



# Metabolic programming of tumor associated macrophages in the context of cancer treatment

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**Abstract:** Tumor associated macrophages (TAMs) are important components of the tumor microenvironment (TME). They are characterized by a remarkable functional plasticity, thereby mostly promoting cancer progression. Changes in immune cell metabolism are paramount for this functional adaptation. Here, we review the functional consequences of the metabolic programming of TAMs and the influence of local and systemic targeted therapies on the metabolic characteristics of the TME that shape the functional phenotype of the TAMs. Understanding these metabolic changes within the context of the cross-talk between the different components of the TME including the TAMs and the tumor cells is an essential step that can pave the way towards identifications of ways to improve responses to different treatments, to overcome resistance to treatments, tumor progression and reduce treatment-specific toxicity.

**Keywords:** Immunotherapy; metabolism; thyroid cancer; tumor associated macrophages; tumor microenvironment (TME)

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## Introduction

Inflammation is an emerging hallmark of malignant tumors. The tumor microenvironment (TME) is involved in the pathogenesis and progression of different cancers in general. Characteristically, the TME is a relative nutrient-poor, hypoxic, acidic milieu that can influence the cellular composition and functional phenotype of local as well as infiltrating immune cells. Metabolic shifts within the immune cells result in changes in the immune cells phenotype and function, thereby influencing their effector functions. Additionally, data suggests that the TME also

differs between different tumor types, and can change depending on the stage and progression of the disease (1). Because of this, it is hard to predict which changes local cells such as tumor associated macrophages (TAMs) and other immune cells undergo within a specific TME and how those metabolic shifts influence the response to specific treatments. Also, different treatment regimens, including local therapies such as radiotherapy, and systemic agents targeting tumor vascularization and tumor cells such as multikinase inhibitors (MKIs) and mechanistic mammalian target of rapamycin (mTOR) inhibitors can induce (metabolic) changes in the TME (2-4). These changes are

likely to influence the function of the immune cells in the TME as well. The important question is whether these phenotypic changes synergistically improve the response to therapy or if they are deleterious, leading to more therapy resistance and subsequently tumor progression. Targeting tumor cell and immune cell metabolism in the right way, at the right time, in conjunction with other therapy modalities could be beneficial in patients and be exploited therapeutically. Therefore, understanding the complex interplay between cancer cells and the immune cells on the one hand and the effects of different therapies on this balance on the other hand is paramount in order to identify ways to improve responses to different treatments.

### The tumor microenvironment and its metabolic characteristics

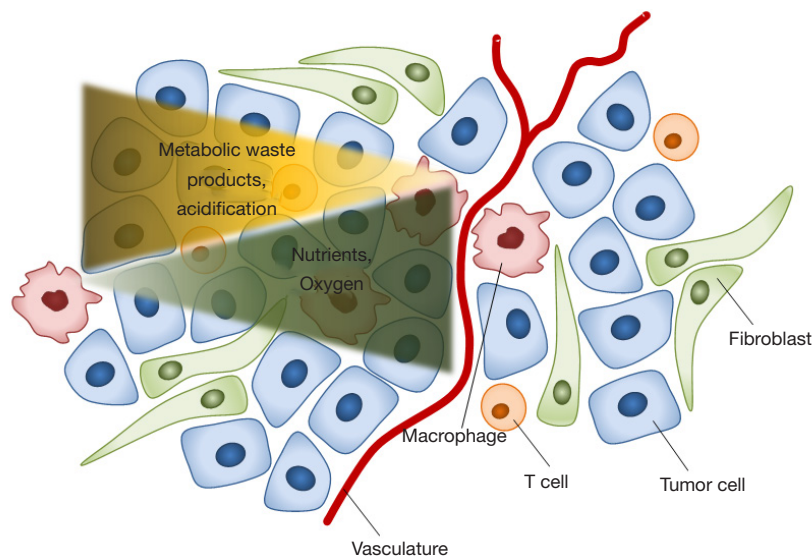
In contrast to normal organs, solid tumors are highly disorganized and heterogeneous structures comprising various different cell types. The TME is defined as the surrounding components of a tumor and include parts of the extracellular matrix (ECM), vascularization, such as blood vessels, as well as endothelial cells, fibroblasts and different types of immune cells. The heterogeneous factors and mediators present at the TME promote tumor progression by providing nutrients and signals that lead to immune suppression and thereby generating an anti-inflammatory milieu (5). In addition to containing immune suppressing factors, the TME of most tumors is hypoxic which favors cancer progression and metastasis by upregulating several important genes such as hypoxia-inducible factor 1- $\alpha$  (HIF-1 $\alpha$ ) (6). During tumor development, the TME is shaped and changed continuously in parallel with the tumor growth. Malignant cells accumulate mutations, leading to clonal diversity and heterogeneity in signal transduction, the epigenome, and gene expression. This induces heterogeneity in differentiation, metabolic and proliferative states across the cancer cell populations (7). Furthermore, tumor cells release various recruitment factors, which result in the infiltration of monocytes and are important mediators in the crosstalk between monocytes and tumor cells (7). Next to these intrinsic changes, other circumstances in the TME, such as proximity to the vasculature, deprivation of and competition for oxygen and nutrients, or the lack of degradation of metabolic waste products, affect the metabolism in both tumor cells and TAMs and the crosstalk between both cell types (*Figure 1*) (7).

