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**Review Series**

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# The impact of hypoxia on tumor-associated macrophages

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The role of tumor-associated macrophages (TAMs) in cancer is often correlated with poor prognosis, even though this statement should be interpreted with care, as the effects of macrophages primarily depend on their localization within the tumor. This versatile cell type orchestrates a broad spectrum of biological functions and exerts very complex and even opposing functions on cell death, immune stimulation or suppression, and angiogenesis, resulting in an overall pro- or antitumoral effect. We are only beginning to understand the environmental cues that contribute to transient retention of macrophages in a specific phenotype. It has become clear that hypoxia shapes and induces specific macrophage phenotypes that serve tumor malignancy, as hypoxia promotes immune evasion, angiogenesis, tumor cell survival, and metastatic dissemination. Additionally, TAMs in the hypoxic niches within the tumor are known to mediate resistance to several anticancer treatments and to promote cancer relapse. Thus, a careful characterization and understanding of this macrophage differentiation state is needed in order to efficiently tailor cancer therapy.

## Macrophages and the tumor microenvironment

Tumor tissue comprises a highly complex network of diverse cell types. In a simplified view, the tumor microenvironment can be subdivided into the cancer cell and the stromal cell compartments, the latter of which harbors many different cell types. A considerable number of innate immune cells reside within the tumor microenvironment, including macrophages, which are the most prevalent cell type in a variety of tumors. Macrophages are primarily considered essential mediators in immune defense and it has been assumed that they might contribute to an antitumor immune response to combat tumor growth; however, mounting evidence indicates that macrophages foster malignancy and tumor progression. The discrepancy between these contrasting observations might stem from the fact that, in general, macrophages are exceptionally plastic cells that respond and adapt to the microenvironment in which they are embedded (1, 2). Moreover, the functional properties of tissue-resident macrophages greatly vary between different organs, not only due to their environmental skewing (3), but also with respect to their specific origin, further complicating a simplified classification of macrophages. For example, brain macrophages arise from the yolk sac (4), Kupffer cells are believed to arise from a mixed lineage that includes the yolk sac and embryonic hematopoietic stem cells (5), and macrophages in the intestine are derived entirely from adult hematopoietic stem cells (6). During adulthood each organ determines to what extent tissue-circulating blood monocytes replace tissue-resident macrophages. However, it still remains an open question as to what extent cell lineage determines macrophage behavior and phenotype (7). A recent study demonstrated for the first time that bone marrow–derived macrophages can give rise to self-renewing tissue-resident macrophages (in this case, Kupffer cells) during the first weeks of life or during tissue injury in adulthood when the niche becomes available. These bone marrow–derived macrophages and their embryonic counterparts were further shown to exhibit a significant phenotypic and transcriptional overlap (8).

Macrophages are equipped to execute a broad repertoire of functions that range from their involvement in tissue homeostasis and wound healing to their role as immune effectors. In a simplified view, there are two main activation states that represent a paradigm for understanding the opposing functions that these cells can carry out: the classical M1 and the alternative M2 macrophage phenotypes, a classification that mirrors the Th1/Th2 polarization of T cells (9). Macrophage responses, which are shaped by these activation modes, are characterized by two contrasting actions: killing and repairing. Proinflammatory cytokines such as TNF-α or IFN-γ, as well as microbial cell wall components, serve as cues that trigger a proinflammatory, antibacterial, and antiangiogenic (M1-like) program, thereby arming macrophages with important effector molecules that allow pathogen recognition and killing as well as recruitment of other immune cells to the site of infection. Generation of ROS and NO, expression of high amounts of IL-12, and low levels of IL-10 are typically associated with an M1 macrophage response. In contrast, cytokines such as IL-4, IL-13, and IL-10 induce macrophages to acquire the ability to execute antiinflammatory, protumorigenic, and proangiogenic (M2-like) functions (10, 11). Under physiological conditions, M2-like macrophages facilitate wound healing by promoting angiogenesis, cell proliferation, and clearing of cellular debris (12, 13). However, as we will discuss in more detail, these capacities are coopted within the tumor microenvironment to fuel tumor growth. In reality, the distinction between these activation states becomes rather blurry, as macrophages might exhibit phenotypes anywhere in between these two extremes. Recent pub-

lications dealing with this issue shed new light on the complexity of macrophage activation states (and ontogeny), proposing new guidelines for nomenclature and experimental standards to describe macrophage heterogeneity (14, 15).

