HHS Public Access

Author manuscript

Oncogene. Author manuscript; available in PMC 2018 May 07.

Published in final edited form as:

Oncogene. 2017 January 26; 36(4): 439–445. doi:10.1038/onc.2016.225.

Hypoxic stress: obstacles and opportunities for innovative immunotherapy of cancer

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Abstract

Tumors use several strategies to evade the host immune response, including creation of an immune-suppressive and hostile tumor environment. Tissue hypoxia due to inadequate blood supply is reported to develop very early during tumor establishment. Hypoxic stress has a strong impact on tumor cell biology. In particular, tissue hypoxia contributes to therapeutic resistance, heterogeneity and progression. It also interferes with immune plasticity, promotes the differentiation and expansion of immune-suppressive stromal cells, and remodels the metabolic landscape to support immune privilege. Therefore, tissue hypoxia has been regarded as a central factor for tumor aggressiveness and metastasis. In this regard, manipulating host-tumor interactions in the context of the hypoxic tumor microenvironment may be important in preventing or reverting malignant conversion. We will discuss how tumor microenvironment-driven transient compositional tumor heterogeneity involves hypoxic stress. Tumor hypoxia is a therapeutic concern since it can reduce the effectiveness of conventional therapies as well as cancer immunotherapy. Thus, understanding how tumor and stromal cells respond to hypoxia will allow for the design of innovative cancer therapies that can overcome these barriers. A better understanding of hypoxia-dependent mechanisms involved in the regulation of immune tolerance could lead to new strategies to enhance antitumor immunity. Therefore, discovery and validation of therapeutic targets derived from the hypoxic tumor microenvironment is of major importance. In this context, critical hypoxia-associated pathways are attractive targets for immunotherapy of cancer. In this review, we summarize current knowledge regarding the molecular mechanisms induced by tumor cell hypoxia with a special emphasis on therapeutic resistance and immune suppression. We emphasize mechanisms of manipulating hypoxic stress and its associated pathways, which may support the development of more durable and successful cancer immunotherapy approaches in the future.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

INTRODUCTION

The field of cancer immunotherapy is witnessing a renaissance over the past 5 years, with rapid development of novel monoclonal antibodies and therapeutic cancer vaccines for multiple tumor types. With both PD-1 and CTLA-4 blocking antibodies earning Food and Drug Administration approval, and promising clinical results using anti-programmed cell death ligand (anti-PD-L1) checkpoint inhibitor monoclonal antibodies in late stage clinical trials, cancer immunotherapy appears poised to become a prominent pillar of oncologic care.[1] Nevertheless, although the advent of new immunotherapy approaches has improved survival for many patients with advanced malignancies, the high degree of non-responders, especially in highly prevalent malignancies, including breast, colon and prostate cancers, serve as a reminder that we possess only a partial understanding of the mechanisms underlying the immune resistance of tumors. It has become clear that tumors use several strategies to evade the host immune response, foremost among them the creation of an immune-suppressive and hostile tumor environment.[2,3]

It is well established that the tumor microenvironment supports tumor growth and limits the effectiveness of solid tumor immunotherapies by promoting neoplastic transformation and cell plasticity and by inducing tumor cell resistance to host immunity.[4,5] Accumulating data suggest that hypoxic stress in the tumor microenvironment promotes several tumor escape mechanisms, including immune suppression and the emergence of tumor variants. Highly aggressive, rapidly growing tumors are exposed to hypoxia or even anoxia that occurs as a consequence of inadequate and/or irregular blood supply. Hypoxia is a common feature of all solid tumors and plays a central role in tumor progression and resistance to therapy by fostering variety of changes in tumor and stromal cell biology. Under hypoxia or pseudohypoxia, cells activate a number of adaptive responses coordinated by various cellular pathways.[6,7] The major mediators of the transcriptional hypoxic response are hypoxia-inducible factors (HIFs). Induction of HIF-1α and/or HIF-2α leads to transcription of hypoxia-responsive genes, which are involved in tumorigenesis and regulation of stroma reactivity.[8,9] Recurrent or long-lasting HIF signaling driven by hypoxia is known to function as an oncogenic stimulus in some settings, as well as to be induced as a consequence of cancer development, invasion and metastasis.[10] While hypoxia signaling is often induced by sensing of oxygen deficiency within certain tumor zones, it can also manifest through genetic mutations that increase HIF activity. Among these alterations, VHL mutations, which are known to be associated with 75% of renal cell carcinoma, are the most common causes of intrinsic induction of HIF-1 and HIF-2.[11]