Macrophages are abundant immune cells in the TME

of most tumors and their presence is thought to be associated with tumor progression and poor prognosis (8). Because of their specific phenotypical character in cancer, those macrophages were named TAMs. Most TAMs originate from circulating bone marrow and spleen derived monocytes and will differentiate in the TME (9-11). Naturally, macrophages show high levels of functional plasticity which is why numerous phenotypes and intermediate phenotypes have been described at different tissue compartments (12). In general, TAMs immunophenotypically differ from circulating macrophages as they usually show high expression of interleukin-10 (IL-10) and low expression of IL-12 as well as higher expression of specific surface receptors such as the mannose receptor (MR, CD206) (13-16). TAMs are often considered M2-like macrophages (or IL-4/IL13-activated) as opposed to the M1-like macrophages (LPS/IFN- $\gamma$ -activated). However, since recent insights reveals a spectrum of macrophage activation states rather than a dichotomous M1/M2 model, we will not use the M1/M2 nomenclature in this review but rather describe them according to their source, activators and specific markers if available as currently recommended (17,18). Given the above mentioned characteristics, TAMs strongly contribute to the anti-inflammatory status of the TME. Additionally, TAMs are known to contribute to angiogenesis and metastasis of tumors by upregulating genes responsible for ECM remodelling such as proteases and matrix metalloproteinases ultimately facilitating the escape of tumor cells (19). Interestingly, several animal studies have shown that depletion of macrophages in malignant tumors resulted in decreased tumor progression, thereby leading to a better outcome and prognosis (20,21).

### Extracellular circumstances affecting the metabolic characteristics of the TME

One of the strongest stimuli, inducing the secretion of tumor-derived factors, is hypoxia. During tumor growth, the level of oxygen is strongly reduced due to abnormal tumor vasculature, especially in the center of the neoplasm. Hypoxia triggers HIF-1 $\alpha$  stabilization in neoplastic cells and consequently the release of pro-angiogenic factors, such as growth factors (VEGF, PDGF, PIGF, ANG-2), chemokines (CXCL8, CXCL12), cytokines, (TNF $\alpha$ , IL1 $\beta$ , TGF $\beta$ ) and metalloproteinases (MMPs), resulting in angiogenesis and thereby cancer cell growth (22,23) as well as monocyte recruitment (24). Furthermore, hypoxia leads to an enhanced glycolytic activity of tumor cells and thereby



**Figure 1** Schematic overview of the tumor microenvironment (TME) containing different cell types that interact with each other. Different gradients regarding the availability of nutrients and oxygen as well as metabolic waste products and the grade of acidification are indicated.

an increased lactate secretion which acidifies the TME and affects the local immune cells (see below) (25).

### Tumor cell metabolism

To sustain the high energy demands of tumor cells required for the high proliferation rate in an oxygen- and nutrient-deprived TME, their metabolism is adapted. It has become evident that metabolic alterations during tumorigenesis encompass all stages of cell-metabolite interaction: (I) increase of metabolite influx, (II) direction of nutrients to metabolic pathways contributing to cellular tumorigenic properties, (III) effect on cellular fate, such as alterations in differentiation by gene-regulation and metabolic interactions with the TME (25).

To fulfill the biosynthetic demands, tumor cells have a high uptake of glucose and glutamine, two principal nutrients that support survival and biosynthesis by maintaining pools of numerous carbon intermediates (26). An elevated consumption of glucose by tumors in comparison to nonproliferating normal cells has been first described by Otto Warburg in 1924 and is now referred to as the “Warburg effect”, entailing the shift from oxidative phosphorylation (OXPHOS) as the main source of energy towards anaerobic glycolysis (26). In this, PI3K/Akt signaling acts as a master regulator of glucose uptake by promoting the expression of plasma membrane glucose

transporter 1 (GLUT1) (25). Upregulation of GLUT1 and the first enzyme of glycolysis, hexokinase (HK), enables the metabolic shift towards an enhanced glucose uptake and glycolysis (27). Next to glucose, a high demand for glutamine in tumor cells has been first described by Harry Eagle in the 1950s. Eagle showed that the optimal growth of cultured HeLa cells requires substantially more glutamine in culture medium relative to other amino acids (28). In line with this, glutamine depletion from the TME as compared to the corresponding normal tissue is described in numerous tumorigenic contexts (29-32).

Interestingly, proliferating tumor cells convert excess pyruvate as product from glycolysis to lactate rather than transporting it into the mitochondria for OXPHOS. Compared to OXPHOS, anaerobic glycolysis is far less efficient in terms of adenosine triphosphate (ATP) production. However, glycolysis is a robust provider of precursor molecules and reducing equivalents in the form of nicotinamide adenine dinucleotide phosphate (NADPH), whereas tricarboxylic (TCA) cycle activity is the major negative regulator of glucose metabolism. By converting pyruvate to lactate, proliferating tumor cells prevent accumulation of cytosolic NADH and reduce ATP production, promoting glycolysis free from feedback repression by excess mitochondrial ATP generation (25).

