mTOR-dependent immunometabolism as Achilles’ heel of anticancer therapy

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Immune cells are important constituents of the tumor microenvironment and essential in eradicating tumor cells during conventional therapies or novel immunotherapies. The mechanistic target of rapamycin (mTOR) signaling pathway senses the intra- and extracellular nutrient status, growth factor supply, and cell stress-related changes to coordinate cellular metabolism and activation dictating effector and memory functions in mainly all hematopoietic immune cells. In addition, the mTOR complex 1 (mTORC1) and mTORC2 are frequently deregulated and become activated in cancer cells to drive cell transformation, survival, neovascularization, and invasion. In this review, we provide an overview of the influence of mTOR complexes on immune and cancer cell function and metabolism. We discuss how mTOR inhibitors aiming to target cancer cells will influence immunometabolic cell functions participating either in antitumor responses or favoring tumor cell progression in individual immune cells. We suggest immunometabolism as the weak spot of anticancer therapy and propose to evaluate patients according to their predominant immune cell subtype in the cancer tissue. Advances in metabolic drug development that hold promise for more effective treatments in different types of cancer will have to consider their effects on the immune system.

Keywords: immunometabolism · mTORC1 · immunotherapy · cancer treatment · tumor microenvironment

Introduction

Tumor development, maintenance, and therapy are tightly linked with the immune system. From an immunological perspective, tumor cells are usually recognized and eradicated via immune surveillance. If tumor cells aim to escape this elimination, they need to acquire mutations that reduce immunogenicity in a process called immunoediting. Once enough cellular alterations occurred to render the tumor cells invisible to the immune system, the tumor progresses to clinically detectable growth [1]. Tumors within tissues do not grow on their own but recruit plenty of heterogeneous stromal cell types such as immune cells, fibroblasts, or endothelial cells, the so-called tumor microenvironment (TME). These stromal cells are being reprogrammed by the tumor cells to support progression and limit their elimination. The microenvironment also contributes to maintaining stemness and promoting angiogenesis, chronic inflammation, and metastasis [2]. It is now established that during chemo- or radiotherapy, the immune system is activated by dying tumor cells and plays an instrumental immunoadjuvant role to support anticancer responses [3–5]. To mount an efficient therapeutic antitumor response, the suppressive phenotype that is imposed on immune cells by the TME needs to be reverted [6,7]. Over the last 10 years, novel antibody-based immunotherapies to block immune and cancer cell interactions successfully developed into the clinic [7–9]. However, especially T cell-targeting therapeutic approaches have...
specific immune-related adverse events (irAEs) that resemble autoimmune responses and can sometimes be life threatening. irAEs are tissue-specific, depend on the molecules targeted and can lead to an autoimmune condition such as hypophysitis, colitis, thyroiditis, pneumonitis, and diabetes mellitus [10,11].

Tumor cells and associated cells of the TME exist in a specific metabolic milieu, where the individual cells compete for scarce nutrients to allow their efficient activation or proliferation during tumor onset and progression [9]. One important sensor that is able to integrate extracellular and intracellular nutrient levels is the serine/threonine kinase mechanistic target of rapamycin (mTOR). The enzyme is a critical hub for many cellular processes such as proliferation, growth, differentiation, metabolism, and inflammation in a variety of cell types and its function is cell type- and tissue-dependent [12,13]. mTOR exists in two known multiprotein complexes, mTOR complex 1 (mTORC1) and mTORC2, which show distinct functions regarding proliferation, cell growth, and energy homeostasis [12–15]. mTORC1 is tightly connected to sensing the nutritional state of the cell and translating this information for appropriate induction of anabolic processes. In the homeostatic context, the two distinct mTOR complexes appear to be rather inactive in vivo. After growth factor or PRR stimulation in the presence of sufficient nutrients, mTORC1 is activated and subsequent PI3K/protein kinase B (Akt) signaling is initialized [16–18]. Stress factors interfering with growth, including hypoxic conditions, DNA damage, and reduced ATP presence, result in the direct or indirect modulation of mTORC1 activity. Abnormal activation of the PI3K-Akt-mTOR axis is frequently observed in tumor cells [12]. Figure 1 gives a summary of processes after receptor activation.