In addition to its established roles in mediating resistance to chemotherapy and radiotherapy, emerging evidence supports the capacity of tumor hypoxia to oppose successful immunotherapy as well. Only by understanding the mechanisms through which hypoxia modulates the stromal composition and malignant phenotype of tumors to foster immune suppression, can we rationally design future interventions that overcome hypoxia-induced resistance.

HYPOXIA INTERFERES WITH T LYMPHOCYTE EFFECTOR FUNCTION

When evaluating the effects of hypoxia on T-cell function, it is critical to separate the intrinsic effects of alterations to hypoxia-sensing pathways and associated downstream signaling from the extrinsic effects of immersion in a hypoxia microenvironment *in vivo*. T cells activate HIF-1α independently of external hypoxia as a consequence of T-cell receptor activation via a PI3K/mTOR dependent pathway.[12] T-cell receptor-activated T cells also increased HIF-1α mRNA synthesis by mechanisms involving protein kinase C and Ca (2+)/ calcineurin.[13] Independently of T-cell receptor stimulation, HIF-1α mRNA is augmented in T cells in the presence of transforming growth factor-β (TGF-β) and/or interleukin-6 (IL-6) by a mechanism involving STAT3.[14] In physiologic conditions, $CD8^+$ T cells from lymphoid organs (spleen, lymph nodes) were found to bind pimonidazole, indicating a hypoxic state within these organs ranging from 1 to 2.5% pO_2.[15] While T cells were functional under these conditions, overall levels of activation were higher in more oxygenated areas. This is consistent with the observation that CD8 T cells activated at a hypoxic pO_2 of 2.5% are more lytic than their counterparts activated at a pO_2 of 20%.[16] Also, constitutive activation of HIF-1 and HIF-2 resulting from deletion of the *vhl* gene in CD8 T cells delays their differentiation into effector cells but increases their cytotoxicity via augmented expression of Granzyme B.[17] The mechanism underlying this enhanced cytotoxicity was further clarified by a recent study showing that HIF-1 regulates, directly or indirectly, Perforin and multiple Granzymes.[18] Dampening of T-cell proliferation under more significant hypoxia (pO_2=1%), in turn, has been explained through downregulation of KV1.3 potassium channel activity which T cells require for expansion.[19]

While hypoxia exposure may support higher cytotoxic effector function, it also dampens production of effector (e.g. IFN-γ) and proliferative (e.g. IL-2) cytokines by both CD4 and CD8 T cells.[16,20] While overexpression of HIF-1 had no effect on cytokine production, *vhl* knockout did augment Th1 type cytokine production from CD8 T cells, suggesting a possible role for HIF-2 or an indirect effect.[17,18]

Although, when measured in isolation, hypoxia may have beneficial effects on T-cell cytotoxicity, it is important to reconcile these observations with the reality that, in most *in vivo* situations, T-cell performance is diminished in hypoxic microenvironments.[21,22] T cells can enter tumors known to be hypoxic,[23] but are often excluded from the actual zones of hypoxia.[24] In most cases, the real culprit in hypoxia-mediated suppression of T-cell responses *in vivo* is not the direct effects of reduced oxygen on T cells, but the metabolic alterations driven by hypoxia that act together to repress T-cell function and even survival.[25] A combination of lactic acid accumulation as a result of the Warburg effect and the action of hypoxia-driven carbonic anhydrases and proton transporters results in the acidification of the extracellular environment of tumors.[26] While mild to moderate hypoxia itself does not significantly impair T-cell function, the acidic pH of tumors, largely a metabolic consequence of hypoxia, potently suppresses T-cell activation, proliferation and cytotoxicity.[27–29] Extracellular pH in hypoxic tumors can be as low as pH 5.8–6.5, yet IL-2 driven T-cell proliferation stalls out at pH 6.7.[30] At the lower range of tumor pH, lymphocytes actually undergo apoptosis while tumors continue to flourish.[31] Lactic acid itself, plentiful in hypoxic microenvironments, can block T-cell proliferation and effector functions.[32,33]