Although glycolysis is often described as a single chain of molecular events, leading to the generation of pyruvate,

several glycolytic intermediates can be shunted into branching pathways as well. The first branch in glycolysis, is the pentose phosphate pathway (PPP), which is often elevated in tumorigenesis and enhances the production of structural components of nucleotides and NADPH. Several other glycolytic intermediates, such as fructose-6-phosphate and dihydroxyacetone phosphate (DHAP), can be utilized for cellular glycosylation reactions as well as heparan sulfate and hyaluronic acid biosynthesis, or in the biosynthesis of phospholipids, respectively (25), enhancing tumor proliferation.

Moreover, tumor cells show a symbiotic metabolism, as in hypoxic regions of a tumor, cancer cells metabolize glucose through anaerobic glycolysis and express monocarboxylate transporter 4 (MCT4) to release lactate, whilst well-oxygenated cancer cells import the lactate produced by hypoxic cancer cells via MCT1 and consume it to fuel mitochondrial metabolism (33,34).

### Metabolism of TAMs

The metabolic profile of TAMs is very dynamic (Figure 2). In a nutrient-deprived TME, TAMs compete with cancer cells for nutrients such as glucose and therefore undergo changes in their cellular metabolism as well.

Under normoxic conditions, several studies indicate that TAMs have an activated aerobic glycolysis (35,36). Furthermore, metabolic changes in TAMs have also been linked to promotion of tumor invasion and metastasis, linked to an increased glycolytic activity (37).

Next to glucose metabolism, TAMs show an increased glutamine uptake and metabolism (38), whereas glutamine depletion restrains programming towards a pro-tumorigenic phenotype (39,40). Furthermore, some TAMs accumulate intracellular lipids, supporting their immunomodulatory functions (41). Multiple factors involved in lipid metabolism, including enzymes and chaperones are deregulated in TAMs (41-45). Transcriptomic profiling showed high gene expression of lipid metabolism pathways in alveolar resident macrophages, including PPAR- $\gamma$  signaling. Another population defined as TAMs selectively expressed chemokine genes and a population resembling tumor-associated monocytes expressed genes involved in matrix remodeling. These findings underscore the diversity of macrophage phenotypes in cancer (46). Interestingly, PPAR- $\gamma$  signaling is associated with a pro-tumorigenic phenotype of TAMs, as described in an ovarian cancer model (47). Furthermore, TAMs are known to express

cyclo-oxygenase 2 (COX2) *in vivo* and *in vitro* (48-50). However, the functional relevance of lipid metabolism in TAMs is still not fully understood.

### Factors involved in the metabolic and functional programming of TAMs

Within the TME, TAMs and tumor cells develop a symbiotic relationship which is the result of both tumor-derived and TAM-derived factors and the intrinsic adaptation of the cellular metabolism of both cells to the metabolically unfavorable TME.

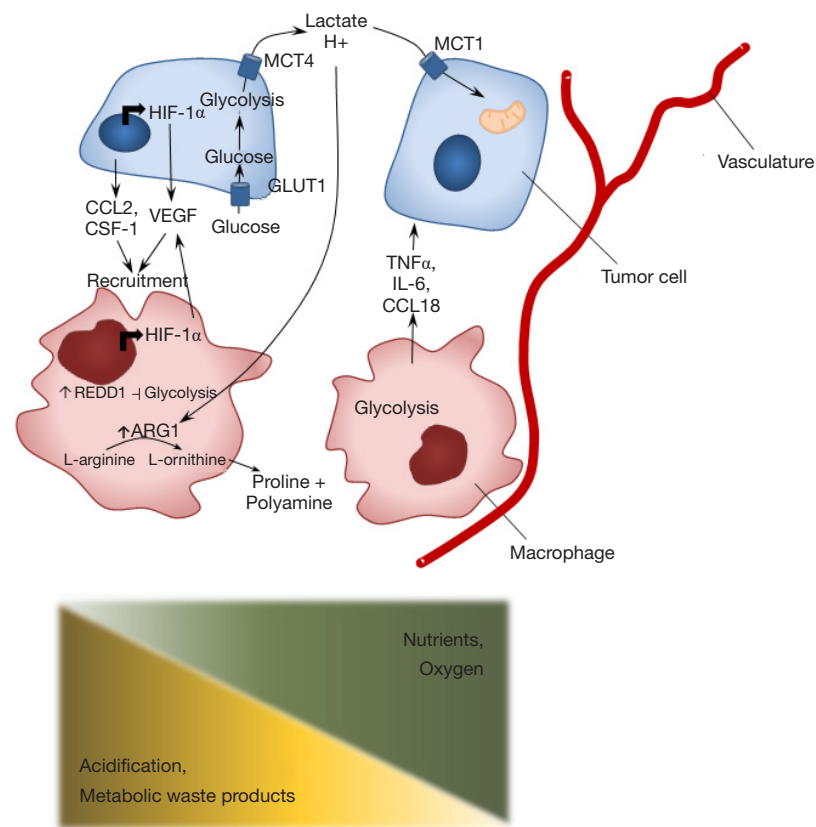
#### Chemokines

Tumor-derived factors are involved in monocyte recruitment, survival, and differentiation within the tumor site. Monocyte chemoattractant protein-1 (MCP1, also known as CCL2) is a tumor- and stromal-derived factor involved in monocyte recruitment (51). Inhibition of the CCL2-CCR2 signaling in a mouse model of breast cancer impaired monocyte infiltration, inhibited metastasis, reduced tumor growth, and depletion of tumor-derived CCL2 inhibited metastatic seeding (52).