Figure 1. mTOR in anabolic signaling. The protein complexes mTORC1 and mTORC2 monitor the environmental state to reconcile this with subsequent pathway activation. mTORC1 is activated when vital factors like nutrients and oxygen are abundantly available. Receptor (co-)stimulation leads to Phosphatidyl-Inositol-4,5-bisphosphate 3-Kinase (PI3K)-dependent phosphatidylinositol-3,4,5-trisphosphate (PIP3) generation and forward the activating signal to phosphoinositide-dependent kinase 1 (PDK1) and its substrate protein kinase B (AKT). Phosphorylated AKT inhibits the TSC complex, consisting of tuberous sclerosis complex 1 (TSC1) and TSC2, and thereby activates mTORC1. Intracellular amino acids and glucose can also activate mTORC1. Counteracting regulation is provided by AMP-activated protein kinase (AMPK), p53, and other tumor suppressor proteins. mTORC1 fosters anabolic signaling and biosynthetic pathways via ribosomal protein S6 kinase 1 (S6K1) and eukaryotic translation initiation factor 4E (eIF4E)-binding protein 1 (4EBP1), besides impairment of cellular catabolism. mTORC2 is mainly triggered by growth factors. mTOR signaling initiates protective mechanisms, such as immune cell activation, but is also exploited by cancer cells. Central metabolic hubs, like glycolysis, are tightly connected to mTOR signaling and contribute to cellular proliferation. Rapamycin and rapalogues allosterically block mTORC1, whereas ATP-competitive inhibitors and RapaLinks interrupt both mTORC1 and mTORC2 signaling. Phosphorylation (P) represents single or multiple phosphorylations of the respective protein. Dashed lines show indirect interactions. Tumor suppressors and other proteins inhibiting mTOR activity are depicted in blue, activators in green. BCR, B-cell receptor; CD28, Cluster of Differentiation 28; ER, endoplasmic reticulum; GLUT1, Solute carrier family 2, facilitated glucose transporter member 1; HIF1α, Hypoxia-inducible factor 1 subunit α; INSR, Insulin receptor; IFN, Interferon; MCT, Monocarboxylic acid transporter; mTOR, Mechanistic target of rapamycin; MYC, Myc proto-oncogene protein; NO, Nitric oxide; OMM, Outer mitochondrial membrane; PDCD4, Programmed cell death protein 4; REDD1, Regulated in the development and DNA damage response 1; Rheb, Ras homolog enriched in brain; SLC, Solute carrier amino acid transporter family; SREBP, Sterol regulatory element-binding protein; TCR, T-cell receptor; TLR, Toll-like receptor.
mTOR signaling as a driver of anabolic metabolism in cancer cells

An altered cellular metabolism in tumor cells emerges as an important hallmark of cancer [9]. Metabolism being a vulnerable spot of a cancer was suggested earlier and termed as the Achilles’ heel of cancer cells [19]. Whereas previous data suggested that the Warburg effect (i.e., the engagement of glycolysis regardless of the oxygen levels) is a specific characteristic of tumor cells, recent data show that tumor metabolism in vivo is very diverse [20–24]. Emerging evidence indicates that there might even be no general metabolic tumor cell program within a single tumor tissue in vivo. Therefore, the most frequent commonalities in tumor cells are discussed in the following.

A cancer cell displays reinforced anabolism to produce energy and biosynthetic precursors necessary for cell proliferation (Figure 2). Many metabolic processes are driven by mTOR signaling, rendering it a promising target for treatments [12]. Although mTORC1 does not seem to be the cause why a cell turns into a tumor cell, it is a supportive hub that induces anabolic processes to generate biomass in response to environmental signals or tumor-promoting mutations [25].

Tumor cells exaggerate glucose uptake and pyruvate synthesis. Under aerobic conditions, the surplus of pyruvate is then metabolized to cytosolic lactate prior to cellular export via monocarboxylate transporters. Permanent mTOR activity after genetic aberrations can lead to continuous lactate production in this scenario [26]. mTOR supports glycolysis via the transcription factors hypoxia-inducible factor 1 subunit α (HIF1α) and Myc proto-oncogene protein (c-Myc). HIF1α expression is mTORC1- and mTORC2-dependent and elevated HIF1α expression is enough to enhance the glycolytic pathway. c-Myc reacts to nutrients and
growth factors and acts as an overall transcriptional activator by reopening chromatin regions and unleashing withheld RNA polymerases, which inevitably opens up transcription of metabolic genes [27].

Abundant amino acids, such as glutamine, are readily used by proliferating cells. Glutamine is an essential nitrogen and carbon source that fuels the tricarboxylic acid (TCA) cycle, maintains redox balance and contributes to lipid and nucleotide synthesis [28–30]. Glutamine and leucine activate mTORC1 signaling and a block in glutaminolysis switches mTORC1 off [31]. In proliferating cells, glutamine is rapidly consumed to support cell growth and tumor cells show a high dependency on this amino acid [30]. Further, the synthesis of α-ketoglutarate from glutamine, an important step in glutaminolysis, is a major anaerobic process to replenish TCA cycle intermediates [29]. Moreover, upregulation of glutamine transporters (SLC family members SLC1, SLC6, SLC7, and SLC38) [32], e-Myc activation, or deletions in Rb protein family members contribute to this stimulatory effect in many tumors [33]. Therefore, glutamine metabolism presents itself as a valuable target in cancer cells and inhibition of glutaminase, the enzyme converting glutamine to glutamate, is already tested in preclinical and clinical trials [34,35].

Amino acids are perceived by mTORC1 on the lysosome and activate mTORC1 to promote protein synthesis and growth [12]. mTORC1 stimulates protein synthesis by phosphorylating the two effector proteins ribosomal protein S6 kinase beta-1 (S6K1) and eukaryotic initiation factor 4E binding proteins [36,37]. Under amino acid-deprived conditions, lysosomal degradation of extracellular proteins can sustain cell survival and also induce activation of mTORC1 [38]. Unlike its growth-promoting activity under amino acid-replete conditions, mTORC1 activation suppresses cell proliferation when cells rely on extracellular proteins as an amino acid source.

