Parkin and subsequent mitophagy. *PLoS Pathog* (2013) 9(3):e1003285. doi:10.1371/journal.ppat.1003285
76. Kim SJ, Khan M, Quan J, Till A, Subramani S, Siddiqui A. Hepatitis B virus disrupts mitochondrial dynamics: induces fission and mitophagy to attenuate apoptosis. *PLoS Pathog* (2013) 9(12):e1003722. doi:10.1371/journal.ppat.1003722
77. Shao BZ, Xu ZQ, Han BZ, Su DF, Liu C. NLRP3 inflammasome and its inhibitors: a review. *Front Pharmacol* (2015) 6:262. doi:10.3389/fphar.2015.00262
78. Shimada K, Crother TR, Karlin J, Dagvadorj J, Chiba N, Chen S, et al. Oxidized mitochondrial DNA activates the NLRP3 inflammasome during apoptosis. *Immunity* (2012) 36(3):401–14. doi:10.1016/j.immuni.2012.01.009
79. Allam R, Lawlor KE, Yu EC, Mildenhall AL, Moujalled DM, Lewis RS, et al. Mitochondrial apoptosis is dispensable for NLRP3 inflammasome activation but non-apoptotic caspase-8 is required for inflammasome priming. *EMBO Rep* (2014) 15(9):982–90. doi:10.15252/embr.201438463
80. Zhou QF, Xu SM, Wang HQ, Xing LM, Fu R, Shao ZH. [Single nucleotide polymorphism of mitochondrial DNA D-LOOP region in peripheral blood lymphocytes of immuno-related pancytopenia patients]. *Zhongguo Shi Yan Xue Ye Xue Za Zhi* (2017) 25(1):186–91. doi:10.7534/j.issn.1009-2137.2017.01.033
81. Seth RB, Sun L, Ea CK, Chen ZJ. Identification and characterization of MAVS, a mitochondrial antiviral signaling protein that activates NF-kappaB and IRF 3. *Cell* (2005) 122(5):669–82. doi:10.1016/j.cell.2005.08.012
82. Buskiewicz IA, Montgomery T, Yasewicz EC, Huber SA, Murphy MP, Hartley RC, et al. Reactive oxygen species induce virus-independent MAVS oligomerization in systemic lupus erythematosus. *Sci Signal* (2016) 9(456):ra115. doi:10.1126/scisignal.aaf1933
83. Park S, Juliana C, Hong S, Datta P, Hwang I, Fernandes-Alnemri T, et al. The mitochondrial antiviral protein MAVS associates with NLRP3 and regulates its inflammasome activity. *J Immunol* (2013) 191(8):4358–66. doi:10.4049/jimmunol.1301170
84. Hee JS, Cresswell P. Viperin interaction with mitochondrial antiviral signaling protein (MAVS) limits viperin-mediated inhibition of the interferon response in macrophages. *PLoS One* (2017) 12(2):e0172236. doi:10.1371/journal.pone.0172236
85. Chao T, Wang H, Ho PC. Mitochondrial control and guidance of cellular activities of T cells. *Front Immunol* (2017) 8:473. doi:10.3389/fimmu.2017.00473
86. Westermann B. Molecular machinery of mitochondrial fusion and fission. *J Biol Chem* (2008) 283(20):13501–5. doi:10.1074/jbc.R800011200

87. Zorzano A, Sebastián D, Segalés J, Palacín M. The molecular machinery of mitochondrial fusion and fission: an opportunity for drug discovery? *Curr Opin Drug Discov Devel* (2009) 12(5):597–606.
88. Khan M, Syed GH, Kim SJ, Siddiqui A. Mitochondrial dynamics and viral infections: a close nexus. *Biochim Biophys Acta* (2015) 1853(10 Pt B):2822–33. doi:10.1016/j.bbamcr.2014.12.040
89. Quintana A, Hoth M. Mitochondrial dynamics and their impact on T cell function. *Cell Calcium* (2012) 52(1):57–63. doi:10.1016/j.ceca.2012.02.005
90. Baixauli F, Martín-Cófreces NB, Morlino G, Carrasco YR, Calabia-Linares C, Veiga E, et al. The mitochondrial fission factor dynamin-related protein 1 modulates T-cell receptor signalling at the immune synapse. *EMBO J* (2011) 30(7):1238–50. doi:10.1038/emboj.2011.25
91. Abarca-Rojano E, Muñiz-Hernández S, Moreno-Altamirano MM, Mondragón-Flores R, Enriquez-Rincón F, Sánchez-García FJ. Re-organization of mitochondria at the NK cell immune synapse. *Immunol Lett* (2009) 122(1):18–25. doi:10.1016/j.imlet.2008.10.008
92. Junger WG. Immune cell regulation by autocrine purinergic signalling. *Nat Rev Immunol* (2011) 11(3):201–12. doi:10.1038/nri2938
93. Singaravelu K, Nelson C, Bakowski D, de Brito OM, Ng SW, Di Capite J, et al. Mitofusin 2 regulates STIM1 migration from the Ca2+ store to the plasma membrane in cells with depolarized mitochondria. *J Biol Chem* (2011) 286(14):12189–201. doi:10.1074/jbc.M110.174029
94. Wang Y, Subramanian M, Yurdagul A Jr, Barbosa-Lorenzi VC, Cai B, de Juan-Sanz J, et al. Mitochondrial fission promotes the continued clearance of apoptotic cells by macrophages. *Cell* (2017) 171(2):331–345.e22. doi:10.1016/j.cell.2017.08.041
95. Cid-Castro C, Hernandez-Espinosa DR, Moran J. ROS as regulators of mitochondrial dynamics in neurons. *Cell Mol Neurobiol* (2018) 38(5):995–1007. doi:10.1007/s10571-018-0584-7
96. Zheng Q, Zhao Y, Guo J, Zhao S, Fei C, Xiao C, et al. Iron overload promotes mitochondrial fragmentation in mesenchymal stromal cells from myelodysplastic syndrome patients through activation of the AMPK/MFF/Drp1 pathway. *Cell Death Dis* (2018) 9(5):515. doi:10.1038/s41419-018-0552-7
97. Tsushima K, Bugger H, Wende AR, Soto J, Jenson GA, Tor AR, et al. Mitochondrial reactive oxygen species in lipotoxic hearts induce post-translational modifications of AKAP121, DRP1, and OPA1 that promote mitochondrial fission. *Circ Res* (2018) 122(1):58–73. doi:10.1161/CIRCRESAHA.117.311307
98. Li N, Wang H, Jiang C, Zhang M. Renal ischemia/reperfusion-induced mitophagy protects against renal dysfunction via Drp1-dependent-pathway. *Exp Cell Res* (2018) 369(1):27–33. doi:10.1016/j.yexcr.2018.04.025
99. Caza TN, Fernandez DR, Talaber G, Oaks Z, Haas M, Madaio MP, et al. HRES-1/Rab4-mediated depletion of Drp1 impairs mitochondrial homeostasis and represents a target for treatment in SLE. *Ann Rheum Dis* (2014) 73(10):1888–97. doi:10.1136/annrheumdis-2013-203794
100. Oaks Z, Winans T, Caza T, Fernandez D, Liu Y, Landas SK, et al. Mitochondrial dysfunction in the liver and antiphospholipid antibody production precede disease onset and respond to rapamycin in lupus-prone mice. *Arthritis Rheumatol* (2016) 68(11):2728–39. doi:10.1002/art.39791
101. Buck MD, O'Sullivan D, Klein Geltink RI, Curtis JD, Chang CH, Sanin DE, et al. Mitochondrial dynamics controls T cell fate through metabolic programming. *Cell* (2016) 166(1):63–76. doi:10.1016/j.cell.2016.05.035
102. Kang YJ, Bang BR, Han KH, Hong L, Shim EJ, Ma J, et al. Regulation of NKT cell-mediated immune responses to tumours and liver inflammation by mitochondrial PGAM5-Drp1 signalling. *Nat Commun* (2015) 6:8371. doi:10.1038/ncomms9371
103. Liu P, Xie Q, Wei T, Chen Y, Chen H, Shen W. Activation of the NLRP3 inflammasome induces vascular dysfunction in obese OLETF rats. *Biochem Biophys Res Commun* (2015) 468(1–2):319–25. doi:10.1016/j.bbrc.2015.10.105
104. Park S, Won JH, Hwang I, Hong S, Lee HK, Yu JW. Defective mitochondrial fission augments NLRP3 inflammasome activation. *Sci Rep* (2015) 5:15489. doi:10.1038/srep15489
105. Zapico SC, Ubelaker DH. mtDNA mutations and their role in aging, diseases and forensic sciences. *Aging Dis* (2013) 4(6):364–80. doi:10.14336/AD.2013.0400364
106. Ribas V, Garcia-Ruiz C, Fernandez-Checa JC. Glutathione and mitochondria. *Front Pharmacol* (2014) 5:151. doi:10.3389/fphar.2014.00151
107. Acosta MJ, Vazquez Fonseca L, Desbats MA, Cerqua C, Zordan R, Trevisson E, et al. Coenzyme Q biosynthesis in health and disease. *Biochim Biophys Acta* (2016) 1857(8):1079–85. doi:10.1016/j.bbabio.2016.03.036
108. Quijano C, Trujillo M, Castro L, Trostchansky A. Interplay between oxidant species and energy metabolism. *Redox Biol* (2016) 8:28–42. doi:10.1016/j.redox.2015.11.010
109. Fraternale A, Brundu S, Magnani M. Glutathione and glutathione derivatives in immunotherapy. *Biol Chem* (2017) 398(2):261–75. doi:10.1515/hsz-2016-0202
110. Mensurado S, Rei M, Lança T, Ioannou M, Gonçalves-Sousa N, Kubo H, et al. Tumor-associated neutrophils suppress pro-tumoral IL-17+ gammadelta T cells through induction of oxidative stress. *PLoS Biol* (2018) 16(5):e2004990. doi:10.1371/journal.pbio.2004990
111. Allen M, Bailey C, Cahatol I, Dodge L, Yim J, Kassissa C, et al. Mechanisms of control of *Mycobacterium tuberculosis* by NK cells: role of glutathione. *Front Immunol* (2015) 6:508. doi:10.3389/fimmu.2015.00508
112. Valdivia A, Ly J, Gonzalez L, Hussain P, Saing T, Islamoglu H, et al. Restoring cytokine balance in HIV-positive individuals with low CD4 T cell counts. *AIDS Res Hum Retroviruses* (2017) 33(9):905–18. doi:10.1089/AID.2016.0303
113. Short JD, Downs K, Tavakoli S, Asmis R. Protein thiol redox signaling in monocytes and macrophages. *Antioxid Redox Signal* (2016) 25(15):816–35. doi:10.1089/ars.2016.6697
114. Allen EM, Mieyal JJ. Protein-thiol oxidation and cell death: regulatory role of glutaredoxins. *Antioxid Redox Signal* (2012) 17(12):1748–63. doi:10.1089/ars.2012.4644
115. Yarosz EL, Chang CH. The role of reactive oxygen species in regulating T cell-mediated immunity and disease. *Immune Netw* (2018) 18(1):e14. doi:10.4110/in.2018.18.e14
116. Gerriets VA, Rathmell JC. Metabolic pathways in T cell fate and function. *Trends Immunol* (2012) 33(4):168–73. doi:10.1016/j.it.2012.01.010
117. Beckermann KE, Dudzinski SO, Rathmell JC. Dysfunctional T cell metabolism in the tumor microenvironment. *Cytokine Growth Factor Rev* (2017) 35:7–14. doi:10.1016/j.cytogfr.2017.04.003
118. Franchina DG, Dostert C, Brenner D. Reactive oxygen species: involvement in T cell signaling and metabolism. *Trends Immunol* (2018) 39(6):489–502. doi:10.1016/j.it.2018.01.005
119. Fracchia KM, Pai CY, Walsh CM. Modulation of T cell metabolism and function through calcium signaling. *Front Immunol* (2013) 4:324. doi:10.3389/fimmu.2013.00324
120. Kouidhi S, Elgaaied AB, Chouaib S. Impact of metabolism on T-cell differentiation and function and cross talk with tumor microenvironment. *Front Immunol* (2017) 8:270. doi:10.3389/fimmu.2017.00270
121. Previte DM, O'Connor EC, Novak EA, Martins CP, Mollen KP, Piganelli JD. Reactive oxygen species are required for driving efficient and sustained aerobic glycolysis during CD4+ T cell activation. *PLoS One* (2017) 12(4):e0175549. doi:10.1371/journal.pone.0175549
122. Sivitz WI, Yorek MA. Mitochondrial dysfunction in diabetes: from molecular mechanisms to functional significance and therapeutic opportunities. *Antioxid Redox Signal* (2010) 12(4):537–77. doi:10.1089/ars.2009.2531
123. Perl A, Gergely P Jr, Puskas F, Banki K. Metabolic switches of T-cell activation and apoptosis. *Antioxid Redox Signal* (2002) 4(3):427–43. doi:10.1089/15230860260196227
124. Laskowski M, Augustynek B, Kulawiak B, Koprowski P, Bednarczyk P, Jarmuszkiewicz W, et al. What do we not know about mitochondrial potassium channels? *Biochim Biophys Acta* (2016) 1857(8):1247–57. doi:10.1016/j.bbabio.2016.03.007
125. Gergely P Jr, Grossman C, Niland B, Puskas F, Neupane H, Allam F, et al. Mitochondrial hyperpolarization and ATP depletion in patients with systemic lupus erythematosus. *Arthritis Rheum* (2002) 46(1):175–90. doi:10.1002/1529-0131(200201)46:1<175::AID-ART10015>3.0.CO;2-H
126. Perl A, Gergely P Jr, Nagy G, Koncz A, Banki K. Mitochondrial hyperpolarization: a checkpoint of T-cell life, death and autoimmunity. *Trends Immunol* (2004) 25(7):360–7. doi:10.1016/j.it.2004.05.001
127. Fernandez D, Perl A. Metabolic control of T cell activation and death in SLE. *Autoimmun Rev* (2009) 8(3):184–9. doi:10.1016/j.autrev.2008.07.041
128. Esparza-Molto PB, Cuezva JM. The role of mitochondrial H(+)-ATP synthase in cancer. *Front Oncol* (2018) 8:53. doi:10.3389/fonc.2018.00053
129. Vojtísková A, Jesina P, Kalous M, Kaplanová V, Houstek J, Tesarová M, et al. Mitochondrial membrane potential and ATP production in primary

disorders of ATP synthase. *Toxicol Mech Methods* (2004) 14(1–2):7–11. doi:10.1080/15376520490257347
130. Barbi de Moura M, Vincent G, Fayewicz SL, Bateman NW, Hood BL, Sun M, et al. Mitochondrial respiration – an important therapeutic target in melanoma. *PLoS One* (2012) 7(8):e40690. doi:10.1371/journal.pone.0040690
131. Park DW, Zmijewski JW. Mitochondrial dysfunction and immune cell metabolism in sepsis. *Infect Chemother* (2017) 49(1):10–21. doi:10.3947/ic.2017.49.1.10
132. Sheu SS, Nauduri D, Anders MW. Targeting antioxidants to mitochondria: a new therapeutic direction. *Biochim Biophys Acta* (2006) 1762(2):256–65. doi:10.1016/j.bbadis.2005.10.007
133. D'Souza GG, Wagle MA, Saxena V, Shah A. Approaches for targeting mitochondria in cancer therapy. *Biochim Biophys Acta* (2011) 1807(6):689–96. doi:10.1016/j.bbabio.2010.08.008
134. Adlam VJ, Harrison JC, Porteous CM, James AM, Smith RA, Murphy MP, et al. Targeting an antioxidant to mitochondria decreases cardiac ischemia-reperfusion injury. *FASEB J* (2005) 19(9):1088–95. doi:10.1096/fj.05-3718com
135. Dare AJ, Bolton EA, Pettigrew GJ, Bradley JA, Saeb-Parsy K, Murphy MP. Protection against renal ischemia-reperfusion injury in vivo by the mitochondria targeted antioxidant MitoQ. *Redox Biol* (2015) 5:163–8. doi:10.1016/j.redox.2015.04.008
136. Reddy PH, Tripathi R, Troung Q, Tirumala K, Reddy TP, Anekonda V, et al. Abnormal mitochondrial dynamics and synaptic degeneration as early events in Alzheimer's disease: implications to mitochondria-targeted antioxidant therapeutics. *Biochim Biophys Acta* (2012) 1822(5):639–49. doi:10.1016/j.bbadis.2011.10.011
137. Wu X, Cheng J, Wang X. Dietary antioxidants: potential anticancer agents. *Nutr Cancer* (2017) 69(4):521–33. doi:10.1080/01635581.2017.1299872
138. Kim HK, Han J. Mitochondria-targeted antioxidants for the treatment of cardiovascular disorders. *Adv Exp Med Biol* (2017) 982:621–46. doi:10.1007/978-3-319-55330-6_32
139. Kalyanaraman B, Cheng G, Hardy M, Ouari O, Lopez M, Joseph J, et al. A review of the basics of mitochondrial bioenergetics, metabolism, and related signaling pathways in cancer cells: therapeutic targeting of tumor mitochondria with lipophilic cationic compounds. *Redox Biol* (2018) 14:316–27. doi:10.1016/j.redox.2017.09.020
140. Mailloux RJ, Treberg JR. Protein S-glutathionlyation links energy metabolism to redox signaling in mitochondria. *Redox Biol* (2016) 8:110–8. doi:10.1016/j.redox.2015.12.010
141. Chen CL, Chen J, Rawale S, Varadharaj S, Kaumaya PP, Zweier JL, et al. Protein tyrosine nitration of the flavin subunit is associated with oxidative modification of mitochondrial complex II in the post-ischemic myocardium. *J Biol Chem* (2008) 283(41):27991–8003. doi:10.1074/jbc.M802691200
142. Espinosa-Diez C, Miguel V, Mennerich D, Kietzmann T, Sánchez-Pérez P, Cadenas S, et al. Antioxidant responses and cellular adjustments to oxidative stress. *Redox Biol* (2015) 6:183–97. doi:10.1016/j.redox.2015.07.008
143. Chouchani ET, Methner C, Nadtochiy SM, Logan A, Pell VR, Ding S, et al. Cardioprotection by S-nitrosation of a cysteine switch on mitochondrial complex I. *Nat Med* (2013) 19(6):753–9. doi:10.1038/nm.3212
144. Bhatt KN, Butler J. Myocardial energetics and heart failure: a review of recent therapeutic trials. *Curr Heart Fail Rep* (2018) 15(3):191–7. doi:10.1007/s11897-018-0386-8
145. Shanmugam MK, Shen H, Tang FR, Arfuso F, Rajesh M, Wang L, et al. Potential role of genipin in cancer therapy. *Pharmacol Res* (2018) 133:195–200. doi:10.1016/j.phrs.2018.05.007
146. Quryshi N, Norwood Toro LE, Ait-Aissa K, Kong A, Beyer AM. Chemotherapeutic-induced cardiovascular dysfunction: physiological effects, early detection-the role of telomerase to counteract mitochondrial defects and oxidative stress. *Int J Mol Sci* (2018) 19(3):E797. doi:10.3390/ijms19030797
147. Dashdorj A, Jyothi KR, Lim S, Jo A, Nguyen MN, Ha J, et al. Mitochondria-targeted antioxidant MitoQ ameliorates experimental mouse colitis by suppressing NLRP3 inflammasome-mediated inflammatory cytokines. *BMC Med* (2013) 11:178. doi:10.1186/1741-7015-11-178
148. Cordero MD, Alcocer-Gómez E, Culic O, Carrión AM, de Miguel M, Díaz-Parrado E, et al. NLRP3 inflammasome is activated in fibromyalgia: the effect of coenzyme Q10. *Antioxid Redox Signal* (2014) 20(8):1169–80. doi:10.1089/ars.2013.5198
149. Morton SU, Prabhu SP, Lidov HGW, Shi J, Anselm I, Brownstein CA, et al. AIFM1 mutation presenting with fatal encephalomyopathy and mitochondrial disease in an infant. *Cold Spring Harb Mol Case Stud* (2017) 3(2):a001560. doi:10.1101/mcs.a001560
150. Fukumoto K, Nguyen TB, Chiba S, Sakata-Yanagimoto M. Review of the biologic and clinical significance of genetic mutations in angioimmunoblastic T-cell lymphoma. *Cancer Sci* (2018) 109(3):490–6. doi:10.1111/cas.13393
151. Moshtaghi A, Vaziri H, Sariri R, Shaigan H. Polymorphism of MnSOD (Val16Ala) gene in pregnancies with blighted ovum: a case-control study. *Int J Reprod Biomed (Yazd)* (2017) 15(8):503–8. doi:10.29252/ijrm.15.8.503

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Mevalonate Metabolism in Immuno-Oncology

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Immuno-oncology not only refers to the multifaceted relationship between our immune system and a developing cancer but also includes therapeutic approaches that harness the body's immune system to fight cancer. The recognition that metabolic reprogramming governs immunity was a key finding with important implications for immuno-oncology. In this review, we want to explore how activation and differentiation-induced metabolic reprogramming affects the mevalonate pathway for cholesterol biosynthesis in immune and cancer cells. Glycolysis-fueled mevalonate metabolism is a critical pathway in immune effector cells, which may, however, be shared by cancer stem cells, complicating the development of therapeutic strategies. Additional engagement of fatty acidy oxidation, as it occurs in regulatory immune cells as well as in certain tumor types, may influence mevalonate pathway activity. Transcellular mevalonate metabolism may play an as yet unanticipated role in the crosstalk between the various cell types and may add another level of complexity. In humans, a subset of γδ T cells is specifically adapted to perform surveillance of mevalonate pathway dysregulation. While the mevalonate pathway remains an important target in immuno-oncology, in terms of personalized medicine, it may be the type or stage of a malignant disease that determines whether mevalonate metabolism requires training or attenuation.

Keywords: mevalonate, metabolism, transcellular, cholesterol, fatty acid oxidation, immune cells, cancer

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MEVALONATE METABOLISM IN IMMUNE CELLS

Glycolysis-Driven Mevalonate Metabolism in Immune Effector Cells

Immune cell activation is associated with shifts in cellular metabolism (1, 2). In contrast to naïve T cells, T helper (Th) cells including type 1 (Th1), type 2 (Th2) as well as type 17 (Th17) display a reprogrammed metabolic phenotype, which is characterized by increased rates of aerobic glycolysis, leading to fatty acid synthesis (FAS) and mevalonate metabolism (**Figure 1**). Glycolysis-driven lipogenesis is induced by Akt signaling and depends on sterol regulatory element-binding protein (SREBP) transcription factors. All these changes are promoted by the metabolic checkpoint kinase

Abbreviations: ABCA1, ATP-binding cassette transporter A1; ACAT-1, acetyl-CoA acetyltransferase 1; apo-AI, apolipoprotein A-I; ATP, adenosine triphosphate; ACL, ATP citrate lyase; BTIC, brain tumor-initiating cell; BTN, butyrophilin; CoA, coenzyme A; CPT1, carnitine palmitoyltransferase 1; CTLA-4, cytotoxic T lymphocyte-associated protein-4; DMAPP, dimethylallyl diphosphate; FA, fatty acid; FAO, fatty acid ß-oxidation; FAS, fatty acid synthesis; FPP, farnesyl diphosphate; GM-CSF, granulocyte/macrophage-colony-stimulating factor; GGPP, geranylgeranyl diphosphate; GPP, geranyl diphosphate; HMG, 3-hydroxy-3-methylglutaryl; HMGCR, HMG-CoA reductase; IFN, interferon; IL, interleukin; IPP, isopentenyl diphosphate; mTOR, mechanistic target of rapamycin; N-BP, nitrogen-containing bisphosphonate; OXPHOS, oxidative phosphorylation; PI3K, phosphoinositide 3-kinase; PP, diphosphate = pyrophosphate; SCAP, SREBP cleavage-activating protein; SREBP, sterol regulatory element-binding protein; TCA, tricarboxylic acid; TCR, T cell receptor; Th, T helper; TKI, tyrosine kinase inhibitor; TLR, toll-like receptor.

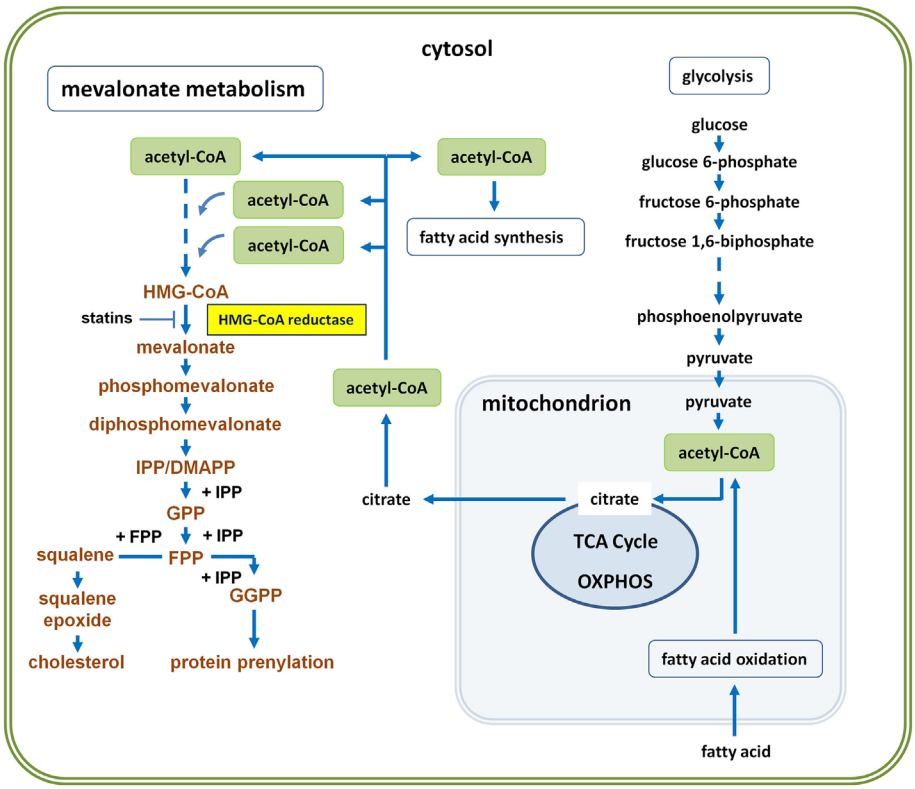

FIGURE 1 | Glycolysis-driven mevalonate metabolism versus fatty acid oxidation (FAO)-driven oxidative phosphorylation (OXPHOS). Glycolysis-derived pyruvate can enter the mitochondrion and fuel the tricarboxylic acid (TCA) cycle to drive OXPHOS. Cells thus generate energy in the form of adenosine triphosphate (ATP). However, activated immune or cancer cells can also export citrate to the cytosol, where it is converted back to acetyl-coenzyme A (acetyl-CoA) by ATP citrate lyase. Abundance of cytosolic acetyl-CoA enables both, fatty acid synthesis (FAS) and mevalonate metabolism, collectively referred to as lipogenesis. Three molecules of acetyl-CoA are required to generate HMG-CoA. HMG-CoA is the substrate of HMG-CoA reductase, the mevalonate-generating enzyme, which catalyzes the first committed step and thus initiates the pathway leading to farnesyl diphosphate, also known as farnesyl pyrophosphate (FPP = branching point). Whereas FPP is the precursor in cholesterol biosynthesis, both, FPP and geranylgeranyl diphosphate (GGPP), represent activated isoprenoid moieties in posttranslational protein prenylation. Concurrent FAO may influence the availability of acetyl-CoA for mevalonate metabolism, because FAO usually serves to drive OXPHOS and thus diverts acetyl-CoA from lipogenic pathways.

mechanistic target of rapamycin (mTOR) that controls protein translation, cell growth, and metabolism (3, 4). The serine/threonine kinase mTOR exists in two complexes, mTORC1 and mTORC2, which have distinct functions. TCR triggering induces in an Akt–mTOR–SREBP-dependent manner the expression of all genes encoding mevalonate-generating and mevalonate-metabolizing enzymes (5), highlighting the importance of this metabolic pathway for T cell activation (6).

M1 macrophages, classically activated by the Th1 cytokine interferon-γ (IFN-γ) plus lipopolysaccharide (LPS), are myeloid effector cells, which are characterized by the expression of high levels of pro-inflammatory cytokines, reactive nitrogen and oxygen intermediates, promotion of Th1 response, and strong microbicidal and tumoricidal activity (7). Like Th cells, M1 macrophages also depend on glycolysis and mevalonate metabolism (2, 8). Finally, dendritic cells (DCs), which encounter bacterial components such as LPS as well as T or NK cell-derived IFN-γ during infection likewise operate glycolytic metabolism (2) and require mevalonate pathway activity for effector cytokine production (9).

M1 macrophages and DCs engage glycolysis-fueled lipogenesis to expand cellular compartments such as the endoplasmic reticulum and the Golgi as well as to prepare the entire secretory machinery for effector responses (10). For this purpose, glucose-derived cytosolic pyruvate enters the citric acid cycle, also known as the Krebs cycle or tricarboxylic acid (TCA) cycle, which takes place in the mitochondria of eukaryotic cells. However, instead of being fully oxidized in the TCA cycle, some of the pyruvate-derived mitochondrial citrate can be exported into the cytosol. ATP citrate lyase, which is phosphorylated by Akt (11), cleaves citrate and thus generates cytosolic acetyl-CoA, the precursor of FAS and mevalonate metabolism (12, 13) (**Figure 1**).

Colony-Stimulating Factors Promote Mevalonate Metabolism during Myelopoiesis and M1 Macrophage Activation

Myelopoiesis is driven by colony-stimulating factors including granulocyte/macrophage-colony-stimulating factor (GM-CSF)

and M-CSF, which are important for the development and function of monocytes and macrophages. In murine myelopiesis, M-CSF stimulation activated mTORC1 and mTORC1-driven glycolysis initiated a transcriptional program involving activation of the protooncogene Myc (14), which is well known to induce metabolic reprogramming, including stimulation of lipogenesis (15). Accordingly, multiple genes involved in mevalonate generation and metabolism toward cholesterol were activated in response to M-CSF treatment (14). Attenuation of cholesterol biosynthesis gene expression by deleting SREBP cleavage-activating protein impaired myelopoiesis, highlighting the crucial role of mevalonate metabolism in macrophage development.

Granulocyte/macrophage-colony-stimulating factor plays a critical role in promoting glycolysis-fueled mevalonate metabolism (8) in M1 macrophages. GM-CSF increases the glycolytic capacity of macrophages and primes them for high levels of acute glycolysis in response to LPS stimulation. LPS has long been known to promote glucose uptake in macrophages (2, 16) and this may in part be due to LPS-induced production of GM-CSF (17). GM-CSF primed macrophages not only contained higher levels of acetyl-CoA but also displayed upregulated mRNA and protein expression of HMG-CoA reductase (8), which is the target of the statins, a class of lipid-lowering drugs widely prescribed for treatment and prophylaxis of coronary heart disease. GM-CSF primed macrophages produced significantly higher levels of the pro-inflammatory cytokines TNF-α, IL-1β, IL-6, and IL-12 in response to LPS and, importantly, simvastatin prevented the GM-CSF priming effect. These observations indicated that mevalonate metabolism instructs the inflammatory response of GM-CSF primed M1 macrophages.