Hypoxia-driven tumor acidification, then, is a formidable barrier to T-cell function in the microenvironment; however, other secondary metabolic consequences of hypoxia act to repress immunity as well.

Tumor hypoxia is known to promote the accumulation of extracellular adenosine, which can be measured at high concentrations in multiple malignancies.[34,35] Free adenosine can inhibit both effector T-cell and NK cell proliferation and cytotoxicity.[36] Hypoxia further contributes to adenosine accumulation through HIF-1α induction of the ATP hydrolyzing ectonucleotidases CD39 and CD73 on FoxP3$^+$ regulatory T cells (Treg).[37–39] Decreasing hypoxia in lung tumors was associated with a concomitant decrease in adenosine and improvement in T-cell accumulation.[24] In addition to adenosine, hypoxia also promotes the generation of reactive nitrogen species, particularly peroxynitrite, through the induction of inducible nitric oxide synthase.[40] Nitration of the T-cell receptor and of CD8 by reactive nitrogen species have been shown to perturb their capacity to recognize cognate MHC:antigen complexes and mediate T-cell activation.[41] Further, nitration of the chemokine CCL2 caused it to lose the capacity to recruit effector lymphocytes but not the ability to chemo-attract suppressive myeloid cells.[42] Adenosine and reactive nitrogen species accumulation provide further examples of mechanisms of T-cell suppression engaged downstream of hypoxia.

Finally, expression of T-cell co-stimulatory and co-inhibitory receptors can be modulated by hypoxia as well. In mice challenged with tumors, intratumoral hypoxia increased expression of the co-stimulatory receptor CD137 at the surface of tumor-infiltrating CD8$^+$ T cells in an HIF-1-dependent manner. The ligation of CD137 by agonistic antibodies increased CD8$^+$ T-cell activity based on *in vitro* increases in production of IFN-γ and TNF-α by CD137$^+$ CD8$^+$ T cells, and on decreased tumor growth *in vivo*.[23] The beneficial effects of CD137 upregulation on tumor progression were found to be tumor specific; however, as some tumors, such as spontaneous breast carcinoma, were resistant to anti-CD137 immunotherapy. Moreover, antigenic stimulation of T cells was necessary for optimal upregulation of CD137 by hypoxia, implying that only in tumors that were already productively infiltrated by antigen-specific T cells could this benefit tumor immunity. Hypoxia, via a HIF-1a-dependent mechanism, has also been recently shown to induce PD-L1 expression on both tumors cells and myeloid-derived suppressor cells (MDSCs).[43,44] Increasing the capacity of tumors and their surrounding stroma expressing PD-L1 to repress T cells via engagement of PD-1 constitutes an additional pathway of T-cell suppression promoted by hypoxia (Figure 1).

HYPOXIA REGULATES NATURAL KILLER AND NATURAL KILLER T-CELL ACTIVITY

Although less studied than T cells, multiple sources of investigation indicate multi-modal suppression of natural killer (NK) and natural killer T-cell (NK-T) responses driven by hypoxia. The activating receptor, NKG2D, plays a critical role in directing NK cell responses against tumors. Yamada *et al.*[45] show that hypoxia, via a HIF-1α-dependent mechanism, promotes downregulation of the NKG2D ligand MICA by tumor cells. NKG2D

itself decreases on NK cells in response to hypoxia, at least partially in response to TGF-β which is upregulated by hypoxia and delivered to NK cells via hypoxia-elaborated tumor Microvesicles.[46,47] These same microvesicles promote downregulation of CD107a, a critical component of cytotoxic granule release by NK cells, via delivery of mIR-23a.[46] Loss of NK cell cytotoxicity in the primary tumor microenvironment has also been described by Sceneay *et al.*,[48] who also showed that loss of NK function helps facilitate establishment of the premetastatic niche which becomes infiltrated by dysfunction NK cells incapable of preventing tumor cell colonization. Beyond tumor systems, hypoxia has also been described to compromise antiviral NK cell responses as well.[49]