Tumor cells elevate lipid synthesis mainly by activating sterol regulatory element-binding protein (SREBP) transcription factors and their target genes (e.g., ATP citrate lyase, acetyl-CoA carboxylase 1, fatty-acid synthase, and transporter proteins) [39]. A stimulated mTORC1-S6K1 axis can drive SREBP activation [40,41], for example, leading to fatty-acid and cholesterol biosynthesis [42]. The mTOR inhibitor rapamycin prevents nuclear entry of SREBP to decrease lipid synthesis and reduces SREBP-targeted gene expression [43].

The distinct functions of mTORC1 and mTORC2 are affected by their different subcellular locations [44]. mTORC2 is associated with the plasma membrane and with ribosomal membranes. The location to the plasma membrane facilitates the proximity to mTORC2 substrates such as Akt. In gliomas and breast cancers, positive for receptor tyrosine-protein kinase erbB-2 (ERBB2, also known as HER2), Rictor overexpression coincides with hyperactive Akt signaling [45,46]. Among the activators of mTORC2 are growth factors and ribosomes, which are commonly upregulated factors in tumors [47]. From a metabolic point of view, mTORC2 is capable of promoting glycolysis, lipogenesis, the pentose phosphate pathway (PPP), purine synthesis, and glutamine metabolism [48]. For example, mTORC2 promotes cancer growth in hepatocellular carcinoma by enhancing fatty acid and lipid synthesis gene expression [49] and targeting these synthetic pathways is already part of clinical trials [50]. Further, mTORC2 is in control of cystine uptake and glutathione metabolism in cancer cell models [51]. Hence, mTORC1 and mTORC2 support tumor growth by feeding various anabolic pathways that are especially required in proliferating cells.

### Three generations of mTOR inhibitors in cancer therapy

The above-mentioned cellular functions regulated by mTORC1 and mTORC2 provided solid ground to inhibit these kinases in tumor medicine. Currently, substances successfully interfering with mTOR signaling fall broadly into three categories: (i) allosteric inhibitors, (ii) ATP-competitive inhibitors; and (iii) third-generation inhibitors. Drugs of the earliest generation of mTOR inhibitors (i) include rapamycin (sirolimus) and rapalogs (e.g., everolimus) and these substances bind directly to FK506-binding protein 12 (FKBP12) [52]. When FKBP12 subsequently binds to the FRB domain of mTOR, mTORC1 phosphorylation activity toward specific substrates is inhibited due to blockade of correct substrate positioning inside the active groove. mTORC2 remains unaffected by these drugs. All in all, the clinical success of rapamycin and rapalogs in cancer therapy is rather underwhelming [53]. One pitfall to explain the lack of efficacy of rapalogs are mTORC1 feedback loops [25,38,54]. ATP-competitive inhibitors (ATP analogs, mTOR-KIs; TORKinibs; e.g., Torin1, PP242, or AZD8055) of the second generation (ii) target the kinase domain of mTOR and prevent ATP from sitting into it. These inhibitors effectively prevent enzymatic activity of both mTORC1 and mTORC2 [53]. Therefore, they were developed to circumvent the feedback loops, especially the reactivation of PI3K/Akt [54,55]. As mTOR inhibitors of the first two generations can cause resistance in the shape of mutations that prevent drug binding [56,57], third-generation inhibitors (iii) (RapaLink, e.g., RapaLink-1) were developed that block the FRB and the kinase domain of mTOR at the same time. Far lower drug concentrations are necessary to yield a cellular effect [56]. Exclusive mTORC2 targeting has remained elusive so far. One interesting nanoparticle-based approach is provided with ternary siRNA polyplexes harboring Rictor siRNA sequences [58]. Clinical trials will outline the clinical success of mTOR inhibitors in the future [59].

### mTOR inhibition and cancer immunity

Although acute inflammatory reactions are indispensable for mounting effective immune responses, chronic and unrestricted inflammation can be a driver for pathologies like cancer and metabolic disorders [60]. When immune cells are activated, they not only change their migration patterns and morphologies but also engage in the production of cytokines, chemokines, and
Figure 3. Effects of mTOR inhibition on different immune cell types in the tumor microenvironment (TME). Active mTOR signaling is required for differentiation of effector lymphocyte populations such as CD8+ CTL. Blocking mTOR in lymphocytes leads to memory T-cell differentiation and not only impairs effector functions in CD4+ and CD8+ cells but further promotes anergy. Treg cells react to mTOR inhibition with enhanced differentiation but lower cholesterol synthesis and mitochondrial activities. A TGF-β-abundant TME expands immunosuppressive Treg populations. In NK cells, mTOR activation results in a cytotoxic and antitumorigenic phenotype, whereas rapamycin blocks these benefits, and this holds mostly true for granulocyte populations. Other professional APCs such as tumor-associated macrophages (TAMs) and DCs react with more diverse phenotypes to the tumor milieu. Although immune cell activation requires mTOR signaling, antigen uptake and costimulatory molecules are often upregulated in patients treated with mTOR inhibitors. Myeloid-derived suppressor cells (MDSCs) contribute to an immunosuppressive environment in tumors, but their expansion can be diminished by blocking mTOR signaling. B lymphocytes react similarly as they downregulate tumor cell-specific antibody production upon mTOR inhibition. Downregulation of mTOR restricts tumor-promoting cytokine production from cancer-associated fibroblasts (CAFs) and the related immune evasion. ARG1, Arginase-1; CTLA-4, Cytotoxic T lymphocyte antigen 4; FAS, Fatty-acid synthesis; IFN-γ, Interferon gamma; iNOS, inducible nitric oxide synthase; TGF-β, Transforming Growth Factor beta; mTOR, Mechanistic target of rapamycin; SMAD, Mothers against decapentaplegic homolog.