Although the maintenance of immature antigen-presenting DCs is facilitated by fatty acid oxidation (FAO)-driven oxidative phosphorylation (OXPHOS) in a steady state (18), maturation into cytokine-producing, immunostimulatory DCs is again driven by glycolysis (2). The increased reliance of activated DCs on glycolysis is reflected by the observation that inhibition of hexokinase by 2-deoxyglucose prevents the DC maturation process (2). A major reason for the metabolic switch of maturing DCs is the increased need for citrate, which determines the levels of cytosolic acetyl-CoA, fueling not only FAS but also mevalonate metabolism (**Figure 1**). In this context, it may be of interest that human monocyte-derived DCs developing in the presence of GM-CSF and IL-4 produce substantial amounts of M-CSF (19). M-CSF synthesis is rapidly induced by GM-CSF during the first 24 h of DC culture and then declines during the 5-day culture period. Given the importance of M-CSF in promoting mevalonate metabolism during myelopoiesis (14), the stimulatory effects of LPS and GM-CSF on glycolysis-fueled mevalonate metabolism may at least in part be mediated by M-CSF, which is induced by LPS and GM-CSF. However, M-CSF on its own is not capable of macrophage priming for enhanced inflammatory responses (20).

OXPHOS Fueled by FAO in Quiescent and Regulatory Immune Cells

In contrast, other immune cell types such as naïve T cells and quiescent CD8 memory T cells, whose major task is to survive, engage OXPHOS driven by FAO (21). In addition, immune cells responsible for the limitation of inflammation to ensure the return to homeostasis such as M2 macrophages (22), which develop in the presence of M-CSF and the Th2 cytokine IL-4, as well as regulatory T (T_{reg}) cells (23) and tolerogenic DCs (24) also operate FAO-driven OXPHOS (2). The role of mevalonate metabolism in these cells is less clear. Interestingly, mTORC1 signaling in T_{reg} cells has been shown to promote cholesterol and lipid metabolism. The mevalonate pathway turned out to be particularly important for coordinated T_{reg} cell proliferation and for the establishment of T_{reg} cell functional competence (25). These findings indicate that regulatory immune cells may concomitantly engage FAO and mevalonate metabolism. However, it seems possible that mitochondrial oxidation of acetyl-CoA for increased ATP synthesis may limit its availability for mevalonate metabolism. If this proves to be true, mevalonate metabolism might represent an Achilles' heel-like target and statins may be used to enhance immunotherapy by relieving cell-mediated immunosuppression.

MEVALONATE METABOLISM IN CANCER CELLS

Uncontrolled growth of tumors is usually promoted by aerobic glycolysis, an observation originally made by Otto Warburg almost a century ago (26). Glycolysis-driven mevalonate metabolism is potentially oncogenic, most likely *via* excessive protein prenylation (27). Physiologically, the tumor suppressor p53 controls mevalonate pathway activity; however, p53 gain-of-function mutation can lead to uncontrolled mevalonate metabolism and subsequently to malignant transformation (28). Other cancers may lack feedback control of HMG-CoA reductase (HMGCR) or overexpress of HMGCR, leading to permanently increased flux through the mevalonate pathway (29, 30). Along the same line, ectopic expression of HMGCR also facilitated malignant transformation (31).

Myc-Driven Mevalonate Metabolism in Cancer Stem Cells

Recently, Myc has been shown to mediate its oncogenic effect by stimulating mevalonate metabolism in cancer stem cells (30) (**Figure 2A**), which share signaling and metabolic pathways with tumor cells upon epithelial–mesenchymal transition (32). Brain tumor-initiating cells (BTICs) were shown to exhibit enhanced mevalonate pathway activity (30). All genes encoding the enzymes that in a series of reactions convert HMG-CoA *via* mevalonate into farnesyl diphosphate (FPP) (**Figure 1**) were shown to be activated in BTIC models and induction of differentiation caused suppression of these mevalonate pathway genes. In addition, targeting the mevalonate pathway in BTICs by RNA interference of HMGCR expression or by pharmacological inhibition of HMGCR activity using statins attenuated proliferation, self-renewal, and tumorigenicity. Moreover, statin treatment of BTICs also reduced Myc expression.

TRANSCELLULAR MEVALONATE METABOLISM

An additional level of complexity has been generated by the observation of extracellular or transcellular mevalonate metabolism.

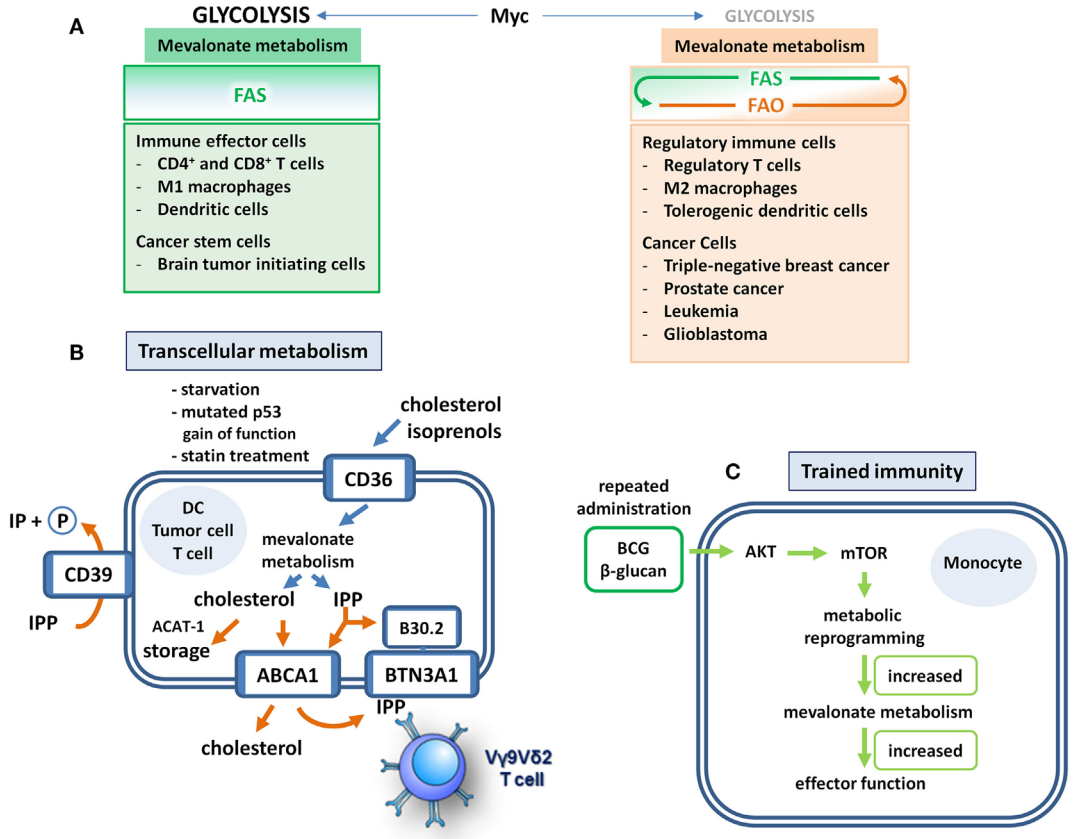

FIGURE 2 | Mevalonate metabolism in immune and cancer cells. **(A)** Fatty acid metabolism makes the difference. Immune cells that realize effector functions depend on Myc-driven glycolysis that fuels lipogenesis. These cells engage mevalonate metabolism and fatty acid synthesis (FAS) but refrain from fatty acid oxidation (FAO). Unfortunately, cancer stem cells may have similar metabolic profiles. Although regulatory immune cells may still require Myc-driven glycolysis and FAS to some extent, they also engage FAO, which may occur at the expense of mevalonate pathway activity. These cells use FAO to realize their suppressive functions and to support survival. Distinct tumor types adopt a similar metabolic profile. In these cells, FAO may limit the availability of acetyl-CoA for mevalonate metabolism rendering it a potential Achilles' heel-like target for therapeutic interventions. **(B)** Transcellular mevalonate metabolism. Starvation or p53 gain-of-function mutations lead to enforced uptake and use of extracellular isoprenoids in tumor cells. Intracellularly accumulating isopentenyl diphosphate (IPP) can bind to the B30.2 domain of butyrophilin 3A1 (BTN3A1), which leads to the activation of Vγ9Vδ2 T cells through a conformational change of the extracellular domain. In dendritic cells (DCs), the cholesterol efflux transporter adenosine triphosphate (ATP)-binding cassette transporter A1 (ABCA1) may export mevalonate-derived IPP into the extracellular space. Extracellular IPP can bind to BTN3A1 on the DC cell surface resulting in the activation of Vγ9Vδ2 T cells. The ecto-ATPase CD39 is able to dephosphorylate IPP thus limiting the duration and strength of IPP-induced γδ T cell responses. The lipid scavenger CD36 may also mediate the uptake of extracellular isoprenoids. Cholesterol storage through ACAT-1-mediated esterification may limit T cell activity. **(C)** Training of mevalonate metabolism. Priming of monocytes with Bacillus Calmette–Guérin (BCG) or β-glucan leads to an Akt–mTOR-driven metabolic reprogramming that empowers these cells to respond to subsequent challenges with increased production of cytokines and reactive oxygen intermediates. This increased responsiveness represents a form of innate memory and is based on enhanced flux through the mevalonate pathway. Significantly upregulated reactions include the production of acetoacetyl-CoA (ACAT-1), phosphomevalonate, farnesyl diphosphate as well as squalene and 2,3 oxidosqualene, the latter being rate-limiting steps of cholesterol biosynthesis. Overall, more than 50% of the genes in the pathway are activated in trained cells.

This term refers to a form of short distance intercellular communication, in which lipid intermediates synthesized and released by one cell type, can be incorporated and further metabolized by another cell type. Such interaction between different cell types by shared metabolism is a well-described phenomenon during eicosanoid biosynthesis (33). Among the secretory products of activated endothelial cells is arachidonic acid, the lipid precursor that initiates the eicosanoid cascade leading to the synthesis of prostaglandins, leukotrienes, and lipoxins. Human monocytes recruited by activated endothelial cells can respond to endothelial cell-derived arachidonic acid by activating not only the eicosanoid cascade but also the *de novo* pathway of FAS. As a consequence, arachidonic acid-pulsed monocytes acquire inflammatory phenotype and function (34).

The concept of transcellular lipid metabolism also applies to the mevalonate pathway. The contribution of extracellular mevalonate to mevalonate pathway activity is usually low (5%) but may increase to >30% during periods of starvation. In addition, unusually high concentrations of extracellular isoprenols (>10 μM) may result in a relative contribution of up to 50% (35). Moreover, tumor cells carrying mutated p53, which enhances mevalonate metabolism instead of suppressing it, increasingly use extracellular isoprenols resulting in a relative contribution of even greater than 50%. Finally, treatment with statins, which blunt mevalonate

metabolism by inhibiting HMG-CoA reductase, increased the use of extracellular isoprenols (35) (**Figure 2B**).

Isopentenyl diphosphate (IPP) can also be exported to the extracellular space. In DCs, the cholesterol efflux transporter ATP-binding cassette transporter A1 (ABCA1) has recently been shown to also mediate the efflux of IPP (36). Since cholesterol efflux serves homeostatic purposes, ABCA1-mediated IPP export will only occur in cells with high flux through the mevalonate pathway. Extracellular IPP may thus function as an indicator of hyperactive mevalonate metabolism that alerts the immune system. IPP efflux to lipid-free apolipoprotein A-I (apoA-I) results in binding of extracellular IPP to butyrophilin 3A1 (BTN3A1) on the cell surface (37, 38). BTN3A1-mediated presentation of IPP subsequently activates Vγ9Vδ2 T cells, which are innate-like T cells with considerable antimicrobial and antitumor potential (39). BTN3A1 has been shown to also serve as a sensor of intracellular IPP levels. Upon binding of IPP to its cytoplasmic B30.2 domain, conformational changes of the BTN3A1 extracellular domain facilitate TCR engagement and Vγ9Vδ2 T cell activation (40). Vγ9Vδ2 T cells activated by either pathway can then kill cells with a hyperactive mevalonate metabolism and thus contribute to the surveillance of infection or oncogenic transformation (**Figure 2B**).

Consistent with a role of extracellular IPP in immune surveillance, the ecto-ATPase CD39 has recently been shown to also dephosphorylate and inactivate IPP and other mevalonate-derived phosphoantigens, thus limiting the duration and strength of phosphoantigen-induced γδ T cell responses (41). Additional evidence for transcellular mevalonate metabolism has been provided by a recent study demonstrating that the lipid scavenger receptor CD36 can also mediate the uptake of extracellular isoprenoids (42). The earlier observation that statin treatment resulted in the upregulation of CD36 had already pointed toward a role of CD36 as an isoprenoid scavenger receptor (43) (**Figure 2B**).

Along the same line, add-back experiments, in which mevalonate metabolism of immune (44–46) and cancer cells (35, 47) could be restored by exogenous isoprenoids during drug-induced pathway inhibition, further confirmed the relevance of transcellular mevalonate metabolism. In such experiments, addition of FPP, or more often of geranylgeranyl diphosphate, was able to reinstate protein prenylation during statin or N-BP-mediated inhibition of mevalonate metabolism (48). An intriguing aspect of transcellular mevalonate metabolism is the possibility of crosstalk not only between immune cell subsets but also between immune cells, stromal cells, and cancer cells (49). The outcome of such shared metabolism is currently unclear and certainly deserves reinforced examination.

THERAPEUTIC TARGETING OF MEVALONATE METABOLISM

Training of Metabolic Skills

As outlined above, mevalonate metabolism is crucial for the inflammatory response of M1 macrophages (2, 8). Intriguingly, mevalonate metabolism can apparently be trained for enhanced innate immune responses, for instance by repetitive administration of Bacillus Calmette–Guérin (BCG) (**Figure 2C**). Live attenuated BCG mycobacteria have a long history as a tuberculosis vaccine and as a cancer therapeutic. In fact, treatment with BCG is among the most effective cancer immunotherapies, and in high-risk, non-muscle-invasive bladder cancer, it is still the standard adjuvant treatment according to the European Association of Urology (EAU) guidelines (50, 51). BCG was used in seminal studies by Mackaness, who coined the term macrophage activation (classical activation) in the context of bacterial infection to describe the non-specifically enhanced, microbicidal activity of macrophages toward BCG (and Listeria) upon secondary pathogen exposure (52). The observation that vaccination with BCG also caused non-specific protective effects against non-related infections renewed the interest in this topic and led to the concept of "trained immunity" (53). At the cellular level, a first treatment with BCG resulted in enhanced responsiveness of monocytes and macrophages, which produced higher levels of cytokines and reactive oxygen species upon a secondary stimulation with BCG or even with non-related pathogens. A similar priming effect has been observed with β-glucan, a major component of the *C. albicans* cell wall (54). This increased responsiveness, which represents a form of innate memory, was a consequence of Akt–mTOR-driven metabolic reprogramming in macrophages and, importantly, increased flux through the mevalonate pathway appeared to be prerequisite for the establishment of trained immunity. The relevance of mevalonate metabolism was demonstrated when statins were shown to prevent the generation of trained immunity. This was consistent with the previous clinical observation that statin therapy has been associated with tumor progression leading to radical cystectomy in patients treated for bladder cancer with BCG (55). In addition, RNA sequencing combined with metabolomics revealed upregulation of multiple steps in the cholesterol synthetic pathway (54) (**Figure 1**).

Trained mevalonate metabolism leads to increased cholesterol biosynthesis, improving innate immunity. In addition, cholesterol is critically required for T cell growth and proliferation (6). T cell fitness has recently been demonstrated to specifically depend on high levels of free cholesterol in T cell membranes (56). Cholesterol esterification for storage purposes can therefore limit T cell activity (**Figure 2B**). Conversely, inhibition of the cholesterol esterification enzyme ACAT-1 was able to improve T cell responses and also improved the efficacy of immune checkpoint blockade by anti-CTLA-4 antibody in a mouse melanoma model (57). These findings collectively confirm the importance of mevalonate metabolism for cholesterol biosynthesis in antitumor immunity. Intriguingly, the efficacy of anti-CTLA-4 in mouse melanoma models depended on the microbiota of these mice (58), raising the important question of how the microbiota affects immunometabolism.

Refraining from Undesirable Metabolism

Myc is obviously not only essential for tumor initiation *via* glycolysis-fueled mevalonate metabolism (30) but also for the maintenance of established tumors *via* FAO-driven OXPHOS (15, 59). For instance, triple-negative breast cancer displays a

Myc-driven bioenergetic reliance on FAO (59). In addition, prostate tumors also exhibit low rates of glucose consumption and display increased OXPHOS driven by FAO (60). Particularly, prostate cancer, that becomes refractory to androgen deprivation therapy (61), critically depends on OXPHOS for growth and metastasis (62, 63). Likewise, leukemia (64) and glioblastoma (65) have been shown to require FAO for growth and survival. Consequently, inhibition of FAO has been suggested as a potential therapeutic strategy for this particular subset of breast cancer and possibly also for prostate cancer, glioblastoma, and leukemia.

The FAO inhibitor etomoxir targets carnitine palmitoyltransferase 1 (CPT1), which catalyzes the cytosolic formation of acyl carnitines at the outer mitochondrial membrane for mitochondrial import and subsequent oxidation of FAs (66). The etomoxir-mediated inhibition of FAO-driven OXPHOS decreases ATP levels and thus tumor cell viability and chemoresistance (64, 65). In addition, CPT1 inactivation in cancer cells resulted in increased sensitivity to oxygen and glucose deprivation as well as decreased tumorigenic potential *in vivo* (67). Unfortunately, however, clinical development of etomoxir has been discontinued because of severe liver toxicity. Currently, other inhibitors of CPT1 are clinically tested although not yet for their antitumor potential.

Although c-Abl-specific tyrosine kinase inhibitors (TKIs) substantially extend the survival of patients with chronic myeloid leukemia (CML), TKIs fail to eliminate leukemic stem cells resulting in minimal residual disease. Recently, primitive CML cells were shown to rely on upregulated OXPHOS for their survival, and intriguingly, combination treatment with the TKI imatinib and tigecycline, an antibiotic that inhibits mitochondrial protein translation, selectively eradicated leukemic stem cells both *in vitro* and in a xenotransplantation model of human CML (68).

As outlined above in the context of regulatory immune cells, additional engagement of FAO may divert acetyl-CoA from mevalonate metabolism (**Figures 1** and **2A**). The mevalonate pathway might thus become an Achilles' heel of such tumor types and might therefore be targeted with statins, possibly as an adjuvant therapy preceding primary treatment. Importantly, statins may exhibit a dual effect in such a setting, since they can inhibit tumor growth or survival as well as hold downregulatory immune cells.

CONCLUDING REMARKS

It is now becoming increasingly clear that mevalonate metabolism governs immune surveillance. However, cancer cells and in particular cancer stem cells may likewise depend on this metabolic pathway. Such a similarity in metabolic orientation between tumor cells and immune effector cells infiltrating the tumor microenvironment inevitably leads to a competition for the nutrients, metabolites, and oxygen that are required for fueling mevalonate metabolism and may ultimately even turn into a struggle for survival. While pharmacological inhibition of mevalonate metabolism in tumor cells may attenuate growth and proliferation, tonic flux through the mevalonate pathway in innate immune cells such as macrophages may contribute to trained immunity.

The additional engagement of FAO as it has been described for breast and prostate cancer cells may limit the availability of acetyl-CoA for mevalonate generation and metabolism. As a consequence, immune cells (T_{reg} cells and M2 macrophages) acquire regulatory function and tumor cells may undergo metastasis. Inhibition of FAO therefore appears to be desirable either as the primary therapeutic approach or as an adjuvant preceding cancer immunotherapy. In addition, the limitation of mevalonate pathway activity resulting from enhanced FAO might increase the sensitivity of tumor cells and regulatory immune cells to statins. Future personalized cancer medicine should include the assessment of the metabolic status of the patients' tumor in order to develop the appropriate therapeutic strategies. Sequential therapy regimens might start with inhibitors of mevalonate metabolism and FAO to directly block tumor cells as well as regulatory immune cells, followed by immunotherapies that induce trained immunity in innate immune cells *via* mevalonate pathway stimulation.

AUTHOR CONTRIBUTIONS

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REFERENCES

1. MacIver NJ, Michalek RD, Rathmell JC. Metabolic regulation of T lymphocytes. *Annu Rev Immunol* (2013) 31:259–83. doi:10.1146/annurev-immunol-032712-095956
2. O'Neill LA, Pearce EJ. Immunometabolism governs dendritic cell and macrophage function. *J Exp Med* (2016) 213(1):15–23. doi:10.1084/jem.20151570
3. Chi H. Regulation and function of mTOR signalling in T cell fate decisions. *Nat Rev Immunol* (2012) 12(5):325–38. doi:10.1038/nri3198
4. Porstmann T, Santos CR, Griffiths B, Cully M, Wu M, Leevers S, et al. SREBP activity is regulated by mTORC1 and contributes to Akt-dependent cell growth. *Cell Metab* (2008) 8(3):224–36. doi:10.1016/j.cmet.2008.07.007
5. Bensinger SJ, Bradley MN, Joseph SB, Zelcer N, Janssen EM, Hausner MA, et al. LXR signaling couples sterol metabolism to proliferation in the acquired immune response. *Cell* (2008) 134(1):97–111. doi:10.1016/j.cell.2008.04.052
6. Thurnher M, Gruenbacher G. T lymphocyte regulation by mevalonate metabolism. *Sci Signal* (2015) 8(370):re4. doi:10.1126/scisignal.2005970
7. Sica A, Mantovani A. Macrophage plasticity and polarization: in vivo veritas. *J Clin Invest* (2012) 122(3):787–95. doi:10.1172/JCI59643
8. Na YR, Gu GJ, Jung D, Kim YW, Na J, Woo JS, et al. GM-CSF induces inflammatory macrophages by regulating glycolysis and lipid metabolism. *J Immunol* (2016) 197(10):4101–9. doi:10.4049/jimmunol.1600745
9. Yilmaz A, Reiss C, Tantawi O, Weng A, Stumpf C, Raaz D, et al. HMG-CoA reductase inhibitors suppress maturation of human dendritic cells: new implications for atherosclerosis. *Atherosclerosis* (2004) 172(1):85–93. doi:10.1016/j.atherosclerosis.2003.10.002
10. Everts B, Amiel E, Huang SC, Smith AM, Chang CH, Lam WY, et al. TLR-driven early glycolytic reprogramming via the kinases TBK1-IKKvarepsilon supports the anabolic demands of dendritic cell activation. *Nat Immunol* (2014) 15(4):323–32. doi:10.1038/ni.2833

11. Berwick DC, Hers I, Heesom KJ, Moule SK, Tavare JM. The identification of ATP-citrate lyase as a protein kinase B (Akt) substrate in primary adipocytes. *J Biol Chem* (2002) 277(37):33895–900. doi:10.1074/jbc.M204681200
12. Goldstein JL, Brown MS. Regulation of the mevalonate pathway. *Nature* (1990) 343(6257):425–30. doi:10.1038/343425a0
13. Motulsky AG. The 1985 Nobel Prize in physiology or medicine. *Science* (1986) 231(4734):126–9. doi:10.1126/science.3510453
14. Karmaus PWF, Herrada AA, Guy C, Neale G, Dhungana Y, Long L, et al. Critical roles of mTORC1 signaling and metabolic reprogramming for M-CSF-mediated myelopoiesis. *J Exp Med* (2017) 214(9):2629–47. doi:10.1084/jem.20161855
15. Dang CV. MYC, metabolism, cell growth, and tumorigenesis. *Cold Spring Harb Perspect Med* (2013) 3(8):a014217. doi:10.1101/cshperspect.a014217
16. Fukuzumi M, Shinomiya H, Shimizu Y, Ohishi K, Utsumi S. Endotoxin-induced enhancement of glucose influx into murine peritoneal macrophages via GLUT1. *Infect Immun* (1996) 64(1):108–12.
17. Lee MT, Kaushansky K, Ralph P, Ladner MB. Differential expression of M-CSF, G-CSF, and GM-CSF by human monocytes. *J Leukoc Biol* (1990) 47(3):275–82.
18. Pearce EJ, Everts B. Dendritic cell metabolism. *Nat Rev Immunol* (2015) 15(1):18–29. doi:10.1038/nri3771
19. Rieser C, Ramoner R, Bock G, Deo YM, Holtl L, Bartsch G, et al. Human monocyte-derived dendritic cells produce macrophage colony-stimulating factor: enhancement of c-fms expression by interleukin-10. *Eur J Immunol* (1998) 28(8):2283–8. doi:10.1002/(SICI)1521-4141(199808)28:08<2283::AID-IMMU2283>3.0.CO;2-X
20. Hayes MP, Enterline JC, Gerrard TL, Zoon KC. Regulation of interferon production by human monocytes: requirements for priming for lipopolysaccharide-induced production. *J Leukoc Biol* (1991) 50(2):176–81.
21. Andrejeva G, Rathmell JC. Similarities and distinctions of cancer and immune metabolism in inflammation and tumors. *Cell Metab* (2017) 26(1):49–70. doi:10.1016/j.cmet.2017.06.004
22. Huang SC, Smith AM, Everts B, Colonna M, Pearce EL, Schilling JD, et al. Metabolic reprogramming mediated by the mTORC2-IRF4 signaling axis is essential for macrophage alternative activation. *Immunity* (2016) 45(4):817–30. doi:10.1016/j.immuni.2016.09.016
23. Berod L, Friedrich C, Nandan A, Freitag J, Hagemann S, Harmrolfs K, 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. doi:10.1038/nm.3704
24. Malinarich F, Duan K, Hamid RA, Bijin A, Lin WX, Poidinger M, et al. High mitochondrial respiration and glycolytic capacity represent a metabolic phenotype of human tolerogenic dendritic cells. *J Immunol* (2015) 194(11):5174–86. doi:10.4049/jimmunol.1303316
25. Zeng H, Yang K, Cloer C, Neale G, Vogel P, Chi H. mTORC1 couples immune signals and metabolic programming to establish T(reg)-cell function. *Nature* (2013) 499(7459):485–90. doi:10.1038/nature12297
26. Warburg O, Wind F, Negelein E. The metabolism of tumors in the body. *J Gen Physiol* (1927) 8(6):519–30. doi:10.1085/jgp.8.6.519
27. Gruenbacher G, Thurnher M. Mevalonate metabolism in cancer. *Cancer Lett* (2015) 356(2):192–6. doi:10.1016/j.canlet.2014.01.013
28. Freed-Pastor WA, Mizuno H, Zhao X, Langerod A, Moon SH, Rodriguez-Barrueco R, et al. Mutant p53 disrupts mammary tissue architecture via the mevalonate pathway. *Cell* (2012) 148(1–2):244–58. doi:10.1016/j.cell.2011.12.017
29. Bjarnadottir O, Romero Q, Bendahl PO, Jirstrom K, Ryden L, Loman N, et al. Targeting HMG-CoA reductase with statins in a window-of-opportunity breast cancer trial. *Breast Cancer Res Treat* (2013) 138(2):499–508. doi:10.1007/s10549-013-2473-6
30. Wang X, Huang Z, Wu Q, Prager BC, Mack SC, Yang K, et al. MYC-regulated mevalonate metabolism maintains brain tumor-initiating cells. *Cancer Res* (2017) 77(18):4947–60. doi:10.1158/0008-5472.CAN-17-0114
31. Clendening JW, Pandyra A, Boutros PC, El Ghamrasni S, Khosravi F, Trentin GA, et al. Dysregulation of the mevalonate pathway promotes transformation. *Proc Natl Acad Sci U S A* (2010) 107(34):15051–6. doi:10.1073/pnas.0910258107
32. Morandi A, Taddei ML, Chiarugi P, Giannoni E. Targeting the metabolic reprogramming that controls epithelial-to-mesenchymal transition in aggressive tumors. *Front Oncol* (2017) 7:40. doi:10.3389/fonc.2017.00040
33. Capra V, Rovati GE, Mangano P, Buccellati C, Murphy RC, Sala A. Transcellular biosynthesis of eicosanoid lipid mediators. *Biochim Biophys Acta* (2015) 1851(4):377–82. doi:10.1016/j.bbalip.2014.09.002
34. Guijas C, Perez-Chacon G, Astudillo AM, Rubio JM, Gil-de-Gomez L, Balboa MA, et al. Simultaneous activation of p38 and JNK by arachidonic acid stimulates the cytosolic phospholipase A2-dependent synthesis of lipid droplets in human monocytes. *J Lipid Res* (2012) 53(11):2343–54. doi:10.1194/jlr.M028423
35. Onono F, Subramanian T, Sunkara M, Subramanian KL, Spielmann HP, Morris AJ. Efficient use of exogenous isoprenols for protein isoprenylation by MDA-MB-231 cells is regulated independently of the mevalonate pathway. *J Biol Chem* (2013) 288(38):27444–55. doi:10.1074/jbc.M113.482307
36. Castella B, Kopecka J, Sciancalepore P, Mandili G, Foglietta M, Mitro N, et al. The ATP-binding cassette transporter A1 regulates phosphoantigen release and Vgamma9Vdelta2 T cell activation by dendritic cells. *Nat Commun* (2017) 8:15663. doi:10.1038/ncomms15663
37. Harly C, Guillaume Y, Nedellec S, Peigne CM, Monkkonen H, Monkkonen J, et al. Key implication of CD277/butyrophilin-3 (BTN3A) in cellular stress sensing by a major human gammadelta T-cell subset. *Blood* (2012) 120(11):2269–79. doi:10.1182/blood-2012-05-430470
38. Vavassori S, Kumar A, Wan GS, Ramanjaneyulu GS, Cavallari M, El Daker S, et al. Butyrophilin 3A1 binds phosphorylated antigens and stimulates human gammadelta T cells. *Nat Immunol* (2013) 14(9):908–16. doi:10.1038/ni.2665
39. Silva-Santos B, Serre K, Norell H. gammadelta T cells in cancer. *Nat Rev Immunol* (2015) 15(11):683–91. doi:10.1038/nri3904
40. Sandstrom A, Peigne CM, Leger A, Crooks JE, Konczak F, Gesnel MC, et al. The intracellular B30.2 domain of butyrophilin 3A1 binds phosphoantigens to mediate activation of human Vgamma9Vdelta2 T cells. *Immunity* (2014) 40(4):490–500. doi:10.1016/j.immuni.2014.03.003
41. Gruenbacher G, Gander H, Rahm A, Idzko M, Nussbaumer O, Thurnher M. Ecto-ATPase CD39 inactivates isoprenoid-derived Vgamma9Vdelta2 T cell phosphoantigens. *Cell Rep* (2016) 16(2):444–56. doi:10.1016/j.celrep.2016.06.009
42. Anderson CM, Kazantzis M, Wang J, Venkatraman S, Goncalves RL, Quinlan CL, et al. Dependence of brown adipose tissue function on CD36-mediated coenzyme Q uptake. *Cell Rep* (2015) 10(4):505–15. doi:10.1016/j.celrep.2014.12.048
43. Ruiz-Velasco N, Dominguez A, Vega MA. Statins upregulate CD36 expression in human monocytes, an effect strengthened when combined with PPAR-gamma ligands putative contribution of Rho GTPases in statin-induced CD36 expression. *Biochem Pharmacol* (2004) 67(2):303–13. doi:10.1016/j.bcp.2003.09.006
44. Gruenbacher G, Gander H, Nussbaumer O, Nussbaumer W, Rahm A, Thurnher M. IL-2 costimulation enables statin-mediated activation of human NK cells, preferentially through a mechanism involving CD56+ dendritic cells. *Cancer Res* (2010) 70(23):9611–20. doi:10.1158/0008-5472.CAN-10-1968
45. Nussbaumer O, Gruenbacher G, Gander H, Komuczki J, Rahm A, Thurnher M. Essential requirements of zoledronate-induced cytokine and gammadelta T cell proliferative responses. *J Immunol* (2013) 191(3):1346–55. doi:10.4049/jimmunol.1300603
46. Nussbaumer O, Gruenbacher G, Gander H, Thurnher M. DC-like cell-dependent activation of human natural killer cells by the bisphosphonate zoledronic acid is regulated by gammadelta T lymphocytes. *Blood* (2011) 118(10):2743–51. doi:10.1182/blood-2011-01-328526
47. van de Donk NW, Kamphuis MM, van Kessel B, Lokhorst HM, Bloem AC. Inhibition of protein geranylgeranylation induces apoptosis in myeloma plasma cells by reducing Mcl-1 protein levels. *Blood* (2003) 102(9):3354–62. doi:10.1182/blood-2003-03-0970
48. Thurnher M, Nussbaumer O, Gruenbacher G. Novel aspects of mevalonate pathway inhibitors as antitumor agents. *Clin Cancer Res* (2012) 18(13):3524–31. doi:10.1158/1078-0432.CCR-12-0489
49. Comito G, Pons Segura C, Taddei ML, Lanciotti M, Serni S, Morandi A, et al. Zoledronic acid impairs stromal reactivity by inhibiting M2-macrophages polarization and prostate cancer-associated fibroblasts. *Oncotarget* (2017) 8(1):118–32. doi:10.18632/oncotarget.9497
50. Babjuk M, Burger M, Zigeuner R, Shariat SF, van Rhijn BW, Comperat E, et al. EAU guidelines on non-muscle-invasive urothelial carcinoma of the bladder: update 2013. *Eur Urol* (2013) 64(4):639–53. doi:10.1016/j.eururo.2013.06.003
51. Pichler R, Gruenbacher G, Culig Z, Brunner A, Fuchs D, Fritz J, et al. Intratumoral Th2 predisposition combines with an increased Th1 functional phenotype in clinical response to intravesical BCG in bladder cancer. *Cancer Immunol Immunother* (2017) 66(4):427–40. doi:10.1007/s00262-016-1945-z