Next to CCL2, tumor cells secrete high levels of the growth factor colony stimulating factor-1 (CSF-1), which is involved in recruitment and differentiation of monocytes (53-55). CSF-1 programs monocyte-derived macrophages towards a pro-tumorigenic phenotype coupled to fatty acid oxidation (FAO) upregulation (56) and secretion of pro-tumorigenic and immunosuppressive factors such as epidermal growth factor (EGF) (57) and IL-10 (58).

#### Hypoxia

Hypoxia has been shown to induce infiltration of TAMs and reprogramming of macrophages toward the pro-tumorigenic phenotype (59-63), promoting tumor cell proliferation and chemoresistance (64). Under hypoxic conditions, TAMs produce angiogenic factors such as vascular endothelial growth factor (VEGFA). VEGFA stimulates chemotaxis of endothelial cells and macrophages (65). Other angiogenic factors released by TAMs include basic fibroblast growth factor, thymidine phosphorylase, urokinase-type plasminogen activator and adrenomedullin (65-67). Macrophages also promote angiogenesis by physically assisting sprouting blood vessels to augment the complexity of the intra-tumorigenic vascular network (68).



**Figure 2** Schematic overview of the metabolic adaptations of tumor cells and (tumor associated) macrophages. Depending on the availability of nutrients and oxygen several metabolic programmes are initiated that allow both cells to adapt to the changing conditions within the TME. Several cytokines and chemokines are involved in these processes and allow both cells to differentiate for adaptation. ARG1, arginase 1; CCL2, monocyte chemoattractant protein-1; CCL18, C-C motif chemokine ligand 18; CSF-1, colony stimulating factor-1; GLUT1, glucose transporter 1; HIF-1 $\alpha$ , hypoxia-inducible factor 1-alpha; IL-6, interleukin-6; MCT, monocarboxylate transporter; REDD1, regulated in development and DNA damage responses 1; TNF $\alpha$ , tumor necrosis factor alpha; VEGF, vascular endothelial growth factor.

Interestingly, under hypoxic conditions, TAMs upregulate REDD1 (regulated in development and DNA damage responses 1), a negative regulator of mTOR. REDD1-mediated mTOR inhibition hinders glycolysis, leaving more glucose for neighboring cells and curtails their excessive angiogenic response, resulting in abnormal blood vessel formation (69).

### Lactate

Extracellular lactate, secreted by tumor cells, functions as signaling molecule which leads the induction of an angiogenic response (70-73). Accumulation of extracellular lactate stimulates the programming of macrophages towards a pro-tumorigenic phenotype and induces expression of VEGF (74-77). Furthermore, the secretion of lactate into

the stroma via MCT1 is co-transported with H<sup>+</sup>, leading to further acidification of the TME. Interestingly, recent *in vivo* animal studies have shown that differences in function of MCT1 transporter on melanoma cells confer different metastatic potential to these cells. The results suggest that the bidirectional, more efficient handling of lactate by the tumor cells results in a more efficient handling of the oxidative stress and may contribute to the higher metastatic potential in melanoma's (78). Interestingly, acidification of the TME enhances an IL-4 driven phenotype in macrophages and induces a pro-tumor phenotype (79).

### Autophagy

Another process involved in differentiation of macrophages into TAMs is autophagy (80,81). It was found that

**Table 1** Effects of local and systemic therapies on the recruitment, survival and functional reprogramming of TAMs through metabolic mechanisms

Therapy	Effect on TAMs				
	Infiltration/recruitment		Increase apoptosis	Reprogramming	
	Increase	Reduce		Anti-tumorigenic phenotype	Pro-tumorigenic phenotype
Radiotherapy	>8–20 Gy (4)		>8–20 Gy (4)	2 Gy (88,89)	
Chemotherapy	Gemcitabine (90); Doxorubicin (91,92) Cyclophosphamides (90,91,93-95); HDAC inhibitors (TMP195) (96); Topoisomerase inhibitors (90,91,93-95); Retinoids (90,91,93-95)	Dual HDAC and GSK3B inhibitors (Metavert) (97)		HDAC inhibitor (TMP195) (96); Leg-3 (98); Fenretinide (99); All-trans-retinoic acid (ATRA) (100); Gemcitabine (93)	Doxorubicin (91,92,95)
Immunotherapy		CSF-1R inhibitors (21,101,102)		CSF-1R inhibitors (21,101,102)	
MKI		Sorafenib ( $\leq 30$ mg/kg) (103)		Sorafenib ( $\leq 30$ mg/kg) (103)	Sorafenib ( $> 30$ mg/kg) (104)
Autophagy inhibitors				Chloroquine (105,106)	
Autophagy activators				Rapamycin (3,107); Baicalin (108); Neferine (109)	
Metabolism activators		Metformin (110-112)	Simvastatin + Fenretinide (113)	Metformin (110-112,114); Simvastatin +/- Fenretinide (113,115)	