Within the cancerous tissue, macrophages are designated as tumor-associated macrophages (TAMs). It is clear that M1-like macrophages can contribute to an antitumor response; however, experimental evidence suggests that during tumor initiation, persistent, proinflammatory (M1-like) macrophage functions might promote malignant transformation (16), supporting the hypothesis that inflammation promotes cancer, which was established by Rudolf Virchow in the 19th century (17). An overwhelming and long-lasting release of inflammation-promoting factors, such as cytokines, chemokines, or free radicals, has been shown to culminate in cell proliferation, mutagenesis, and oncogene activation, all of which are determinants of cell transformation (16). Thus, chronic inflammatory conditions are characterized by a greater risk of tumor incidence. In an established tumor, macrophages are educated to a proangiogenic tumor-promoting (M2-like) phenotype. Tumor hypoxia is thought to play a pivotal role in the phenotypic control of TAMs, as hypoxic TAMs release factors that assist in tumor growth, cancer immunosuppression, and angiogenesis (18, 19). It is therefore easy to conceive that phenotypic switches in TAMs occur in a spatiotemporal fashion, as they are dependent on microenvironmental cues such as hypoxia in conjunction with cytokine availability. It should be noted that it is the concerted action of hypoxia and cytokines that ultimately shapes macrophage responses, as hypoxia alone is a common feature in many pathophysiological settings (20–22) in which the respective macrophage phenotype might differ strongly. LPS in sepsis is an M1 stimulus and hypoxic macrophages in sepsis will most likely possess an M1-like phenotype, whereas hypoxic TAMs are more strongly associated with an M2-like response (18), illustrating how a combination of factors ultimately directs the macrophage response.

## Tumor hypoxia

Oxygen homeostasis is indispensable for the survival of all aerobic organisms. Molecular oxygen has evolved as the terminal electron acceptor in the mitochondrial electron transport chain — a deadly dependency, as every disturbance of oxygen availability endangers the respiratory capacity of the cell and, consequently, cellular survival (23). Thus, different mechanisms have evolved to restore oxygen homeostasis in order to protect against tissue damage (24, 25). Importantly, a considerable number of pathophysiological conditions are linked with tissue hypoxia, which frequently favors disease progression (18, 26).

Perturbation in oxygen homeostasis is a common feature of solid tumors, in which preclinical evidence has demonstrated that hypoxia correlates with a poor prognosis (27–29). However, in clinical settings the lack of an accurate and approved method to evaluate tumor hypoxia accounts for the limited capacity to intervene with a personalized hypoxia-based therapy. Thus, further studies are needed to determine if hypoxia can indeed serve as a reliable prognostic marker in clinical settings; contrary to the accepted opinion that hypoxia causes resistance to chemotherapy, some hypoxia-induced alterations might actually sensitize cells to chemotherapy (30).

Tumor hypoxia occurs when uncontrolled cell proliferation prevails such that blood vessel growth and the supply of oxygen and nutrition become limiting. The hypoxic response initiates a program to restore oxygen availability. On the cellular level this is reflected by angiogenesis induction, metabolic reprogramming, proliferation, self-renewal, and autophagy, which are among multiple mechanisms to counteract oxygen shortage; however, these are also detrimental processes that are exploited to foster tumor progression and metastatic dissemination (31). Therefore, the consequences of oxygen shortage are multifaceted, but exhibit a commonality in that they contribute to a hostile microenvironment that selects for a more aggressive cancer phenotype (32).

The hypoxic response is mediated by, among others, the hypoxia-inducible transcription factors HIF1$\alpha$ and HIF2$\alpha$, making it apparent how perturbations in oxygen availability can alter cellular responses. M1 and M2 macrophages differentially express HIF1$\alpha$ and HIF2$\alpha$ as well as inducible nitric oxide synthase (iNOS) and arginase 1 (ARG1) (33). Myeloid-specific loss of HIF1$\alpha$ reduced tumor growth and retarded tumor progression in the murine MMTV-PyMT model (34). Loss of HIF2$\alpha$ in macrophages had a favorable outcome in hepatocellular and colitis-associated colon carcinomas (35).