52. Mackaness GB. Cellular resistance to infection. *J Exp Med* (1962) 116:381–406. doi:10.1084/jem.116.3.381
53. Netea MG, van der Meer JW. Trained immunity: an ancient way of remembering. *Cell Host Microbe* (2017) 21(3):297–300. doi:10.1016/j.chom.2017.02.003
54. Arts RJ, Novakovic B, Ter Horst R, Carvalho A, Bekkering S, Lachmandas E, et al. Glutaminolysis and fumarate accumulation integrate immunometabolic and epigenetic programs in trained immunity. *Cell Metab* (2016) 24(6):807–19. doi:10.1016/j.cmet.2016.10.008
55. Hoffmann P, Roumeguere T, Schulman C, van Velthoven R. Use of statins and outcome of BCG treatment for bladder cancer. *N Engl J Med* (2006) 355(25):2705–7. doi:10.1056/NEJMc062714
56. Kidani Y, Bensinger SJ. Modulating cholesterol homeostasis to build a better T cell. *Cell Metab* (2016) 23(6):963–4. doi:10.1016/j.cmet.2016.05.015
57. Yang W, Bai Y, Xiong Y, Zhang J, Chen S, Zheng X, et al. Potentiating the antitumour response of CD8(+) T cells by modulating cholesterol metabolism. *Nature* (2016) 531(7596):651–5. doi:10.1038/nature17412
58. Vetizou M, Pitt JM, Daillere R, Lepage P, Waldschmitt N, Flament C, et al. Anticancer immunotherapy by CTLA-4 blockade relies on the gut microbiota. *Science* (2015) 350(6264):1079–84. doi:10.1126/science.aad1329
59. Camarda R, Zhou AY, Kohnz RA, Balakrishnan S, Mahieu C, Anderton B, et al. Inhibition of fatty acid oxidation as a therapy for MYC-overexpressing triple-negative breast cancer. *Nat Med* (2016) 22(4):427–32. doi:10.1038/nm.4055
60. Zaidi N, Lupien L, Kuemmerle NB, Kinlaw WB, Swinnen JV, Smans K. Lipogenesis and lipolysis: the pathways exploited by the cancer cells to acquire fatty acids. *Prog Lipid Res* (2013) 52(4):585–9. doi:10.1016/j.plipres.2013.08.005
61. Sun Y, Wang BE, Leong KG, Yue P, Li L, Jhunjhunwala S, et al. Androgen deprivation causes epithelial-mesenchymal transition in the prostate: implications for androgen-deprivation therapy. *Cancer Res* (2012) 72(2):527–36. doi:10.1158/0008-5472.CAN-11-3004
62. Liu Y. Fatty acid oxidation is a dominant bioenergetic pathway in prostate cancer. *Prostate Cancer Prostatic Dis* (2006) 9(3):230–4. doi:10.1038/sj.pcan.4500879
63. Giannoni E, Taddei ML, Morandi A, Comito G, Calvani M, Bianchini F, et al. Targeting stromal-induced pyruvate kinase M2 nuclear translocation impairs oxphos and prostate cancer metastatic spread. *Oncotarget* (2015) 6(27):24061–74. doi:10.18632/oncotarget.4448
64. Samudio I, Harmancey R, Fiegl M, Kantarjian H, Konopleva M, Korchin B, et al. Pharmacologic inhibition of fatty acid oxidation sensitizes human leukemia cells to apoptosis induction. *J Clin Invest* (2010) 120(1):142–56. doi:10.1172/JCI38942
65. Pike LS, Smift AL, Croteau NJ, Ferrick DA, Wu M. Inhibition of fatty acid oxidation by etomoxir impairs NADPH production and increases reactive oxygen species resulting in ATP depletion and cell death in human glioblastoma cells. *Biochim Biophys Acta* (2011) 1807(6):726–34. doi:10.1016/j.bbabio.2010.10.022
66. Galluzzi L, Kepp O, Vander Heiden MG, Kroemer G. Metabolic targets for cancer therapy. *Nat Rev Drug Discov* (2013) 12(11):829–46. doi:10.1038/nrd4145
67. Zaugg K, Yao Y, Reilly PT, Kannan K, Kiarash R, Mason J, et al. Carnitine palmitoyltransferase 1C promotes cell survival and tumor growth under conditions of metabolic stress. *Genes Dev* (2011) 25(10):1041–51. doi:10.1101/gad.1987211
68. Kuntz EM, Baquero P, Michie AM, Dunn K, Tardito S, Holyoake TL, et al. Targeting mitochondrial oxidative phosphorylation eradicates therapy-resistant chronic myeloid leukemia stem cells. *Nat Med* (2017) 23(10):1234–40. doi:10.1038/nm.4399

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REVIEW
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The Aryl Hydrocarbon Receptor and Tumor Immunity

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The aryl hydrocarbon receptor (AhR) is an important cytosolic, ligand-dependent transcription factor. Emerging evidence suggests the promoting role of the AhR in the initiation, promotion, progression, invasion, and metastasis of cancer cells. Studies on various tumor types and tumor cell lines have shown high AhR expression, suggesting that AhR is activated constitutively in tumors and facilitates their growth. Interestingly, immune evasion has been recognized as an emerging hallmark feature of cancer. A connection between the AhR and immune system has been recognized, which has been suggested as an immunosuppressive effector on different types of immune cells. Certain cancers can escape immune recognition *via* AhR signaling pathways. This review discusses the role of the AhR in tumor immunity and its potential mechanism of action in the tumor microenvironment.

Keywords: aryl hydrocarbon receptor, tumor immunity, tumor development, immune surveillance, cancer immunotherapy

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INTRODUCTION

In 2011, Hanahan and Weinberg proposed eight hallmarks of cancer: self-sufficiency in growth signals; blockade of antigrowth signals; limitless replicative potency; sustained angiogenesis; anti-apoptosis; metabolic reprogramming; tumor infiltration and metastasis; and evasion of the immune system (1). Increasing evidence suggests that the development and progression of cancer cells result from a cancer-induced immunosuppressive situation, one that the immune system cannot recognize. "Immune evasion" is an emerging hallmark feature of cancer (2).

The aryl hydrocarbon receptor (AhR) is an important cytosolic, ligand-activated receptor expressed in various mammals (3, 4). This receptor was studied first as a receptor to the exogenous ligand 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) (5). A connection between the AhR and the immune system had been recognized through a pathway reacting with TCDD, which had been reported to be an immunosuppressive effector on T cells and dendritic cells (DCs) in animals and humans (6).

In 2005, Funatake and colleagues hypothesized that regulatory T cells can be generated through an AhR-dependent mechanism (7). Some studies showed that AhR-deficient mice are prone to autoimmunity (8, 9), whereas AhR-responsive mouse strains with constitutive expression of the AhR have been shown to be more susceptible to developing malignancies (10). Some studies suggested that several types of human cancer cells showed higher numbers of copies of the AhR than normal cells (11). The potential function of the AhR in carcinogenesis in different types of cancer has been explored for several years (12, 13). The AhR may affect the proliferation, tissue invasion, metastasis, and angiogenesis of cancer cells. In addition, certain cancer types can escape from immune recognition *via* an AhR pathway, as shown in malignant gliomas by Opitz and colleagues (14). A tumor-promoting role of the AhR as well as its function in the immune system have been recognized. However, studies on the role of the AhR in tumor immunity are scarce.

Here, we present a brief overview of recent investigations on the role of the AhR and potential mechanism of action (MoA) in tumor immunity. We hope our review serves as a "roadmap" to guide future studies and even future therapeutic perspectives for malignancies.

BACKGROUND OF THE AhR

Fundamental Information of the AhR

The AhR belongs to basic helix–loop–helix/Per-ARNT-Sim (bHLH-PAS) transcription factor families (5). Poland and Knutson stated that TCDD, benzo(a)pyrene, and polycyclic aromatic hydrocarbons (PAHs) exert their biologic actions by binding directly to the AhR, a cytosolic receptor (15). The AhR is a unique member of the bHLH-PAS family known to be in an activated state by integrating with exogenous or endogenous ligands (16, 17).

The functional structure of the AhR protein comprises three parts: the bHLH motif, the PAS domains, and a Q-rich domain. The basic domain of the bHLH motif is located at the N-terminal region of the AhR protein. The latter binds the AhR to the promoter region of target genes at consistent regulatory sequences termed "aryl hydrocarbon response elements" (AHREs), as well as at dioxin-response elements (DREs). The PAS domains help the formation of a heterozygous protein complex by connecting with the AhR nuclear translocator (ARNT) and binding with the ligand. At the C-terminal region of the protein is a Q-rich domain that affects the recruitment and transcriptional activation of the motif (**Figure 1**).

In the absence of ligands, the AhR is located in the cytoplasm as one part of a protein complex comprising heat shock protein 90, p23, and AhR-interacting protein (18–20). Upon binding to ligands such as TCDD, 6-formylindolo[3,2-b]carbazole (FICZ), kynurenine, or 2-(1′H-indole-3′-carbonyl)-thiazole-4-carboxylic acid methyl ester (ITE), the AhR complex is activated. This action is followed by translocation to the nucleus, release from chaperone proteins, and interaction with ARNT. The chaperone proteins can protect the AhR from proteolysis and retain a propitious construction for ligand binding (21). The AhR–ARNT heterodimer correlates with signaling factors (e.g., chromatin remodeling factors, histone acetyltransferases, and transcriptional factors) and finally binds to DREs or AHREs to promote transcriptional regulation (22, 23). Classical AhR target genes include cytochrome P450 (Cyp)1a1, Cyp1a2, Cyp1b1, and AhR repressor (**Figure 2**).

The AhR is distributed in almost all tissues in humans and expressed abundantly in the placenta, liver, and lungs (24, 25). The AhR can be activated in epithelial cells, Langerhans cells, microglias, T cells, B cells, natural killer (NK) cells, DCs, and macrophages (26–32).

AhR Ligands

The AhR is activated or inhibited by various types of exogenous and endogenous ligands that bind to it. Different types of ligand interactions with the AhR protein result in different effects (33).

Exogenous/Xenobiotic Ligands

The best-characterized high-affinity exogenous/xenobiotic ligands for the AhR are environmental contaminants such as halogenated aromatic hydrocarbons, polychlorinated biphenyls, and PAHs. A well-known prototypic exogenous ligand for the AhR is TCDD, an environmental pollutant with high toxicity. TCDD is a specific epigenetic carcinogen and a potential tumor promoter (12, 34). Exposure to TCDD can produce diverse specific toxic (immunotoxicity, hepatotoxicity, tumor promotion, cardiotoxicity, reproductive toxicity, dermal toxicity, teratogenesis, wasting syndrome, lethality, and endocrine disruption) and biologic effects (35). A rich body of evidence (*in vivo* and *in vitro*) supports these phenomena. $AhR^{(-/-)}$ mice are not sensitive to the toxic activities of TCDD or TCDD-like toxicants (36–38).

Endogenous Ligands

It is reasonable to suspect that endogenous ligands must exist for the AhR because it can be activated in some cell types without an exogenous ligand being present (39). Different types of endogenous ligands have been isolated from mammalian tissues, such as indigo and indirubin from human urinary products (40), ITE from the lungs (39), kynurenine and kynurenic acid from the brain (41), and others such as equilenin, arachidonic acid metabolites, and FICZ (42).

Almost all of the endogenous/natural ligands that depend on DRE have been proposed to be AhR agonists. Indigo and indirubin compete for receptor occupancy with TCDD and upregulate the activity of Cyp1a1 monooxygenase in human hepatoma cell lines and in rodent models (43, 44). Equilenin is an estrogen produced by pregnant mares and has been recognized as an AhR

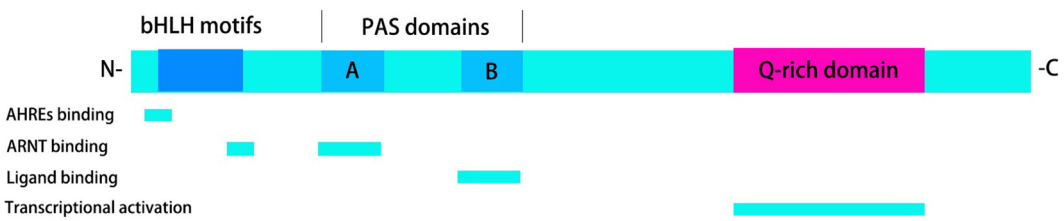

FIGURE 1 | Functional structure of the aryl hydrocarbon receptor (AhR). The functional structure of the AhR protein consists of three parts: the basic helix–loop–helix (bHLH) motifs, the Per-ARNT-Sim (PAS) domains, and a Q-rich domain. bHLH motifs are involved in the activity of aryl hydrocarbon response elements (AHREs) binding and AhR nuclear translocator (ARNT) binding. PAS domains are required for ARNT binding and ligand binding. Transcriptional activation can be observed in Q-rich domain.

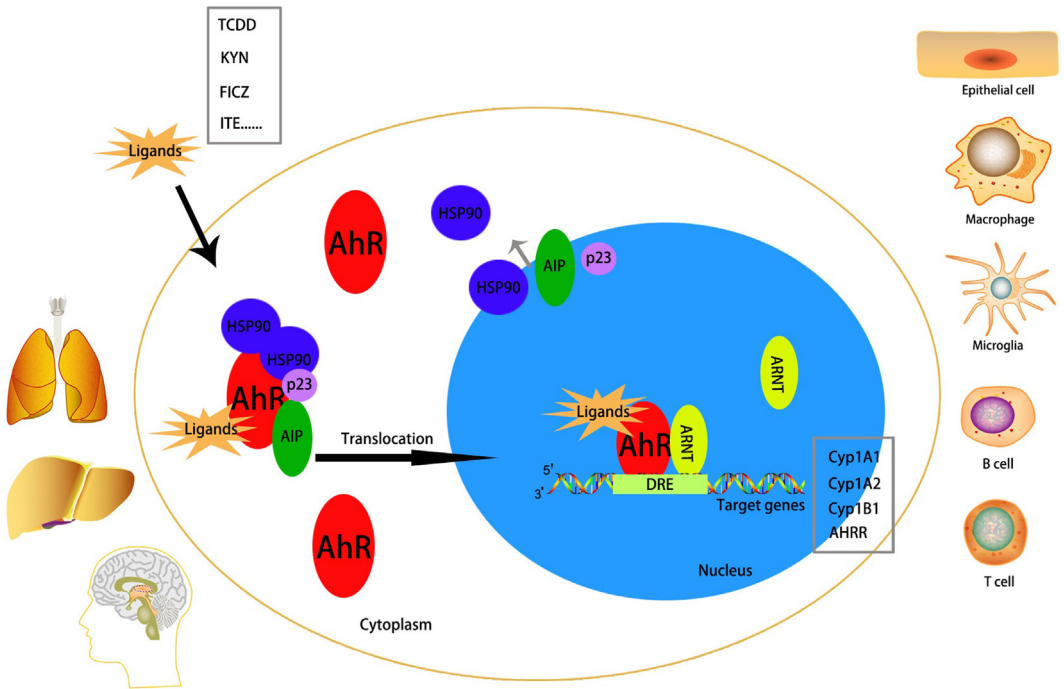

FIGURE 2 | Mechanism of activation of the aryl hydrocarbon receptor (AhR). The AhR is abundantly expressed in lung, liver, and brain. It can be activated in many cell types, including epithelial cell, microglia, macrophage, B cell, T cell, etc. Without a ligand, AhR is inactivated in the cytoplasm as a part of a complex with heat shock protein (HSP)90, AhR-interacting protein (AIP), and p23. After binding with an exo/endogenous ligand, the AhR will be activated and translocates to the nucleus to interact with AhR nuclear translocator (ARNT) and simultaneously detaches from the complex. The AhR/ARNT heterodimer finally binds to the dioxin-response elements (DREs), which is called the promoter region of target genes [classical target genes include cytochrome P450 (Cyp)1a1, Cyp1a2, Cyp1b1, and AHRR], to promote transcriptional activation.

agonist. Equilenin has been studied in human HepG2 cells; a half-maximal response (EC_{50}) of 30 μM of equilenin can produce a considerable increase in expression of Cyp1a1 mRNA and DRE-mediated reporter activity (45). Potential endogenous AhR ligands from metabolites of arachidonic acid include lipoxin A4 and prostaglandins (PGs). AhR activation by lipoxin A4 induces expression of Cyp1a1 and Cyp1a2 monooxygenases, and lipoxin A4 also serves as a substrate of these enzymes. This phenomenon has been shown in mouse hepatoma cells (46). Furthermore, PG-G2 function has been examined in a murine hepatoma cell line by dose–response assays, indicating that it can induce DRE-dependent transcription with a higher EC_{50} than that elicited by lipoxin A4. PG-G2 may be a weak ligand of the AhR (47). Moreover, heme metabolites could be candidate endogenous ligands for the AhR, of which bilirubin has been suggested to be the most important. Sinal and Bend demonstrated that the gene expression and enzymatic activity of Cyp1a1 can be modulated directly by bilirubin *via* an AhR pathway in mouse hepatoma cells (48). Among the endogenous ligands mentioned earlier, ITE and kynurenine have garnered more attention from immunologists and oncologists in recent years.

2-(1′H-Indole-3′-Carbonyl)-Thiazole-4-Carboxylic Acid Methyl Ester

2-(1′H-Indole-3′-carbonyl)-thiazole-4-carboxylic acid methyl ester (ITE) was extracted from porcine lung tissues and shown to be an agonist of the AhR by Song and colleagues in 2002 (39). They extracted and purified ITE by ultraviolet spectroscopy, electron-impact mass spectrometry, Fourier-transform infrared spectroscopy, and proton nuclear magnetic resonance spectroscopy. Upon exposure to ITE, the AhR binds to the DRE domain and induces increased expression of Cyp1a1 mRNA and DRE-dependent reporter activity, showing that ITE is a ligand and agonist of the AhR. Competitive-binding studies and experiments based on sucrose gradient sedimentation have suggested that ITE competes with TCDD for binding with the AhR from human, murine, killifish, and zebrafish. Its binding affinity (K_i) to the AhR (3 nM) has been shown to be slightly lower than that of the classical AhR ligand TCDD ($K_i = 0.5$ nM).

The biologic function of ITE in the immune system has been studied. Quintana and colleagues showed that the progression of experimental autoimmune encephalomyelitis (EAE) is inhibited effectively by ITE treatment *in vivo* and that ITE acts on DCs and T cells through binding with the AhR (49). Nugent LF et al. also studied ITE for its capacity to suppress the development of experimental autoimmune uveitis (EAU) and relevant immune responses. They showed that ITE can suppress EAU development and immune-cell responses against the uveitogenic antigen, reduce the proportion of cells expressing interleukin (IL)-10, interferon (IFN)-γ, and IL-17, and increase the proportion of forkhead box P3 (Foxp3)$^+$ cells (50). Kai et al. suggested that the AhR is expressed abnormally in different histotypes in human

ovarian cancers and that ITE inhibits the proliferation and migration of OVCAR-3 and SKOV-3 cells through an AhR pathway. Unlike TCDD, ITE is hypotoxic or even non-toxic *in vitro* and *in vivo*, so ITE could be developed as a potent immunosuppressant agent for the treatment of immune diseases and ovarian cancer (51, 52).

Kynurenine

The tryptophan metabolites kynurenine, FICZ, and kynurenic acid have been described as natural endogenous AhR ligands that mediate immunosuppressive functions. Kynurenine appears to be the most intriguing tryptophan metabolite in several types of cancers. In numerous cell types, most of the tryptophan is metabolized *via* a kynurenine pathway (53, 54). Kynurenine triggers nuclear translocation of the AhR, thereby enabling activation of its target genes. Indoleamine-2,3-dioxygenase (IDO)1, IDO2, and tryptophan-2,3-dioxygenase 2 (TDO-2) (55) have been shown to be the significant rate-limiting enzymes metabolizing tryptophan to kynurenine. The expression of IDO1 and TDO-2 has been shown to be controlled by the AhR. Such enzymatic activity leads to the exhaustion of tryptophan in the local microenvironment, suppression of antigen-specific T-cell responses, and promotion of the differentiation of T regulatory (Treg) cells during tumor development (56). Emerging evidence suggests that increased expression of IDO in many types of cancers is accompanied with immune escape and cancer-associated inflammation in their microenvironment (57). Opitz et al. demonstrated that kynurenine derived from TDO-2-mediated tryptophan metabolites can inhibit antitumor immune responses and promote the survival and motility of tumor cells in an autocrine, AhR-dependent manner (14).

THE AhR AND THE TUMOR MICROENVIRONMENT

The physiological effects of AhR activation have been suggested to have significant roles in immune modulation and carcinogenesis. The AhR is expressed at high levels and is chronically active in blood tumors (58, 59), such as T-cell leukemia (60) and lymphoma (61), as well as in solid tumors such as glioblastoma (14), ovarian cancer (51), lung cancer (62), liver cancer (63), and head and neck carcinomas (58). Murray et al. suggested that detection of AhR activity in the microenvironment can serve as a potent diagnostic indicator for tumor aggressiveness (64). Depending on the cancer type, two types of results are associated with AhR activity and the prognosis. Saito et al. indicated that, in hormone-dependent breast cancers, AhR activation is associated with attenuated aggressiveness and a better prognosis (65). However, higher AhR activity has been suggested as being correlated with increased aggressiveness and a poor prognosis in non-small-cell lung cancer (66).

The AhR and Tumor Development

Strong evidence suggests that constitutively high AhR expression and nuclear localization can be observed in invasive tumor tissues and malignant tumor cell lines (67, 68). The AhR may have important roles in various stages of tumorigenesis owing to its involvement in the inflammatory response and cell-cycle progression (64, 69, 70). The underlying MoA of the AhR in cancers was reviewed in detail by Feng and colleagues (17). With regard to abnormal activation of the AhR with exogenous/endogenous stimulation, certain physiological and pathological processes are disturbed: the proliferation and differentiation of cells, apoptosis, extracellular matrix (ECM) remodeling, angiogenesis, metabolism, and survival. In this way, expression of the target genes is not regulated, and malignant tumors are formed.

The AhR has been suggested to affect cell proliferation in different tumor models and cancer cell lines. In the Hepa1c1c7 cell line, an AhR-defective variant showed delayed progression through the G1 phase in comparison with a wild-type counterpart (71). Studies in a human adenocarcinoma (A549) cell line revealed that DNA binding with the AhR was necessary for the cell cycle and that interaction with an AhR agonist could transform the AhR to its DNA-binding form that stimulated the growth of cancer cells (72). Another study using flow cytometry found that, in AhR-overexpressing cancer cells, 10% were in the S phase and none were in the G2/M phase and that increased expression of transcription factors, replication factors as well as proliferation of cell-nucleus antigens was observed. These results suggested that the AhR can promote proliferation of malignant tumor cells (73). The AhR can also induce cell-cycle arrest. TCDD shows its suppressive effects on gastric cancer cells, breast cancer cells, and retinoblastoma cells to induce growth arrest at the G1-S phase, which can be modulated by persistent phosphorylation of the retinoblastoma tumor suppressor protein *via* cluster of differentiation (CD) K4/6 complexes (74, 75).

Tissue invasion and metastasis are hallmarks of aggressive malignancies. Loss of cell–cell contact triggers the progression and promotion of tumor cells. Increased expression of the AhR is associated with deregulation of cell–cell contact and tumor malignancy. For example, after exposure to the xenobiotic ligand TCDD, cell–cell contact is destroyed, and cell migration and epithelial–mesenchymal transformation (EMT) induced *via* a c-Jun N-terminal kinase-dependent pathway and loss of E-cadherin expression (13). Owens et al. suggested that dissociation of sarcoma (Src) kinase from the AhR complex disrupts cadherin-dependent cell–cell contact (76). Hence, the AhR can reduce cell–cell contact and adhesion and increase the motility and invasiveness of cancer cells, which finally results in the invasion and metastasis of cancer cells.

Another key element involved in the pathogenesis and metastasis of tumor cells is the ECM. Studies have shown that ECM remodulation-associated proteolytic enzymes such as cathepsins (77), urokinase plasminogen activator (uPA) (78), and matrix metalloproteinases (MMPs) (79) are intriguing components of an AhR pathway. Son and Rozman showed, in mouse hepatoma cells, that AhR activation by ligand binding induced expression of uPA protease (80). Similar results have been demonstrated in the studies of Villano and colleagues (81) and Haque and coworkers (82), suggesting that activation of an AhR pathway can enhance MMP expression and result in tumor invasiveness.

Angiogenesis is the physiological process through which new blood vessels form from pre-existing vessels. Angiogenesis and the provision of nutrients and oxygen to support the proliferation

of cancer cells also have roles in the aggressiveness and metastasis of tumor cells. During this period, AhR–ARNT heterodimers interact with hypoxia-inducible factor (HIF)-1α to counteract oxygen deprivation and simultaneously upregulate the expression of HIF-1, IL-8, and vascular endothelial growth factor (VEGF) and downregulate expression of transforming growth factor (TGF)-β (83, 84). Angiogenesis is impaired in $AhR^{(-/-)}$ endothelial cells and in AhR-null mice as shown in experiments involving aortic rings. However, this situation can be rescued by VEGF addition. Furthermore, addition of anti-VEGF or knocking out of VEGF in $AHR^{(+/+)}$ cell types results in a reduction of angiogenesis. Experiments on TGF-β in stroma cells elicit the opposite result. The data mentioned earlier suggest that the AhR could be an intriguing regulator and potential therapeutic target for angiogenesis and metastasis during tumor development.

Tumor suppressors regulate the orientation of tumor cells to proliferation or to senescence and apoptosis. Among numerous tumor suppressors, p53 protein shows an obvious interaction with the AhR according to experiments *in vitro and in vivo* (85, 86). In the HepG2 cell line, exposure to TCDD and hypoxia result in inhibition of p53 expression and activation *via* a pathway involving estrogen receptor (ER)α and human double minute-2 and, finally, promotion of tumor progression (67, 87). Furthermore, the AhR has also been reported to affect cancer stem cells and crosstalk with an ER- and inflammatory factor-associated signaling pathway in the pathologic phase of carcinogenesis (88, 89).

Inflammation is also a common feature of tumors. Studies have shown an interaction between AhR activation and expression of inflammatory signaling molecules such as IL-6, IL-10, TGF-β, VEGF-A, signal transducer and activator of transcription (STAT) 6, and nuclear factor-kappa B (NF-κB) (90–93). Kolasa and colleagues found, in a human breast cancer cell (MCF-7) line, that simultaneous exposure to environmental PAHs and tumor necrosis factor (TNF)-α induced increased expression of IL-6 and that this effect could be counteracted by silencing the AhR, implying that AhR may have a key role in IL-6 regulation within the tumor microenvironment (93). The MoA was suggested to be driven by occupancy of AhR–ARNT complexes in DREs, which mediated displacement of histone deacetylase-1 with the IL-6 promoter and subsequently acetylated NF-κB. Dinatale and colleagues presented similar results in head and neck squamous cell carcinoma (HNSCC) lines (58). In the presence of lipopolysaccharide (LPS) in bone marrow dendritic cells (BMDCs), secretion of IL-6, IL-10, and IL-22 has been shown to be regulated through AhR activation (94). Furthermore, the AhR has been shown to bind to NF-κB subunit RelB and that interaction of RelB and AhR in the breast cancer cell lines MCF-7 and MDA-MB-436 induced IL-8 expression (95). John and coworkers observed, in an inflammatory environment in HNSCC lines, that the AhR was likely to regulate the expression or function of several growth factors directly (96). The research mentioned earlier suggests that AhR activation may contribute to inflammatory signaling within a tumor microenvironment through multiple MoAs.

The AhR has been suggested to be a promoter for the initiation and progression of tumor cells, but this view is controversial. Several studies have demonstrated that the AhR may be a tumor suppressor under certain circumstances (74, 97). Wang et al. reported that ITE inhibited the proliferation and migration of ovarian cancer cells *in vitro* and in mice models through the AhR pathway. They also found that TCDD could suppress the proliferation of cancer cells in an AhR-dependent manner at a certain dose (51). Iida and colleagues suggested that N-nitrosobutyl(4-hydroxybutyl)amine suppressed the AhR signaling pathway and finally induced bladder cancer (98).

The AhR and the Immune System

The AhR has been reported to take part in the modulation of innate immunity and adaptive immunity, which may be involved in tumorigenesis and tumor immune surveillance.