other messenger molecules. In the TME, the tumor cells reprogram immune cells to render them immunosuppressive and not only less harmful but even beneficial for the tumor [61]. A logical consequence for immunotherapy is to aim at reactivating the immune cells. However, distinct immune cells, such as T cells and macrophages, depend on specific metabolic pathways like glycolysis or glutamine metabolism for efficient immune and antitumor responses [28,61]. Hence, therapeutically interfering with cancer cell metabolism by, for example, targeting mTORC1 and mTORC2 will also have varying effects on the immune system that may support but also block antitumor effects.

A wide range of intracellular processes is conducted through the mTORC1-mTORC2 network in immune cells [13,62]. Initially, mTOR inhibitors, such as rapamycin, were clinically applied to avert detrimental immune activation for the prevention of kidney transplant rejection [63]. In the era of immunotherapy, it might seem surprising at first sight to use an immunosuppressive medicine as a promising cancer drug, but rapamycin affects many immune cells with variable effects [54] (Figure 3). mTOR inhibitors enable T-cell anergy under concurrent costimulation and antigen presentation [64] leading to immunosuppressive effects of mTOR inhibitors in vivo. Since forcing T cells to become anergic/exhaustive is a prominent mechanism of tumors to evade the immune system, activating mTORC1 signaling might be more advantageous in this context [12]. In the following chapters, we discuss the role of mTOR in shaping potent immune responses in individual immune cell subtypes.

**CD8+ T cells**

Effector CD8+ CTLs are essential for the immune response against intracellular pathogens and tumors. CTLs kill transformed or infected cells directly via granule release or indirectly via IFN-γ and TNF secretion. Naive CD8+ cells are, in turn, stimulated
by type I IFN and IL-12 to become mature, terminally differentiated effector CD8\(^+\) T cells. After defense, most CTLs undergo apoptosis to establish homeostasis, and the remaining minorities adjust their transcriptional features to become protective, long-lived CD8\(^+\) memory T cells that can react immediately once the same antigen reoccurs [65]. Therefore, maintaining a CD8\(^+\) memory fosters tumor control in the long run [61]. Evidence provided by in vitro and in vivo studies showed a tight connection between mTOR and the development of CTLs [66,67]. Naïve CD8\(^+\) T cells emerging from the thymus gradually become more sensitive to metabolic cues when circulating in the periphery [68]. The initial low flux through aerobic glycolysis is the result of sparse mTORC1/c-Myc signaling [69,70]. During activation, mTOR controls transcriptional processes to generate effector CD8\(^+\) T cells. Deletion of the mTORC1 suppressor TSC2 in T cells entails not only mTORC1 upregulation but also strengthens CD8\(^+\) effector function [69]. In contrast, decreasing mTOR signaling inhibits effector functions of T cells [71] and rapamycin promotes CD8\(^+\) memory T-cell generation [72]. The chemokine receptor CCR7 and 1-selectin (CD62L) are highly present in naive and memory T cells but sparsely expressed in effector CTLs [73]. They are negatively regulated by PI3K and mTOR signaling and to regain homing capability to secondary lymphoid tissues, rapamycin treatment is sufficient [74]. A lack of CCR7 results in defective priming of lymphocytes that would otherwise attack the tumor [75]. mTORC2 negatively affects CTL memory responses as well, because deletion of rapamycin-insensitive companion of mammalian target of rapamycin (Rictor) enhances CTL memory formation [69,76]. CD8\(^+\) memory T cells maintain their function by enhancing the metabolic flow through fatty-acid oxidation (FAO) and the mitochondria rather than implementing aerobic glycolysis and mTOR [72,77].

Unlike memory CTLs, glycolysis has a more prominent role in supporting effector functions. Glycolysis is induced for secondary effector cell differentiation from CD8\(^+\) memory T cells and subsequent proliferation in a secondary response [77]. Reactivation requires an mTOR-linked glycolytic switch to synthesize IFN-γ [78], although a TLR-mediated activation and IFN-γ production of resting bystander T cells via mitochondrial respiration was also reported [24]. This glycolytic switch, in turn, presupposes an increase of Glut1 on the surface of T cells [77]. B-cell leukemias can prevent glycolytic flux in T cells and this can be rescued by artificially increasing Glut1 expression or mTORC1 signaling [79]. In a recently conducted study, mice lacking the glycolytic enzyme lactate dehydrogenase A showed diminished PI3K signaling and, therefore, impaired effector T-cell activation. This positive feedback loop points out the importance of signaling from metabolic enzymes to regulate growth factor signaling in cancer cells and not mainly vice versa [80].