CSF-1R, colony stimulating factor-1 receptor; GSK3B, glycogen synthase kinase 3 beta; Gy, gray; HDAC, histone deacetylase; MKI, multikinase inhibitors; TAMs, tumor-associated macrophages.

autophagy, induced by toll-like receptor 2 (TLR2) signaling, could differentiate bone marrow-derived macrophages into a pro-tumorigenic phenotype in the presence of hepatoma tumor cell condition medium (82). In another study, myeloid-cell specific autophagy was shown to impair anti-tumorigenic immune responses and promote the survival and accumulation of pro-tumorigenic macrophages in tumor tissues, a process modulated via CSF-1 and transforming growth factor (TGF $\beta$ ) (83). Wen *et al.* show that tumor cell-released autophagosomes differentiated macrophages into an immunosuppressive phenotype characterized by the expression of programmed cell death protein ligand-1 (PD-L1) and IL-10 (84).

Importantly, the effects of metabolic effects of cancer cells on TAMs is not unidirectional. TAMs secrete multiple cytokines with metabolic functions, including IL-6, tumor necrosis factor alpha (TNF $\alpha$ ) and CCL18 (85-87). TAM-

derived IL-6, TNF $\alpha$  and CCL18 promote tumor cell glycolysis and proliferation (85-87).

### Effects of local and systemic therapies on the cross-talk between tumor cells and TAMs and their metabolic reprogramming

Different local and systemic cancer therapies influence the composition of the TME and the cross-talk between the cellular components of the TME. Some of these effects can be attributed to changes of the metabolic characteristics of the TME through induction of ischemia and hypoxia or through direct effects of these drugs on the cellular metabolism or other intracellular signaling pathways (Table 1). The effect of these therapies on TAMs is not well understood and only scarcely studied, particularly with respect to immunometabolism.

### Radiotherapy

Upon irradiation, immunosuppressive pathways are activated in the TME and radiation-induced inflammation is caused, subsequently affecting tumor progression. TAMs are recruited and programmed by increased expression of CSF-1 and the destruction of vascularization upon irradiation (4). As immunosuppressive cells, TAMs are involved in the process of radiation-induced inflammation and may promote tumor recurrence (116). HIF-1 $\alpha$  and interferon gamma (IFN- $\gamma$ ) signaling in the TME induces the expression of PD-L1 in TAMs, suppressing anti-tumor immunity (117). Conversely, regression of tumors at distant sites from the irradiated lesion, called “abscopal” effect, suggest an activation of host immunity (118). Furthermore, radiation-induced tumor cell apoptosis drives differentiation of macrophages into a pro-tumorigenic, anti-inflammatory phenotype (119,120). Interestingly, the effects seem to be dose-dependent, as high doses of irradiation (>8 Gy) may promote an anti-inflammatory phenotype of macrophages: a dose of 20 Gy induces a phenotype of TAMs with tolerogenic properties (4), whereas low-dose irradiation (2 Gy) skew macrophages towards an anti-tumorigenic phenotype, inducing effective T cell immunity (88) and influences angiogenic responses in tumor endothelial cells (89).

### Chemotherapeutics

Chemotherapeutic agents are used to directly or indirectly inhibit the proliferation of rapidly growing cells, typically in the context of malignancy. Next to the effects on neoplastic cells, different chemotherapeutic agents among which antimetabolites (e.g., gemcitabine), anti-tumor antibiotics (e.g., anthracyclines), alkylating reagents (e.g., cyclophosphamides), histone deacetylase (HDAC) inhibitors, topoisomerase inhibitors and retinoids have been shown to influence the recruitment and the functional phenotype of TAMs as well. Various chemotherapeutics, including gemcitabine have been reported to induce the recruitment of myeloid cells (90,91,93-95). The immunogenic cell death of neoplastic cells upon chemotherapy leads to modulation of an adaptive immune response (121), which is in line with the discovery that distinct mouse tumors only show an optimal response to anthracyclines in the presence of an intact immune system (122). Interestingly, gemcitabine, even induces a reprogramming of TAMs toward an immunostimulatory

phenotype in a pancreatic murine tumor model (93).

In contrast, there are discrepant findings in the role of TAMs to the anthracycline Doxorubicin. Whereas some studies show that treatment induces macrophage recruitment, angiogenesis and pro-tumor effects or polarization (91,92), others show that blocking monocyte recruitment by the administration of a CSF-1R-signaling antagonist in a preclinical breast cancer model, enhanced the therapeutic efficacy of chemotherapy (91,123).

