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Review

Macrophage Responses to Hypoxia

Implications for Tumor Progression and Anti-Cancer Therapies

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The presence of multiple areas of hypoxia (low oxygen tension) is a hallmark feature of human and experimental tumors. Monocytes are continually recruited into tumors, differentiate into tumor-associated macrophages (TAMs), and then accumulate in these hypoxic areas. A number of recent studies have shown that macrophages respond to the levels of hypoxia found in tumors by up-regulating such transcription factors as hypoxia-inducible factors 1 and 2, which in turn activate a broad array of mitogenic, proinvasive, proangiogenic, and prometastatic genes. This could explain why high numbers of TAMs correlate with poor prognosis in various forms of cancer. In this review, we assess the evidence for hypoxia activating a distinct, protumor phenotype in macrophages and the possible effect of this on the growth, invasion, angiogenesis, and immune evasion of tumors. We also discuss current attempts to selectively target TAMs for destruction or to use them to deliver gene therapy specifically to hypoxic tumor sites. *(Am J Pathol 2005, 167:627–635)*

Macrophages are highly versatile immune effector cells that are derived from bone marrow progenitors, which continually proliferate and release promonocytes into the bloodstream. The promonocytes circulate briefly, differentiate into monocytes, and then migrate from the blood into tissues, where they differentiate further into resident macrophages. Here they protect the body from infection by bacteria, viruses, and other pathogens. Extensive monocyte extravasation is also an early event in the onset of inflammation, wound healing, and various diseases, in which they exhibit a tissue-specific range of functions that include phagocytosis, antigen presentation to T cells, and the release of a wide array of cytokines, chemokines, growth factors and enzymes, reactive oxygen and nitrogen species, complement components, coagulation factors, and prostaglandins.[1]

Macrophages are often prominent in tumor tissues, comprising up to 80% of the cell mass in breast carcinoma.[2] A number of tumor-derived chemoattractants have been implicated in monocyte uptake into tumors including CCL2 (formally monocyte chemoattractant protein-1), macrophage-colony stimulating factor (M-CSF or CSF-1), and vascular endothelial growth factor (VEGF). When monocytes are recruited into malignant tumors, they rapidly differentiate into tumor-associated macrophages (TAMs). Tumor levels of chemoattractant proteins often correlate positively with TAM numbers in human tumors.[3]

It has been suggested that TAMs retain a relatively immature macrophage phenotype that is characterized by low expression of differentiation-associated macrophage antigens such as carboxypeptidase M and CD51, high constitutive expression of interleukin (IL)-1 and IL-6, and low levels of tumor necrosis factor (TNF)-α.[2,4] Furthermore, although macrophages derived from healthy or inflamed tissues are capable of lysing tumor cells, expressing immunostimulatory cytokines, and presenting tumor-associated antigens to stimulate the proliferation and anti-tumor functions of T and NK cells *in vitro*,[1] TAMs show reduced levels of these activities. This may be due in part to their exposure to the tumor-derived anti-inflammatory molecules IL-4, IL-10, transforming growth factor-β1, and prostaglandin E_2.[2,5] Indeed, this prompted Mantovani and colleagues[6] to suggest that exposure to IL-4 and IL-10 may induce monocytes in tumors to develop

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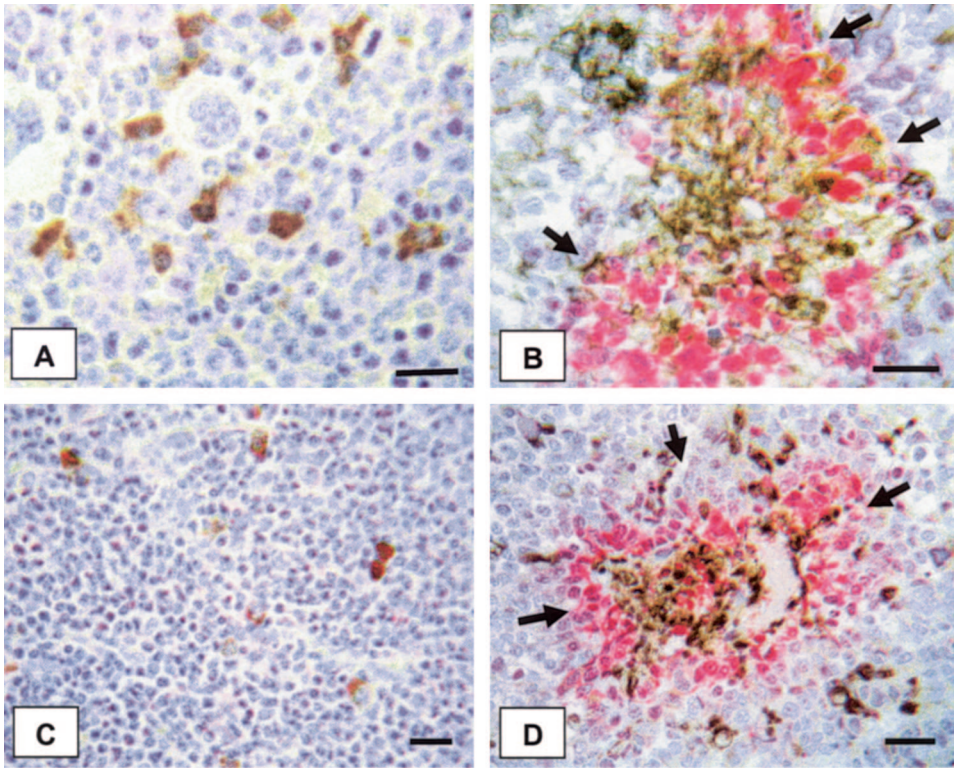