When lymphocytes are continuously stimulated with antigens, an exhaustion of CD8\(^+\) T cells as well as CD4\(^+\) T cells, NK cells, and B cells occurs, also termed lymphocyte exhaustion [81–84]. This process is known to be present in chronic infections and cancer and characterized in CTLs by the expression of the immunosuppressive surface molecules programmed cell death protein 1 (PD-1) and cytotoxic T lymphocyte antigen 4 (CTLA-4) [85]. Interestingly, exhausted T cells have low mTORC1 activity that seems to contribute to the exhausted phenotype [86]. The expression of the PD-1 ligands PD-L1 and PD-L2 on the surface of many different cell types in the TME might contribute to CTL anergy [87]. In immunotherapy, the main goal is to reactivate the cytolytic functions of exhausted CD8\(^+\) T cells by blocking negative checkpoints. Several studies showed that mTOR activates PD-L1 expression, in part, because mTOR-induced IFN-γ production initiates PD-L1 transcription [88], and rapamycin blocks PD-L1 on monocytes, macrophages, and endothelial cells [88–90]. In the context of different susceptibilities to blocking PD-1/PD-L1 signaling in patients [87,91], targeting only this pathway might not be enough to restore CTL activities [92]. But future studies are needed to clarify which parameters actually induce tolerance in the tumor context [93,94]. In conclusion, inhibition of mTOR restrains effector responses in CD8\(^+\) T cells but stimulates memory formation and may promote exhaustion.

**CD4\(^+\) T cells**

CD4\(^+\) T cells help stimulating immune responses after DC contact but have also been suggested of killing tumor cells and influencing the TME. They can promote co-stimulation and antigen presentation in DCs and are important supporters of CD8\(^+\) T-cell activation [95]. The functions of mTORC1 and mTORC2 have been well defined in CD4\(^+\) Th. mTOR activation supports Th1-/Th17-cell differentiation of CD4\(^+\) T cells [96,97] to promote an inflammatory response. As in CD8\(^+\) T cells, decreased mTORC1 signaling abrogates effector T-cell differentiation [71]. Active mTORC2 signaling does not affect T-cell survival but enhances Th1 and Th2 CD4\(^+\) lymphocyte differentiation [98].

Metabolically, antitumorogenic CD4\(^+\) effector T cells are very similar to their CD8\(^+\) counterpart as they show the high activity of glycolysis, oxidative phosphorylation (OXPHOS), amino acid turnover, and PPP [61]. Glut1 upregulation by mTOR serves inflammation-induced effector CD4\(^+\) lymphocyte functions. Both glycolysis and OXPHOS are capable of amplifying proliferation of the CD4\(^+\) compartment but complete effector function (i.e., IFN-γ production) is provided exclusively via aerobic glycolysis and can be abrogated by its absence [99].

Active mTORC1 signaling promotes immunity against cancer via enhanced Th1 differentiation [97,99] and this subtype combats tumors mainly by secreting cytokines and supporting CTL activation [61,95]. Interestingly, inhibiting glutaminolysis by deletion of glutaminase sensitizes Th17 cells to IL-2-induced mTORC1 activation, while Th17 cells do not enhance mTORC1 signaling in response to IL-2 due to epigenetic reprogramming [100]. The direct inhibition of mTORC1 in CD4\(^+\) T cells might generally promote Treg cell differentiation but also renders CD4\(^+\) T cells more susceptible to TGF-β [101,102], which has pro- and antitumorogenic effects [103].

Treg cells are indispensable for tolerance to self-antigens and for restoring tissue homeostasis after triggering an immune
response [104]. Human tumors with a high presence of Treg cells, such as renal cell and hepatocellular carcinomas, tumors of the gastrointestinal tract and other hormone-related tumors show a poor prognosis. Characteristic of CD4+ Treg cells is the expression of the IL-2 receptor (IL-2R) subunit CD25 and the transcription factor forkhead box protein P3 (FOXP3). Treg-mediated immunosuppression acts on DC and effector T-cell populations via several mechanisms: release of granocyte B and perforin causes apoptosis in effector cells [105], secretion of inhibitory cytokines, such as TGF-β, IL-10, and IL-35 that provoke an immunosuppressive response in other immune cells [106], and utilization of inhibitory surface receptors, such as CTLA-4, to counteract costimulatory receptor signaling [107]. CTLA-4 binding to B7 receptors on DCs results in conversion of tryptophan to kynurenine via IDO within the APC. This immunomodulatory enzyme acts in the rate-limiting step in tryptophan degradation. Upon sensing tryptophan depletion, effector T cells are likely to initiate apoptosis or at least tolerance effects [108–110]. mTORC1 inhibition by either downregulation of essential AA-consuming enzymes or rapamycin leads to de novo induction of FOXP3 expression [111]. Overall, Treg generation can be enhanced by mTOR inhibitors, but mTORC1 activity seems to be essential for Treg suppressive functions. For instance, deleting mTORC1 activity does not affect FOXP3 levels in Treg cells but it lowers the synthesis of cholesterol which is essential for suppressive functions of Treg cells in vivo [112]. FOXP3 downregulates c-Myc and PI3K-Akt-mTORC1 signaling to render the cells more adapted to a lactate-high and glucose-deprived environment [113,114]. Interestingly, autophagy is active in Treg cells and contributes to their suppressive function by inhibiting c-Myc, mTORC1, and glycolytic signaling [114,115]. Hence, inhibition of mTORC1 in the context of the TME may inhibit effective CD4+ T-cell responses and promote Treg-cell induction.