The AhR and Innate Immunity

The AhR and NK Cells

Natural killer cells are important components of the innate immune system and contribute substantially to antitumor immune responses. Many aspects of the biology of NK cells have been shown to be tightly linked with immune surveillance (99). Upon activation, NK cells can "wipe out" tumor cells by: (i) recognizing tumor-induced immune-activating ligands on host cells *via* receptor activation; (ii) responding to tumor cells without the major histocompatibility complex or other immune-suppressive ligands; (iii) activating cytokines secreted by tumor cells or tumor cell-stimulated immune cells; and (iv) interacting with tumor-infiltrating immune cells such as DCs and macrophages (100).

Increasingly, the AhR has been shown to regulate subsets of immune cells with regard to differentiation and activation *via* cytokine stimulation. However, studies focusing on the relationship between the AhR and NK cells are scarce. Emerging evidence supports a role for NK cells in tumor surveillance (101). Shin and colleagues showed, using *in vivo* experiments, that the cytolytic activity and capacity to suppress formation of RMA-S tumors of NK cells is impaired in the absence of the AhR. AhR activation with the endogenous ligand FICZ can potentiate NK cells to increase IFN-γ secretion and simultaneously enhance cytolytic activity and antitumor activity in an NK cell-dependent manner (100). Wagage and colleagues suggested that AhR activation in NK cells is required for IL-10 production. They isolated NK cells from *Toxoplasma gondii*-infected $AhR^{(-/-)}$ mice and found that IL-10 secretion was impaired and associated with increased resistance to such infection (32). Zhang et al. demonstrated that liver-resident NK cells expressed the AhR constitutively and that in $AhR^{(-/-)}$ mice *in vivo*, deficiency of the AhR in NK cells resulted in increased susceptibility to cytokine-induced cell death (102). These data suggest that the AhR may affect NK cells *via* inflammatory signaling pathways to induce tumor development and immune surveillance.

The AhR and Macrophages

Macrophages induce innate immune responses to pathogens through toll-like receptors. Tumor-associated macrophages (TAMs) are critical components of the tumor microenvironment. Masuda et al. demonstrated that interaction between the AhR and STAT1 negatively regulated IL-6 production by inhibiting NF-κB activation and that AhR–specific protein 1

complexes suppress histamine production in macrophages upon LPS stimulation (28). Climaco-Arvizu et al. showed that the AhR can affect the balance between the inflammatory M1 phenotype and anti-inflammatory M2 phenotype by comparing AhR-null mice with wild-type mice: the AhR gene altered macrophage polarization. Activated M2 macrophages have been regarded as being pro-tumor phenotypes in many tumor types (103). Yeung et al. suggested that M2 macrophages contributed to a poor prognosis in hepatocellular carcinoma (HCC) and promoted tumor invasiveness through C–C motif chemokine 22-induced EMT (104). Partecke et al. showed M2 macrophages to be promoters of tumor growth in pancreatic cancer. C57BL/6 mice were injected orthotopically with murine pancreatic cancer cells (6606PDA), and macrophages were depleted by clodronate liposomes. Treatment with M2 macrophages induced tumor growth (105). Zhang et al. investigated the role of M2 macrophages in the progression of colon cancer and found that M2 macrophage-conditioned medium induced the migration of SW480 cells and CD47 expression (106). However, the role of the AhR in TAMs has not been explored. Taken together, these data suggest that the AhR affects tumor development and immune responses within tumor environments *via* TAMs.

The AhR and DCs

Considerable attention has been paid recently to the immunoregulatory role of the AhR in DCs. Nguyen et al. showed that LPS and CpG oligonucleotides stimulated BMDCs to express the AhR. $AhR^{(-/-)}$ mature BMDCs induced immune responses with a reduction in expression of kynurenine and IL-10 by treatment with LPS or CpG compared with mature wild-type BMDCs. Upon coculture with BMDCs and naïve T cells, differentiation from naïve T cells to Treg cells was found to be inhibited in $AhR^{(-/-)}$ mature BMDCs. Treatment with L-kynurenine to the system stated earlier rescued this situation. Nguyen et al. concluded that the AhR regulated DC immunogenicity negatively *via* a kynurenine-dependent MoA (107). Thatcher et al. demonstrated that AhR-knockout mice developed intense allergic responses to the allergen ovalbumin with increased activation of DCs. Deficiency of the AhR resulted in enhanced activation of T cells by pulmonary DCs and intense pro-inflammatory allergic responses (108). Wang and colleagues explored the influence of AhR activation by ITE and FICZ on the differentiation, maturation, and function of monocyte-derived DCs (MODCs) in patients with Behçet's disease. AhR activation by FICZ or ITE inhibited DC biology with reduced production of TNF-α, IL-1β, IL-6, and IL-23 and increased secretion of IL-10 (109). Vogel et al. showed that the AhR modified maturation of BMDCs accompanied with increased expression of IDO and altered secretion of cytokines, chemokines, and DC-specific surface markers and receptors (110). Kado et al. demonstrated that the AhR modulated toll-like receptor-induced expression of cytokines and DC-specific surface markers in human MODCs involving NF-κB RelB and the immune regulatory factor caudal type homeobox-2 by treatment with ligands, such as TCDD, FICZ, and I3C, but not kynurenine (111). Taken together, these studies suggest that AhR activation through exogenous or endogenous ligands affects the function and differentiation of DCs with regard to maintaining immune homeostasis.

Dendritic cells also act as antigen-presenting cells in terms of initiating adaptive immune responses, including the differentiation and polarization of T cells and B cells. Jurado-Manzano et al. found that FICZ activated the AhR in MODCs, promoted the differentiation and maturation of DCs, and induced naïve T cells to differentiate into $CD4^+CD25^+Foxp3^+$ Treg-like cells to cause immune tolerance (112). Ping and colleagues showed, in allergic rhinitis (AR) patients, that the AhR modulated the increased secretion of IL-10 in DCs and $CD4^+$ T cells, reduced expression of IL-1β and IL-6 in DCs and IL-17 in $CD4^+$ T cells, *via* ITE treatment, and subsequently inhibited the response of T-helper (Th)17 cells to suppress the AR (113). De Araújo et al. demonstrated the fundamental role of the IDO–AhR axis in adjusting the balance between Th17 cells and Treg cells in pulmonary paracoccidioidomycosis through its effects on plasmacytoid DCs (114). The research results mentioned earlier were confined to inflammatory or autoimmune diseases. However, there are very few reports on the relationship between the AhR and DCs in tumors, and further investigations are warranted.

The AhR and Adaptive Immunity

An adaptive immune response is triggered *via* activation, differentiation, and clonal expansion of lymphoid lineage cells (T and B cells). Studies have shown that tumors "escape" from immune surveillance *via* inactivation or deletion of self-reactive T cells and B cells, which is an important early event in tumor development (114, 115).

AhR and T Cells

Type-1 regulatory T cells (Tr1), thymus-derived Treg cells, and Th17 cells have central roles in mediating immunosuppressive effects within the tumor microenvironment by suppressing the proliferation and cytokine secretion of effector cells (116). The AhR regulates $CD4^+$ T-cell differentiation, and thus, AhR levels are increased in this process (117, 118). The AhR can induce Treg cells and Th17 cells based on the TCDD concentration in EAE, suggesting that the balance between Treg cells and Th17 cells has a key role in autoimmune disease (8). Considering the immunosuppressive effects of AhR ligands on autoimmune disease, it is rational to propose that AhR activation in the tumor microenvironment is associated with an increased proportion of Treg cells and may explain (at least in part) the tumor-promoting properties of TCDD.

On account of the phenomena observed in models of autoimmune disease, one could infer that TDO-derived kynurenine induces the differentiation of Treg cells and suppresses tumor-specific $CD8^+$ T cells. For instance, Opitz et al. suggested that kynurenine affected the proliferation of $CD4^+$ and $CD8^+$ T cells in a concentration-dependent manner and verified that kynurenine suppressed antitumor immune responses through the AhR in sections of human gliomas (14, 119). Presence of the AhR is necessary in T cells for optimal generation of $Foxp3^+$ Treg cells and kynurenine induces generation of $Foxp3^+$ Treg cells in an AhR-dependent manner (30). The AhR is also crucial for the formation of Tr1 cells in mice and humans, which inhibit

autoimmune responses by interaction with the transcriptional factor macrophage-activating factor to enhance expression of IL-10, IL-21, and IL-27 (120). AhR activation is also involved in the promotion of Th17 to Tr1 transdifferentiation (98).

Whether the AhR can modulate antigen-specific $CD8^+$ T-cell responses is controversial. Winans et al. demonstrated that AhR deficiency affected primary $CD8^+$ T-cell responses with altered patterns of DNA methylation in a cell-extrinsic manner during infection with an influenza virus (121). The role of the AhR in tumor immunity has not been explored in depth and merits further investigation.

The AhR can regulate the apoptosis of process of T cells *via* modulation of expression of Fas and Fas ligand by exposure to TCDD or other endogenous ligands. Several key pathway molecules in the tryptophan pathway, including IDO-1, TDO-2, and kynurenine, participate in controlling immune tolerance and promoting tumor escape by regulating T cells and the proliferation, differentiation, and apoptosis of tumor cells *via* the AhR (122). However, the underlying molecular MoA is incompletely understood, and deeper investigations are needed.

The AhR and B Cells

Recent data have suggested the potential role of the AhR in regulation of the function of B cells involved in the tumor microenvironment (123, 124). The AhR has been reported to mediate B-cell differentiation from hematopoietic stem cells into pro-B cells, mature B cells, and plasma cells (61). For example, B cells are sensitive targets for TCDD. In LPS-activated CH12LX B lymphoma cells with simultaneous TCDD treatment, the DNA binding and transcriptional activity of activated protein-1 and immunoglobulin expression were repressed markedly, which revealed possible associations between the AhR and the genes pivotal to the maturation and function of B cells (123). More than 1,000 human cancer cell lines generated at the Broad Institute of the Massachusetts Institute of Technology and Harvard University have undergone microarray analyses. Data revealed that myelomas and B-cell lymphomas (diffuse large phenotype and unspecified phenotype) showed reduced expression of the AhR, but notably increased expression in chronic lymphocytic leukemias and Hodgkin's lymphomas (61). Interaction between the AhR and TCDD and other ligands contribute to cancers derived from B-cell lineages by affecting the growth and survival of cells *via* the tumor microenvironment. Further studies on the function of the AhR in B cell-associated microenvironment in solid tumors must be explored.

THE AhR AND SOLID TUMORS

The AhR and Malignant Gliomas

Emerging evidence has demonstrated the role of the AhR and its ligands in brain tumors. The generation of reactive oxygen species may have a role in the underlying MoA of AhR-mediated glioma, as well as the activation of glutamate receptors, histone acetylation, signal transducers, peroxisome proliferator-activated receptors, and transcription activators (125). Gramatzki et al. explored AhR expression and its MoA in human glioma cells and found that AhR inhibition downregulated expression of the TGF-β/Smad [mothers against decapentaplegic homolog 1 (Drosophila)] pathway (59). Silginer and colleagues identified a signaling network composed of the AhR, integrins, and TGF-β. They showed integrin inhibition to be a prospective strategy to tumor inhibit angiogenesis and to restrain the AhR- and TGF-β-controlled characteristics of malignancy in glioblastomas (126). Dever and Opanashuk explored the function of the AhR in a medulloblastoma cell line (DAOY). They suggested that abnormal activation or suppression of the AhR could dysregulate the cycle of granule neuron precursor (GNP) cells and that the AhR could promote the proliferation of medulloblastoma cells (127). Adams et al. provided a new perspective on how dysregulation of the kynurenine pathway affects antitumor immune responses. Tryptophan metabolites such as 3-hydroxyanthranilic acid, quinolinic acid, and kynurenine as well as regulatory enzymes such as IDO-1 and TDO-2 have central roles in antitumor immunity and are dependent on the AhR signaling pathway (128). Bostian et al. demonstrated that activation of the AhR pathway *via* TDO-2 increased expression of kynurenine and human Y-family polymerase κ, which resulted in genomic instability and high levels of replication stress in glioblastomas (129). Opitz et al. found that the TDO–kynurenine–AhR pathway is closely associated with malignant progression and a poor prognosis. They found antitumor immune responses to be suppressed and the proliferation and survival of tumor cells to be promoted by TDO-derived kynurenine in an autocrine/paracrine AhR-mediated manner (14). Taken together, these data suggest that the AhR may affect the growth of brain tumors and antitumor immune responses.

The AhR and Breast Cancer

Constitutive activation of the AhR has been examined in different models of breast cancer in mice and humans (75, 130, 131). The AhR is involved in several cell-signaling pathways, including interaction with cytokines, tyrosine kinases, and growth factors. Belguise and colleagues and Vogel et al. suggested a connection between AhR activity and upregulation of the transcriptional genes associated with the invasion and survival of cancer cells (132, 133). Goode and coworkers and Parks et al. demonstrated that knockdown or inhibition of the AhR led to downregulation of expression of tumor cells and metastasis-associated genes, as well as inhibition of the invasion and migration of cancer cells (130, 134). In addition, hyperactivation of the AhR with exogenous and endogenous ligands may induce different signaling pathways and lead to reduced invasion of breast cancer cells (70, 135, 136). Abnormal tryptophan metabolism and increased production of tryptophan metabolites have also been documented in human breast cancers. D'Amato et al. demonstrated that a TDO2–AhR signaling axis in a kynurenine pathway promoted anoikis resistance *via* NF-κB and highlighted that TDO-2 could be an intriguing target for the treatment of triple-negative breast cancer (137). Bekki et al. explored the anti-apoptotic function of the AhR in breast cancer cells by exposure to ultraviolet light, TCDD, and kynurenine, respectively. They suggested that TCDD and kynurenine mediate tumor immunity *via* the suppression of apoptosis, accompanied with the induction of expression of the COX-2 and

NF-κB subunit RelB (138). Saito and colleagues demonstrated the AhR to be a newly defined prognostic factor for breast cancers. They showed that AhR$^+$ breast cancer patients had a relatively better prognosis than those with AhR$^-$ breast cancer because of the effects of activating AhR on cell proliferation and expression of MMPs genes (65). Most of these studies were confined to the tumor itself and more attention should be paid to role of the AhR in the microenvironment of breast cancers.

The AhR and Lung Cancer

Exposure to cigarette smoke and environmental pollutants is the dominant pathogenesis for lung cancer. PAHs are exogenous ligands that bind to the AhR, which reacts with PAHs *via* Phase I CYP enzymes to sequentially influence the initiation, promotion, and progression of lung cancer. Oyama et al. tested 78 non-small-cell lung cancer samples and observed a direct correlation between expression of the AhR and downstream expression of CYP1a1, most notably in adenocarcinomas (139). Lin et al. also reported that lung adenocarcinoma tissues and cell lines expressed increased AhR at mRNA and protein levels (140). Gao et al. found AhR overexpression to be associated with an increase in nuclear translocation of RelA, the AhR–RelA complex, and NF-κB activity, giving rise to upregulation of IL-6 secretion (which is critical for lung cancer initiation) (141). Lung carcinogenesis has also been hypothesized to be *via* crosstalk with nuclear factor (erythroid-derived 2)-like 2 and ER, thereby providing effective targets for the AhR to prevent and treat lung cancer.

The AhR and Liver Cancer

The AhR conduces the regulation of the communications, adhesion, migration, and proliferation of cells in liver carcinogenesis. Fan et al. showed that AhR activation led to arrest of the G0–G1 phase in the cell cycle and diminished the competency for DNA replication and suppression of cell proliferation. They also found that the AhR was a cancer-suppressor gene in the absence of a xenobiotic ligand and that its silencing may be linked with cancer progression (97). de Tomaso et al. demonstrated that AhR was a mediator in the extracellular signal-regulated kinase1/2 signaling pathway and contributed to the regulation of the cell cycle in hexachlorobenzene-treated HepG2 cells (142). Andrysik et al. investigated if toxic AhR agonists may synchronously relieve contact inhibition and reduce gap junctional intercellular communication *via* regulation of connexin-43 (143). Terashima et al. suggested that the AhR induced VEGF expression by activation of activating transcription factor 4 during glucose deprivation in the HepG2 cell line, which affected the malignancy of liver cancer (144). Kennedy et al. found that tumor promotion by TCDD was attributed to activation of the AhR and TNF/IL-1 receptors in liver cancer (145). Koch et al. observed that flutamide activated the TGF-β1 signaling pathway *via* the AhR and influenced some biologic characteristics in human HCCs (146).

The AhR and Solid Tumors in Children

Neuroblastoma is the most common malignant solid tumor of infancy. Wu et al. found AhR expression to be correlated negatively with N-myc proto-oncogene (MYCN) expression and

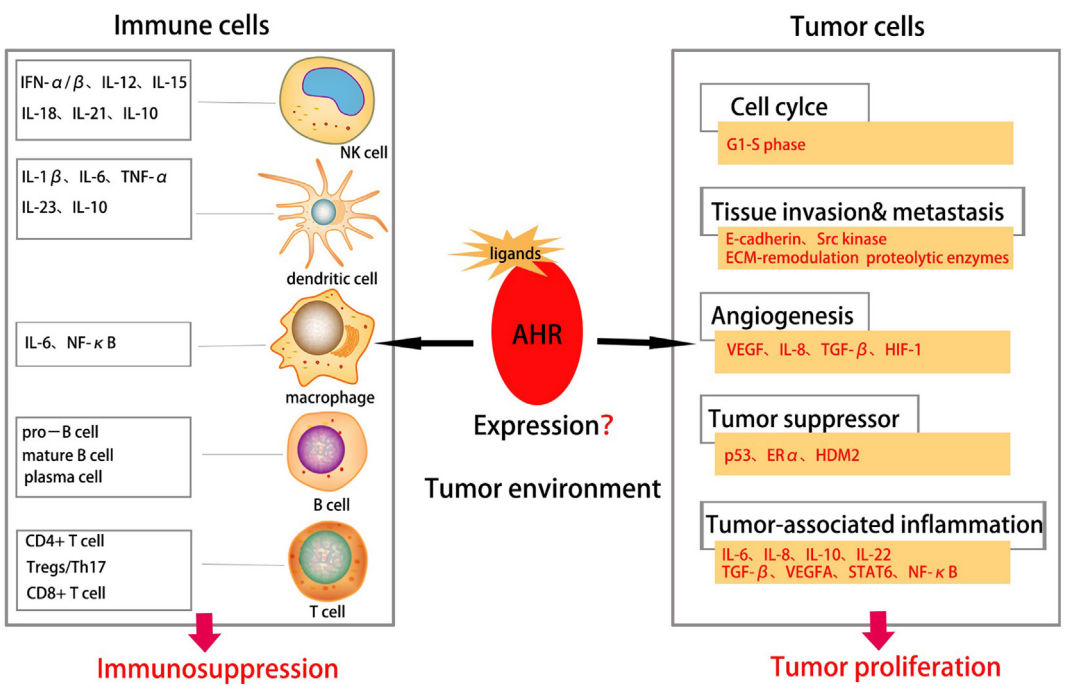

FIGURE 3 | Aryl hydrocarbon receptor (AhR) in tumor environment. Abnormal expression of AhR in tumor environment affects both the immune cells and tumor cells in tumor environment. The AhR plays an important role in various stages of tumorigenesis, including cell proliferation, tissue invasion, angiogenesis, tumor-associated inflammation, metastasis etc., suggesting AhR as a promoter for tumor initiation. It can also suppress proliferation and function of immune cells thus suppressing tumor immune responses.

highly correlated with the histology grade of differentiation in human neuroblastoma tissues, suggesting that the AhR regulates the expression and function of MYCN upstream through modulation of E2F transcription factor 1 (147). Huang et al. demonstrated that knockdown of micro-RNA-124 promoted the differentiation, cell-cycle arrest, and apoptosis of the neuroblastoma cell line SK-N-SH *via* the AhR signaling pathway (148). Little is known about medulloblastoma and the AhR. Dever and Opanashuk reported that the AhR was overexpressed in the GNP cells from the developing cerebellum and that abnormal activation/suppression of the AhR led to aberrant regulation of their cell cycle and maturation, suggesting that the AhR stimulates the growth of medulloblastomas (127).

Other Solid Tumors

The AhR is also constitutively active in prostate cancer, melanoma, ovarian cancer, colon cancer, and gastric cancer (51, 149, 150). Xie et al. found that Src-mediated crosstalk between the AhR and epidermal growth factor receptor stimulated proliferation of colon cancer cells (151). Villano et al. investigated how AhR activation affected several melanoma cell lines and normal human melanocytes. They hypothesized that expression of the AhR and ARNT activated by TCDD in the transformed melanoma cell line A2058 resulted in increased expression and enhanced activity of MMP-1, MMP-2, and MMP-9 (81).

CONCLUDING REMARKS AND FUTURE PERSPECTIVES

Overexpression and constitutive activation of the AhR have been observed in various tumor types and is associated with histology grade. The AhR occupies an important place in multiple stages of the development and progression of cancer cells; whether it functions as an oncogene or suppressor gene merits further investigations. Tumor immunity is one of the most important and promising fields in oncology. The AhR is not only a transcription factor responding to toxins but also crucial in the physiological functions of immune-cell compartments. Additional in-depth investigations of AhR function in the tumor microenvironment, including tumor cells and immune cells, and the relationship between immunity and tumors are warranted (**Figure 3**). Among AhR signaling and other pathways, the kynurenine pathway is a new and prospective way to link the immune system and tumors. The immunosuppressive role of the AhR in tumors suggests that targeting the AhR and associated signaling pathways may provide a novel therapeutic strategy for cancer.

AUTHOR CONTRIBUTIONS

PX, the first author, contributed to collection of references and manuscript preparation. JF and YZ contributed to the modification of the manuscript.

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REFERENCES

1. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. *Cell* (2011) 144(5):646–74. doi:10.1016/j.cell.2011.02.013
2. O'Sullivan T, Dunn GP, Lacoursiere DY, Schreiber RD, Bui JD. Cancer immunoediting of the NK group 2D ligand H60a. *J Immunol* (2011) 187(7):3538–45. doi:10.4049/jimmunol.1100413
3. Hahn ME. Aryl hydrocarbon receptors: diversity and evolution. *Chem Biol Interact* (2002) 141(1–2):131–60. doi:10.1016/S0009-2797(02)00070-4
4. Chiba T, Chihara J, Furue M. Role of the Arylhydrocarbon Receptor (AhR) in the pathology of asthma and COPD. *J Allergy (Cairo)* (2012) 2012:372384. doi:10.1155/2012/372384
5. Denison MS, Pandini A, Nagy SR, Baldwin EP, Bonati L. Ligand binding and activation of the Ah receptor. *Chem Biol Interact* (2002) 141(1–2):3–24. doi:10.1016/S0009-2797(02)00063-7
6. Vorderstrasse BA, Kerkvliet NI. 2,3,7,8-tetrachlorodibenzo-p-dioxin affects the number and function of murine splenic dendritic cells and their expression of accessory molecules. *Toxicol Appl Pharmacol* (2001) 171(2):117–25. doi:10.1006/taap.2000.9119
7. Funatake CJ, Marshall NB, Steppan LB, Mourich DV, Kerkvliet NI. Cutting edge: activation of the aryl hydrocarbon receptor by 2,3,7,8-tetrachlorodibenzo-p-dioxin generates a population of CD4+ CD25+ cells with characteristics of regulatory T cells. *J Immunol* (2005) 175(7):4184–8. doi:10.4049/jimmunol.175.7.4184
8. Quintana FJ, Basso AS, Iglesias AH, Korn T, Farez MF, Bettelli E, et al. Control of T(reg) and T(H)17 cell differentiation by the aryl hydrocarbon receptor. *Nature* (2008) 453(7191):65–71. doi:10.1038/nature06880
9. Esser C, Rannug A, Stockinger B. The aryl hydrocarbon receptor in immunity. *Trends Immunol* (2009) 30(9):447–54. doi:10.1016/j.it.2009.06.005
10. Wang T, Wyrick KL, Pecka MR, Wills TB, Vorderstrasse BA. Mechanistic exploration of AhR-mediated host protection against *Streptococcus pneumoniae* infection. *Int Immunopharmacol* (2012) 13(4):490–8. doi:10.1016/j.intimp.2012.05.008
11. Saunders V, Breysse P, Clark J, Sproles A, Davila M, Wills-Karp M. Particulate matter-induced airway hyperresponsiveness is lymphocyte dependent. *Environ Health Perspect* (2010) 118(5):640–6. doi:10.1289/ehp.0901461
12. Knerr S, Schrenk D. Carcinogenicity of 2,3,7,8-tetrachlorodibenzo-p-dioxin in experimental models. *Mol Nutr Food Res* (2006) 50(10):897–907. doi:10.1002/mnfr.200600006
13. Dietrich C, Kaina B. The aryl hydrocarbon receptor (AhR) in the regulation of cell-cell contact and tumor growth. *Carcinogenesis* (2010) 31(8):1319–28. doi:10.1093/carcin/bgq028
14. Opitz CA, Litzenburger UM, Sahm F, Ott M, Tritschler I, Trump S, et al. An endogenous tumour-promoting ligand of the human aryl hydrocarbon receptor. *Nature* (2011) 478(7368):197–203. doi:10.1038/nature10491
15. Poland A, Knutson JC. 2,3,7,8-Tetrachlorodibenzo-p-dioxin and related halogenated aromatic hydrocarbons: examination of the mechanism of toxicity. *Annu Rev Pharmacol Toxicol* (1982) 22:517–54. doi:10.1146/annurev.pa.22.040182.002505
16. Bersten DC, Sullivan AE, Peet DJ, Whitelaw ML. bHLH-PAS proteins in cancer. *Nat Rev Cancer* (2013) 13(12):827–41. doi:10.1038/nrc3621
17. Feng S, Cao Z, Wang X. Role of aryl hydrocarbon receptor in cancer. *Biochim Biophys Acta* (2013) 1836(2):197–210. doi:10.1016/j.bbcan.2013.05.001
18. Perdew GH. Association of the Ah receptor with the 90-kDa heat shock protein. *J Biol Chem* (1988) 263(27):13802–5.
19. Kazlauskas A, Poellinger L, Pongratz I. Evidence that the co-chaperone p23 regulates ligand responsiveness of the dioxin (Aryl hydrocarbon) receptor. *J Biol Chem* (1999) 274(19):13519–24. doi:10.1074/jbc.274.19.13519