Under oxygen deprivation the angiogenic process is induced to ensure oxygen availability; however, the excessive release of angiogenic factors within the tumor microenvironment under hypoxic conditions culminates in a rather tortuous vascular network that does not effectively restore the blood supply. This aberrant vascular structure further contributes to spatiotemporal changes in oxygen delivery, thereby aggravating the hypoxic phenotype of tumors. Additionally, hypoxic cells are subjected to selection pressure, with the most aggressive cells surviving these hostile growth conditions and driving tumor growth (36). Oxygen shortage results in electron leakage and the generation of ROS, which subsequently oxidize proteins and cause DNA damage. As a net outcome, hypoxic cells experience genomic instability, which might further foster the accumulation of oncogenic drivers that then accelerate malignant progression (37).

From a therapeutic point of view, an inoperable vascular network limits the response to irradiation and reduces the efficacy of chemotherapeutics through different mechanisms such as insufficient distribution of the drug or a decrease in therapeutic cytotoxicity due to low oxygen levels, and/or the presence of a more acidic microenvironment (38–40).

A self-sustaining capacity of the tumor microenvironment also includes immune regulatory function, as suppression of the immune cell executer function and evasion of the immune response play a pivotal role in tumor progression (41). Hypoxia is intertwined in this response, as it contributes to a general shift from an antitumoral Th1-type response to a protumoral Th2-type response. Macrophages massively infiltrate tumor tissue and they are found in normoxic and hypoxic tumor compartments, albeit in different polarization states (42). The remainder of this review will focus on macrophages and elaborate on how hypoxia influences the TAM phenotype with respect to its contribution to disease progression.

## Protumoral function of hypoxic TAMs

Double staining of hypoxia and macrophage markers reveals that macrophages massively infiltrate hypoxic/necrotic regions in
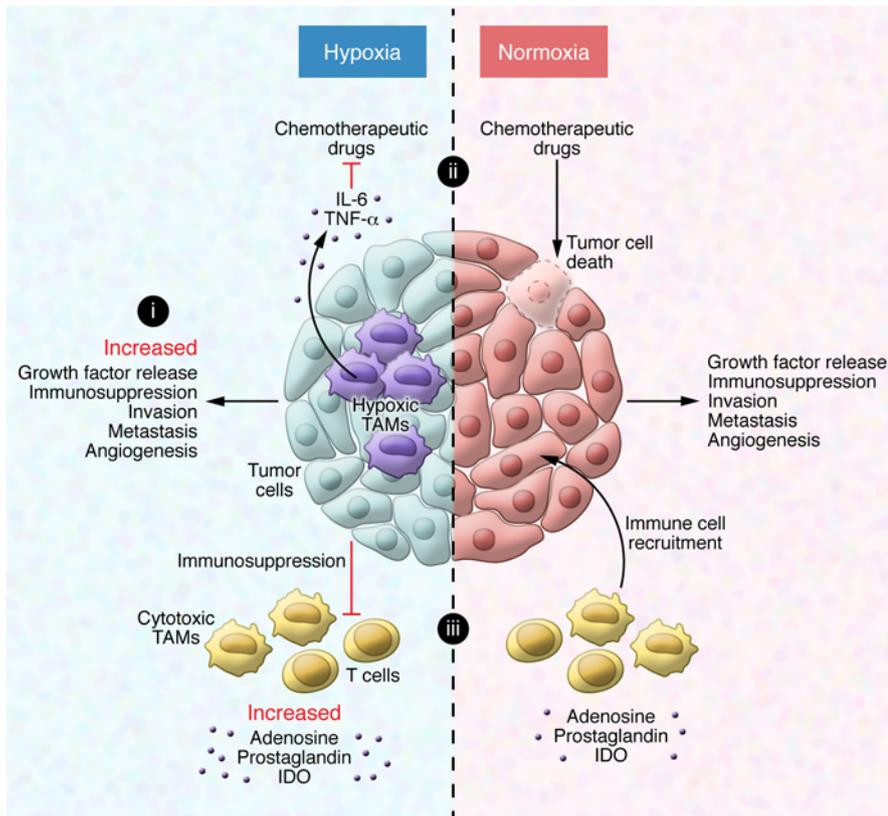
**Figure 1. Hypoxic regulation of TAMs in cancer progression and therapy.** (i) Hypoxia-induced release of chemoattractants results in enhanced TAM recruitment, which further amplifies the protumoral response. (ii) TAMs release survival factors for cancer cells, which protect them from chemotherapeutics. (iii) The hypoxic tumor environment is immunosuppressive and prevents an antitumor response.