Whereas the efficacy of anthracycline-based neoadjuvant chemotherapy in breast cancer patients is associated with the expression levels of the cytokines YKL-39 and CCL18, it is not associated with the amount of TAMs present (124). In line with this, compounds packed in liposomes, such as Long-circulating liposomes (LCL)-encapsulated doxorubicin (Doxil) and LCL-encapsulated prednisolone phosphate (LCL-PLP) have anti-angiogenic and anti-inflammatory effects on TAMs. Interestingly, only LCL-PLP inhibited tumor growth through strong suppressive effects on pro-angiogenic functions of TAMs (125). Furthermore, a doxorubicin-based prodrug (leg-3) selectively ablated TAMs and resulted in a significant reduction of angiogenic factors and related tumor vessel growth (98).

A selective class IIa HDAC inhibitor, TMP195, induces the recruitment and differentiation of highly phagocytic and stimulatory macrophages. Combining TMP195 with chemotherapy regimens or T-cell checkpoint blockade significantly enhanced the durability of tumor reduction in different studies (96), underscoring a potential role of macrophages in treatment duration. Metavert, a dual inhibitor of glycogen synthase kinase 3 beta (GSK3B) and HDACs, increases killing of drug-resistant pancreatic ductal adenocarcinoma cells by paclitaxel and gemcitabine. Administration of Metavert *in vivo* leads to beneficial effects such as, decreased TAM infiltration, tumor growth inhibition, prevention of tumor metastasis and a decrease of pro-tumorigenic cytokines (97).

Furthermore, Etoposide, a topoisomerase II-inhibitor, increases cancer cell apoptosis in cells co-cultured with IFN- $\gamma$ /LPS induced macrophages, but reduces apoptosis in the presence of IL-4/IL-13 induced macrophages (126).

Retinoic acid (RA) altered the ability of monocytes to contribute in the tumor angiogenesis process and decreased the ability of TAMs to secrete IL-8 and VEGF (127). All-trans-retinoic acid (ATRA) abrogated the ability of prostate cancer cell-derived factors to induce a pro-tumorigenic TAM-like phenotype characterized by increased

proliferation and increased expression of pro-angiogenic, immunosuppressive and pro-metastatic factors. ATRA also inhibited the cancer cell-stimulated proliferation of the pro-tumorigenic macrophages and restored their cytotoxic capacity towards prostate cancer cells (100). Furthermore, fenretinide, a synthetic retinoid, suppresses IL-4/IL-13 induced programming of macrophages, shown by the reduced expression of pro-tumorigenic surface markers, the down-regulation of marker genes and the inhibition of pro-tumorigenic macrophage-promoted angiogenesis (99).

Taken together, anthracyclines and retinoids rather have anti-angiogenic effects on TAMs, whereas the effects of HDAC and topoisomerase inhibitors are only scarcely investigated.

### **Immunotherapies**

In the past decades, substantial evidence is arising that immunotherapy against a broad range of cancers is a suitable alternative to conventional therapies such as chemo- or radiotherapy. In general, this therapeutic approach makes use of existing immunological pathways and cellular interactions to improve the anti-tumorigenic immune response or by blocking undesired interactions of immune cells with cancer cells. To date, several therapies that target specific immune checkpoints have been approved and were proven effective in different forms of cancer. One such checkpoint is the inhibitory interaction between PD-L1 (CD274), expressed by cancer cells, and its receptor PD-1 (CD279) present on different immune cells. T-lymphocyte associated protein-4 (CTLA-4, CD152) is another immune checkpoint efficiently targeted in cancer immunotherapy. The effects of immune checkpoint inhibitors on the TAMs metabolism is largely unknown. Nonetheless, recently, Palaskas *et al.* showed that PD-1 signaling broadly influences the primary human T-cell metabolism *in vitro*, shifting it from aerobic glycolysis and glutaminolysis towards utilization of alternative substrates to fuel the TCA cycle. Moreover, the glucose-derived carbon was not derived towards FAO. Furthermore, the study uncovered a block in the nucleoside phosphate *de novo* synthesis pathway and a reduced mTORC1 signaling (128). Another recent study suggests that immune cell metabolites could be relevant additional markers to predict response to PD-1 blockade therapy (129). Apart from targeting immune checkpoints, inhibiting recruitment of TAMs by blocking the CSF-1/CSF-1R pathway represents a different

approach. CSF-1R inhibitors not only have been shown to reduce the number of TAMs, but also reprogramming of TAMs from pro-tumorigenic towards an anti-tumorigenic phenotype (21,101,102).