20. Ma Q, Whitlock JJ. A novel cytoplasmic protein that interacts with the Ah receptor, contains tetratricopeptide repeat motifs, and augments the transcriptional response to 2,3,7,8-tetrachlorodibenzo-p-dioxin. *J Biol Chem* (1997) 272(14):8878–84. doi:10.1074/jbc.272.14.8878
21. Cox MB, Miller CR. Cooperation of heat shock protein 90 and p23 in aryl hydrocarbon receptor signaling. *Cell Stress Chaperones* (2004) 9(1):4–20. doi:10.1379/460.1
22. Tomita S, Jiang HB, Ueno T, Takagi S, Tohi K, Maekawa S, et al. T cell-specific disruption of arylhydrocarbon receptor nuclear translocator (Arnt) gene causes resistance to 2,3,7,8-tetrachlorodibenzo-p-dioxin-induced thymic involution. *J Immunol* (2003) 171(8):4113–20. doi:10.4049/jimmunol.171.8.4113
23. Schrenk D. Impact of dioxin-type induction of drug-metabolizing enzymes on the metabolism of endo- and xenobiotics. *Biochem Pharmacol* (1998) 55(8):1155–62.
24. Funk CD, Furci L, FitzGerald GA, Grygorczyk R, Rochette C, Bayne MA, et al. Cloning and expression of a cDNA for the human prostaglandin E receptor EP1 subtype. *J Biol Chem* (1993) 268(35):26767–72.
25. Dolwick KM, Swanson HI, Bradfield CA. In vitro analysis of Ah receptor domains involved in ligand-activated DNA recognition. *Proc Natl Acad Sci U S A* (1993) 90(18):8566–70. doi:10.1073/pnas.90.18.8566
26. Kimura A, Naka T, Nohara K, Fujii-Kuriyama Y, Kishimoto T. Aryl hydrocarbon receptor regulates Stat1 activation and participates in the development of Th17 cells. *Proc Natl Acad Sci U S A* (2008) 105(28):9721–6. doi:10.1073/pnas.0804231105
27. Kimura A, Naka T, Nakahama T, Chinen I, Masuda K, Nohara K, et al. Aryl hydrocarbon receptor in combination with Stat1 regulates LPS-induced inflammatory responses. *J Exp Med* (2009) 206(9):2027–35. doi:10.1084/jem.20090560
28. Masuda K, Kimura A, Hanieh H, Nguyen NT, Nakahama T, Chinen I, et al. Aryl hydrocarbon receptor negatively regulates LPS-induced IL-6 production through suppression of histamine production in macrophages. *Int Immunol* (2011) 23(10):637–45. doi:10.1093/intimm/dxr072
29. Monteiro P, Gilot D, Le Ferrec E, Lecureur V, N'Diaye M, Le Vee M, et al. AhR- and c-maf-dependent induction of beta7-integrin expression in human macrophages in response to environmental polycyclic aromatic hydrocarbons. *Biochem Biophys Res Commun* (2007) 358(2):442–8. doi:10.1016/j.bbrc.2007.04.111
30. Mezrich JD, Fechner JH, Zhang X, Johnson BP, Burlingham WJ, Bradfield CA. An interaction between kynurenine and the aryl hydrocarbon receptor can generate regulatory T cells. *J Immunol* (2010) 185(6):3190–8. doi:10.4049/jimmunol.0903670
31. Thurmond TS, Staples JE, Silverstone AE, Gasiewicz TA. The aryl hydrocarbon receptor has a role in the in vivo maturation of murine bone marrow B lymphocytes and their response to 2,3,7,8-tetrachlorodibenzo-p-dioxin. *Toxicol Appl Pharmacol* (2000) 165(3):227–36. doi:10.1006/taap.2000.8942
32. Wagage S, John B, Krock BL, Hall AO, Randall LM, Karp CL, et al. The aryl hydrocarbon receptor promotes IL-10 production by NK cells. *J Immunol* (2014) 192(4):1661–70. doi:10.4049/jimmunol.1300497
33. Smith KJ, Murray IA, Tanos R, Tellew J, Boitano AE, Bisson WH, et al. Identification of a high-affinity ligand that exhibits complete aryl hydrocarbon receptor antagonism. *J Pharmacol Exp Ther* (2011) 338(1):318–27. doi:10.1124/jpet.110.178392
34. Bock KW, Kohle C. Ah receptor- and TCDD-mediated liver tumor promotion: clonal selection and expansion of cells evading growth arrest and apoptosis. *Biochem Pharmacol* (2005) 69(10):1403–8. doi:10.1016/j.bcp.2005.02.004
35. Denison MS, Soshilov AA, He G, DeGroot DE, Zhao B. Exactly the same but different: promiscuity and diversity in the molecular mechanisms of action of the aryl hydrocarbon (dioxin) receptor. *Toxicol Sci* (2011) 124(1):1–22. doi:10.1093/toxsci/kfr218
36. Kerkvliet NI. AHR-mediated immunomodulation: the role of altered gene transcription. *Biochem Pharmacol* (2009) 77(4):746–60. doi:10.1016/j.bcp.2008.11.021
37. Kopec AK, Burgoon LD, Ibrahim-Aibo D, Burg AR, Lee AW, Tashiro C, et al. Automated dose-response analysis and comparative toxicogenomic evaluation of the hepatic effects elicited by TCDD, TCDF, and PCB126 in C57BL/6 mice. *Toxicol Sci* (2010) 118(1):286–97. doi:10.1093/toxsci/kfq236
38. Kopf PG, Scott JA, Agbor LN, Boberg JR, Elased KM, Huwe JK, et al. Cytochrome P4501A1 is required for vascular dysfunction and hypertension induced by 2,3,7,8-tetrachlorodibenzo-p-dioxin. *Toxicol Sci* (2010) 117(2):537–46. doi:10.1093/toxsci/kfq218
39. Song J, Clagett-Dame M, Peterson RE, Hahn ME, Westler WM, Sicinski RR, et al. A ligand for the aryl hydrocarbon receptor isolated from lung. *Proc Natl Acad Sci U S A* (2002) 99(23):14694–9. doi:10.1073/pnas.232562899
40. Adachi J, Mori Y, Matsui S, Takigami H, Fujino J, Kitagawa H, et al. Indirubin and indigo are potent aryl hydrocarbon receptor ligands present in human urine. *J Biol Chem* (2001) 276(34):31475–8. doi:10.1074/jbc.C100238200
41. Nishiumi S, Yamamoto N, Kodoi R, Fukuda I, Yoshida K, Ashida H. Antagonistic and agonistic effects of indigoids on the transformation of an aryl hydrocarbon receptor. *Arch Biochem Biophys* (2008) 470(2):187–99. doi:10.1016/j.abb.2007.11.021
42. Nguyen LP, Bradfield CA. The search for endogenous activators of the aryl hydrocarbon receptor. *Chem Res Toxicol* (2008) 21(1):102–16. doi:10.1021/tx7001965
43. Peter GF, Martin MV, McCormick WA, Nguyen LP, Glover E, Bradfield CA. Aryl hydrocarbon receptor response to indigoids in vitro and in vivo. *Arch Biochem Biophys* (2004) 423(2):309–16. doi:10.1016/j.abb.2004.01.002
44. Sugihara K, Kitamura S, Yamada T, Okayama T, Ohta S, Yamashita K, et al. Aryl hydrocarbon receptor-mediated induction of microsomal drug-metabolizing enzyme activity by indirubin and indigo. *Biochem Biophys Res Commun* (2004) 318(2):571–8. doi:10.1016/j.bbrc.2004.04.066
45. Jinno A, Maruyama Y, Ishizuka M, Kazusaka A, Nakamura A, Fujita S. Induction of cytochrome P450-1A by the equine estrogen equilenin, a new endogenous aryl hydrocarbon receptor ligand. *J Steroid Biochem Mol Biol* (2006) 98(1):48–55. doi:10.1016/j.jsbmb.2005.07.003
46. Schaldach CM, Riby J, Bjeldanes LF. Lipoxin A4: a new class of ligand for the Ah receptor. *Biochemistry* (1999) 38(23):7594–600. doi:10.1021/bi982861e
47. Seidel SD, Winters GM, Rogers WJ, Ziccardi MH, Li V, Keser B, et al. Activation of the Ah receptor signaling pathway by prostaglandins. *J Biochem Mol Toxicol* (2001) 15(4):187–96. doi:10.1002/jbt.16
48. Sinal CJ, Bend JR. Aryl hydrocarbon receptor-dependent induction of cyp1a1 by bilirubin in mouse hepatoma hepa 1c1c7 cells. *Mol Pharmacol* (1997) 52(4):590–9. doi:10.1124/mol.52.4.590
49. Quintana FJ, Murugaiyan G, Farez MF, Mitsdoerffer M, Tukpah AM, Burns EJ, et al. An endogenous aryl hydrocarbon receptor ligand acts on dendritic cells and T cells to suppress experimental autoimmune encephalomyelitis. *Proc Natl Acad Sci U S A* (2010) 107(48):20768–73. doi:10.1073/pnas.1009201107
50. Nugent LF, Shi G, Vistica BP, Ogbeifun O, Hinshaw SJ, Gery I. ITE, a novel endogenous nontoxic aryl hydrocarbon receptor ligand, efficiently suppresses EAU and T-cell-mediated immunity. *Invest Ophthalmol Vis Sci* (2013) 54(12):7463–9. doi:10.1167/iovs.12-11479
51. Wang K, Li Y, Jiang YZ, Dai CF, Patankar MS, Song JS, et al. An endogenous aryl hydrocarbon receptor ligand inhibits proliferation and migration of human ovarian cancer cells. *Cancer Lett* (2013) 340(1):63–71. doi:10.1016/j.canlet.2013.06.026
52. Li Y, Wang K, Jiang YZ, Chang XW, Dai CF, Zheng J. 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) inhibits human ovarian cancer cell proliferation. *Cell Oncol (Dordr)* (2014) 37(6):429–37. doi:10.1007/s13402-014-0206-4
53. Wirthgen E, Hoeflich A. Endotoxin-induced tryptophan degradation along the kynurenine pathway: the role of indolamine 2,3-dioxygenase and aryl hydrocarbon receptor-mediated immunosuppressive effects in endotoxin tolerance and cancer and its implications for immunoparalysis. *J Amino Acids* (2015) 2015:973548. doi:10.1155/2015/973548
54. Takikawa O. Biochemical and medical aspects of the indoleamine 2,3-dioxygenase-initiated L-tryptophan metabolism. *Biochem Biophys Res Commun* (2005) 338(1):12–9. doi:10.1016/j.bbrc.2005.09.032
55. Ball HJ, Sanchez-Perez A, Weiser S, Austin CJ, Astelbauer F, Miu J, et al. Characterization of an indoleamine 2,3-dioxygenase-like protein found in humans and mice. *Gene* (2007) 396(1):203–13. doi:10.1016/j.gene.2007.04.010
56. Platten M, von Knebel DN, Oezen I, Wick W, Ochs K. Cancer immunotherapy by targeting IDO1/TDO and their downstream effectors. *Front Immunol* (2014) 5:673. doi:10.3389/fimmu.2014.00673
57. Prendergast GC, Smith C, Thomas S, Mandik-Nayak L, Laury-Kleintop L, Metz R, et al. Indoleamine 2,3-dioxygenase pathways of pathogenic

inflammation and immune escape in cancer. *Cancer Immunol Immunother* (2014) 63(7):721–35. doi:10.1007/s00262-014-1549-4
58. DiNatale BC, Schroeder JC, Perdew GH. Ah receptor antagonism inhibits constitutive and cytokine inducible IL6 production in head and neck tumor cell lines. *Mol Carcinog* (2011) 50(3):173–83. doi:10.1002/mc.20702
59. Gramatzki D, Pantazis G, Schittenhelm J, Tabatabai G, Kohle C, Wick W, et al. Aryl hydrocarbon receptor inhibition downregulates the TGF-beta/Smad pathway in human glioblastoma cells. *Oncogene* (2009) 28(28):2593–605. doi:10.1038/onc.2009.104
60. Hayashibara T, Yamada Y, Mori N, Harasawa H, Sugahara K, Miyanishi T, et al. Possible involvement of Aryl hydrocarbon Receptor (AhR) in adult T-cell leukemia (ATL) leukemogenesis: constitutive activation of AhR in ATL. *Biochem Biophys Res Commun* (2003) 300(1):128–34. doi:10.1016/S0006-291X(02)02793-6
61. Sherr DH, Monti S. The role of the aryl hydrocarbon receptor in normal and malignant B cell development. *Semin Immunopathol* (2013) 35(6):705–16. doi:10.1007/s00281-013-0390-8
62. Chang JT, Chang H, Chen PH, Lin SL, Lin P. Requirement of aryl hydrocarbon receptor overexpression for CYP1B1 up-regulation and cell growth in human lung adenocarcinomas. *Clin Cancer Res* (2007) 13(1):38–45. doi:10.1158/1078-0432.CCR-06-1166
63. Liu Z, Wu X, Zhang F, Han L, Bao G, He X, et al. AhR expression is increased in hepatocellular carcinoma. *J Mol Histol* (2013) 44(4):455–61. doi:10.1007/s10735-013-9495-6
64. Murray IA, Patterson AD, Perdew GH. Aryl hydrocarbon receptor ligands in cancer: friend and foe. *Nat Rev Cancer* (2014) 14(12):801–14. doi:10.1038/nrc3846
65. Saito R, Miki Y, Hata S, Takagi K, Iida S, Oba Y, et al. Aryl hydrocarbon receptor in breast cancer-a newly defined prognostic marker. *Horm Cancer* (2014) 5(1):11–21. doi:10.1007/s12672-013-0160-z
66. Su JM, Lin P, Chang H. Prognostic value of nuclear translocation of aryl hydrocarbon receptor for non-small cell lung cancer. *Anticancer Res* (2013) 33(9):3953–61.
67. Yang X, Solomon S, Fraser LR, Trombino AF, Liu D, Sonenshein GE, et al. Constitutive regulation of CYP1B1 by the aryl hydrocarbon receptor (AhR) in pre-malignant and malignant mammary tissue. *J Cell Biochem* (2008) 104(2):402–17. doi:10.1002/jcb.21630
68. DiNatale BC, Smith K, John K, Krishnegowda G, Amin SG, Perdew GH. Ah receptor antagonism represses head and neck tumor cell aggressive phenotype. *Mol Cancer Res* (2012) 10(10):1369–79. doi:10.1158/1541-7786.MCR-12-0216
69. Gasiewicz TA, Henry EC, Collins LL. Expression and activity of aryl hydrocarbon receptors in development and cancer. *Crit Rev Eukaryot Gene Expr* (2008) 18(4):279–321. doi:10.1615/CritRevEukarGeneExpr.v18.i4.10
70. Safe S, Lee SO, Jin UH. Role of the aryl hydrocarbon receptor in carcinogenesis and potential as a drug target. *Toxicol Sci* (2013) 135(1):1–16. doi:10.1093/toxsci/kft128
71. Ma Q, Whitlock JJ. The aromatic hydrocarbon receptor modulates the Hepa 1c1c7 cell cycle and differentiated state independently of dioxin. *Mol Cell Biol* (1996) 16(5):2144–50. doi:10.1128/MCB.16.5.2144
72. Shimba S, Komiyama K, Moro I, Tezuka M. Overexpression of the Aryl hydrocarbon Receptor (AhR) accelerates the cell proliferation of A549 cells. *J Biochem* (2002) 132(5):795–802. doi:10.1093/oxfordjournals.jbchem.a003289
73. Strobeck MW, Fribourg AF, Puga A, Knudsen ES. Restoration of retinoblastoma mediated signaling to Cdk2 results in cell cycle arrest. *Oncogene* (2000) 19(15):1857–67. doi:10.1038/sj.onc.1203510
74. Peng TL, Chen J, Mao W, Liu X, Tao Y, Chen LZ, et al. Potential therapeutic significance of increased expression of aryl hydrocarbon receptor in human gastric cancer. *World J Gastroenterol* (2009) 15(14):1719–29. doi:10.3748/wjg.15.1719
75. Barhoover MA, Hall JM, Greenlee WF, Thomas RS. Aryl hydrocarbon receptor regulates cell cycle progression in human breast cancer cells via a functional interaction with cyclin-dependent kinase 4. *Mol Pharmacol* (2010) 77(2):195–201. doi:10.1124/mol.109.059675
76. Owens DW, McLean GW, Wyke AW, Paraskeva C, Parkinson EK, Frame MC, et al. The catalytic activity of the Src family kinases is required to disrupt cadherin-dependent cell-cell contacts. *Mol Biol Cell* (2000) 11(1):51–64. doi:10.1091/mbc.11.1.51
77. Wang F, Samudio I, Safe S. Transcriptional activation of cathepsin D gene expression by 17beta-estradiol: mechanism of aryl hydrocarbon receptor-mediated inhibition. *Mol Cell Endocrinol* (2001) 172(1–2):91–103. doi:10.1016/S0303-7207(00)00379-8
78. Mizuyachi K, Son DS, Rozman KK, Terranova PF. Alteration in ovarian gene expression in response to 2,3,7,8-tetrachlorodibenzo-p-dioxin: reduction of cyclooxygenase-2 in the blockage of ovulation. *Reprod Toxicol* (2002) 16(3):299–307. doi:10.1016/S0890-6238(02)00024-2
79. Peng TL, Chen J, Mao W, Song X, Chen MH. Aryl hydrocarbon receptor pathway activation enhances gastric cancer cell invasiveness likely through a c-Jun-dependent induction of matrix metalloproteinase-9. *BMC Cell Biol* (2009) 10:27. doi:10.1186/1471-2121-10-27
80. Son DS, Rozman KK. 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) induces plasminogen activator inhibitor-1 through an aryl hydrocarbon receptor-mediated pathway in mouse hepatoma cell lines. *Arch Toxicol* (2002) 76(7):404–13. doi:10.1007/s00204-002-0354-6
81. Villano CM, Murphy KA, Akintobi A, White LA. 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) induces matrix metalloproteinase (MMP) expression and invasion in A2058 melanoma cells. *Toxicol Appl Pharmacol* (2006) 210(3):212–24. doi:10.1016/j.taap.2005.05.001
82. Haque M, Francis J, Sehgal I. Aryl hydrocarbon exposure induces expression of MMP-9 in human prostate cancer cell lines. *Cancer Lett* (2005) 225(1):159–66. doi:10.1016/j.canlet.2004.11.043
83. Maltepe E, Schmidt JV, Baunoch D, Bradfield CA, Simon MC. Abnormal angiogenesis and responses to glucose and oxygen deprivation in mice lacking the protein ARNT. *Nature* (1997) 386(6623):403–7. doi:10.1038/386403a0
84. Roman AC, Carvajal-Gonzalez JM, Rico-Leo EM, Fernandez-Salguero PM. Dioxin receptor deficiency impairs angiogenesis by a mechanism involving VEGF-A depletion in the endothelium and transforming growth factor-beta overexpression in the stroma. *J Biol Chem* (2009) 284(37):25135–48. doi:10.1074/jbc.M109.013292
85. Su JM, Lin P, Wang CK, Chang H. Overexpression of cytochrome P450 1B1 in advanced non-small cell lung cancer: a potential therapeutic target. *Anticancer Res* (2009) 29(2):509–15.
86. Papoutsis AJ, Lamore SD, Wondrak GT, Selmin OI, Romagnolo DF. Resveratrol prevents epigenetic silencing of BRCA-1 by the aromatic hydrocarbon receptor in human breast cancer cells. *J Nutr* (2010) 140(9):1607–14. doi:10.3945/jn.110.123422
87. Ambolet-Camoit A, Bui LC, Pierre S, Chevallier A, Marchand A, Coumoul X, et al. 2,3,7,8-tetrachlorodibenzo-p-dioxin counteracts the p53 response to a genotoxicant by upregulating expression of the metastasis marker agr2 in the hepatocarcinoma cell line HepG2. *Toxicol Sci* (2010) 115(2):501–12. doi:10.1093/toxsci/kfq082
88. Hirabayashi Y, Inoue T. Aryl hydrocarbon receptor biology and xenobiotic responses in hematopoietic progenitor cells. *Biochem Pharmacol* (2009) 77(4):521–35. doi:10.1016/j.bcp.2008.09.030
89. Okino ST, Pookot D, Basak S, Dahiya R. Toxic and chemopreventive ligands preferentially activate distinct aryl hydrocarbon receptor pathways: implications for cancer prevention. *Cancer Prev Res (Phila)* (2009) 2(3):251–6. doi:10.1158/1940-6207.CAPR-08-0146
90. DiNatale BC, Schroeder JC, Francey LJ, Kusnadi A, Perdew GH. Mechanistic insights into the events that lead to synergistic induction of interleukin 6 transcription upon activation of the aryl hydrocarbon receptor and inflammatory signaling. *J Biol Chem* (2010) 285(32):24388–97. doi:10.1074/jbc.M110.118570
91. Hanahan D, Coussens LM. Accessories to the crime: functions of cells recruited to the tumor microenvironment. *Cancer Cell* (2012) 21(3):309–22. doi:10.1016/j.ccr.2012.02.022
92. Sekine H, Mimura J, Oshima M, Okawa H, Kanno J, Igarashi K, et al. Hypersensitivity of aryl hydrocarbon receptor-deficient mice to lipopolysaccharide-induced septic shock. *Mol Cell Biol* (2009) 29(24):6391–400. doi:10.1128/MCB.00337-09
93. Kolasa E, Houlbert N, Balaguer P, Fardel O. AhR- and NF-kappaB-dependent induction of interleukin-6 by co-exposure to the environmental contaminant benzanthracene and the cytokine tumor necrosis factor-alpha in human mammary MCF-7 cells. *Chem Biol Interact* (2013) 203(2):391–400. doi:10.1016/j.cbi.2013.01.005
94. Furman DP, Oshchepkova EA, Oshchepkov DY, Shamanina MY, Mordvinov VA. Promoters of the genes encoding the transcription factors regulating

the cytokine gene expression in macrophages contain putative binding sites for aryl hydrocarbon receptor. *Comput Biol Chem* (2009) 33(6):465–8. doi:10.1016/j.compbiolchem.2009.10.004
95. Vogel CF, Sciullo E, Matsumura F. Involvement of RelB in aryl hydrocarbon receptor-mediated induction of chemokines. *Biochem Biophys Res Commun* (2007) 363(3):722–6. doi:10.1016/j.bbrc.2007.09.032
96. John K, Lahoti TS, Wagner K, Hughes JM, Perdew GH. The Ah receptor regulates growth factor expression in head and neck squamous cell carcinoma cell lines. *Mol Carcinog* (2014) 53(10):765–76. doi:10.1002/mc.22032
97. Fan Y, Boivin GP, Knudsen ES, Nebert DW, Xia Y, Puga A. The aryl hydrocarbon receptor functions as a tumor suppressor of liver carcinogenesis. *Cancer Res* (2010) 70(1):212–20. doi:10.1158/0008-5472.CAN-09-3090
98. Iida K, Mimura J, Itoh K, Ohyama C, Fujii-Kuriyama Y, Shimazui T, et al. Suppression of AhR signaling pathway is associated with the down-regulation of UDP-glucuronosyltransferases during BBN-induced urinary bladder carcinogenesis in mice. *J Biochem* (2010) 147(3):353–60. doi:10.1093/jb/mvp169
99. Naumova E, Pawelec G, Mihaylova A. Natural killer cells, ageing and cancer. *Cancer Immunol Immunother* (2016) 65(4):367–70. doi:10.1007/s00262-016-1817-6
100. Shin JH, Zhang L, Murillo-Sauca O, Kim J, Kohrt HE, Bui JD, et al. Modulation of natural killer cell antitumor activity by the aryl hydrocarbon receptor. *Proc Natl Acad Sci U S A* (2013) 110(30):12391–6. doi:10.1073/pnas.1302856110
101. Chiba S, Ikushima H, Ueki H, Yanai H, Kimura Y, Hangai S, et al. Recognition of tumor cells by dectin-1 orchestrates innate immune cells for anti-tumor responses. *Elife* (2014) 3:e04177. doi:10.7554/eLife.04177
102. Zhang LH, Shin JH, Haggadone MD, Sunwoo JB. The aryl hydrocarbon receptor is required for the maintenance of liver-resident natural killer cells. *J Exp Med* (2016) 213(11):2249–57. doi:10.1084/jem.20151998
103. Climaco-Arvizu S, Dominguez-Acosta O, Cabanas-Cortes MA, Rodriguez-Sosa M, Gonzalez FJ, Vega L, et al. Aryl hydrocarbon receptor influences nitric oxide and arginine production and alters M1/M2 macrophage polarization. *Life Sci* (2016) 155:76–84. doi:10.1016/j.lfs.2016.05.001
104. Yeung OW, Lo CM, Ling CC, Qi X, Geng W, Li CX, et al. Alternatively activated (M2) macrophages promote tumour growth and invasiveness in hepatocellular carcinoma. *J Hepatol* (2015) 62(3):607–16. doi:10.1016/j.jhep.2014.10.029
105. Partecke LI, Gunther C, Hagemann S, Jacobi C, Merkel M, Sendler M, et al. Induction of M2-macrophages by tumour cells and tumour growth promotion by M2-macrophages: a quid pro quo in pancreatic cancer. *Pancreatology* (2013) 13(5):508–16. doi:10.1016/j.pan.2013.06.010
106. Zhang Y, Sime W, Juhas M, Sjolander A. Crosstalk between colon cancer cells and macrophages via inflammatory mediators and CD47 promotes tumour cell migration. *Eur J Cancer* (2013) 49(15):3320–34. doi:10.1016/j.ejca.2013.06.005
107. Nguyen NT, Kimura A, Nakahama T, Chinen I, Masuda K, Nohara K, et al. Aryl hydrocarbon receptor negatively regulates dendritic cell immunogenicity via a kynurenine-dependent mechanism. *Proc Natl Acad Sci U S A* (2010) 107(46):19961–6. doi:10.1073/pnas.1014465107
108. Thatcher TH, Williams MA, Pollock SJ, McCarthy CE, Lacy SH, Phipps RP, et al. Endogenous ligands of the aryl hydrocarbon receptor regulate lung dendritic cell function. *Immunology* (2016) 147(1):41–54. doi:10.1111/imm.12540
109. Wang C, Ye Z, Kijlstra A, Zhou Y, Yang P. Activation of the aryl hydrocarbon receptor affects activation and function of human monocyte-derived dendritic cells. *Clin Exp Immunol* (2014) 177(2):521–30. doi:10.1111/cei.12352
110. Vogel CF, Wu D, Goth SR, Baek J, Lollies A, Domhardt R, et al. Aryl hydrocarbon receptor signaling regulates NF-kappaB RelB activation during dendritic-cell differentiation. *Immunol Cell Biol* (2013) 91(9):568–75. doi:10.1038/icb.2013.43
111. Kado S, Chang WL, Chi AN, Wolny M, Shepherd DM, Vogel CF. Aryl hydrocarbon receptor signaling modifies toll-like receptor-regulated responses in human dendritic cells. *Arch Toxicol* (2017) 91(5):2209–21. doi:10.1007/s00204-016-1880-y
112. Jurado-Manzano BB, Zavala-Reyes D, Turrubiartes-Martinez EA, Portales-Perez DP, Gonzalez-Amaro R, Layseca-Espinosa E. FICZ generates human tDCs that induce CD4+ CD25high Foxp3+ Treg-like cell differentiation. *Immunol Lett* (2017) 190:84–92. doi:10.1016/j.imlet.2017.07.013
113. Wei P, Hu GH, Kang HY, Yao HB, Kou W, Liu H, et al. An aryl hydrocarbon receptor ligand acts on dendritic cells and T cells to suppress the Th17 response in allergic rhinitis patients. *Lab Invest* (2014) 94(5):528–35. doi:10.1038/labinvest.2014.8
114. de Araujo EF, Feriotti C, Galdino NAL, Preite NW, Calich VLG, Loures FV, et al. The IDO-AhR Axis Controls Th17/Treg Immunity in a Pulmonary Model of Fungal Infection. *Front Immunol* (2017) 8:880.
115. Corthay A. Does the immune system naturally protect against cancer? *Front Immunol* (2014) 5:197. doi:10.3389/fimmu.2014.00197
116. Mottet C, Golshayan D. CD4+CD25+Foxp3+ regulatory T cells: from basic research to potential therapeutic use. *Swiss Med Wkly* (2007) 137(45–46):625–34.
117. Darrasse-Jeze G, Podsypanina K. How numbers, nature, and immune status of foxp3(+) regulatory T-cells shape the early immunological events in tumor development. *Front Immunol* (2013) 4:292. doi:10.3389/fimmu.2013.00292
118. Marshall NB, Vorachek WR, Steppan LB, Mourich DV, Kerkvliet NI. Functional characterization and gene expression analysis of CD4+ CD25+ regulatory T cells generated in mice treated with 2,3,7,8-tetrachlorodibenzo-p-dioxin. *J Immunol* (2008) 181(4):2382–91. doi:10.4049/jimmunol.181.4.2382
119. Cella M, Colonna M. Aryl hydrocarbon receptor: linking environment to immunity. *Semin Immunol* (2015) 27(5):310–4. doi:10.1016/j.smim.2015.10.002
120. Peng L, Mayhew CN, Schnekenburger M, Knudsen ES, Puga A. Repression of Ah receptor and induction of transforming growth factor-beta genes in DEN-induced mouse liver tumors. *Toxicology* (2008) 246(2–3):242–7. doi:10.1016/j.tox.2008.01.002
121. Winans B, Nagari A, Chae M, Post CM, Ko CI, Puga A, et al. Linking the aryl hydrocarbon receptor with altered DNA methylation patterns and developmentally induced aberrant antiviral CD8+ T cell responses. *J Immunol* (2015) 194(9):4446–57. doi:10.4049/jimmunol.1402044
122. Platten M, Litzenburger U, Wick W. The aryl hydrocarbon receptor in tumor immunity. *Oncoimmunology* (2012) 1(3):396–7. doi:10.4161/onci.19071
123. Suh J, Jeon YJ, Kim HM, Kang JS, Kaminski NE, Yang KH. Aryl hydrocarbon receptor-dependent inhibition of AP-1 activity by 2,3,7,8-tetrachlorodibenzo-p-dioxin in activated B cells. *Toxicol Appl Pharmacol* (2002) 181(2):116–23. doi:10.1006/taap.2002.9403
124. Boverhof DR, Tam E, Harney AS, Crawford RB, Kaminski NE, Zacharewski TR. 2,3,7,8-Tetrachlorodibenzo-p-dioxin induces suppressor of cytokine signaling 2 in murine B cells. *Mol Pharmacol* (2004) 66(6):1662–70. doi:10.1124/mol.104.002915
125. Korashy HM, Abuohashish HM, Maayah ZH. The role of aryl hydrocarbon receptor-regulated cytochrome P450 enzymes in glioma. *Curr Pharm Des* (2013) 19(40):7155–66. doi:10.2174/13816128113199990583
126. Silginer M, Burghardt I, Gramatzki D, Bunse L, Leske H, Rushing EJ, et al. The aryl hydrocarbon receptor links integrin signaling to the TGF-beta pathway. *Oncogene* (2016) 35(25):3260–71. doi:10.1038/onc.2015.387
127. Dever DP, Opanashuk LA. The aryl hydrocarbon receptor contributes to the proliferation of human medulloblastoma cells. *Mol Pharmacol* (2012) 81(5):669–78. doi:10.1124/mol.111.077305
128. Adams S, Braidy N, Bessede A, Brew BJ, Grant R, Teo C, et al. The kynurenine pathway in brain tumor pathogenesis. *Cancer Res* (2012) 72(22):5649–57. doi:10.1158/0008-5472.CAN-12-0549
129. Bostian AC, Maddukuri L, Reed MR, Savenka T, Hartman JH, Davis L, et al. Kynurenine signaling increases DNA polymerase kappa expression and promotes genomic instability in glioblastoma cells. *Chem Res Toxicol* (2016) 29(1):101–8. doi:10.1021/acs.chemrestox.5b00452
130. Goode G, Pratap S, Eltom SE. Depletion of the aryl hydrocarbon receptor in MDA-MB-231 human breast cancer cells altered the expression of genes in key regulatory pathways of cancer. *PLoS One* (2014) 9(6):e100103. doi:10.1371/journal.pone.0100103
131. Li ZD, Wang K, Yang XW, Zhuang ZG, Wang JJ, Tong XW. Expression of aryl hydrocarbon receptor in relation to p53 status and clinicopathological parameters in breast cancer. *Int J Clin Exp Pathol* (2014) 7(11):7931–7.
132. Belguise K, Guo S, Yang S, Rogers AE, Seldin DC, Sherr DH, et al. Green tea polyphenols reverse cooperation between c-Rel and CK2 that induces the aryl hydrocarbon receptor, slug, and an invasive phenotype. *Cancer Res* (2007) 67(24):11742–50. doi:10.1158/0008-5472.CAN-07-2730

133. Vogel CF, Li W, Wu D, Miller JK, Sweeney C, Lazennec G, et al. Interaction of aryl hydrocarbon receptor and NF-kappaB subunit RelB in breast cancer is associated with interleukin-8 overexpression. *Arch Biochem Biophys* (2011) 512(1):78–86. doi:10.1016/j.abb.2011.05.011
134. Parks AJ, Pollastri MP, Hahn ME, Stanford EA, Novikov O, Franks DG, et al. In silico identification of an aryl hydrocarbon receptor antagonist with biological activity in vitro and in vivo. *Mol Pharmacol* (2014) 86(5):593–608. doi:10.1124/mol.114.093369
135. Hall JM, Barhoover MA, Kazmin D, McDonnell DP, Greenlee WF, Thomas RS. Activation of the aryl-hydrocarbon receptor inhibits invasive and metastatic features of human breast cancer cells and promotes breast cancer cell differentiation. *Mol Endocrinol* (2010) 24(2):359–69. doi:10.1210/me.2009-0346
136. Zhao S, Kanno Y, Nakayama M, Makimura M, Ohara S, Inouye Y. Activation of the aryl hydrocarbon receptor represses mammosphere formation in MCF-7 cells. *Cancer Lett* (2012) 317(2):192–8. doi:10.1016/j.canlet.2011.11.025
137. D'Amato NC, Rogers TJ, Gordon MA, Greene LI, Cochrane DR, Spoelstra NS, et al. A TDO2-AhR signaling axis facilitates anoikis resistance and metastasis in triple-negative breast cancer. *Cancer Res* (2015) 75(21):4651–64. doi:10.1158/0008-5472.CAN-15-2011
138. Bekki K, Vogel H, Li W, Ito T, Sweeney C, Haarmann-Stemmann T, et al. The aryl hydrocarbon receptor (AhR) mediates resistance to apoptosis induced in breast cancer cells. *Pestic Biochem Physiol* (2015) 120:5–13. doi:10.1016/j.pestbp.2014.12.021
139. Oyama T, Uramoto H, Kagawa N, Yoshimatsu T, Osaki T, Nakanishi R, et al. Cytochrome P450 in non-small cell lung cancer related to exogenous chemical metabolism. *Front Biosci (Schol Ed)* (2012) 4:1539–46. doi:10.2741/s350
140. Lin P, Chang H, Ho WL, Wu MH, Su JM. Association of aryl hydrocarbon receptor and cytochrome P4501B1 expressions in human non-small cell lung cancers. *Lung Cancer* (2003) 42(3):255–61. doi:10.1016/S0169-5002(03)00359-3
141. Gao SG, Dong XY, Wang LJ, He J. [The study on the chromosome aneuploidy in human lung cancer]. *Zhonghua Yi Xue Za Zhi* (2007) 87(24):1701–3.
142. de Tomaso PA, Caimi GR, Sanchez M, Chiappini F, Randi AS, Kleiman DPD, et al. Hexachlorobenzene induces cell proliferation, and aryl hydrocarbon receptor expression (AhR) in rat liver preneoplastic foci, and in the human hepatoma cell line HepG2. AhR is a mediator of ERK1/2 signaling, and cell cycle regulation in HCB-treated HepG2 cells. *Toxicology* (2015) 336:36–47. doi:10.1016/j.tox.2015.07.013
143. Andrysik Z, Prochazkova J, Kabatkova M, Umannova L, Simeckova P, Kohoutek J, et al. Aryl hydrocarbon receptor-mediated disruption of contact inhibition is associated with connexin43 downregulation and inhibition of gap junctional intercellular communication. *Arch Toxicol* (2013) 87(3):491–503. doi:10.1007/s00204-012-0963-7
144. Terashima J, Tachikawa C, Kudo K, Habano W, Ozawa S. An aryl hydrocarbon receptor induces VEGF expression through ATF4 under glucose deprivation in HepG2. *BMC Mol Biol* (2013) 14:27. doi:10.1186/1471-2199-14-27
145. Kennedy GD, Nukaya M, Moran SM, Glover E, Weinberg S, Balbo S, et al. Liver tumor promotion by 2,3,7,8-tetrachlorodibenzo-p-dioxin is dependent on the aryl hydrocarbon receptor and TNF/IL-1 receptors. *Toxicol Sci* (2014) 140(1):135–43. doi:10.1093/toxsci/kfu065
146. Koch DC, Jang HS, O'Donnell EF, Punj S, Kopparapu PR, Bisson WH, et al. Anti-androgen flutamide suppresses hepatocellular carcinoma cell proliferation via the aryl hydrocarbon receptor mediated induction of transforming growth factor-beta1. *Oncogene* (2015) 34(50):6092–104. doi:10.1038/onc.2015.55
147. Wu PY, Liao YF, Juan HF, Huang HC, Wang BJ, Lu YL, et al. Aryl hydrocarbon receptor downregulates MYCN expression and promotes cell differentiation of neuroblastoma. *PLoS One* (2014) 9(2):e88795. doi:10.1371/journal.pone.0088795
148. Huang TC, Chang HY, Chen CY, Wu PY, Lee H, Liao YF, et al. Silencing of miR-124 induces neuroblastoma SK-N-SH cell differentiation, cell cycle arrest and apoptosis through promoting AHR. *FEBS Lett* (2011) 585(22):3582–6. doi:10.1016/j.febslet.2011.10.025
149. Richmond O, Ghotbaddini M, Allen C, Walker A, Zahir S, Powell JB. The aryl hydrocarbon receptor is constitutively active in advanced prostate cancer cells. *PLoS One* (2014) 9(4):e95058. doi:10.1371/journal.pone.0095058
150. Yin XF, Chen J, Mao W, Wang YH, Chen MH. Downregulation of aryl hydrocarbon receptor expression decreases gastric cancer cell growth and invasion. *Oncol Rep* (2013) 30(1):364–70. doi:10.3892/or.2013.2410
151. Xie G, Peng Z, Raufman JP. Src-mediated aryl hydrocarbon and epidermal growth factor receptor cross talk stimulates colon cancer cell proliferation. *Am J Physiol Gastrointest Liver Physiol* (2012) 302(9):G1006–15. doi:10.1152/ajpgi.00427.2011

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REVIEW
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Impact of Metabolism on T-Cell Differentiation and Function and Cross Talk with Tumor Microenvironment

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The immune system and metabolism are highly integrated and multilevel interactions between metabolic system and T lymphocyte signaling and fate exist. Accumulating evidence indicates that the regulation of nutrient uptake and utilization in T cells is critically important for the control of their differentiation and manipulating metabolic pathways in these cells can shape their function and survival. This review will discuss some potential cell metabolism pathways involved in shaping T lymphocyte function and differentiation. It will also describe show subsets of T cells have specific metabolic requirements and signaling pathways that contribute to their respective function. Examples showing the apparent similarity between cancer cell metabolism and T cells during activation are illustrated and finally some mechanisms being used by tumor microenvironment to orchestrate T-cell metabolic dysregulation and the subsequent emergence of immune suppression are discussed. We believe that targeting T-cell metabolism may provide an additional opportunity to manipulate T-cell function in the development of novel therapeutics.