cooperation with an increasing gradient of migratory stimulating factors such as CCL2, CCL5, colony-stimulating factor 1 (CSF1, also known as macrophage colony-stimulating factor, M-CSF), VEGF, semaphorin 3A (SEMA3A), endothelial cell monocyte-activating polypeptide-II (EMAP-II), endothelin, stromal cell-derived factor 1α (SDF1α), eotaxin, and oncosatin M (19, 43–45). Hypoxia induces the expression of VEGF, EMAP-II, endothelin, SEMA3A, SDF1α, eotaxin, and oncosatin M, thus explaining the enhanced availability of these factors within oxygen-deprived regions (19, 44–49). The cytokine-rich tumor microenvironment further provides factors such as CCL2, CCL5, or CSF1 produced by many different cell types such as tumor cells, fibroblasts, endothelial cells, or TAMs themselves (43). Apart from these chemoattractants, signals released from necrotic cells may also trigger the recruitment of innate immune cells through damage-associated molecular pattern (DAMP) receptors and macrophages to clear cellular debris (50, 51). Once macrophages arrive in these tumor compartments their mobility is slowed down via hypoxia-dependent mechanisms. In conjunction with the decrease in oxygen, macrophage expression of CCR2, CCR5, and neuropilin-1 (NRP1) is markedly diminished, disrupting these signaling pathways to halt macrophage mobility and trap TAMs within the hypoxic niche (19, 52, 53). Furthermore, MAPK phosphatase 1 (MPK1), which is upregulated under hypoxic conditions, dephosphorylates enzymes such as MEK, ERK1/2, and p38 MAPK, blocking pathways that are triggered by migration-stimulating factors (54, 55). Thus, two plausible mechanisms might account for the accumulation of macrophages in hypoxic/necrotic regions: (a) the attraction of macrophages by a cytokine gradient and (b) the hampered mobility of macrophages within these areas.

Once entrapped, macrophages are primed to serve protumoral functions (Figure 1). We have shown that impeding TAM entry into hypoxic tumor regions by genetically targeting *Nrp1* prevents angiogenesis and cancer immune escape, providing evidence that hypoxia can fuel TAM-driven vessel formation and immunosuppression (19). The means by which hypoxic TAMs promote tumor progression include the upregulation of growth factors such as FGF2, PDGF, and VEGF, which support the growth of tumor cells in nutrient-deprived regions (56, 57). However, most of our knowledge concerning these factors stems from in vitro studies; thus, the in vivo situation still needs to be validated. Which growth factor predominates depends on the tumor type. For example, EGF acts in a paracrine manner to induce production of CSF1 in breast cancer, ensuring macrophage survival as well as stimulating the growth of tumor cells (58, 59). Macrophages release proteolytic enzymes that destroy the extracellular matrix, thereby contributing to cancer cell invasion. Depending on the specific microenvironmental cues, macrophages secrete a number of different matrix metalloproteinases (MMPs) (60). Hypoxic TAMs specifically release MMP7 (61), which enhances tumor cell migration and invasion. Within the hypoxic microenvironment tumor cells increase expression of endothelin 1 and 2 (62), which concomitantly stimulate the release of MMP2 and MMP9 from macrophages (63), further assisting an effective migratory response. Moreover, hypoxic macrophages achieve a proangiogenic response either by directly upregulating angiogenic molecules (VEGF, FGF2, CXCL8, IL-8, type I receptor for VEGF, angiopoietin) or through upregulation of angiogenic modulators (COX2, iNOS, MMP7) (64).