### **Multikinase inhibitors (MKI), selective kinase inhibitors and VEGFR targeting drugs**

MKIs have become a promising treatment strategy, since increased intracellular, paracrine and autocrine kinase signaling play a key role in many cancers (2). MKIs often influence tumor vascularization by targeting VEGF receptor (VEGFR) and thereby affect tumor oxygenation (2,130-132). This can also alter the TME to a more immunosuppressive state and inhibit drug uptake in the tumor (2,130-132). Furthermore, VEGFR inhibition can directly affect TAMs, because VEGFR-1 is mainly expressed on anti-inflammatory macrophages and its suppression causes reprogramming towards a more pro-inflammatory activated phenotype (133). There are several examples of interactions between kinases and VEGFR inhibition and the functional programming of TAMs within the TME, some of which possibly also involving metabolic pathways. The immunomodulatory effects of some MKIs, such as sorafenib, which is broadly used for treatment of hepatocellular carcinoma (HCC), renal cell carcinoma (RCC) and non-medullary thyroid carcinoma (DTC), seem to be dose-dependent (134). As such, treatment of hepatocellular carcinoma (HCC) with sorafenib in low doses ( $\leq 30$  mg/kg), was mostly associated with TAM programming towards a more pro-inflammatory phenotype, possibly through VEGF reduction, miR-101 inhibition or enhanced CCL2 expression (103). Furthermore, sorafenib can inhibit the release of the growth factors mCD163, insulin-like growth factor 1 (IGF-1) and TGF $\beta$  by anti-inflammatory TAMs. Additionally, low dose sorafenib treatment could decrease TAM percentages and increase their expression of four activation markers (FAS-L, chemokine (C-X-C motif) ligand 9 (CXCL-9), CD31, and CD105) (103). However, high doses ( $>30$  mg/kg) impelled immunosuppressive effects in HCC through TAMs by increasing the PD-L1 expression (104). Even though, these high doses might not be achievable in humans, TME and immune changes have often been involved in challenging sorafenib resistance (103). Initially, sorafenib dose-dependently inhibits HIF-1 $\alpha$  synthesis (135) leading to downregulated VEGF expression and decreased tumor vascularization.



However, the hypoxic TME, caused by sustained sorafenib treatment, leads to upregulation of the SDF1- $\alpha$ /CXCR4 axis (136), HIF-1 $\alpha$  and HIF-2 $\alpha$  (137). The increase of HIF-1 $\alpha$  and HIF-2 $\alpha$  has been associated with sorafenib resistance in myeloid leukemia, renal, gastric and hepatoma, because it enhances the expression of target genes involved in mitophagy (BNIP3, NIX), angiogenesis (VEGF), glycolysis (GLUT1, HK2, LDH, PDK1) and proliferation (cyclin D1, c-Myb/PCNA, TGF $\alpha$  /EGFR) (137). Even without hypoxia, sorafenib is known to increase aerobic glycolysis, decrease OXPHOS activity and increase reactive oxygen species (ROS) levels in a dose-dependent manner in hepatoma cells *in vitro*. This results in higher glucose uptake and lactate production (138,139), thereby altering the TME. It remains to be investigated if sorafenib can also directly shift the metabolism of TAMs in a similar manner.

The immunological effects of vandetanib, the most recently approved MKI for medullary thyroid cancer (MTC), are still mostly unknown. Nonetheless, interesting metabolic effects in highly glycolytic hereditary leiomyomatosis and RCC cells have been reported by Sourbier *et al.* Vandetanib inhibits the phosphorylation of ABL1, a non-receptor tyrosine kinase, which leads to a reduction of the mTOR-HIF-1 $\alpha$ -dependent aerobic glycolysis. In contrast to sorafenib, vandetanib leads to an alteration of the TME by reduced lactate production and glucose uptake, through downregulated Glut1 and Glut4. Additionally, suppressed ABL1 function diminishes the NRF2-dependent antioxidant response which is essential for the survival of ROS-producing tumor cells (140). Future research could clarify whether TAM function is affected by vandetanib, directly or through TME modifications.

### **Autophagy modulators**

Autophagy is known to have both pro-tumorigenic and anti-tumorigenic effects in cancer (141). The role of autophagy in cancer might differ in different stages of carcinogenesis, different tumor types and could be related to changes in the TME and treatment. Autophagy activation results in intracellular lysosomal degradation and recycling of proteins and organelles. This process is controlled by autophagy-related genes, regulated by nutrient, energy and stress sensing mechanisms, thereby recycling intracellular components to maintain energy homeostasis and mitochondrial metabolic function inside the cell (141).

### **Autophagy inhibition**

Chloroquine (CQ), an autophagy inhibitor, was able to reprogram pro-tumorigenic macrophages to an anti-tumorigenic phenotype. The reprogrammed macrophages presented higher phagocytotic activity towards Hep-2 laryngeal tumor cells and re-sensitized Hep-2 cells to cisplatin treatment *in vitro* (105). Another study by Li *et al.* showed that CQ abrogated the abnormal angiogenic efficacy of pro-tumorigenic macrophage-conditioned media, thereby decreasing abnormal angiogenesis and lung carcinogenesis (106).

### **Autophagy stimulation**

Whereas previously mentioned studies demonstrate a beneficial effect of autophagy inhibition on the reprogramming of TAMs, others have shown that the induction of autophagy is the key to reprogramming of TAMs. Shan *et al.* demonstrate inhibition of programming of mouse RAW 264.7 macrophages to pro-tumorigenic macrophages by autophagy inducer rapamycin. Not only pro-tumorigenic expression was down-regulated, but intracellular ROS generation was also blocked by autophagy induction (3). Another group showed that upregulation of TAM autophagy using rapamycin exhibited effective inhibition of colony formation and proliferation in colorectal cancer cells while inducing apoptosis (107). Like rapamycin, Baicalin, a natural flavonoid, elevated autophagy programmed TAMs towards an anti-tumorigenic phenotype and promoted pro-inflammatory cytokine production. Co-culture of HCC cells with baicalin-treated TAMs resulted in reduced proliferation and motility (108).