Natural killer cells

NK cells are type 1 innate lymphoid cytotoxic cells and specialized in early defense mechanisms that monitor their host for intracellular viral infections and tumor cells. NK cells exert potent antitumor functions, because of direct tumor cell lysis and their secretion of IFN-γ [116]. The main cytokine responsible for activation and differentiation of NK cells is IL-15 and treatments with this cytokine were already tested in clinical trials [117]. IL-15 activates mTORC1 and subsequently leads to proliferation and production of IFN-γ and granzyme B. Rapamycin treatment blocks these beneficial features in mice and humans [118,119]. Metabolically, mTORC1 activation promotes glucose uptake and glycolysis and an interference with glycolysis is sufficient to abrogate NK cell cytotoxicity. Like other lymphocyte populations, NK cells are prone to develop functional exhaustion after continuous exposure to activating stimuli, especially under constant IL-15 supply [119]. Experiments in PBMC-derived NK cells exposed to long-term (longer than 48 h) IL-15 stimulation showed that excessive mTOR signaling was a key to their functional exhaustion and this effect could be rescued with rapamycin [119]. Therefore, it might be reasonable to assume that mTOR inhibitors harm NK cells in the healthy context but counteract an exhaustion phenotype in the TME.

Granulocytes

Granulocytes (neutrophils, eosinophils, basophils, and mast cells) are often part of an activated inflammatory tumor context [120]. Neutrophils can exert contradictory roles in the TME due to their numerous distinct activation states. Their pro- and antitumorigenic actions, though, depend highly on their cellular interaction partners at the tumor site and their released factors [121]. For example, neutrophil-released oncostatin M (OSM) renders macrophages protumorigenic via mTORC2 [122]. Usually, neutrophils are considered purely glycolytic cells [123], however, in the TME, they highly engage in FAO and oxidative phosphorylation to maintain immunosuppressive properties that support tumor growth [123,124]. Mostly in advanced cancer stages, high granulocyte numbers correlate with poor prognosis and promotion of metastasis [125,126]. In contrast, high eosinophil numbers, degranulation, and the associated cytotoxicity mainly seem to be beneficial for cancer patients [127]. As evidenced by many studies, mast cells act protumorigenic and their deletion delays malignant transformations [128]. Overall, mTOR supports activation, effector functions, and migration of granulocytes and inhibition of mTOR may be an effective antitumorigenic approach [13].

Tumor-associated macrophages

Macrophages are vital to maintain tissue homeostasis and immune surveillance. In the healthy tissue, macrophages restore homeostatic conditions via removing debris and apoptotic cells toward the end of an inflammatory process. Their phagocytic and antigen-presenting features are of critical importance in coordinating the adaptive immune response after exposure to foreign antigens, cellular debris, or abnormal intercellular signaling and are considered as key therapeutic targets for many pathogenic developments. As highly plastic cells, macrophages continually adapt to metabolic changes in their environment, which results in polarization to different effector phenotypes [129]. After TLR- or nod-like receptor-mediated activation, macrophages focus on aerobic glycolysis for M1-like pro-inflammatory cytokine expression [130]. In the course of an immune response, the accompanying lactate production results in switching back the macrophage program to a more M2-like resolving and repairing phenotype [131]. Regarded as being lactate-high, the TME can polarize macrophages toward an M2-like tumor-supportive phenotype, characterized by vascular endothelial growth factor expression [132]. Among the phenotype spectrum, M2-like macrophages have the highest glutamine metabolism rate [133] and upregulated glycolysis for carbon metabolism [14,134]. They show elevated scavenger receptor CD36 expression and lysosomal
lipolysis [130]. Most of the current tumor-associated macrophage (TAM)-targeting approaches aim to reprogram these macrophages toward an M1-like phenotype or focus on depletion of M2-like TAMs, although many uncertainties remain in the complex interplay within the TME [135].

A myeloid-specific Tsc1 gene deletion in mice results in activating mTORC1 signaling that prevents the differentiation toward the promalignant M2-like phenotype [136]. On the other hand, a myeloid deletion of Tsc2, which also activates mTORC1 signaling, leads to a CDK4-mediated upregulation in glycolysis and M2-like gene expression but downregulation of apoptosis and NF-κB signaling [14]. An inhibition of mTORC1 in this scenario initiates apoptosis in these macrophages and contributes to the view that TSC2 balances macrophage proliferation and apoptosis [14]. A lack of amino acids limits IL-4-mediated mTORC1 activation in M2 polarization [133]. In hypoxic TAMs, a deficiency of the mTOR suppressor protein regulated in development and DNA damage response 1 (REDD1) increases cellular glycolytic flux [137,138]. REDD1 is induced upon various signals of stressed cells and might act protective in the TME context.

Active mTORC2 signaling promotes polarization of M2-like anti-inflammatory macrophages, and deletion of Rictor in macrophages leads to a stronger M1 polarization in the TME [139,140]. This lowers proliferation of B16 melanoma tumor cells in Rictor-deficient mice [139]. However, enhanced M1 polarization of macrophages outside of a TME in myeloid Rictor-deficient mice can enhance inflammation-induced colitis, which then promotes tumor growth in a colitis-induced colorectal tumor model. mTORC2 activity in macrophages was important to reduce inflammation and stimulate repair during colitis and inhibited colitis-induced tumor formation by induction of the cytokine osteopontin. Interestingly, treatment with the second-generation mTOR inhibitor AZD8055 reduced mTORC2 signaling in macrophages and, therefore, the tumor burden in this colorectal cancer (CRC) model was significantly increased. Even more interesting, mTORC2 signaling was not observed in mouse and human CRC tumors in vivo, for example, in CRC patients [140]. Hence, inhibiting mTORC2 may mainly impact macrophage functions, but may not interfere with cancer cell function in vivo in this context.