Previously, Baginska *et al.*[50] provided evidence indicating that hypoxia-induced autophagy impairs NK-mediated lysis through Granzyme B degradation in hypoxic targets. Very recently, we demonstrated that hypoxic microenvironment negatively affects the immune surveillance of tumors by NK cells through the selective degradation of synaptic connexin-43 protein.[51]

The effects of hypoxia on NK-T cells have been less well studied; however, HIF-2a seems to drive multiple pathways of immune suppression in this population.[52] Using conditional HIF-2a knockout mice, Zhang *et al.*[52] show that HIF-2 helps suppress these cells by downregulating their Fas-ligand expression while at the same time inducing their expression of Adenosine A2A receptors. Potential mechanisms to reverse hypoxia-induced suppression of NK and NK-T cells remain to be discovered; however, Sarkar *et al.*[47] do provide evidence that hypoxia-induced NK dysfunction can be reversed by exogenous IL-2 treatment.

HYPOXIA INDUCES RESISTANCE TO CELL-MEDIATED CYTOTOXICITY

The ultimate goal of most cancer immunotherapy strategies is to induce a strong cytotoxic T lymphocyte (CTL) response. The prevailing view is that generation of a sufficiently high frequency of CTL will result in tumor regression. It has become increasingly apparent in both preclinical models and patient trials, however, that tumors can efficiently evade or inactivate even high-frequency immune responses by denying T cells access to the tumor, by establishing a metabolically hostile microenvironment, and through selection of immune-resistant tumor cell variants. Recently, we showed that hypoxia-induced autophagy impairs CTL-mediated tumor cell lysis by regulating phospho-STAT3 in target cells. Autophagy inhibition in hypoxic cells decreases phospho-STAT3 and restores CTL-mediated tumor cell killing by a mechanism involving the ubiquitin proteasome system and SQSTM1/p62. Simultaneously boosting the CTL response, using a TRP-2-peptide vaccination strategy, and targeting autophagy in hypoxic tumors, improves the efficacy of this cancer vaccine and promotes tumor regression *in vivo*.[53,54] More recently, we have reported that attenuation of miR-210 in hypoxic cells significantly restored susceptibility to autologous CTL-mediated lysis, independent of tumor cell recognition and CTL reactivity. A comprehensive approach using transcriptome analysis, Argonaute protein immunoprecipitation and a luciferase reporter assay revealed that the genes *PTPN1*, *HOXA1* and *TP53I11* were miR-210 target genes regulated in hypoxic cells. Silencing of *PTPN1*, *HOXA1* and *TP53I11* dramatically decreased tumor cell susceptibility to CTL-mediated lysis.[55] These findings show how miR-210 induction links hypoxia to immune escape from CTL-mediated lysis.[46,54]

Recent data have shown that hypoxia-induced autophagy is an important regulator of the innate and adaptive antitumor immunity mediated by NK cells and CTL, respectively.[53,54] *In vivo* inhibition of autophagy improves the antitumor effect of a TRP-2-based vaccine.[53,54] More importantly, it has been reported that hypoxic tumor cells can escape NK-mediated immune surveillance by activating autophagy under hypoxia.[50,56] At the mechanistic level, it was shown that Granzyme B is selectively degraded upon activation of autophagy in hypoxic cells, thereby inhibiting NK-mediated target cell apoptosis. More recently, the role of autophagy in regulating NK-mediated immune responses was investigated in a clear cell renal cell carcinoma cell model displaying mutation in *VHL* gene and known to be resistant to NK-mediated killing. We found evidence indicating that the accumulation of inositol 1,4,5-trisphosphate receptor, type 1, an intracellular channel that mediates calcium release from the endoplasmic reticulum and plays a role in stress induced apoptosis, in 786-O renal cells was associated with the ability of these cells to activate autophagy triggered by a signal derived from NK cells, as targeting inositol 1,4,5-trisphosphate receptor, type 1 in these cells abrogates the ability of NK cells to activate autophagy.[57] Taken together, these results suggest that inhibiting inositol 1,4,5-trisphosphate receptor, type 1/autophagy in tumors improves their elimination by NK cells *in vivo*.[11,58]