Neferine, a bisbenzylisoquinoline alkaloid, induced autophagy via inhibition of the mTOR/p70S6K pathway in human umbilical vein endothelial cells and also possessed the ability to suppress pro-tumorigenic macrophage programming (109).

### **Metabolism modulators**

#### **Metformin**

Metformin, well known for its use in the treatment of diabetes, has been intensively studied for its anti-tumorigenic properties. Despite the numerous studies, using metformin in clinical trials provided conflicting data (142,143). Metformin-use not only affects the tumor cells, but also influences the TME and TAMs present in the TME. Several studies demonstrate a role of metformin

in the pro-tumorigenic programming of macrophages. Metformin suppressed IL-13-induced pro-tumorigenic macrophages *in vitro*, as indicated by a reduced expression of CCL2 and marker CD206 (114). Metformin has also been shown to reduce TAM infiltration, inhibit tumor growth and angiogenesis by skewing TAM programming towards an anti-tumorigenic phenotype (110-112).

### Simvastatin

Simvastatin, a member of the group 3-hydroxy-3-methylglutaryl (HMG)-CoA reductase inhibitors, is clinically used for the reduction of hypercholesterolemia. Similar to metformin, simvastatin, has also been studied in relation to cancer. In anaplastic thyroid cancer (ATC), simvastatin has been shown to inhibit proliferation and migration of ATC cells *in vitro* (144). Additionally, Simvastatin has been shown to have the potential to re-sensitize drug-resistant cancer cells via reprogramming of TAMs. Simvastatin was able to reprogram TAMs *in vitro* via cholesterol-associated LXR/ABCA1 regulation, re-sensitizing non-small cell lung cancer cells to paclitaxel (115). Finally, Simvastatin weakened the TAM-mediated resistance of pancreatic ductal adenocarcinoma cells to gemcitabine (145). A drug combination of simvastatin and fenretinide reprogrammed TAMs *in vitro* via regulation of the STAT6 pathway as well as induced ROS-mediated mitochondrial apoptosis by inhibiting the Ras/Raf/p-Erk pathway in glioma cells (113).

### Future research directions and outstanding questions

The functional consequences of metabolic programming of TAMs and tumor cells present in the TME has attracted increased attention. Understanding the dynamic metabolic changes and how these affect the functional phenotype of the tumor-infiltrating TAMs is important for the development and improvement of therapies. The same holds true for other immune and non-immune cells which coexist and interplay within the inflammatory TME. This will provide not only a better understanding on the pathophysiology of tumor development and progression, but potentially presents new opportunities for therapy and monitoring of patients with malignant tumors. Next to the conventional therapies, such as radiotherapy, chemotherapy and immunotherapy, novel approaches to target macrophages in the TME could complement the armamentarium of anti-cancer therapy. The latter include

strategies to inhibit recruitment and survival of TAMs in the TME, and induce functional reprogramming of these cells towards anti-tumorigenic phenotypes. Such strategies are currently being explored in clinical and translational settings. Despite several limitations related to therapy resistance and loss of sensitivity due to compensatory immunosuppressive mechanisms and toxicity, such approaches show promising results and will most likely be further investigated in clinical trials (146).

Targeting immune cell metabolism to synergize the effect of other systemic or local treatments and overcome therapy resistance is interesting, but at this time it still faces several challenges.

One of the challenges involves the targeting of specific metabolic pathways to the different cell types. Indeed, future work towards the design of delivery systems that could target the metabolic modulators directly towards the desired cell type would be crucial, with the recent progress in nanotechnologies being promising. These developments would automatically help for the second challenge of these therapies: depending on the cell type, what may be favorable for one cell or process, may be deleterious for another, thereby creating either a synergistic or counteracting effect of potential therapeutic metabolic modulators. Finally, as metabolic mediators could affect inadvertently bystander organs and cells, this could lead to side effects, that could be also tackled by targeting the drug to specific cells.

In conclusion, during therapeutic interventions changes in the metabolic landscape of the tumor occur. These changes could result in reprogramming of TAMs into a more anti-tumorigenic phenotype that could be harnessed therapeutically. Future studies are warranted to discover the specific role of cellular metabolic pathways in various cell types in TC, to discover new modulators of these pathways with therapeutic potential, to deliver these molecular modulators to the appropriate cell type, and finally to test and implement these new approaches in patients. In this respect, modulators of immune cell metabolism could be combined with other forms of immunotherapies, such as checkpoint inhibitors, to identify new and more efficient therapeutic strategies. One example of such an approach could be the inhibition of transcription factor c-MAF inducing metabolic programming of TAMs towards a pro-tumoral phenotype, which has been shown to overcome anti-PD-1 therapy resistance in a Lewis lung carcinoma model (147). Only after these studies are performed in the coming years the full potential of metabolic modulators of the immune system will be realized.

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