Keywords: immune system, T-lymphocytes, tumor cell metabolism, cancer, hypoxia, tumor microenvironment

INTRODUCTION

It is well admitted that one of the mechanisms by which immune cells integrate the signals required for their proliferation, migration, differentiation, and effector functions is through the modulation of their metabolic activity (1). In this regard, T cells metabolically reprogram and upregulate glucose and amino acid, to allow the synthesis of the new macromolecules required

Abbreviations: AKT, serine/threonine-specific protein kinase; AMPK, AMP-activated protein kinase; ATP, adenosine triphosphate; CTLs, cytolytic T cells; CTLA-4, cytotoxic T-lymphocyte antigen; FAO, fatty acid oxidation; GLUT, glucose transporters; HIF, hypoxia-inducible factor; IFN-γ, interferon-γ; MHC, major-histocompatibility complex; mTOR, mammalian target of rapamycin; c-Myc, avian myelocytomatosis virus oncogene cellular homolog; OXPHOS, oxidative phosphorylation; PD-1, programmed death-1; PD-L1, programmed death ligand-1; PI3K, phosphatidylinositol-3 kinase; PPP, pentose phosphate pathway; PTEN, phosphatqase and tensin homolog; ROS, reactive oxygen species; TAA, tumor associated antigen; TCA, tricarboxylic acid; TCR, T-cell receptor; T_{eff}, effector T cells; Th, helper T cells; TNF-α, tumor necrosis factor; T_{reg}, regulatory T cells.

for their proliferation and effector function (2, 3). Furthermore, beyond these key nutrients, iron uptake is also critical for T-cell function (4). Indeed, development and differentiation of antigen-specific T cells depend on iron uptake and internalization via type I transferrin receptor (5). Several previous studies suggested that iron deficiency impaired T-cell proliferation and cytokine production in activated T cells. Conversely, less is known about the effect of iron overload on T-cell function (6).

Nevertheless, how metabolism regulates immune T-cell differentiation, function, and plasticity remains very challenging and how immune cells function in terms of their intracellular metabolism and how these metabolic pathways affect the phenotype and activation of immune cells is attracting a lot of attention at present. Tumor progression is characterized by a tangled network of relationships among different cell types that collectively exploit a metabolic reprogramming and mutually influence their functionality and, in particular, T-cell functions. Our recent knowledge of T-cell molecules involved in the regulation of antitumor T-cell responses has led to the development of several monoclonal antibody-based therapies, against molecules like cytotoxic T-lymphocyte antigen (CTLA-4) or programmed death-1 (PD-1) (7). Although these treatments have shown unprecedented responses in some patients suffering from several cancers (8–10), the response rates are usually low and transient. This is likely due to multiple mechanisms suppressing antitumor immune functions within an unfavorable tumor milieu and metabolism. The metabolic activity of T cells in the context of tumor microenvironment could be one of the key mechanisms.

It should be noted that the dynamic and reciprocal interactions between tumor cells, metabolites, and a variety of cells including immune cells from the tumor microenvironment orchestrate several events, which are critical for tumor evolution toward metastasis. In this context, many cellular and molecular elements of the tumor ecosystem are emerging as attractive targets for therapeutic approaches. Among these targets, hypoxia, which is a hallmark of solid tumors, is strongly associated with advanced disease stage and poor clinical outcome. This is, in part, due to inappropriate local immune reaction and resistance of hypoxic tumor cells to cytotoxic treatments. In fact, most human tumors develop a pathophysiological microenvironment during growth, characterized by an irregular microvascular network and regions of chronically and transiently hypoxic cells. We and others provided evidence that hypoxia plays a crucial role in tumor promotion and immune escape by conferring tumor resistance (11) immunosuppression (12) and tumor heterogeneity (13), which contributes to the generation of diverse cancer invasion programs and enhanced stroma plasticity (11, 14). Therefore, it is of major interest to understand how immune cell intracellular metabolism and some metabolic pathways influence the acquisition of their phenotype, the regulation of their activation and effector function. The metabolic activity of T cells in the context of tumor microenvironment, its heterogeneity, and complexity is therefore an important consideration in immunotherapy. Clearly, if T cells play the music during an adaptive immune response, the metabolic tumor microenvironment calls the tune. Indeed, a better understanding of these metabolic related issues in relationship with T-cell activity may offer new therapeutic strategies in future to better control their plasticity and effector function and boost their efficacy and potential use in cancer immunotherapy approaches.

BASIC OVERVIEW OF METABOLISM IN T CELLS

Metabolism is the process whereby cells can either break down molecules to generate energy in the form of adenosine triphosphate (ATP) or synthesize several macromolecules. Metabolism could be divided into two complex pathways: the catabolic processes, critical for cellular proliferation and functions and the anabolic process, important for cellular growth.

Consistent studies focused on the molecular mechanisms that dictate metabolic reprogramming in the immune cells (15). It is now widely appreciated that T-cell metabolic remodeling plays a key role to shape immune response, in particular, antitumor immunity. Profound metabolic changes occur under tight regulation allowing T cells to maintain energy balance between anabolic and catabolic metabolism, which support adequate immune responses (16, 17).

During quiescence, T cells require energy-oriented oxidative metabolism and relatively small amounts of glucose, amino acids and fatty acids to maintain basic energetic, primarily anabolic and minimal replacement biosynthesis demands. Encounter with cognate antigen activation, T-cell stimulation by T-cell receptor (TCR) ligation and binding with costimulatory molecules induce metabolic remodeling (18, 19). In fact, metabolism shifts to glycolysis to support rapid growth and to biosynthesis for differentiation into effector T cells (T_{eff}) (1, 20, 21) (**Figure 1A**). Albeit, aerobic glycolysis is less efficient than oxidative phosphorylation (OXPHOS) at yielding ATP, it generates metabolic intermediates which are important for cell growth and proliferation as well as for cytotoxicity and cytokine production. Nevertheless, glycolytic pathway generates macromolecule precursors required in the pentose phosphate pathway (PPP) for cell growth and NAD phosphate (NADPH) production important for anabolic pathways and maintaining redox balance (22).

After pathogen clearance, most T cells go through apoptosis while few of them remain as long-lived memory cells responsible for enhanced immunity against upcoming pathogens or tumors re-exposure (23).

DISTINCT METABOLIC PROGRAMS FOR T CELLS DIFFERENTIATION AND FUNCTION

T lymphocytes (T cells) that undergo an immune response constitute an ideal system to study the rapid shift from quiescent to active state that belongs to growth, proliferation, and differentiation into largely heterogeneous T-cell subsets. Emerging concepts in immunology suggest that lymphocyte activation is intricately linked to metabolic reprogramming (24–26). In fact, metabolism fundamentally underpins T-cell function and lymphocytes

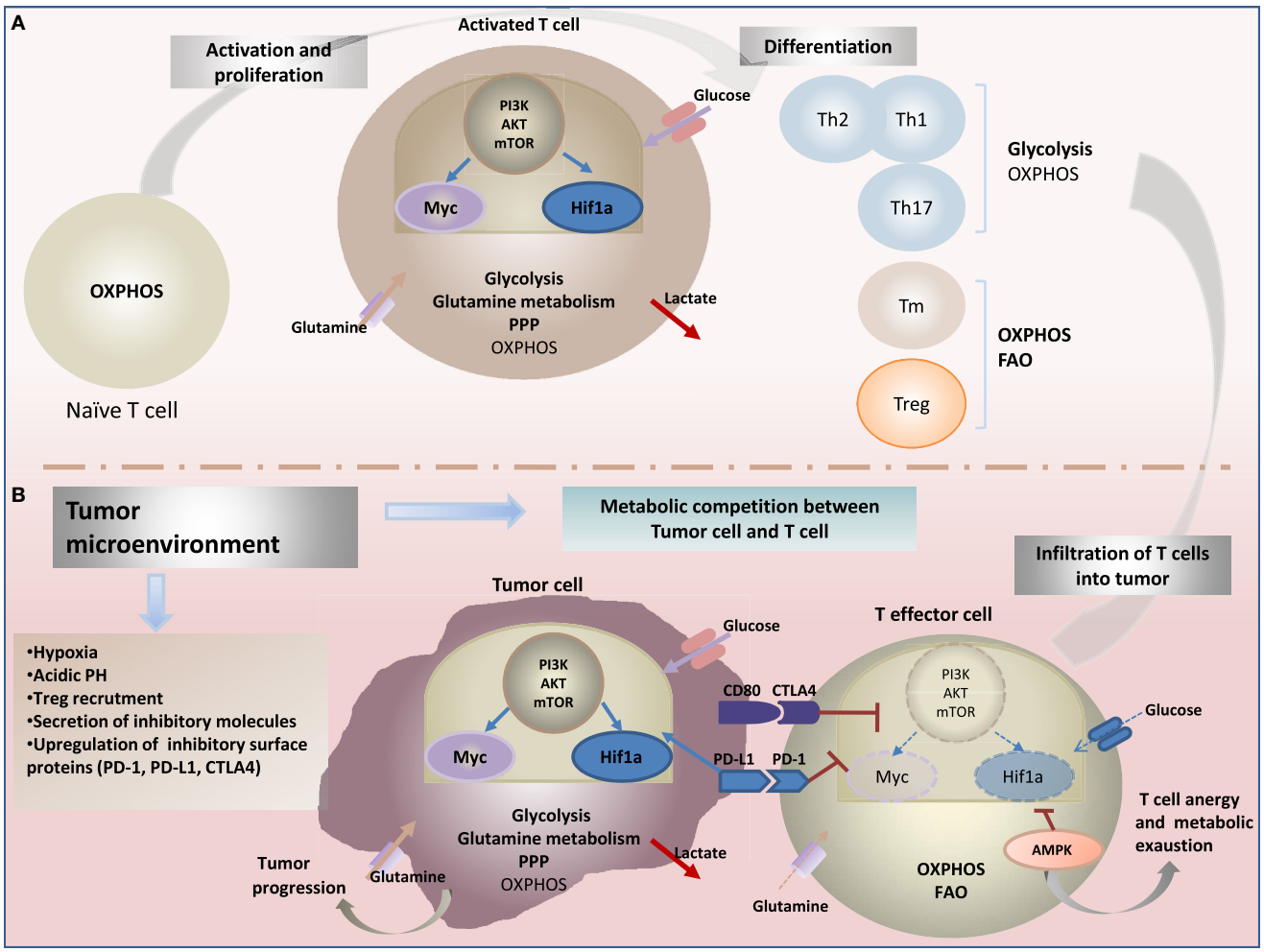

FIGURE 1 | **Metabolic reprogramming drives: (A) T-cell fate and function and (B) antitumor-immune response**. **(A)** Upon activation through T-cell receptor (TCR) and costimulatory signals, T cells engage in growth, and differentiation into different cytotoxic, regulatory T cells (T_{reg}), helper T (Th), and memory T (T_m) subsets cells. Metabolic reprogramming has been shown to intimately support T-cell activation and differentiation. While naïve T cells rely on oxidative phosphorylation (OXPHOS) to maintain energy demand; activated T cells engage increased aerobic glycolysis and glutaminolysis consuming massive amount of glucose and glutamine, enabling to generate effector cytokines, including interferon-γ (IFN-γ) and IL-2. In contrast to cytotoxic and effector Th cells, the metabolic profile of T_{reg} and T_m cells rely on OXPHOS and fatty acid oxidation (FAO) to support their survival and differentiation. The central energy-monitoring system underlying this metabolic remodeling is the phosphoinositide 3-kinase/protein kinase B/mammalian target of rapamycin (PI3K/AKT/mTOR) pathway that boosts glycolytic activity in T cells via activation of transcription factors hypoxia-inducible factor-1α (HIF-1α) and Myc pathways. **(B)** Tumor-specific T cells are often rendered dysfunctional due to an immunosuppressive microenvironment. Infiltrating T cells are reprogrammed by the tumor favoring its survival and immune escape. Cancer cells induce several metabolic changes in the microenvironment. Tumor-mediated decreased extracellular nutrients levels cause impaired glycolysis capacity and IFN-γ production in T cells. Cancer cells also generate a hypoxic microenvironment. Hypoxia stabilizes HIF-1α and enhance glycolysis in tumor cells, a phenomenon recognized as "the Warburg effect." HIF-1α also enhances constitutive expression of programmed death ligand-1 (PD-L1) leading to activation of Akt/mTOR pathway. Activating immune checkpoints and PD-L1 signaling through binding to its receptor programmed death-1 (PD-1) leads to dampening of the Akt-mTOR pathway and reduced T-cell glycolysis. Collectively, tumor environment affects metabolic fitness of infiltrating immune cells and drives impairment of antitumor effector function and increased tumor progression.

metabolism is dynamically regulated depending on their separate phases of development: (1) naïve or resting, (2) effector or activated, and (3) memory T (T_m) cells (27).

Naïve T cells are activated to rapidly respond to foreign pathogens or inflammation through a tight interaction with the TCR and major-histocompatibility complex. Further, T cells enter the effector phase of rapid growth, proliferation, and differentiation. T_{eff} could be divided into cytolytic T cells, secreting granzyme B, perforin, interferon-γ (IFN-γ), into helper T cells (Th) including the type-1 (Th1), type-2 (Th2), and type-17 (Th17) producing characteristic cytokines or into regulatory T cells (T_{reg}) (28, 29). T_{eff} play a pivotal role to mediate antitumor immunity. Hence, T_{reg} obstruct T_{eff} activity and suppress immunity, showing a poor prognosis in many cancers (30). After expansion phase and antigen clearance, most clonally differentiated T cells undergo apoptosis, while a small fraction

become quiescent T_m cells, responsible for enhanced immunity after re-exposure to the pathogen (31, 32). The differences in functional and phenotypic characteristics of quiescent T cells and activated T cells are supported by differential metabolic requirements (17). Each subset of T-cell demonstrates unique metabolic demands and signaling pathways that contribute to its fate and function (25).

Quiescent T cells and activated T-cell fate are characterized by different metabolic pathways (33–35). Globally, activated T cells adopt an anabolic metabolism supporting rapid proliferation whereas quiescent T cells engage catabolic metabolism (36). T_{eff} subtypes switch their metabolic program to robust aerobic glycolysis, but increased glycolytic rates occurred much higher in Th1, Th2, and Th17 cells than in T_{reg} cells (24). T_{reg} cells sustain enhanced fatty acid oxidation (FAO) metabolism as a major source of energy to maintain their survival (37–39). Upon antigen encounter, upregulation of aerobic glycolysis in extensive proliferating T cells is accompanied with glutaminolysis, PPP, not only to support ATP generation, but also to enhance biosynthesis of crucial intermediates and precursors necessary for subsequent macromolecules that are incorporated into cellular biomass (40, 41) (**Figure 1A**). Th17 cells rely, in particular, on increased glycolysis. Hence, inhibiting glycolysis during Th17 cell differentiation re-enforce T_{reg} generation (42). Nevertheless, consistent data suggest that mitochondrial reactive oxygen species (ROS) produced during OXPHOS is also crucial to activate T-cell and to enhance antigen-specific proliferation. However, excessive ROS levels are toxic for T cells and leads to apoptosis (43). CD4 + regulatory T lineage cells exhibit a mixed metabolic program involving mainly FAO and OXPHOS and low level of glycolysis (44). T_{reg} favor FA catabolism via b-oxidation and prioritize oxidative ATP to meet their energetic demands, an important metabolic phenotype for the differentiation of T_{reg} (38).

After the clearance of pathogens, the remaining antigen-specific T cells (T_m cells) as a quiescent T-cell population share common metabolic requirements with other nonproliferating cells. T_m cells maintain catabolic profile with lower nutrient uptake and biomass synthesis and predominantly engage mitochondrial OXPHOS and FAO metabolism for long-term persistence, ATP production and the capacity to vigorously respond to antigen stimulation (45). Several studies revealed that maintaining mitochondrial mass is critical for T_m cells development since it offers the opportunity to use a wide range of substrates responsible for energy generation, like fatty acids (46, 47). FAO constitute a preferred fuel source for T_m cells as this lipid oxidation generates intermediate of tricarboxylic acid (TCA) cycle related to OXPHOS metabolism. However, their detailed metabolic profiles remain to be explored (25).

Metabolic regulation of T-cell fate and function involves a network of molecular regulators. The main induced signaling pathways underlying the activation through the TCR with CD28 costimulation, is the phosphoinositide 3-kinase/protein kinase B/mammalian target of rapamycin (PI3K/Akt/mTOR) (48–50). Increasing evidences suggest that the mammalian target of rapamycin (mTOR) is a central regulator of cell metabolism. Interestingly, T-cell differentiation to effectors or T_m cells is governed in part by asymmetric partitioning of fate determining proteins (51, 52). Recent study demonstrates that asymmetric division of T cells generates two sets of daughter cells with differential mTORC1 activity (53). The first set exhibits increased activity mTORC1, as well as high levels of glycolysis and effector molecules expression. Besides, the second T-cell set shows decrease in mTORC1 activity associated with enhanced rates of lipid metabolism and antiapoptotic molecules. Behind, the latter daughter cells display enhanced long-term survival and differentiate to T_m cells (53). This pathway plays key transcriptional and post-transcriptional roles to promote anabolic gene expression and intracellular trafficking of nutrient transporters (54). mTOR is the downstream target of the PI3K–AKT signaling and a central player governing metabolic reprogramming and fate of T-cell (55–57). Two major transcription factors are upregulated by mTOR: avian myelocytomatosis virus oncogene cellular homolog (c-Myc) and hypoxia-inducible factor-1α (HIF-1α) (58, 59) (**Figure 1A**). It has been shown that c-Myc is crucial to activate glucose transporters (GLUT) and key enzymes for enhancing glucose influx and glycolysis that accompany early stage of T-cell growth, proliferation, and the transition from a naïve T-cell to a T_{eff} cell (60). Furthermore, c-Myc is responsible for enhanced glutaminolysis by inducing glutamine transporters and glutaminase1 expression to sustain cell growth and proliferation (60, 61).

Hypoxia-inducible factor-1α is another master transcription factor monitoring glycolytic enzymes expression (62, 63). HIF-1α acts also to downregulate mitochondrial oxygen consumption and inhibit TCA cycle. At later times of differentiation, the role of HIF-1α appears more complex to mediate T-cell fate and function (64). HIF-1α is reported to play a more selective role in inflammatory Th17 CD4 T-cell subsets (42) and cytolytic CD8 T (58). In addition, HIF-1α appears to influence the balance of Th17:T_{reg} cells (65). Indeed, it directly promotes glycolysis in differentiating Th17 cells and reciprocally increases Th17 differentiation and decreases T_{reg} differentiation (66). However, *in vitro* and *in vivo* studies demonstrate that a lack of HIF-1α strongly impair Th17 cell development and drives T_{reg} cell differentiation and FAO. T_{reg} cells unlike other T_{eff} cells mainly display increased FAO metabolism and enhance AMP-activated protein kinase (AMPK) activation (67). The utilization of lipid oxidation by T_{reg} cells might play a central role in their survival advantage over T_{eff} cells and in the maintenance of a stable pool of pro-tumor (68, 69).

Finally, the mechanisms regulating the transition of T cells from effector to memory states remain to be elucidated. Recent studies demonstrated that mitochondrial FAO in T_m cells require stimulation of tumor necrosis factor receptor-associated factor 6 pathway (70). Further, memory CD8+ T-cell development is also supported by activating the energy sensor AMPK pathway (71, 72). FAO has clinical implications for memory CD8+ T as well as for T_{reg} cells (73). In fact, administration of metformin or the mTOR inhibitor rapamycin, reduce mTOR activity and induce AMPK phophorylation that in turn perform lipid oxidation and enhance the formation of T_m cells after infection and increase T_{reg} responses in asthma model (74, 75).

FUELING T-CELL PROLIFERATION

Increasing data suggest that regulation of metabolic fuels uptake is a critical component of T-cell activation to accomplish their functional requirements. Yet, limiting conditions could suppress the suitable access to nutrients, causing a barrier to T-cell function. To maintain a proper response, T-cell activation requires the upregulation of both glucose and amino acid transporters (1, 76). Several metabolic pathways that are imminent for lymphocyte proliferation are supported by the availability of these fuels (24).

Glucose

Glucose is the most used nutrient predominantly existing in the surrounding environment, and glucose metabolism, in particular, is essential for T cells for normal survival and function. Glucose is a critical substrate for energy production, and its deprivation prevents T-cell function despite the presence of other alternative carbon source (77, 78). When T_{eff} are activated, glucose uptake raises to maintain aerobic glycolysis and subsequently to support growth and proliferation, whereas glucose use via OXPHOS is decreased (79). Further, the expression and trafficking patterns of GLUT are upregulated allowing T cells to enrich their intracellular glucose. The GLUT consists of 14 different members (GLUT1–14) relying on diverse substrate specificities (80). GLUT2 and GLUT3 are expressed in resting human peripheral blood T cells, while GLUT1 is expressed at a low level in naïve T cells, but rapidly induced upon T-cell activation. Consequently, overexpression of GLUT1 after TCR activation leads to increased glucose uptake and enhanced expression and activity of glycolytic enzymes. During glycolysis, glucose is not fully oxidized in the mitochondria but rather broken down into pyruvate that is converted into lactate even though in presence of sufficient oxygen (81). Glucose could be also derived toglucose-6phosphate and further directed into the PPP, providing precursors for the synthesis of nucleotides and aromatic amino acids (77).

It has also been reported that T-cell cytokine production is also relying on glucose. In fact, data showed enhanced T-cell cytokine production such as IL-2 and IFN-γ in transgenic model expressing GLUT1 specifically in T cells (78). In contrast, glucose deprivation has been shown to strongly inhibit cytokine production and to decrease cytolytic activity of CD8+ T cells, marked by reduced granzyme and perforin production. Thus, failure to properly upregulate glucose metabolism during T-cell activation can lead to impaired proliferation. As a consequence, T cells can enter to anergy if they survive this metabolic stress, or they die by apoptosis. Collectively, glucose is fundamental to support proliferation and effector functions that accompany clonal expansion of T_{eff}. Besides, T_{reg} cells do not depend on high rates of glucose as they express low levels of GLUT1 and rely on lipid oxidation for energy (39).

Glutamine

Glutamine is a nonessential amino acid and the most abundant nutrient in the blood. Glutamine constitutes also a critical substrate for T cells activation and growth process. Following T-cell activation through efficient TCR signaling, the uptake and biosynthesis of amino acids or amino acid transporter expression are dramatically increased (82, 83).

Glutamine catabolism is dramatically induced in active T cells providing intermediate molecules necessary for different pathways of biosynthesis and substrates for mitochondria (84, 85). During glutaminolysis, glutamine carbon backbone can be converted to α-ketoglutarate to maintain homeostasis of the TCA, or to lactate that generates NAD and NADPH (86). During T-cell activation glutamine can be used, providing pyruvates to overcome intense aerobic glycolysis levels (87). Further, activated T cells selectively increase glutamine uptake. This increase has been suggested to be concomitant with induced expression of glutamine transporters, recognized as members of the sodium-dependent neutral amino acid transporter (SNAT) family. In fact, the previous study demonstrates rapidly enhanced mRNA expression of SNAT1 and SNAT2 isoforms after *in vivo* stimulation of T cells (82). However, lack of glutamine can result in profound inhibition of cell growth, proliferation, and cytokine production (88). Since T-cell activation is strongly impacted by glutamine, thus different aspects of glutamine metabolism could serve as novel targets for immune modulation.

Tryptophan and Arginine

In addition to glutamine, other limiting amino acids such as tryptophan and arginine have been suggested to be crucial for T-cell activation and function. This concept has gained interest especially in cancer context, where tumor-induced extracellular depletion of these amino acids alters T-cell activity and causes their anergy. Tryptophan is an essential amino acid required for the production of several important molecules and its catabolism through the kynurenine pathway generate metabolites such as kynurenine, kynurenic acid, 3-hydroxy-kynurenine, and 3-hydroxy-anthranilic acid (89). Numerous studies showed that tryptophan plays a key role in T-cell survival and activation whereas its metabolites eliminate T-cell function and are able to induce T-cell apoptosis (90). T_{eff} are affected by the decrease in tryptophan concentrations and high rates of toxic tryptophan-metabolites induced by mature antigen-presenting cells expressing enzymes that catabolize tryptophan (91). Tryptophan degradation is one of a resistance mechanisms adopted by tumors to avoid immune suppression (92, 93). Three enzymes were identified to control tryptophan degradation through the kynurenine pathway: tryptophan-2,3-dioxygenase, indoleamine 2,3-dioxygenase 1, and indoleamine 2,3-dioxygenase. Hence, T-cell cycle progression is prevented and T_{eff} cells shift to anergy and apoptosis. In hostile tumor microenvironment context, such inhibition is resulting in suppression of antitumor immune responses (94).

In addition to tryptophan, arginine has gained much attention as an important amino acid in T-cell function. Arginine is a versatile amino acid engaged in protein synthesis and in generating many metabolites precursors including, polyamines, and nitric oxide involved in immunometabolism (95). Indeed, deficiency in extracellular arginine or in enzymes responsible of *de novo* synthesizing arginine [argininosuccinate 1 (ASS1)], has been found to critical during activation (96). Low levels of arginine impair T-cell proliferation, aerobic glycolysis and reduce cytokine

production and expression of activation markers such as CD25 and CD28 (97, 98). Further, deletion of ASS1 blunt *in vitro* Th1 and Th17 cell polarization, even in the presence of extracellular arginine (99). Interestingly, recent study showed that increased arginine levels display improved survival capacity of T memory cells and antitumor activity (95). Taken together and according to the beneficial effect of arginine and tryptophan on T-cell metabolic adaptation and antitumor activity, both amino acids would be exploited as an attractive target for therapeutic intervention in antitumor response (96).

WARBURG EFFECT OR HOW CANCER CELL REWIRE METABOLIC PROGRAM

It is well established that cancer cells must reprogram cellular pathways to enable their growth and proliferation. Tumor cells reprogram their metabolic pathways and rely upon increased glucose uptake and high rate lactate production, principally through aerobic glycolysis (100), regardless of the level of oxygen (101). Metabolic switch of cancer cell supports biosynthesis of essential macromolecules (nucleic acids, lipids, and amino acids), through interconnected pathways. This metabolic program was recognized since 1920s by Otto Warburg as the "Warburg effect" (102, 103), a strategic metabolic adaptation enhancing rapid tumor growth, proliferation, and to dampen antitumor immunity, thus representing one additional hallmark of cancers. Since 1923, Otto Warburg has reported that cancer cells acquire irreversible switch of their energy-producing machinery from mitochondrial OXPHOS respiration, to aerobic glycolysis (104). Glycolysis is a predominant energy source for cancer cells, occurring either under aerobic or hypoxic conditions to produce large amounts of lactate, and much less efficient than OXPHOS for producing ATP (105, 106) (**Figure 1B**). This reprogramming of cancer cell metabolism has been acknowledged recently as a hallmark of cancer with many faces (107, 108). By analogy to immune cells, similar metabolic features with T cells during activation are observed. But, despite an apparent similarity, there is deep down a wide difference between glycolysis in activated T cells and cancer cells. Such metabolic transitions in T cells are part of a physiological adaptation process. However, intrinsic genetic mutations and external responses to the tumor microenvironment monitor the metabolic phenotype of tumor cells (109, 110). Cellular dysregulation of oncogenic signaling pathways are the result of the loss of tumor suppressors (such as p53) or the activation of oncoproteins (such as PI3K) (111). As a consequence, cancer cells thereby gain selective growth and survival (112).