HYPOXIA INDUCES IMMUNE SUPPRESSION (TREG, TUMOR-ASSOCIATED MACROPHAGE AND MDSC) AND CONTRIBUTES TO IMMUNE TOLERANCE

Accumulating evidence indicate that hypoxic zones in tumors attract immunosuppressive cells such as MDSCs, tumor-associated macrophages (TAMs) and Treg cells.[7] Very recently, we have demonstrated that hypoxia selectively upregulates PD-L1 on MDSCs via HIF-1α binding to an HRE in the PD-L1 proximal promoter. Blockade of PD-L1 under hypoxic conditions enhanced MDSC-mediated T-cell activation by attenuating MDSC secretion of IL-6 and IL-10.[44] Under hypoxic stress and in the presence of TGF-β, $CD4^+$ T cells upregulate Foxp3 through direct binding of HIF-1 to Foxp3 promoter region, inducing Treg formation.[59] In the absence of TGF-β, however, HIF-1 promotes the degradation of FoxP3 and pushes T cells toward a Th17 phenotype through induction of RORγt.[14] The skewing of tumor T cells toward the Treg phenotype and away from Th17 in HIF-1α knockout animals could be mimicked by blocking T-cell glycolysis.[60] In this context, HIF-1 also prolonged the survival of Th17 cells through induction of survival factors.[61] On the other hand, Foxp3-restricted VHL deletion in Tregs, which resulted in constitutive HIF-1 stabilization, skewed Treg cells into a Th1-like phenotype.[62] These Tregs exhibited high levels of IFN-γ production by direct binding of HIF-1 to IFNγ promoter, and a negligible increase in IL-17 production. Tumor hypoxia also attracts Tregs inside the tumor bed by impacting the cytokine profile inside the microenvironment. Facciabene *et al.*[63] have recently reported that hypoxic stress increases the expression and secretion of CCL28 by ovarian tumor cells. CCL28 act as a chemoattractant for Treg cells, whose immunosuppressive functions on $CD8^+$ T cells are well documented.

TAMs support tumor survival, expansion, invasion and metastasis and are critical to the establishment and maintenance of tumor immune suppression.[64] Hypoxia, via HIF-1, induces production of VEGF, SDFα, IL-8 and granulocyte colony stimulating factor all of

which contribute to mobilization and recruitment of immature myeloid cells to the tumor microenvironment where they can be converted to TAM and MDSCs.[65–69] These cells often express Semaphorin 3A, which mediates their entry into hypoxic zones of tumors via Neuropilin-1 binding; however, HIF-1 then downregulates Semaphorin 3A causing them to be retained and to concentrate in hypoxia.[70] This helps explain the frequent observation that M2-polarized macrophages are localized in hypoxic zones of tumors while M1 inflammatory ones are found in normoxic zones.[71] A number of factors enriched in this environment, including IL-4, IL-6, TGF-β, prostaglandin E2, VEGF and reactive oxygen species, favor the differentiation of these cells into suppressive M2-type macrophages or MDSC. High levels of HIF-1α have been shown to favor TAM differentiation and induction of inducible nitric oxide synthase expression, while HIF-2α may favor Arginase expression.[72] HIF-1α critically regulates the suppressive phenotype in both TAM and MDSC. MDSC lacking HIF-1α, for example, lose markers of suppressive myeloid cells and acquire dendritic cell features instead.[73] Also, TAMs lacking in HIF-1α lose both the capacity to suppress T-cell responses and to promote tumor progression.[74] Besides secreting immunosuppressive cytokines, TAMs also induce the expression of the matrix metalloproteinase-7 metalloprotease in hypoxic areas of tumors.[75] Matrix metalloproteinase-7 is known to cleave Fas from neighboring cells creating a soluble decoy that protects tumor cells from Fas-ligand mediated lysis by NK and T cells.[76]