The hypoxic tumor microenvironment plays a fundamental role in immune evasion (Figure 1). By releasing prostaglandin E2 and IL-10, TAMs create an immune-suppressive environment that halts the immune response through several mechanisms (65–67). The function of immune effector cells such as T cells is impaired through the release of these suppressive factors, but also through a diminished activation of T cells by TAMs. For example, IL-6 and IL-10 induce expression of programmed death-ligand 1 (PD-L1) (68). Moreover HIF1α directly induces PD-L1 expression on macrophages. PD-L1 subsequently inhibits T cell effector function by binding its receptor, programmed death-1 (PD-1), on T cells (69). Additionally, TAMs attract CCR4-expressing Tregs to the tumor through the secretion of cytokines such as CCL17 and CCL22 (70, 71). Notably, Treg enrichment correlates with reduced survival in ovarian cancer (70). Macrophages cocultured with hypoxic tumor cells upregulate indoleamine 2,3-dioxygenase (IDO) expression, resulting in inhibition of T cell proliferation and IFN-γ production by $CD4^+$ and $CD8^+$ T cells. Moreover, $CD25^+Foxp3^+$ Tregs increase when IDO-expressing macrophages are cocultured with $CD4^+CD25^-$ effector T cells (72).

Further experimental evidence for the protumoral function of hypoxic macrophages stems from the findings that two different TAM subsets, which derive from the same $Ly6C^{hi}$ monocyte population, reside in different intratumoral regions (73). TAMs with low MHC-II expression (MHC-II$^{lo}$) populate hypoxic areas, whereas TAMs with high MHC-II expression (MHC-II$^{hi}$) populate normoxic areas. While hypoxia does not seem to influence differentiation of these two TAM subsets or MHC-II expression levels, hypoxia does influence the MHC-II$^{lo}$ macrophage phenotype by fine-tuning hypoxia-responsive genes that are involved in metabolism, angiogenesis, and metastasis, thereby fostering tumor progression (74). The underlying mechanism for this phenotypic switch in MHC-II$^{lo}$ compared to MHC-II$^{hi}$ macrophages, as well as why MHC-II$^{lo}$ macrophages specifically reside in hypoxic areas, remains an open question; nevertheless, the gene expression profile specifically affected in these macrophages by hypoxia, such as increased availability of *VEGFA*, lactate dehydrogenase A (*LDHA*), or urokinase-type plasminogen activator receptor (*uPAR*) underscores the contribution of MHC-II$^{lo}$ macrophages to tumor progression. Thus, it is very likely that the modulation of HIFs in these populations impinges on their specific phenotype, as all of these genes have been reported to be HIF targets (75–77).

## TAMs and cancer therapy

Tumor cells located in hypoxic tumor areas undergo a selection process in order to survive adverse growth conditions. As a result, they display a more aggressive phenotype and may acquire resistance to radiotherapy (30), while irradiation results in demolished or even hypoxic tissue. Macrophages recruited to clear the damaged tissue might counterproductively be skewed within these hypoxic areas towards a tumor-promoting phenotype, contributing to tumor relapse (Figure 1). Moreover, a specific subset of M2-type macrophages ($MRC1^+TIE2^{hi}CXCR4^{hi}$) accumulates in perivascular regions following chemotherapy and accounts for high *VEGFA* expression in these regions (78). Thus, it is conceivable that these macrophages might initiate an angiogenic response that restores tumor vascularization. In contrast, low-dose irradia-

tion primes T cells towards an antitumor response. This is accomplished through antigen release by dying tumor cells (79), as well as through M1 skewing (specifically characterized by NO release) of TAMs, which subsequently enhances T effector cell recruitment into the tumor (80).

The protumoral phenotypic skewing of macrophages within the hypoxic/necrotic tumor areas is further supported by the finding that the number of macrophages within the hypoxic area correlates with a bad prognosis (81). TAMs localize in high numbers in hypoxic tumor regions (18, 82, 83) and, in general, TAM abundance within the tumor is correlated with a poor prognosis in a variety of tumor types (2, 84). However, this statement cannot be applied universally, as in several cases it is important to discriminate the specific tumor niche where TAMs accumulate or to evaluate specific macrophage subtypes. For example, TAM infiltration at the invasive front (in colon cancer) or at the tumor nest (in gastric cancer) has been associated with a promising disease outcome (85–87). In lung cancer, staining for the M2-specific macrophage marker CD204, which is expressed on alternatively activated alveolar macrophages, and for CD68, a pan-macrophage marker, has shown that it is not the total number of TAMs but rather a specific macrophage subset that correlates with prognosis. In this setting, accumulation of $CD204^+$ M2 macrophages correlated with a worse disease outcome, whereas the overall $CD68^+$ macrophage number did not correlate with disease progression (88). Therefore, despite the fact that clodronate-mediated macrophage depletion, which targets all macrophage subtypes as well as other phagocytic cells, is beneficial in several tumor models, a body of evidence shows that the cytotoxic ability of macrophages is actually very important to abate tumor growth (80, 89). Findings in the RIP1-Tag5 (RT5) mouse model of spontaneous pancreatic islet carcinogenesis demonstrate that disease outcome is determined by macrophage functionality rather than the presence or absence of macrophages (80). This conclusion is supported by a study showing that histidine-rich glycoprotein (HRG) inhibits tumor growth and metastasis in different murine tumor models by inducing macrophage polarization from an M2 to an M1 phenotype (90).