Cancer cells use the Warburg effect as strategic metabolic adaptation to satisfy their urgent requirements for growth and proliferation under tumor microenvironmental limitations for oxygen and nutrients (113, 114). Under hypoxic conditions, cancer cells accelerate metabolism that lead to increased NADPH rate to cope with higher ROS levels (115, 116). Thus, the Warburg effect also supports tightly controlled redox balance for cancer cells, considered as important survival mechanism (117).

Glucose is considered as prominent player in the alterations of metabolism and energetic of cancer cells (118). Increased glucose uptake lead to upregulated glycolysis and thus more pyruvate is produced even in normoxia conditions. Under limited oxygen availability (hypoxia), more pyruvate avoids TCA cycle and generates excess of lactate secreted thereby in the tumor microenvironment (118, 119). In addition to its central role as a carbohydrate nutrient for ATP synthesis, new evidence revealed that high glucose uptake is also important for biomass synthesis needed for rapidly proliferating cancer cells. Upregulation of glycolysis increased several metabolic intermediates that may be shunted to interconnected pathways, as PPP (120, 121). The resulting glycolytic intermediates such fructose-6-phosphate, glyceraldehyde-3-phosphate, and 3-phosphoglycerate are critical for *de novo* synthesis of ribonucleotides, amino acids, and phospholipids, respectively (122).

Glutamine is the most abundant free amino acid and essential source of carbohydrate for proliferating cells. Cancer cells display increased glutamine demand and consumption. Interestingly, the glutamine dependence extends beyond protein synthesis to other important requirements (123). Rapidly proliferating, cancer cells use glutamine to fuel biosynthesis of nucleotides, to replenish TCA cycle intermediates through a process called anaplerosis, or to be taken from the mitochondria and then modified into lactate (glutaminolysis) (124, 125). Glutamine metabolism occurs in cancer cells, in general, with concomitant production of NADPH that not only maintains cellular redox but also reduces agent in varied biosynthetic pathways–underlying *de novo* fatty acid synthesis (126).

The molecular drivers that lead to the shift of cancer cell from oxidative to glycolytic metabolism are distinct and tend to happen simultaneously. Cancer metabolism adaptation to the anabolic program has been suggested to be under direct management by various transcription factors, such as Myc and hypoxia-inducible factor 1 (HIF-1) (127, 128).

Myc is a transcription factor upregulated in tumors and considered as master regulator of normoxic cancer cell reprogramming (129). Indeed, Myc contributes to cancer cells switch to aerobic metabolism by facilitating cellular glucose uptake and activating the expression of numerous genes essential for glycolysis. Furthermore, Myc plays important role to promote macromolecules synthesis and mitochondrial biogenesis, critical for fast developing cancer cells (130, 131).

Upon rapid proliferation, hypoxia becomes a key mediator of the Warburg effect and a common feature of human tumors. Extensive studies have provided evidence that cancer cells utilize hypoxia as physiological adaptation pathway that promotes metabolic changes in fast growing tumors (132). Indeed, under hypoxic tumor microenvironment, the uptake of glucose and the glycolytic flux are increased. This metabolic adaptation is mainly orchestrated through the upregulation of the transcription factor, HIF-1α. HIF-1α is induced by low oxygen conditions and recognized as independent marker of poor prognosis (133, 134). The activated tumor glycolytic flux involving HIF-1α implies upregulation and increased activity of several glycolytic protein including key glycolytic enzymes (HK2, PFK-L, PKM2, and LDH-A) and GLUT (GLUT1 and GLUT3) (135, 136). In contrast to Myc, HIF-1 strongly inhibits mitochondrial respiration and biogenesis (111).

Furthermore, PI3K/Akt/mTOR is one of the most frequently altered signaling pathway known to play an important role in glycolysis, cancer metabolism and cancer cell proliferation (137, 138) (**Figure 1B**). It is well known that this pathway is activated under the loss of function of the tumor suppressor gene phosphatqase and tensin homolog. The best studied driver of tumor glycolytic program in such pathway. The latter has been reported to induce GLUT expression and to stimulate phosphorylation of key glycolytic enzymes (139). In addition, AKT1 strongly activates mTOR signaling pathway. Hence, mTOR is constitutively activated during tumorigenesis (140) and constitutes a key metabolic issue, coupling cell growth to protein, and lipid biosynthesis (141).

TUMOR MICROENVIRONMENT ABROGATES T-CELL METABOLIC AND IMMUNE CHECKPOINTS

Immuno-metabolism plays a key role of adaptive immunity and is particularly central to effective antitumor T-cell responses. T cells, following the metabolic strategies of growing tumors, have to start their effector programs. However, most of human tumors proliferate in spite of the presence of tumor associated antigen-specific T cells. In fact, tumor microenvironment may impose several limitations to dampen T-cell immunity (142) and deplete crucial nutrient availability and handling, such as glucose or amino acids (143). It can also stimulate conserved negative feedback mechanisms, such as through PD-1 (144). Besides, tumor cells must evade the checkpoint controls under such stressful metabolic conditions.

Tumor microenvironment is a forbidding environment that can pose significant metabolic challenges for infiltrating T cells to impair the effectiveness T-cell response. It is likely that T cells undergo immune suppressive networks that impair their specific functions and thereby enable tumor escape (145, 146). Many different molecular and cellular mechanisms have been proposed to contribute to the failure of T cells in tumor eradication. Recent studies have started to reveal that the feature and function of T_{eff} in tumors are severely influenced by the tumor microenvironment context (147). Indeed, tumor microenvironment components form a very complex immunosuppressive network in cancer (148), lead to metabolic and immune checkpoints abrogation, which limits T-cell activation and induces T-cell dysfunction (149, 150). However, the exact mechanisms remain insufficiently understood.

Evidence is beginning to emerge suggesting that alterations of the T-cell metabolic pathways are critical to impair antitumor immunity, supporting immune escape (151). Cancer cells are recognized to be the most important players in tumor microenvironment mediating immune suppression. In fact, metabolic interplay and nutrient (glucose and glutamine) competition between cancer cells and T cells exist. Such competition is recognized as a key driver of cancer progression (152, 153). Due to high demand for energy and increased glucose addiction and glycolysis rate, fast growing cancer cells consumes most nutrients and specifically increases rate of glucose intake, from the surrounding environment (154). As a consequence, tumor-imposed metabolic restrictions can mediate T-cell hyporesponsiveness during cancer. T cells dramatically reduced glycolysis and become unable to produce cytokines and to develop into tumor-specific T_{eff} cells, leading to a state of anergy (155) (**Figure 1B**). Thus, T_{reg} cells differentiation is favored to inhibit antitumor immune response, instead of expansion of tumor-specific T cells (156, 157). As a contrast to T_{eff} that suffer from a hostile tumor microenvironment, T_{reg} cells, feel comfortable with a similar environment (158). This is possibly the result of to the flow in growth factors (such as transforming growth factor-β) and chemokines (such as CCL22) promoting T_{reg} differentiation and recruitment (156, 159). One molecular explanation is that alteration of functional fate of T cells due to nutrient limitation could occur through modulation of metabolically sensitive signaling pathways. Under tumoral context, the balance between T_{eff} and T_{reg} may be directly disturbed when AMPK signaling pathway inhibits mTORC (56, 160). Opposing to mTORC, AMPK is activated in conditions where nutrients are limiting and promote oxidative metabolism (161) (**Figure 1B**). AMPK can be highly phosphorylated and activated in T_{reg}. Consequently, T_{eff} function is impaired while T_{reg} cells are promoted. Furthermore, T_{reg} cells have also been reported to be induced under hypoxic tumor microenvironment, through over activated HIF-1α (12, 162). The presence of T_{reg} in solid tumors essentially correlates with poor prognosis (27).

Immunosuppressive tumor microenvironment is also characterized by elevated rates of ROS (115). Besides cancer cells, tumor-infiltrating leukocytes, including myeloid-derived suppressor cells, tumor-associated macrophages, and T_{reg}, also generate excessive ROS (163). It has been demonstrated that high level of ROS in the tumor microenvironment downregulates T-cell activity and enhanced T-cell apoptosis, inhibiting subsequently antitumor immune response (164). However, although high levels of ROS impair T-cell metabolism and function, ROS at a low or moderate-concentration is indispensable for T-cell activation and effector function (165). Considering the paradoxal effect of ROS on T-cell function a tight balance between production and consumption of ROS should be accomplished to potentiate antitumor activity compromising T_{eff} function.

Under immunosuppressive tumor microenvironment T cells acquire an "exhausted" phenotype highlighted by upregulation of inhibitory receptors. Interestingly, to eradicate effectiveness of antitumor immune response, tumor hostile environment act not only to impair metabolic checkpoints of T_{eff} cells encountering tumor antigens, but also to abrogate immune checkpoints. Indeed, several negative feedback mechanisms are stimulated, such as PD-1 and CTLA4 pathways (166, 167), which can both promote T cells exhaustion (**Figure 1B**). Hence, further research is needed to identify new target to reverse exhaustion in addition to PD-1 and CTLA4.

Programmed death-1 is the major inhibitory receptor in T cells regulating T-cell exhaustion. Interaction of PD-1 with its ligand programmed death ligand-1 (PD-L1), allows the tumor to evade immune system by inhibiting T-cell function (168, 169). Recently, it has been reported that upon ligation, T cells receiving PD-1 signals can lower the capacity of T cells to express GLUT1,

uptake glucose, and become unable to engage in glycolysis, glutaminolysis, or metabolism of branched-chain amino acids (144). Interestingly, PD-1 displayed an increased rate of FAO of endogenous lipids, and lipolysis is indicated by elevation of the lipase ATGL and by release of fatty acids (144). PD-1 signaling is associated with reduced cMyc expression and inhibition of activity of the PI3K/Akt/mTOR pathway, necessary for effector function (50, 170, 171). Besides, PD-L1 directly regulates tumor metabolism. Surface expressed PD-L1 is important for Akt/mTOR signaling to promote mTOR activity and glycolytic metabolism in tumor cells (172, 173).

Nonetheless, CTLA4 signaling also plays a key role in tumor immune escape since it inhibits CD28-mediated costimulation of T_{eff} and favors T_{reg} expansion (174, 175). Subsequently, CTLA4 may broadly impair T_{eff} cell activation against antigenic stimulation in part by reducing the capability of Akt to enhance GLUT1 expression, glucose uptake, and aerobic glycolysis, but without enhanced FAO as for PD-1 pathway (151).

IMMUNE CHECKPOINTS TARGETING FOR ENHANCING T-CELL FUNCTION: RELATIONSHIP WITH METABOLISM

Metabolic reprogramming plays a pivotal role for appropriate T-cell activation that supports antitumor immunity. However, T-cell function is compromised by the immunosuppressive tumor microenvironment. Nevertheless, multiple mechanisms that instruct the development of immune suppression may exist to prevent effective antitumor response, but remain largely unclear. Metabolic and functional pathways in T cells may uncover new targets and challenges for cancer therapy (176). Therefore, manipulating metabolism may be a way to beneficially enhance or temper antitumor immunity. Current attractive therapeutic approaches which specially target T-cell metabolism are meant to use immunotherapy directed against several negative immunologic regulators CTLA-4 and PD-1/PD-L1 pathway (177–179).

In recent studies, it has been reported that mice exhibiting or transplanted with tumors were treated with checkpoint blockade therapy, such blockade increased the glucose concentrations in the extracellular tumor milieu and TILs from these mice had increased glucose uptake, glycolytic rates, activated mTORC1 pathway, and IFN-γ production (152). The same effects were reported after PD-L1 blockade or RNA interference directed against PD-L1 in cultured tumor cells (152). More importantly, T cells in allogeneic PD-$L1^{-/-}$ bone marrow transplant recipients had elevated levels of GLUT1 and lactate production, suggesting a normal *in vivo* role for PD-1 signaling to restrain T-cell glucose metabolism (180).

Although there is a promising efficacy of immunotherapy, the clinical benefit has been restricted by tumor-derived immunosuppression and its related coinhibitory signals. Indeed, to escape antitumor immune response, tumors develop different strategies including secretion of immunosuppressive cytokines and chemokines (TGF-β, IL-10, VEGF, CCL2, and CCL12) (181) or immunosuppressive converting tryptophan and arginine enzymes [indoleamine-2,3-dioxygenase (IDO) and arginase, respectively] (98, 182, 183). In light of this, it would be reasonable to combine immunotherapy with an immunosuppression-blocking protocol. In particular, IDO is an attractive area for exploitation to potentiate immunotherapy, since it is highly expressed in the microenvironments of various tumors (184). Recently, preclinical studies demonstrate the efficiency of two IDO inhibitors to attenuate tumor growth (185, 186). Currently, IDO inhibitors entered clinical trials (187). Interestingly, *in vivo* study has been conducted on mouse melanoma model where synergistic immunotherapy strategy that locally targets PD-1 and IDO for the treatment of melanoma has been developed. The preliminary results are quite encouraging and showed enhanced T_{eff} cells and antitumor efficacy (188).

Therefore, the use of metabolism-targeting drugs working with checkpoint inhibitors might not only change the activation and differentiation program of tumor-specific T cells but also prohibit the generation of exhausted T cells. Currently, there is a lack of data taking into consideration the metabolic consequences occurring in T cells and/or tumor cells by targeting these immune checkpoint pathways. Nevertheless, combined immunotherapeutic strategies would be exciting and show promise to improve the anticancer efficacy of immunotherapy in the future.

CONCLUDING REMARKS

It is widely admitted that tumors are not autonomous masses of cells but function as organs composed of many interdependent cells supporting malignant cell survival, growth and progression. To ensure tumor growth and immune evasion, the tumor stromal components undergo numerous metabolic adaptations, reprogramming the mode of energy generation. T cells play key role in the orchestration of the immune response and T-cell metabolic adaptation acts as crucial checkpoint hijacked by tumors to dampen antitumor immunity as T cells are rendered dysfunctional, unable to carry out their effector functions. Accumulating evidence indicate that the diverse functions of the immune system require several bioenergetic processes and that T-cell metabolic reprograming relies upon the activation of distinct transcriptional and signaling pathways. In the context of tumor microenvironment, tumors impose several limitations to dampen T-cell immunity as T cells, experiencing the metabolic framework of growing tumors, fail to activate distinct pathways to accomplish their functional requirements. Tumor microenvironmental hypoxia is in this regard a relevant example demonstrating how the tumor microenvironment of a tumor can paralyze and neutralize T-cell functions. In fact, O_2 is a master regulator of the CD8+ T-cell response and T lymphocytes face pathologically low O_2 tensions within the tumor bed at which they will have to function. It has become clear that tumor-imposed metabolic restrictions may result in an impairment of T-cell function and that either some programmed changes or pathologic manifestations can inhibit the required energy essential for their several functions. Accordingly, attempts are made to identify approaches aiming at manipulating the reprogramming of T-cell metabolic pathways for therapeutic purposes, in particular, antitumor immunity.

AUTHOR CONTRIBUTIONS

All authors listed have made substantial, direct, and intellectual contribution to the work and approved it for publication.

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REFERENCES

1. MacIver NJ, Michalek RD, Rathmell JC. Metabolic regulation of T lymphocytes. *Annu Rev Immunol* (2013) 31:259–83. doi:10.1146/annurev-immunol-032712-095956
2. Ramsay G, Cantrell D. Environmental and metabolic sensors that control T cell biology. *T Cell Biol* (2015) 6:99. doi:10.3389/fimmu.2015.00099
3. Macintyre AN, Gerriets VA, Nichols AG, Michalek RD, Rudolph MC, Deoliveira D, et al. The glucose transporter Glut1 is selectively essential for CD4 T cell activation and effector function. *Cell Metab* (2014) 20(1):61–72. doi:10.1016/j.cmet.2014.05.004
4. Cherayil BJ. Iron and immunity: immunological consequences of iron deficiency and overload. *Arch Immunol Ther Exp (Warsz)* (2010) 58(6):407–15. doi:10.1007/s00005-010-0095-9
5. Kemp JD, Thorson JA, Gomez F, Smith KM, Cowdery JS, Ballas ZK. Inhibition of lymphocyte activation with anti-transferrin receptor Mabs: a comparison of three reagents and further studies of their range of effects and mechanism of action. *Cell Immunol* (1989) 122(1):218–30. doi:10.1016/0008-8749(89)90162-7
6. Jason J, Archibald LK, Nwanyanwu OC, Bell M, Jensen RJ, Gunter E, et al. The effects of iron deficiency on lymphocyte cytokine production and activation: preservation of hepatic iron but not at all cost. *Clin Exp Immunol* (2001) 126(3):466–73. doi:10.1046/j.1365-2249.2001.01707.x
7. Pardoll DM. The blockade of immune checkpoints in cancer immunotherapy. *Nat Rev Cancer* (2012) 12(4):252–64. doi:10.1038/nrc3239
8. Brahmer JR, Tykodi SS, Chow LQM, Hwu W-J, Topalian SL, Hwu P, et al. Safety and activity of anti–PD-L1 antibody in patients with advanced cancer. *N Engl J Med* (2012) 366(26):2455–65. doi:10.1056/NEJMoa1200694
9. Topalian SL, Hodi FS, Brahmer JR, Gettinger SN, Smith DC, McDermott DF, et al. Safety, activity, and immune correlates of anti–PD-1 antibody in cancer. *N Engl J Med* (2012) 366(26):2443–54. doi:10.1056/NEJMoa1200690
10. Dai C, Lin F, Geng R, Ge X, Tang W, Chang J, et al. Implication of combined PD-L1/PD-1 blockade with cytokine-induced killer cells as a synergistic immunotherapy for gastrointestinal cancer. *Oncotarget* (2016) 7(9):10332–44. doi:10.18632/oncotarget.7243
11. Noman MZ, Messai Y, Carré T, Akalay I, Méron M, Janji B, et al. Microenvironmental hypoxia orchestrating the cell stroma cross talk, tumor progression and antitumor response. *Crit Rev Immunol* (2011) 31(5):357–77. doi:10.1615/CritRevImmunol.v31.i5.10
12. Facciabene A, Peng X, Hagemann IS, Balint K, Barchetti A, Wang L-P, et al. Tumour hypoxia promotes tolerance and angiogenesis via CCL28 and T(reg) cells. *Nature* (2011) 475(7355):226–30. doi:10.1038/nature10169
13. Keith B, Simon MC. Hypoxia-inducible factors, stem cells, and cancer. *Cell* (2007) 129(3):465–72. doi:10.1016/j.cell.2007.04.019
14. Chouaib S, Messai Y, Couve S, Escudier B, Hasmim M, Noman MZ. Hypoxia promotes tumor growth in linking angiogenesis to immune escape. *Front Immunol* (2012) 3:21. doi:10.3389/fimmu.2012.00021
15. Wang R, Green DR. Metabolic reprogramming and metabolic dependency in T cells. *Immunol Rev* (2012) 249(1):14–26. doi:10.1111/j.1600-065X.2012.01155.x
16. Slack M, Wang T, Wang R. T cell metabolic reprogramming and plasticity. *Mol Immunol* (2015) 68(2 Pt C):507–12. doi:10.1016/j.molimm.2015.07.036
17. Pearce EL, Pearce EJ. Metabolic pathways in immune cell activation and quiescence. *Immunity* (2013) 38(4):633–43. doi:10.1016/j.immuni.2013.04.005
18. Sanchez-Lockhart M, Rojas AV, Fettis MM, Bauserman R, Higa TR, Miao H, et al. T cell receptor signaling can directly enhance the avidity of CD28 ligand binding. *PLoS One* (2014) 9(2):e89263. doi:10.1371/journal.pone.0089263
19. Beyersdorf N, Kerkau T, Hünig T. CD28 co-stimulation in T-cell homeostasis: a recent perspective. *Immunotargets Ther* (2015) 4:111–22. doi:10.2147/ITT.S61647
20. Sukumar M, Liu J, Ji Y, Subramanian M, Crompton JG, Yu Z, et al. Inhibiting glycolytic metabolism enhances CD8+ T cell memory and antitumor function. *J Clin Invest* (2013) 123(10):4479–88. doi:10.1172/JCI69589
21. Liu H, Yang H, Chen X, Lu Y, Zhang Z, Wang J, et al. Cellular metabolism modulation in T lymphocyte immunity. *Immunology* (2014). doi:10.1111/imm.12321
22. Anastasiou D, Poulogiannis G, Asara JM, Boxer MB, Jiang J, Shen M, et al. Inhibition of pyruvate kinase M2 by reactive oxygen species contributes to cellular antioxidant responses. *Science* (2011) 334(6060):1278–83. doi:10.1126/science.1211485
23. Buck MD, O'Sullivan D, Pearce EL. T cell metabolism drives immunity. *J Exp Med* (2015) 212(9):1345–60. doi:10.1084/jem.20151159
24. Wang R, Green DR. Metabolic checkpoints in activated T cells. *Nat Immunol* (2012) 13(10):907–15. doi:10.1038/ni.2386
25. Marelli-Berg FM, Fu H, Mauro C. Molecular mechanisms of metabolic reprogramming in proliferating cells: implications for T-cell-mediated immunity. *Immunology* (2012) 136(4):363–9. doi:10.1111/j.1365-2567.2012.03583.x
26. Nguyen HD, Chatterjee S, Haarberg KM, Wu Y, Bastian D, Heinrichs J, et al. Metabolic reprogramming of alloantigen-activated T cells after hematopoietic cell transplantation. *J Clin Invest* (2016) 126(4):1337–52. doi:10.1172/JCI82587
27. Gerriets VA, Rathmell JC. Metabolic pathways in T cell fate and function. *Trends Immunol* (2012) 33(4):168–73. doi:10.1016/j.it.2012.01.010
28. Chaplin DD. Overview of the immune response. *J Allergy Clin Immunol* (2010) 125(2 Suppl 2):S3–23. doi:10.1016/j.jaci.2009.12.980
29. Petrova G, Ferrante A, Gorski J. Cross-reactivity of T cells and its role in the immune system. *Crit Rev Immunol* (2012) 32(4):349–72. doi:10.1615/CritRevImmunol.v32.i4.50
30. Chaudhry A, Rudra D, Treuting P, Samstein RM, Liang Y, Kas A, et al. CD4+ regulatory T cells control Th17 responses in a Stat3-dependent manner. *Science* (2009) 326(5955):986–91. doi:10.1126/science.1172702
31. Obar JJ, Lefrançois L. Memory CD8+ T cell differentiation. *Ann N Y Acad Sci* (2010) 1183:251–66. doi:10.1111/j.1749-6632.2009.05126.x
32. Arens R, Schoenberger SP. Plasticity in programming of effector and memory CD8 T-cell formation. *Immunol Rev* (2010) 235(1):190–205. doi:10.1111/j.0105-2896.2010.00899.x
33. Yang K, Chi H. mTOR and metabolic pathways in T cell quiescence and functional activation. *Semin Immunol* (2012) 24(6):421–8. doi:10.1016/j.smim.2012.12.004
34. Chisolm DA, Weinmann AS. TCR-signaling events in cellular metabolism and specialization. *Front Immunol* (2015) 6:292. doi:10.3389/fimmu.2015.00292
35. Delgoffe GM, Kole TP, Zheng Y, Zarek PE, Matthews KL, Xiao B, et al. mTOR differentially regulates effector and regulatory T cell lineage commitment. *Immunity* (2009) 30(6):832–44. doi:10.1016/j.immuni.2009.04.014
36. Araujo L, Khim P, Mkhikian H, Mortales CL, Demetriou M. Glycolysis and glutaminolysis cooperatively control T cell function by limiting metabolite supply to N-glycosylation. *Elife* (2017) 6:e21330. doi:10.7554/eLife.21330
37. Smith PM, Howitt MR, Panikov N, Michaud M, Gallini CA, Bohlooly-Y M, et al. The microbial metabolites, short chain fatty acids, regulate colonic Treg cell homeostasis. *Science* (2013) 341(6145):569–73. doi:10.1126/science.1241165
38. Michalek RD, Gerriets VA, Jacobs SR, Macintyre AN, MacIver NJ, Mason EF, 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. doi:10.4049/jimmunol.1003613
39. Barbi J, Pardoll D, Pan F. Metabolic control of the Treg/Th17 axis. *Immunol Rev* (2013) 252(1):52–77. doi:10.1111/imr.12029

40. Lane AN, Fan TW. Regulation of mammalian nucleotide metabolism and biosynthesis. *Nucleic Acids Res* (2015) 7:gkv047. doi:10.1093/nar/gkv047
41. Arts RJ, Novakovic B, Ter Horst R, Carvalho A, Bekkering S, Lachmandas E, et al. Glutaminolysis and fumarate accumulation integrate immunometabolic and epigenetic programs in trained immunity. *Cell Metab* (2016) 24(6):807–19. doi:10.1016/j.cmet.2016.10.008
42. Shi LZ, Wang R, Huang G, Vogel P, Neale G, Green DR, et al. HIF1α-dependent glycolytic pathway orchestrates a metabolic checkpoint for the differentiation of TH17 and Treg cells. *J Exp Med* (2011) 208(7):1367–76. doi:10.1084/jem.20110278
43. Sena LA, Li S, Jairaman A, Prakriya M, Ezponda T, Hildeman DA, et al. Mitochondria are required for antigen-specific T cell activation through reactive oxygen species signaling. *Immunity* (2013) 38(2):225–36. doi:10.1016/j.immuni.2012.10.020
44. Berod L, Friedrich C, Nandan A, Freitag J, Hagemann S, Harmrolfs K, 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. doi:10.1038/nm.3704
45. van der Windt GJ, O'Sullivan D, Everts B, Huang SC, Buck MD, Curtis JD, 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. doi:10.1073/pnas.1221740110
46. Pearce EL, Walsh MC, Cejas PJ, Harms GM, Shen H, Wang L-S, et al. Enhancing CD8 T cell memory by modulating fatty acid metabolism. *Nature* (2009) 460(7251):103–7. doi:10.1038/nature08097
47. van der Windt GJ, Everts B, Chang CH, Curtis JD, Freitas TC, Amiel E, et al. Mitochondrial respiratory capacity is a critical regulator of CD8+ T cell memory development. *Immunity* (2012) 36(1):68–78. doi:10.1016/j.immuni.2011.12.007
48. Chen L, Flies DB. Molecular mechanisms of T cell co-stimulation and co-inhibition. *Nat Rev Immunol* (2013) 13(4):227–42. doi:10.1038/nri3405
49. Delgoffe GM, Kole TP, Zheng Y, Zarek PE, Matthews KL, Xiao B, et al. The mTOR kinase differentially regulates effector and regulatory T cell lineage commitment. *Immunity* (2009) 30(6):832–44. doi:10.1016/j.immuni.2009.04.014
50. Waickman AT, Powell JD. mTOR, metabolism, and the regulation of T-cell differentiation and function. *Immunol Rev* (2012) 249(1):43–58. doi:10.1111/j.1600-065X.2012.01152.x
51. Pham K, Shimoni R, Charnley M, Ludford-Menting MJ, Hawkins ED, Ramsbottom K, et al. Asymmetric cell division during T cell development controls downstream fate. *J Cell Biol* (2015) 210(6):933–50. doi:10.1083/jcb.201502053
52. Verbist KC, Guy CS, Milasta S, Liedmann S, Kamiński MM, Wang R, et al. Metabolic maintenance of cell asymmetry following division in activated T lymphocytes. *Nature* (2016) 532(7599):389–93. doi:10.1038/nature17442
53. Pollizzi KN, Sun I-H, Patel CH, Lo Y-C, Oh M-H, Waickman AT, et al. Asymmetric inheritance of mTORC1 kinase activity during division dictates CD8+ T cell differentiation. *Nat Immunol* (2016) 17(6):704–11. doi:10.1038/ni.3438
54. Sauer S, Bruno L, Hertweck A, Finlay D, Leleu M, Spivakov M, et al. T cell receptor signaling controls Foxp3 expression via PI3K, Akt, and mTOR. *Proc Natl Acad Sci U S A* (2008) 105(22):7797–802. doi:10.1073/pnas.0800928105
55. Hamilton KS, Phong B, Corey C, Cheng J, Gorentla B, Zhong X, et al. T cell receptor-dependent activation of mTOR signaling in T cells is mediated by carma1 and MALT1, but not Bcl10. *Sci Signal* (2014) 7(329):ra55–55. doi:10.1126/scisignal.2005169
56. Chi H. Regulation and function of mTOR signalling in T cell fate decisions. *Nat Rev Immunol* (2012) 12(5):325–38. doi:10.1038/nri3198
57. Yang K, Shrestha S, Zeng H, Karmaus PWF, Neale G, Vogel P, et al. T cell exit from quiescence and differentiation into Th2 cells depend on raptor-mTORC1-mediated metabolic programming. *Immunity* (2013) 39(6):1043–56. doi:10.1016/j.immuni.2013.09.015
58. Finlay DK, Rosenzweig E, Sinclair LV, Feijoo-Carnero C, Hukelmann JL, Rolf J, et al. PDK1 regulation of mTOR and hypoxia-inducible factor 1 integrate metabolism and migration of CD8+ T cells. *J Exp Med* (2012) 209(13):2441–53. doi:10.1084/jem.20112607
59. Wouters BG, Koritzinsky M. Hypoxia signalling through mTOR and the unfolded protein response in cancer. *Nat Rev Cancer* (2008) 8(11):851–64. doi:10.1038/nrc2501
60. Wang R, Dillon CP, Shi LZ, Milasta S, Carter R, Finkelstein D, et al. The transcription factor Myc controls metabolic reprogramming upon T lymphocyte activation. *Immunity* (2011) 35(6):871–82. doi:10.1016/j.immuni.2011.09.021
61. Gao P, Tchernyshyov I, Chang TC, Lee YS, Kita K, Ochi T, et al. c-Myc suppression of miR-23a/b enhances mitochondrial glutaminase expression and glutamine metabolism. *Nature* (2009) 458(7239):762–5. doi:10.1038/nature07823
62. Semenza GL, Roth PH, Fang HM, Wang GL. Transcriptional regulation of genes encoding glycolytic enzymes by hypoxia-inducible factor 1. *J Biol Chem* (1994) 269(38):23757–63.
63. Discher DJ, Bishopric NH, Wu X, Peterson CA, Webster KA. Hypoxia regulates beta-enolase and pyruvate kinase-M promoters by modulating Sp1/Sp3 binding to a conserved GC element. *J Biol Chem* (1998) 273(40):26087–93. doi:10.1074/jbc.273.40.26087
64. Roman J, Rangasamy T, Guo J, Sugunan S, Meednu N, Packirisamy G, et al. T-cell activation under hypoxic conditions enhances IFN-gamma secretion. *Am J Respir Cell Mol Biol* (2010) 42(1):123–8. doi:10.1165/rcmb.2008-0139OC
65. Dang EV, Barbi J, Yang H-Y, Jinasena D, Yu H, Zheng Y, et al. Control of TH17/Treg balance by hypoxia-inducible factor 1. *Cell* (2011) 146(5):772–84. doi:10.1016/j.cell.2011.07.033
66. Shi LZ, Wang R, Green D, Chi H. Metabolic control of T cell fate decision: the HIF1α-glycolysis axis in the differentiation of TH17 and iTreg cells. *J Immunol* (2012) 188(1 Suppl):17–163.
67. Gualdoni GA, Mayer KA, Göschl L, Boucheron N, Ellmeier W, Zlabinger GJ. The AMP analog AICAR modulates the Treg/Th17 axis through enhancement of fatty acid oxidation. *FASEB J* (2016) 30(11):3800–9. doi:10.1096/fj.201600522R
68. Mockler MB, Conroy MJ, Lysaght J. Targeting T cell immunometabolism for cancer immunotherapy; understanding the impact of the tumor microenvironment. *Front Oncol* (2014) 4:107. doi:10.3389/fonc.2014.00107
69. Loftus RM, Finlay DK. Immunometabolism: cellular metabolism turns immune regulator. *J Biol Chem* (2016) 291(1):1–10. doi:10.1074/jbc.R115.693903
70. Lochner M, Berod L, Sparwasser T. Fatty acid metabolism in the regulation of T cell function. *Trends Immunol* (2015) 36(2):81–91. doi:10.1016/j.it.2014.12.005
71. Araki K, Ahmed R. AMPK: a metabolic switch for CD8+ T-cell memory. *Eur J Immunol* (2013) 43(4):878–81. doi:10.1002/eji.201343483
72. Rolf J, Zarrouk M, Finlay DK, Foretz M, Viollet B, Cantrell DA. AMPKα1: a glucose sensor that controls CD8 T-cell memory. *Eur J Immunol* (2013) 43(4):889–96. doi:10.1002/eji.201243008
73. Sun Y, Tian T, Gao J, Liu X, Hou H, Cao R, et al. Metformin ameliorates the development of experimental autoimmune encephalomyelitis by regulating T helper 17 and regulatory T cells in mice. *J Neuroimmunol* (2016) 292:58–67. doi:10.1016/j.jneuroim.2016.01.014
74. Araki K, Youngblood B, Ahmed R. The role of mTOR in memory CD8 T-cell differentiation. *Immunol Rev* (2010) 235(1):234–43. doi:10.1111/j.0105-2896.2010.00898.x
75. Zarrouk M, Finlay DK, Foretz M, Viollet B, Cantrell DA. Adenosine-monophosphate-activated protein kinase-independent effects of metformin in T cells. *PLoS One* (2014) 9(9):e106710. doi:10.1371/journal.pone.0106710
76. Michalek RD, Rathmell JC. The metabolic life and times of a T-cell. *Immunol Rev* (2010) 236:190–202. doi:10.1111/j.1600-065X.2010.00911.x
77. Maciver NJ, Jacobs SR, Wieman HL, Wofford JA, Coloff JL, Rathmell JC. Glucose metabolism in lymphocytes is a regulated process with significant effects on immune cell function and survival. *J Leukoc Biol* (2008) 84(4):949–57. doi:10.1189/jlb.0108024
78. Jacobs SR, Herman CE, Maciver NJ, Wofford JA, Wieman HL, Hammen JJ, et al. Glucose uptake is limiting in T cell activation and requires CD28-mediated Akt-dependent and independent pathways. *J Immunol* (2008) 180(7):4476–86. doi:10.4049/jimmunol.180.7.4476
79. Greiner EF, Guppy M, Brand K. Glucose is essential for proliferation and the glycolytic enzyme induction that provokes a transition to glycolytic energy production. *J Biol Chem* (1994) 269(50):31484–90.
80. Mueckler M, Thorens B. The SLC2 (GLUT) family of membrane transporters. *Mol Aspects Med* (2013) 34(0):121–38. doi:10.1016/j.mam.2012.07.001