**Dendritic cells**

DCs are the most potent APCs that instruct adaptive immune responses [141]. Their inherent expression of PRRs as such as TLRs enables them to react to inflammation- or pathogen-related signals. This receptor-mediated activation enhances antigen presentation via MHC molecules and promotes the expression of chemokine and costimulatory receptors as well as cytokine production. Mature DCs migrate to the draining LN for T-cell priming [142]. In vitro experiments of BM-derived DCs grown in presence of GM-CSF showed that these cells are highly committed to glycolysis for ATP production after activation by TLR agonists and in later activation stages [143]. This is due to the expression of iNOS and the resulting nitric oxide (NO) production in mouse DCs because this reaction poisons the mitochondrial respiratory chain [143,144]. Further, mTOR-mediated HIF1-α activation after TLR stimulation fosters maintained reliance on glycolysis [145]. This metabolic switch to glycolysis after TLR stimulation does not occur after blocking mTOR [146]. Nevertheless, rapamycin has immunostimulatory effects on DCs resulting in amplified activation of T cells. Costimulatory membrane protein CD86 shows higher expression upon mTOR restriction and the immunosuppressive protein PD-L1 downregulated [13,146]. Moreover, rapamycin downregulates iNOS expression and activity [147]. Antigen uptake and presentation by professional APCs were elevated in rapamycin-treated transplant recipients [148]. Under conditions of chronic inflammation in the intestine, mTORC1 in DCs acts has anti-inflammatory activity due to production of IL-10 [149]. DCs can be reprogrammed by the TME to tolerogenic DCs with an immature and inactive phenotype [150], hence, short-term inhibition of mTORC1 might reverse this tolerogenic phenotype.

**Myeloid-derived suppressor cells**

Myeloid-derived suppressor cells (MDSCs) comprise a heterogeneous group of immature myeloid precursor cells that accumulate in pathogenic conditions, such as cancer, acute and chronic infections, autoimmune disorders, and chronic inflammatory conditions [151]. One of their main activities is the release of IL-10 to suppress other immune cell populations and promote a Treg cell-abundant environment [152]. MDSCs facilitate tumor progression and metastasis through hindering NK cell and CD8+ responses [153,154]. Other mechanisms used by activated MDSCs comprise ROS production, arginase-1, and iNOS expression, activation of Treg cells and TGF-β release [151]. A deficiency in lysosomal acid lipase, an enzyme involved in the development and maintenance of MDSCs, promotes an expansion of immature MDSCs. This expansion is due to excess mTOR signaling in MDSCs to promote cellular growth and metastasis [155]. In addition, G-CSF is secreted in an mTOR-dependent fashion by mammary tumors to foster accumulation of MDSCs [156]. In contrast, MDSCs exert a protective role in inflammatory bowel diseases in counteracting disease progression. This was shown in several experimental models [157] and the oral mTORC1/2 dual-inhibitor INK128 helps to attenuate the proinflammatory effects of DSS-induced colitis through MDSC-mediated Treg-cell expansion [158].

**B cells**

B lymphocytes participate in the adaptive immune response with antigen presentation, activation of T cells, and secretion of antibodies and cytokines [159]. mTOR complex signaling is initiated in B cells through B-cell receptor, TLR4, or costimulatory signaling [160]. Follicular B cells use mTORC1 to induce a coordinated
unfolded protein response prior to antibody production in plasma cell development [161]. In immunization experiments, mTORC1 deletion in mature B cells resulted in hampered plasma cell differentiation and antibody synthesis [162]. Hypoxic conditions that occur physiologically in GC light zones impair antibody production by disruption of mTORC1 signaling [163]. On the other hand, chronic hyperactive mTORC1 signaling after B-cell-specific TSC1 deletion in mice results in diminished maturation of splenic B cells [164]. The tendency of regulatory B cells to act immunosuppressive in the TME and to impair the antitumor response highlights them as targets [165]. Mechanistically, cancer cells can activate PPARα signaling in B cells leading to high FAO and reprogramming toward immunosuppression [166]. B lymphocytes often accumulate around the draining LNs of the tumor [167]. Combined drug therapies, including mTOR inhibitors, have been suggested to target B-cell malignancies as a personalized therapeutic approach [168,169].

Cancer-associated fibroblasts

Fibroblasts are the predominant cell type in connective tissue and become activated when tissue remodeling (e.g., wound healing) is required. In the wound healing process, the immunoregulatory cytokine TGF-β secreted by many leukocytes activates fibroblasts for proliferation and tissue regeneration. Fibroblasts in the tumor stroma are reprogrammed under the influence of cancer cells to cancer-associated fibroblasts (CAFs) by TGF-β and other growth factors [170]. A high presence of CAFs correlates with poor prognosis in cancer, for example, in CRC patients [171,172]. In pancreatic tumors, CAFs secrete M-CSF to promote a tumor supportive phenotype in macrophages [173]. The activation of CAFs seems to be irreversible and their production of IL-22 promotes invasive conditions via mTOR activation, as shown in lung cancer cell lines [174]. In the TME, CAFs reduce the expression of p62, which leads to diminished mTORC1 activation, but counterintuitively increases tumorigenesis of epithelial prostate cancer cells [175]. This is mediated via enhanced expression of IL-6 that is negatively regulated by mTORC1 in CAFs. On the other hand, in 3D-coculture models of colorectal tumors and fibroblasts, a high antitumorigenic and apoptotic effect was yielded with a combination of mTOR (rapamycin), MEK1, and Akt inhibition [176].