IMPAIRMENT OF T-CELL INFILTRATION BY HYPOXIC STRESS

When T cells infiltrate the tumor environment they encounter a myriad of metabolic stressors including hypoxia. We have previously shown that hypoxia-induced Nanog favors the intratumoral infiltration of regulatory T cells and macrophages via direct regulation of TGF-β1. Inhibition of hypoxia resulted in an increase in $CD8^+$ T effector cells in the tumor bed in part by modulating TGF-β1 production.[77] Poorly vascularized zones within solid tumors are characterized by severe hypoxia which is perpetuated by a host of factors originating in these zones which foster abnormal angiogenesis and a tumor-supportive metabolic state.[78] These disordered vessels lack expression of proteins necessary to support T-cell adhesion, attraction and extravastion into the tumor.[79] Multiple therapeutic efforts are focused on inhibiting production of angiogenic factors such as VEGF by the tumor to normalize these vessels.[80] Recently it has been shown that a trio of factors enriched in hypoxic zones of tumors, VEGF, IL-10 and PGE2, can induce tumor vessels to express Fas-ligand and induce apoptosis on T cells that arrest there.[81] Thus, overcoming irregular hypoxia-induced angiogenesis may be necessary both to ensure access to and survival within tumor cores.

Previously we have shown that combination blockade of the T-cell co-inhibitory receptors CTLA-4 and PD-1 could cure a majority of animals of pre-implanted melanomas.[82] In the clinic, antibodies that block CTLA-4 and PD-1 are now Food and Drug Administration approved for melanoma and non-small-cell lung cancer (PD-1 only), as is combination blockade of both checkpoints which produces a 54% response rate and 90% two-year survival in metastatic melanoma.[83] Despite these successes, many solid tumors such as castration-resistant prostate cancer, pancreatic cancer and colorectal cancer (except for mismatch repair mutant subtypes) fail to respond to checkpoint blockade.[84,85] These are

known to be profoundly hypoxic cancers, and finding mechanisms by which the suppressive impact of hypoxia can be minimized will likely be critical to sensitizing these tumors to immunotherapeutic interventions such as checkpoint blockade, cancer vaccines and adoptive cell therapy.

HYPOXIA PLAYS A KEY ROLE IN SHAPING TUMOR HETEROGENEITY

Intratumoral heterogeneity in human tumors is a widespread phenomenon of critical importance for tumor progression and the response to therapeutic intervention. Hypoxia is a crucial contributor to heterogeneity of cancer, influencing treatment response and the propensity to develop features of aggressive disease such as genetic instability, metastases, stemness, angiogenesis and altered metabolism. In this regard, hypoxia has been reported to contribute to melanoma heterogeneity by triggering HIF-1α-dependent phenotype switching. It is one of the microenvironmental influences driving metastatic progression by promoting a switch from a proliferative to an invasive phenotype.[86] In addition, poorly vascularized tumors contain hypoxic regions with undifferentiated ‘stem-like’ tumor cells that survive under control of HIFs.[87] In this regard, Yeung *et al.*[88] used three-dimensional cell culture to demonstrate that hypoxia inhibits differentiation of colon cancer cells and maintains a stem-like phenotype. More importantly, in response to hypoxia, HIF-1α accumulates and induces epithelial–mesenchymal transition in tumor cells which facilitates tissue invasion, metastatic spread and suppressive immune education of the surrounding stroma.[89]