81. Feron O. Pyruvate into lactate and back: from the Warburg effect to symbiotic energy fuel exchange in cancer cells. *Radiother Oncol* (2009) 92(3):329–33. doi:10.1016/j.radonc.2009.06.025
82. Carr EL, Kelman A, Wu GS, Gopaul R, Senkevitch E, Aghvanyan A, et al. Glutamine uptake and metabolism are coordinately regulated by ERK/MAPK during T lymphocyte activation. *J Immunol* (2010) 185(2):1037–44. doi:10.4049/jimmunol.0903586
83. van der Windt GJ, Pearce EL. Metabolic switching and fuel choice during T-cell differentiation and memory development. *Immunol Rev* (2012) 249(1):27–42. doi:10.1111/j.1600-065X.2012.01150.x
84. Newsholme EA, Crabtree B, Ardawi MS. Glutamine metabolism in lymphocytes: its biochemical, physiological and clinical importance. *Q J Exp Physiol* (1985) 70(4):473–89. doi:10.1113/expphysiol.1985.sp002935
85. Newsholme P, Curi R, Curi TCP, Murphy CJ, Garcia C, de Melo MP. Glutamine metabolism by lymphocytes, macrophages, and neutrophils: its importance in health and disease 1. *J Nutr Biochem* (1999) 10(6):316–24. doi:10.1016/S0955-2863(99)00022-4
86. DeBerardinis RJ, Mancuso A, Daikhin E, Nissim I, Yudkoff M, Wehrli S, et al. Beyond aerobic glycolysis: transformed cells can engage in glutamine metabolism that exceeds the requirement for protein and nucleotide synthesis. *Proc Natl Acad Sci U S A* (2007) 104(49):19345–50. doi:10.1073/pnas.0709747104
87. Blagih J, Coulombe F, Vincent EE, Dupuy F, Galicia-Vázquez G, Yurchenko E, et al. The energy sensor AMPK regulates T cell metabolic adaptation and effector responses in vivo. *Immunity* (2015) 42(1):41–54. doi:10.1016/j.immuni.2014.12.030
88. Hammami I, Chen J, Bronte V, DeCrescenzo G, Jolicoeur M. L-glutamine is a key parameter in the immunosuppression phenomenon. *Biochem Biophys Res Commun* (2012) 425(4):724–9. doi:10.1016/j.bbrc.2012.07.139
89. Chen Y, Guillemin GJ. Kynurenine pathway metabolites in humans: disease and healthy states. *Int J Tryptophan Res* (2009) 2:1–19.
90. Munn DH, Shafizadeh E, Attwood JT, Bondarev I, Pashine A, Mellor AL. Inhibition of T cell proliferation by macrophage tryptophan catabolism. *J Exp Med* (1999) 189(9):1363–72. doi:10.1084/jem.189.9.1363
91. Mellor AL, Munn DH. Ido expression by dendritic cells: tolerance and tryptophan catabolism. *Nat Rev Immunol* (2004) 4(10):762–74. doi:10.1038/nri1457
92. van Baren N, Van den Eynde BJ. Tryptophan-degrading enzymes in tumoral immune resistance. *Front Immunol* (2015) 6:34. doi:10.3389/fimmu.2015.00034
93. Uyttenhove C, Pilotte L, Théate I, Stroobant V, Colau D, Parmentier N, et al. Evidence for a tumoral immune resistance mechanism based on tryptophan degradation by indoleamine 2,3-dioxygenase. *Nat Med* (2003) 9(10):1269–74. doi:10.1038/nm934
94. Muller AJ, Sharma MD, Chandler PR, DuHadaway JB, Everhart ME, Johnson BA, et al. Chronic inflammation that facilitates tumor progression creates local immune suppression by inducing indoleamine 2,3 dioxygenase. *Proc Natl Acad Sci U S A* (2008) 105(44):17073–8. doi:10.1073/pnas.0806173105
95. Geiger R, Rieckmann JC, Wolf T, Basso C, Feng Y, Fuhrer T, et al. L-arginine modulates T cell metabolism and enhances survival and anti-tumor activity. *Cell* (2016) 167(3):829.e–42.e. doi:10.1016/j.cell.2016.09.031
96. Qiu F, Huang J, Sui M. Targeting arginine metabolism pathway to treat arginine-dependent cancers. *Cancer Lett* (2015) 364(1):1–7. doi:10.1016/j.canlet.2015.04.020
97. Rodriguez PC, Zea AH, DeSalvo J, Culotta KS, Zabaleta J, Quiceno DG, et al. L-arginine consumption by macrophages modulates the expression of CD3 zeta chain in T lymphocytes. *J Immunol* (2003) 171(3):1232–9. doi:10.4049/jimmunol.171.3.1232
98. Rodriguez PC, Quiceno DG, Zabaleta J, Ortiz B, Zea AH, Piazuelo MB, et al. Arginase I production in the tumor microenvironment by mature myeloid cells inhibits T-cell receptor expression and antigen-specific T-cell responses. *Cancer Res* (2004) 64(16):5839–49. doi:10.1158/0008-5472.CAN-04-0465
99. Tarasenko TN, Gomez-Rodriguez J, McGuire PJ. Impaired T cell function in argininosuccinate synthetase deficiency. *J Leukoc Biol* (2015) 97(2):273–8. doi:10.1189/jlb.1AB0714-365R
100. Li C, Zhang G, Zhao L, Ma Z, Chen H. Metabolic reprogramming in cancer cells: glycolysis, glutaminolysis, and Bcl-2 proteins as novel therapeutic targets for cancer. *World J Surg Oncol* (2016) 14(1):15. doi:10.1186/s12957-016-0769-9
101. Zheng J. Energy metabolism of cancer: glycolysis versus oxidative phosphorylation (review). *Oncol Lett* (2012) 4(6):1151–7. doi:10.3892/ol.2012.928
102. Koppenol WH, Bounds PL, Dang CV. Otto Warburg's contributions to current concepts of cancer metabolism. *Nat Rev Cancer* (2011) 11(5):325–37. doi:10.1038/nrc3038
103. Yi M, Xiang B, Li X, Li G. [Metabolic reprogramming in cancer: the art of balance]. *Zhong Nan Da Xue Xue Bao Yi Xue Ban* (2013) 38(11):1177–87. doi:10.3969/j.issn.1672-7347.2013.11.016
104. Amoêdo ND, Valencia JP, Rodrigues MF, Galina A, Rumjanek FD. How does the metabolism of tumour cells differ from that of normal cells. *Biosci Rep* (2013) 33(6):e00080. doi:10.1042/BSR20130066
105. Sheng H, Tang W. Glycolysis inhibitors for anticancer therapy: a review of recent patents. *Recent Pat Anticancer Drug Discov* (2016) 11(3):297–308. doi:10.2174/1574892811666160415160104
106. Yu L, Chen X, Wang L, Chen S. The sweet trap in tumors: aerobic glycolysis and potential targets for therapy. *Oncotarget* (2016) 7(25):38908–26. doi:10.18632/oncotarget.7676
107. Cantor JR, Sabatini DM. Cancer cell metabolism: one hallmark, many faces. *Cancer Discov* (2012) 2(10):881–98. doi:10.1158/2159-8290.CD-12-0345
108. Nakajima EC, Van Houten B. Metabolic symbiosis in cancer: refocusing the Warburg lens. *Mol Carcinog* (2013) 52(5):329–37. doi:10.1002/mc.21863
109. Brock A, Krause S, Ingber DE. Control of cancer formation by intrinsic genetic noise and microenvironmental cues. *Nat Rev Cancer* (2015) 15(8):499–509. doi:10.1038/nrc3959
110. Scott JG, Hjelmeland AB, Chinnaiyan P, Anderson AR, Basanta D. Microenvironmental variables must influence intrinsic phenotypic parameters of cancer stem cells to affect tumourigenicity. *PLoS Comput Biol* (2014) 10(1):e1003433. doi:10.1371/journal.pcbi.1003433
111. Courtnay R, Ngo DC, Malik N, Ververis K, Tortorella SM, Karagiannis TC. Cancer metabolism and the Warburg effect: the role of HIF-1 and PI3K. *Mol Biol Rep* (2015) 42(4):841–51. doi:10.1007/s11033-015-3858-x
112. Cairns RA, Harris IS, Mak TW. Regulation of cancer cell metabolism. *Nat Rev Cancer* (2011) 11(2):85–95. doi:10.1038/nrc2981
113. Vander Heiden MG, Cantley LC, Thompson CB. Understanding the Warburg effect: the metabolic requirements of cell proliferation. *Science* (2009) 324(5930):1029–33. doi:10.1126/science.1160809
114. Liberti MV, Locasale JW. The Warburg effect: how does it benefit cancer cells? *Trends Biochem Sci* (2016) 41(3):211–8. doi:10.1016/j.tibs.2015.12.001
115. Qutub AA, Popel AS. Reactive oxygen species regulate hypoxia-inducible factor 1α differentially in cancer and ischemia. *Mol Cell Biol* (2008) 28(16):5106–19. doi:10.1128/MCB.00060-08
116. Liemburg-Apers DC, Willems PH, Koopman WJ, Grefte S. Interactions between mitochondrial reactive oxygen species and cellular glucose metabolism. *Arch Toxicol* (2015) 89(8):1209–26. doi:10.1007/s00204-015-1520-y
117. Upadhyay M, Samal J, Kandpal M, Singh OV, Vivekanandan P. The Warburg effect: insights from the past decade. *Pharmacol Ther* (2013) 137(3):318–30. doi:10.1016/j.pharmthera.2012.11.003
118. Hay N. Reprogramming glucose metabolism in cancer: can it be exploited for cancer therapy? *Nat Rev Cancer* (2016) 16(10):635–49. doi:10.1038/nrc.2016.77
119. Granja S, Pinheiro C, Reis RM, Martinho O, Baltazar F. Glucose addiction in cancer therapy: advances and drawbacks. *Curr Drug Metab* (2015) 16(3):221–42. doi:10.2174/1389200216666150602145145
120. Jiang P, Du W, Wu M. Regulation of the pentose phosphate pathway in cancer. *Protein Cell* (2014) 5(8):592–602. doi:10.1007/s13238-014-0082-8
121. Lucarelli G, Galleggiante V, Rutigliano M, Sanguedolce F, Cagiano S, Bufo P, et al. Metabolomic profile of glycolysis and the pentose phosphate pathway identifies the central role of glucose-6-phosphate dehydrogenase in clear cell-renal cell carcinoma. *Oncotarget* (2015) 6(15):13371–86. doi:10.18632/oncotarget.3823
122. Chen X, Qian Y, Wu S. The Warburg effect: evolving interpretations of an established concept. *Free Radic Biol Med* (2015) 79:253–63. doi:10.1016/j.freeradbiomed.2014.08.027

123. Wise DR, Thompson CB. Glutamine addiction: a new therapeutic target in cancer. *Trends Biochem Sci* (2010) 35(8):427–33. doi:10.1016/j.tibs.2010.05.003
124. Weinberg SE, Chandel NS. Targeting mitochondria metabolism for cancer therapy. *Nat Chem Biol* (2015) 11(1):9–15. doi:10.1038/nchembio.1712
125. Cassago A, Ferreira AP, Ferreira IM, Fornezari C, Gomes ER, Greene KS, et al. Mitochondrial localization and structure-based phosphate activation mechanism of glutaminase C with implications for cancer metabolism. *Proc Natl Acad Sci U S A* (2012) 109(4):1092–7. doi:10.1073/pnas.1112495109
126. Li Y, Erickson JW, Stalnecker CA, Katt WP, Huang Q, Cerione RA, et al. Mechanistic basis of glutaminase activation: a key enzyme that promotes glutamine metabolism in cancer cells. *J Biol Chem* (2016) 291(40):20900–10. doi:10.1074/jbc.M116.720268
127. Matijevic Glavan T, Cipak Gasparovic A, Vérillaud B, Busson P, Pavelic J. Toll-like receptor 3 stimulation triggers metabolic reprogramming in pharyngeal cancer cell line through Myc, MAPK, and HIF. *Mol Carcinog* (2016). doi:10.1002/mc.22584
128. Dang CV, Kim J, Gao P, Yustein J. The interplay between MYC and HIF in cancer. *Nat Rev Cancer* (2008) 8(1):51–6. doi:10.1038/nrc2274
129. Dang CV, Le A, Gao P. MYC-induced cancer cell energy metabolism and therapeutic opportunities. *Clin Cancer Res* (2009) 15(21):6479–83. doi:10.1158/1078-0432.CCR-09-0889
130. Dang CV. MYC, metabolism, cell growth, and tumorigenesis. *Cold Spring Harb Perspect Med* (2013) 3(8):a014217. doi:10.1101/cshperspect.a014217
131. Gabay M, Li Y, Felsher DW. MYC activation is a hallmark of cancer initiation and maintenance. *Cold Spring Harb Perspect Med* (2014) 4(6):a014241. doi:10.1101/cshperspect.a014241
132. Lu H, Forbes RA, Verma A. Hypoxia-inducible factor 1 activation by aerobic glycolysis implicates the Warburg effect in carcinogenesis. *J Biol Chem* (2002) 277(26):23111–5. doi:10.1074/jbc.M202487200
133. LaGory EL, Giaccia AJ. The ever-expanding role of HIF in tumour and stromal biology. *Nat Cell Biol* (2016) 18(4):356–65. doi:10.1038/ncb3330
134. Masson N, Ratcliffe PJ. Hypoxia signaling pathways in cancer metabolism: the importance of co-selecting interconnected physiological pathways. *Cancer Metab* (2014) 2:3. doi:10.1186/2049-3002-2-3
135. Ros S, Schulze A. Balancing glycolytic flux: the role of 6-phosphofructo-2-kinase/fructose 2,6-bisphosphatases in cancer metabolism. *Cancer Metab* (2013) 1:8. doi:10.1186/2049-3002-1-8
136. Lu H, Li X, Luo Z, Liu J, Fan Z. Cetuximab reverses the Warburg effect by inhibiting HIF-1-regulated LDH-A. *Mol Cancer Ther* (2013) 12(10):2187–99. doi:10.1158/1535-7163.MCT-12-1245
137. Zhao X, Jiang P, Deng X, Li Z, Tian F, Guo F, et al. Inhibition of mTORC1 signaling sensitizes hepatocellular carcinoma cells to glycolytic stress. *Am J Cancer Res* (2016) 6(10):2289–98.
138. Lien EC, Lyssiotis CA, Cantley LC. Metabolic reprogramming by the PI3K-Akt-mTOR pathway in cancer. *Recent Results Cancer Res* (2016) 207:39–72. doi:10.1007/978-3-319-42118-6_3
139. Makinoshima H, Takita M, Saruwatari K, Umemura S, Obata Y, Ishii G, et al. Signaling through the phosphatidylinositol 3-kinase (PI3K)/mammalian target of rapamycin (mTOR) axis is responsible for aerobic glycolysis mediated by glucose transporter in epidermal growth factor receptor (EGFR)-mutated lung adenocarcinoma. *J Biol Chem* (2015) 290(28):17495–504. doi:10.1074/jbc.M115.660498
140. Rodrik-Outmezguine VS, Okaniwa M, Yao Z, Novotny CJ, McWhirter C, Banaji A, et al. Overcoming mTOR resistance mutations with a new-generation mTOR inhibitor. *Nature* (2016) 534(7606):272–6. doi:10.1038/nature17963
141. Agani F, Jiang BH. Oxygen-independent regulation of HIF-1: novel involvement of PI3K/AKT/mTOR pathway in cancer. *Curr Cancer Drug Targets* (2013) 13(3):245–51. doi:10.2174/1568009611313030003
142. Molon B, Calì B, Viola A. T cells and. *Front Immunol* (2016) 7:20. doi:10.3389/fimmu.2016.00020
143. Frey AB. Suppression of T cell responses in the tumor microenvironment. *Vaccine* (2015) 33(51):7393–400. doi:10.1016/j.vaccine.2015.08.096
144. Patsoukis N, Bardhan K, Chatterjee P, Sari D, Liu B, Bell LN, et al. PD-1 alters T-cell metabolic reprogramming by inhibiting glycolysis and promoting lipolysis and fatty acid oxidation. *Nat Commun* (2015) 6:6692. doi:10.1038/ncomms7692
145. Vinay DS, Ryan EP, Pawelec G, Talib WH, Stagg J, Elkord E, et al. Immune evasion in cancer: mechanistic basis and therapeutic strategies. *Semin Cancer Biol* (2015) 35(Suppl):S185–98. doi:10.1016/j.semcancer.2015.03.004
146. Kim R, Emi M, Tanabe K. Cancer immunoediting from immune surveillance to immune escape. *Immunology* (2007) 121(1):1–14. doi:10.1111/j.1365-2567.2007.02587.x
147. Gajewski TF, Fuertes M, Spaapen R, Zheng Y, Kline J. Molecular profiling to identify relevant immune resistance mechanisms in the tumor microenvironment. *Curr Opin Immunol* (2011) 23(2):286–92. doi:10.1016/j.coi.2010.11.013
148. Bianchi G, Borgonovo G, Pistoia V, Raffaghello L. Immunosuppressive cells and tumour microenvironment: focus on mesenchymal stem cells and myeloid derived suppressor cells. *Histol Histopathol* (2011) 26(7):941–51. doi:10.14670/HH-26.941
149. Taylor ES, McCall JL, Girardin A, Munro FM, Black MA, Kemp RA. Functional impairment of infiltrating T cells in human colorectal cancer. *Oncoimmunology* (2016) 5(11):e1234573. doi:10.1080/2162402X.2016.1234573
150. Quail D, Joyce J. Microenvironmental regulation of tumor progression and metastasis. *Nat Med* (2013) 19(11):1423–37. doi:10.1038/nm.3394
151. Herbel C, Patsoukis N, Bardhan K, Seth P, Weaver JD, Boussiotis VA. Clinical significance of T cell metabolic reprogramming in cancer. *Clin Transl Med* (2016) 5:29. doi:10.1186/s40169-016-0110-9
152. Chang C-H, Qiu J, O'Sullivan D, Buck MD, Noguchi T, Curtis JD, et al. Metabolic competition in the tumor microenvironment is a driver of cancer progression. *Cell* (2015) 162(6):1229–41. doi:10.1016/j.cell.2015.08.016
153. Zhang Y, Ertl HCJ. Starved and asphyxiated: how can CD8(+) T cells within a tumor microenvironment prevent tumor progression. *Front Immunol* (2016) 7:32. doi:10.3389/fimmu.2016.00032
154. Nakaigawa N, Kondo K, Ueno D, Namura K, Makiyama K, Kobayashi K, et al. The acceleration of glucose accumulation in renal cell carcinoma assessed by FDG PET/CT demonstrated acquisition of resistance to tyrosine kinase inhibitor therapy. *BMC Cancer* (2017) 17(1):39. doi:10.1186/s12885-016-3044-0
155. Crespo J, Sun H, Welling TH, Tian Z, Zou W. T cell anergy, exhaustion, senescence, and stemness in the tumor microenvironment. *Curr Opin Immunol* (2013) 25(2):214–21. doi:10.1016/j.coi.2012.12.003
156. Chaudhary B, Elkord E. Regulatory T cells in the tumor microenvironment and cancer progression: role and therapeutic targeting. *Vaccines (Basel)* (2016) 4(3):E28. doi:10.3390/vaccines4030028
157. Yaqub S, Henjum K, Mahic M, Jahnsen FL, Aandahl EM, Bjørnbeth BA, et al. Regulatory T cells in colorectal cancer patients suppress anti-tumor immune activity in a COX-2 dependent manner. *Cancer Immunol Immunother* (2008) 57(6):813–21. doi:10.1007/s00262-007-0417-x
158. Chaudhary B, Abd Al Samid M, al-Ramadi BK, Elkord E. Phenotypic alterations, clinical impact and therapeutic potential of regulatory T cells in cancer. *Expert Opin Biol Ther* (2014) 14(7):931–45. doi:10.1517/14712598.2014.900539
159. Ruter J, Barnett BG, Kryczek I, Brumlik MJ, Daniel BJ, Coukos G, et al. Altering regulatory T cell function in cancer immunotherapy: a novel means to boost the efficacy of cancer vaccines. *Front Biosci (Landmark Ed)* (2009) 14:1761–70. doi:10.2741/3338
160. Shaw RJ. LKB1 and AMPK control of mTOR signalling and growth. *Acta Physiol (Oxf)* (2009) 196(1):65–80. doi:10.1111/j.1748-1716.2009.01972.x
161. Chaube B, Bhat MK. AMPK, a key regulator of metabolic/energy homeostasis and mitochondrial biogenesis in cancer cells. *Cell Death Dis* (2016) 7(1):e2044. doi:10.1038/cddis.2015.404
162. Woo EY, Chu CS, Goletz TJ, Schlienger K, Yeh H, Coukos G, et al. Regulatory CD4(+)CD25(+) T cells in tumors from patients with early-stage non-small cell lung cancer and late-stage ovarian cancer. *Cancer Res* (2001) 61(12):4766–72.
163. Wei J, Zhang M, Zhou J. Myeloid-derived suppressor cells in major depression patients suppress T-cell responses through the production of reactive oxygen species. *Psychiatry Res* (2015) 228(3):695–701. doi:10.1016/j.psychres.2015.06.002
164. Chen X, Song M, Zhang B, Zhang Y. Reactive oxygen species regulate T cell immune response in the tumor microenvironment. *Oxid Med Cell Longev* (2016) 2016:1580967. doi:10.1155/2016/1580967

165. Devadas S, Zaritskaya L, Rhee SG, Oberley L, Williams MS. Discrete generation of superoxide and hydrogen peroxide by T cell receptor stimulation: selective regulation of mitogen-activated protein kinase activation and fas ligand expression. *J Exp Med* (2002) 195(1):59–70. doi:10.1084/jem.20010659
166. Bengsch B, Johnson AL, Kurachi M, Odorizzi PM, Pauken KE, Attanasio J, 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. doi:10.1016/j.immuni.2016.07.008
167. Postow MA, Callahan MK, Wolchok JD. Immune checkpoint blockade in cancer therapy. *J Clin Oncol* (2015) 33(17):1974–82. doi:10.1200/JCO.2014.59.4358
168. Keir ME, Butte MJ, Freeman GJ, Sharpe AH. PD-1 and its ligands in tolerance and immunity. *Annu Rev Immunol* (2008) 26(1):677–704. doi:10.1146/annurev.immunol.26.021607.090331
169. Spranger S, Spaapen RM, Zha Y, Williams J, Meng Y, Ha TT, et al. Up-regulation of PD-L1, IDO, and Tregs in the melanoma tumor microenvironment is driven by CD8+ T cells. *Sci Transl Med* (2013) 5(200):200ra116. doi:10.1126/scitranslmed.3006504
170. Celada LJ, Rotsinger JE, Young A, Shaginurova G, Shelton D, Hawkins C, et al. Programmed death-1 inhibition of PI3K/AKT/mTOR signaling impairs sarcoidosis CD4+ T cell proliferation. *Am J Respir Cell Mol Biol* (2016) 56(1):74–82. doi:10.1165/rcmb.2016-0037OC
171. Waickman AT, Powell JD. Mammalian target of rapamycin integrates diverse inputs to guide the outcome of antigen recognition in T cells. *J Immunol* (2012) 188(10):4721–9. doi:10.4049/jimmunol.1103143
172. Lastwika KJ, Wilson W, Li QK, Norris J, Xu H, Ghazarian SR, et al. Control of PD-L1 expression by oncogenic activation of the AKT–mTOR pathway in non-small cell lung cancer. *Cancer Res* (2016) 76(2):227–38. doi:10.1158/0008-5472.CAN-14-3362
173. Dong L, Lv H, Li W, Song Z, Li L, Zhou S, et al. Co-expression of PD-L1 and p-AKT is associated with poor prognosis in diffuse large B-cell lymphoma via PD-1/PD-L1 axis activating intracellular AKT/mTOR pathway in tumor cells. *Oncotarget* (2016) 7(22):33350–62. doi:10.18632/oncotarget.9061
174. McCoy KD, Le Gros G. The role of CTLA-4 in the regulation of T cell immune responses. *Immunol Cell Biol* (1999) 77(1):1–10. doi:10.1046/j.1440-1711.1999.00795.x
175. Wells AD, Walsh MC, Bluestone JA, Turka LA. Signaling through CD28 and CTLA-4 controls two distinct forms of T cell anergy. *J Clin Invest* (2001) 108(6):895–903. doi:10.1172/JCI13220
176. Chen L, Han X. Anti-PD-1/PD-L1 therapy of human cancer: past, present, and future. *J Clin Invest* (2015) 125(9):3384–91. doi:10.1172/JCI80011
177. Gubin MM, Zhang X, Schuster H, Caron E, Ward JP, Noguchi T, et al. Checkpoint blockade cancer immunotherapy targets tumour-specific mutant antigens. *Nature* (2014) 515(7528):577–81. doi:10.1038/nature13988
178. Pardoll D, Drake C. Immunotherapy earns its spot in the ranks of cancer therapy. *J Exp Med* (2012) 209(2):201–9. doi:10.1084/jem.20112275
179. Hamanishi J, Mandai M, Matsumura N, Abiko K, Baba T, Konishi I. PD-1/PD-L1 blockade in cancer treatment: perspectives and issues. *Int J Clin Oncol* (2016) 21(3):462–73. doi:10.1007/s10147-016-0959-z
180. Saha A, Aoyama K, Taylor PA, Koehn BH, Veenstra RG, Panoskaltsis-Mortari A, et al. Host programmed death ligand 1 is dominant over programmed death ligand 2 expression in regulating graft-versus-host disease lethality. *Blood* (2013) 122(17):3062–73. doi:10.1182/blood-2013-05-500801
181. Rabinovich GA, Gabrilovich D, Sotomayor EM. Immunosuppressive strategies that are mediated by tumor cells. *Annu Rev Immunol* (2007) 25:267–96. doi:10.1146/annurev.immunol.25.022106.141609
182. Eleftheriadis T, Pissas G, Antoniadi G, Spanoulis A, Liakopoulos V, Stefanidis I. Indoleamine 2,3-dioxygenase increases p53 levels in alloreactive human T cells, and both indoleamine 2,3-dioxygenase and p53 suppress glucose uptake, glycolysis and proliferation. *Int Immunol* (2014) 26(12):673–84. doi:10.1093/intimm/dxu077
183. Chang CI, Liao JC, Kuo L. Macrophage arginase promotes tumor cell growth and suppresses nitric oxide-mediated tumor cytotoxicity. *Cancer Res* (2001) 61(3):1100–6.
184. Platten M, Wick W, Van den Eynde BJ. Tryptophan catabolism in cancer: beyond IDO and tryptophan depletion. *Cancer Res* (2012) 72(21):5435–40. doi:10.1158/0008-5472.CAN-12-0569
185. Muller AJ, DuHadaway JB, Donover PS, Sutanto-Ward E, Prendergast GC. Inhibition of indoleamine 2,3-dioxygenase, an immunoregulatory target of the cancer suppression gene Bin1, potentiates cancer chemotherapy. *Nat Med* (2005) 11(3):312–9. doi:10.1038/nm1196
186. Hou D-Y, Muller AJ, Sharma MD, DuHadaway J, Banerjee T, Johnson M, et al. Inhibition of indoleamine 2,3-dioxygenase in dendritic cells by stereoisomers of 1-methyl-tryptophan correlates with antitumor responses. *Cancer Res* (2007) 67(2):792–801. doi:10.1158/0008-5472.CAN-06-2925
187. Vacchelli E, Aranda F, Eggermont A, Sautès-Fridman C, Tartour E, Kennedy EP, et al. Trial watch: IDO inhibitors in cancer therapy. *Oncoimmunology* (2014) 3(10):e957994. doi:10.4161/21624011.2014.957994
188. Ye Y, Wang J, Hu Q, Hochu GM, Xin H, Wang C, et al. Synergistic transcutaneous immunotherapy enhances antitumor immune responses through delivery of checkpoint inhibitors. *ACS Nano* (2016) 10(9):8956–63. doi:10.1021/acsnano.6b04989

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