Conclusions and perspectives

Hyperactivated mTOR signaling represents a feature of many cancers. Since aberrations in the mTOR pathway are intertwined with cancer progression, mTOR inhibitor therapy provides a promising treatment option in clinical studies. Nevertheless, tumor treatment involving an mTOR block with single or combined mechanisms had so far limited success. Apart from the heterogeneous and cell type-dependent kinase activity in tumor cells, mTOR (especially mTORC1) is a central hub for the immune system and its cellular metabolism. Therefore, mTOR has so far refused to be categorized simply as pro- or anti-tumorigenic and pro- or anti-inflammatory when considering all cells residing in the tumor environment [12,177]. Beyond rapamycin and other rapalogos, substances targeting mTORC1 and mTORC2 simultaneously in patients may more frequently lead to irAEs. mTORC2 activity may be restricted to immune but not tumor cells in some cancers and its targeting can be protumorigenic [140].

Escaping the immune system constitutes a critical feature for tumor survival and for immunotherapies. We propose to evaluate mTOR inhibitors according to the six recently-identified immune subtypes that are present in cancer independently of the cancer type [178]. These six immune subtypes are characterized by differences in macrophage or lymphocyte signatures, Th1:Th2 cell ratio, overall cell proliferation, and other features [178]. Moreover, recent evidence suggests that immune cell heterogeneity in the TME impacts immunotherapy and other anticancer therapies [179]. Different immune features of the individual subtypes can be correlated to high or low mTOR activity as discussed in the previous sections and, therefore, we suggest that these subtypes might be rather sensitive or resistant to mTOR inhibitors. For example, subtype C1 (wound healing) might respond well to mTOR inhibition because mTOR activity strongly contributes to the defining features angiogenic gene expression and cell proliferation. Patients with subtypes C2 (high M1/M2 macrophages, high CD8+ T-cell signal), may be at least in part resistant to mTOR inhibitors, since the expanded M1 and CD8+ responses indicate that mTOR signaling is already low in those tumors as described earlier. Hence, subtypes C4 and C5 that present high M2 macrophages may be better responsive to mTOR inhibitors, because M2 macrophages seem to be overall more dependent on mTORC1 activity than M1 macrophages, owing to their enhanced lipid metabolism [180]. Additionally, it will be important to consider whether T cells in the TME are exhausted, because low mTOR activity drives chronic long-term T-cell exhaustion. However, short-term inhibition of mTOR may still be beneficial [181]. Hence, the effects of mTOR inhibitors are likely to be contingent on the immune subtypes in cancer in consideration of the person’s individual immune repertoire.

Metabolic targeting of cancer cells holds great therapeutic promise. However, cancer cells seem to be much more flexible in regard to the need for special nutrients than immune cells [182] and metabolic adaptability beyond the Warburg effect is a crucial factor in tumor progression in different stages and treatment failure [39]. Hence, combination therapies of metabolic inhibitors with established treatment protocols will have to consider their effects on the immune system. Additionally, interfering with diet by depleting individual nutrients emerges as an attractive therapeutic strategy against tumors, but needs to avoid general malnutrition that is well known to promote immunodeficiency and may limit antitumor responses. In that regard, mTOR inhibitors may actually support cancer cell survival and proliferation by helping tumor cells to degrade extracellular proteins as nutrients from an otherwise amino-acid-restricted TME [38]. Therefore, novel ways...
to target individual mTOR complexes and individual immune cells more selectively may be required to make mTOR-inhibitor-based cancer immunotherapy a big success. As mentioned earlier [58], nanoparticle-based siRNA approaches may offer more selective targeting in the future. In addition, mTOR inhibitors encapsulated by nanoparticles or covalently linked to TOR inhibitors or cell-type-specific antibodies may allow for an optimized and selective cell-type-specific drug delivery in vivo [183]. Inhibiting mTOR complexes and cancer cell metabolism will likely provide clinical benefit to many patients when their responses on the immune system are considered.

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Abbreviations: CAFs: cancer-associated fibroblasts · c-Myc: Myc protooncogene protein · CTLA-4: cytotoxic T-lymphocyte antigen 4 · CRC: colorectal cancer · FAO: fatty-acid oxidation · FOXP3: forkhead box protein P3 · HIF1α: hypoxia-inducible factor 1 subunit α · irAEs: immune-related adverse events · MDSCs: myeloid-derived suppressor cells · mTOR: mechanistic target of rapamycin · OXPHOS: oxidative phosphorylation · PD-1: programmed cell death protein 1 · PPP: pentose phosphate pathway · SREBP: sterol regulatory element-binding protein · TAM: tumor-associated macrophage · TCA: tricarboxylic acid · TME: tumor microenvironment

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