TOWARDS COMBINING THE USE OF CRYPTIC ANTIGENS AND HYPOXIA TARGETING

A new class of tumor antigens has recently been described, the neo-antigens that are created by point mutations of tumor-expressing proteins and are recognized by the immune system as non-self. Neo-antigens exhibit two main properties: they are not involved in the immune self-tolerance process and are immunogenic.[90] However, hypoxia may interfere with DNA repair, tumor mutational burden and the subsequent expression of neo-antigens. This raises the question of whether neo-antigen-like vaccines could be used in the design of cancer vaccine innovative approaches. In this regard, optimized cryptic peptides from TAA are neo-antigen-like peptides.[91,92] Optimized cryptic peptides are recognized by the immune system as non-self because they target self-cryptic peptides that escape self-tolerance; in addition, they are strongly immunogenic because their sequence is modified in order to enhance their affinity for the HLA molecule. In this regard, we believe that combining hypoxia targeting with the use of cryptic antigens could be an innovative approach for cancer immunotherapy.

CONCLUSION

Tumor hypoxia predicts poor outcome across all cancers,[93] and serves as a center for recruitment, polarization and expansion of immune-suppressive stromal cell populations.[14,73,94,95] Antibody blockade of the T-cell immune checkpoint receptors PD-1 and CTLA-4 can potently reverse T-cell anergy and suppression in 'hot' tumors with pre-existing immune infiltrates; however, immunotherapy performs poorly in 'cold' tumors in which T cells are

sparse or absent from the tumor microenvironment. Hypoxia-driven modulation of tumor angiogenesis promotes T-cell exclusion and even apoptosis helping to maintain this 'cold' state. Even where T cells are able to enter hypoxic tumors, they face multi-layered challenges to their survival and effector function, many of which are driven directly or indirectly by hypoxia-orchestrated mechanisms. For example, hypoxia promotes acidification of the extracellular milieu of tumors to a level which promotes tumor invasiveness while blocking the capacity of T cells to expand, perform essential cytotoxic effector functions or even simply survive. Suppressive myeloid cells, both TAM and MDSC, are recruited more efficiently, expanded and concentrated to higher densities, and phenotypically programmed for higher suppressive capacity in more hypoxic tumors. Taken together, the prominent role of hypoxia in establishing and maintaining tumor immune privilege, even in the context of immunotherapy, is clear. In addition, accumulating evidence indicates that hypoxia and activation of HIF-dependent signaling at the primary tumor and distant site promote metastatic dissemination and colonization. It has been reported by Sceneay *et al.*[96] that factors secreted by hypoxic tumor cells condition premetastatic niches by recruiting CD11b+/Ly6Cmed/Ly6G+ myeloid cells and suppressing natural killer cell functions.

Understanding the impact of hypoxia on these many facets of tumor immune suppression is an important first step toward designing combination therapies that can overcome these barriers and potentiate partnered immunotherapeutics. Currently, there are multiple hypoxia-activated prodrugs designed to kill selectively in hypoxic zones of tumors, as well as oxygen-carrier compounds designed to reverse the effects of irregular tumor vasculature.[97,98] Small-molecule inhibitors of both HIF-1 and HIF-2 are also available or in development which can dampen the anti-immune effects of hypoxia, particularly in suppressive myeloid cells and tumors.[99,100] Specific inhibitors of suppressive enzymes induced in myeloid cells including Arginase, inducible nitric oxide synthase and indoleamine 2 3-dioxygenase are now also widely available. The challenge ahead is to determine experimentally which of these approaches act to diminish hypoxia-induced immune suppression without also impeding some aspect of antitumor immunity.