Based on the studies described above, repolarization of macrophages to an antitumor phenotype might serve as a new angle of attack for tumor therapy. However, this view might also be too simplified, as currently there is limited evidence that repolarization of macrophages towards a tumoricidal phenotype is always beneficial for disease outcome. Further investigation will be required to determine whether the hypoxic microenvironment does not overrule the antitumor function of macrophages once they are repolarized towards an M1-like phenotype. An elegant study using a CD40 agonist in a genetically engineered mouse model of pancreatic ductal adenocarcinoma (PDAC) supports the findings that the activation of macrophages via this pathway might be enough to initiate an antitumor effect without $CD4^+$ and $CD8^+$ T cells. This study defines the mechanisms underlying a clinical trial of a combined CD40 agonist treatment with gemcitabine chemotherapy in a small cohort of PDAC patients (91). However, it is not known if this is a tumor type–specific response. Another report in hepatocellular carcinoma further supports the notion that macrophages may be key players in the antitumor response. Ex vivo IL-12 treat-

ment evoked intratumoral macrophage infiltration and a T cell– and NK cell–independent antitumor response (92).

Due to the limited success of conventional chemotherapeutics, which are mainly directed against cancer cells, other therapeutic interventions such as antiangiogenic agents or immune therapeutics have shifted the focus to cells in the stroma. Combined approaches utilizing both conventional chemotherapeutics and stroma-targeting agents appear to be superior to monotherapy. As outlined in more detail below, the beneficial effects of targeting multiple processes likely stem from different mechanisms of action, as well as changes in the nature of the tumor microenvironment. In vitro studies have already demonstrated that the presence of macrophages can account for resistance towards chemotherapeutics, enhancing cancer cell survival (93). Thus, it is likely that combinatory approaches will be successful in the clinic. Indeed, to allow chemotherapeutic regimens to reach their highest cytotoxic effect, macrophage functions conferring cancer cell resistance to these drugs should be disabled so that the chemotherapeutics can function without being detoxified by the surrounding TAMs.

The mechanisms by which macrophages participate in therapy resistance are not fully understood. The secretion of cytokines such as IL-6 activates STAT3 signaling in neighboring tumor cells and may promote survival; however, the link between TAMs, IL-6 secretion, and therapy resistance in vivo has not been investigated (93). In melanoma, macrophage-dependent TNF-α release was shown to protect tumor cells from MEK and BRAF inhibitors (ref. 94 and Figure 1). Moreover, chemotherapeutics can trigger the release of macrophage chemoattractants from tumor cells, thus recruiting more monocytes/macrophages into the tumor, which promote tumor progression. CSF1 regulates monocyte-to-macrophage differentiation as well as macrophage recruitment and proliferation (95). Agents targeting CSF1 or its receptor (CSF1R) are in clinical development; however, as a monotherapy, such agents will most likely not be sufficient, and several combinatory approaches have been investigated or are still under evaluation, including combinations of CSF1/CSF1R-targeting agents with chemotherapeutics (96–98), irradiation (99), immune check point inhibitors (100), and antiangiogenic agents (101–103), among others. In animal breast cancer models, anti-CSF1R treatment in combination with paclitaxel diminishes tumor growth and metastasis formation and shifts the tumor from a CD4^{+} T cell–enriched environment towards a CD8^{+} T cell–enriched environment (96).

There are several other examples of successful combinations of well-established treatment regimens with immunotherapy. In B16 melanoma and 9464D neuroblastoma, multidrug chemotherapy (vincristine, cyclophosphamide, and doxorubicin) combined with immunotherapy (anti-CD40 and cytosine-phosphate-guanosine–containing oligodeoxynucleotide 1826 [CpG-ODN]) enhanced NO, IFN-γ, and IL-12p40 secretion by macrophages, leading to a strong antitumor response (104).