ABBREVIATIONS

CSC	Cancer stem cells
CTC	Circulating tumor cells
CTL	Cytotoxic T lymphocytes
DC	Dendritic cells
EMT	Epithelial-to-mesenchymal transition
HIF	Hypoxia-inducible factor
MDSC	Myeloid-derived suppressive cells
NK	Natural killer cells

PD-L1	Programmed death-ligand 1
TAMs	Tumor associated macrophages
TGF-β	Transforming growth factor-β
Treg	T regulatory cells.
Treg	T regulatory cells
VEGF	vascular endothelial growth factor

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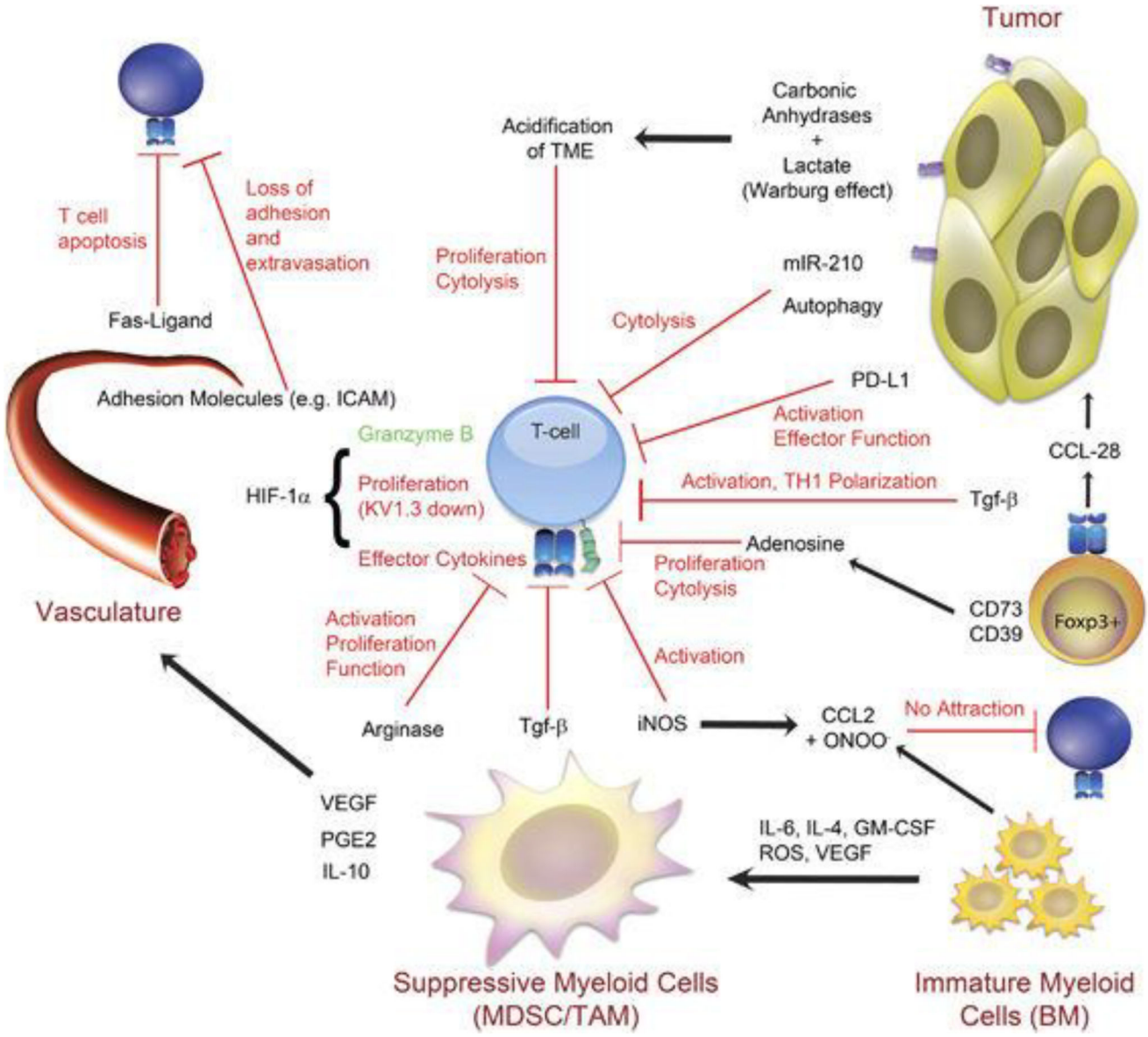

Figure 1.