Other options for treatment have arisen from the observation that metabolic reprogramming can contribute to macrophage plasticity and function. An increase in glycolysis, reprogramming of the TCA cycle, and reduced oxidative phosphorylation can ultimately enhance formation of ROS and NO in order to promote a proinflammatory macrophage response. Moreover, an amplified arginosuccinate/citrulline/arginine cycle further contributes to a proinflammatory response (105). A recent report supports the idea that glycolytic cancer cells polarize TAMs to an M2 phenotype via their secretion of lactic acid (106). In a murine model of mammary carcinoma, treatment with zoledronic acid and subsequent repolarization of macrophages to an M1-like phenotype further highlights that a change in the polarization state can be achieved through very different mechanisms (107). Zoledronic acid is a nitrogen-containing bisphosphonate that inhibits farnesyl pyrophosphate (FPP) synthase, an enzyme in the mevalonate pathway. This decreases the prenylation of GTPase signaling proteins, thereby altering cellular function (108, 109). We are only beginning to understand how metabolic reprogramming in macrophages can impact macrophage plasticity and function, and future studies on these aspects will allow new treatment options for cancer therapy.

Overall, the lack of an approved method to measure intratumoral hypoxia still represents an obstacle to better categorization and characterization of hypoxic TAMs in cancer patients. During the last several years, tumor specimens have been characterized with different macrophage markers and inconsistent analytical methods, which has led to confusion about the role of TAMs in tumors. Too little is known about the specific role of the hypoxic subtype in tumor progression, even though hypoxia is likely to markedly impact disease outcomes. For example, the immunosuppressive signaling molecule adenosine controls macrophage functionality. Hypoxia induces adenosine signaling via different mechanisms. HIF1α transcriptionally upregulates the adenosine receptor A2B (110), thereby enhancing adenosine signaling under hypoxic conditions. Moreover, oxygen deprivation increases the extracellular conversion of ATP to adenosine by CD39 and CD73 (111, 112) and enhances ATP availability (113–116). Adenosine-mediated immunosuppression seems to be a protective feedback mechanism to counter the hypoxia-driven inflammatory response in many pathological conditions. Regarding macrophages, adenosine both halts the proinflammatory cytokine release of M1 macrophages via A2A and A2B receptors (117) and enhances the release of the antiinflammatory cytokine IL-10, while M2 macrophages exhibit enhanced activation in the presence of adenosine mediated by increased expression of arginase-1, tissue inhibitor of metalloproteinase-1 (TIMP-1), and macrophage galactose-type C-type lectin-1 (Mgl-1) (118). With respect to its therapeutic relevance, adenosine also augments VEGF expression in an A2A receptor–dependent manner, enhancing tumor vascularization (119). Thus, apart from its well-known inhibitory function on effector T cells and NK cells, adenosine fuels protumor activities of macrophages (Figure 1). Further studies are needed to dissect the specific roles of the respective adenosine receptors in tumor growth in order to design new treatment strategies.

## Conclusion

Hypoxic TAMs play a prominent role in tumorigenesis and could potentially serve as a new therapeutic target for cancer therapy. Because macrophages are a very heterogeneous population that encompasses different phenotypes, it is necessary to thoroughly characterize the specific macrophage polarization states in different tumor types and at different time points of cancer progression,

and to determine how polarization state affects cross-talk with the other tumor components, including cancer cells, T cells, and endothelial cells. Macrophages are important gatekeepers in the immune response; however, during tumor progression they support processes that promote tumor growth and ultimately destroy the organism. Tumors are referred to as wounds that do not heal (120) because they are characterized by persistent inflammation; thus, disrupting inflammation will be critical to eliminating tumors. During wound healing, M2 macrophages are only transiently present, whereas they are persistently abundant in the tumor, possibly explaining why these wounds cannot heal. The immune system is equipped with highly plastic macrophages in order to balance the immune response such that an overwhelming proinflammatory response can be countered by an antiinflammatory response. As this balancing act is accomplished by diverse polarization/differentiation states of the same cell type, it is conceivable that broad depletion of macrophages might not be favorable; rather, phenotypic conversion of macrophages may be beneficial. Thus, a close characterization of macrophage diversity within specific microenvironments might offer important information for new treatment regimens.

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