Figure 1. TAM localization in hypoxic areas of murine mammary (**A**, **B**) and human breast (**C**, **D**) tumors. Tumor hypoxia was visualized by injecting mice bearing 4T1 mammary tumors or breast cancer patients with the hypoxic cell marker pimonidazole (PIMO) before surgical removal of tumors. PIMO retained by hypoxic cells in tumor sections was then detected using a rabbit anti-PIMO antibody (red staining, **arrows** in **B** and **D**). Macrophages were co-localized in these sections using a monoclonal antibody to the pan macrophage markers F4/80 (**B**) and CD68 (**D**) (brown staining). Although TAMs can be seen in both normoxic (**A**, **C**) and hypoxic (**B**, **D**) areas of tumors, they accumulated at highest density in the latter. Hematoxylin staining of nuclei is shown in blue. Scale bars, 50 μm.

into polarized type II (alternatively activated) or M2 macrophages. These macrophages have poor antigen-presenting ability and produce factors that suppress T-cell proliferation and activity. They are better adapted to scavenging for debris, promoting angiogenesis, and repairing and remodeling wounded/damaged tissues. This contrasts markedly with the phenotype of classically activated type I or M1 macrophages that are efficient immune effector cells able to kill microorganisms and tumor cells, present antigen, and produce high levels of immunostimulatory cytokines.[6]

The level of TAMs in tumors appears to be affected by hypoxia (low oxygen tension), a trait commonly found in these tissues. TAM numbers are generally higher in tumors containing high overall levels of hypoxia, as seen in primary human breast carcinomas[7] and various animal tumors.[8] Interestingly, TAM numbers also correlate positively with overall hypoxia (as assessed using the hypoxic cell marker carbonic anhydrase IX) in liver metastases from breast and colorectal tumors.[9] These findings suggest that hypoxic tumors secrete higher amounts of chemoattractants and/or other factors that enhance monocyte attachment to and migration through the tumor vasculature.

Once targeted to hypoxic sites, TAM functions are greatly affected by hypoxia-related factors. Here, we review the evidence for hypoxia being an important intratumoral signal that stimulates TAMs to secrete a range of mitogenic factors, proangiogenic cytokines and enzymes, and immunosuppressive agents. Collectively, these effects may explain, in part, why the presence of large numbers of TAMs correlate with poor prognosis in carcinoma of the breast,[10] cervix,[11] stomach,[12] bladder,[13] uterus,[14] prostate,[15] and kidney.[16]

Macrophage Accumulation in Hypoxic Areas of Tumors: Mechanisms and Biological Significance

New blood vessels in tumors are usually disorganized and prone to collapse, resulting in areas of inadequate perfusion and hypoxia. Additionally, rapid tumor cell proliferation in some areas may outpace the rate of new blood vessel growth, causing hypoxic areas to form.[17,18] Use of such hypoxic cell markers as pimonidazole has allowed identification of both transient (avascular, non-necrotic) and chronic (peri-necrotic) areas of hypoxia in both human and experimental animal tumors (Figure 1).[4,17–19] The correlation between overall hypoxia and TAM accumulation in tumors has prompted some to suggest that an *in vivo* assessment of TAM accumulation might have utility in the detection of metastases and/or monitoring of hypoxia levels in tumors.

Recent evidence has shown that TAMs may accumulate in high numbers in hypoxic/necrotic areas of endometrial,[20] breast,[8,10] prostate,[21] and ovarian[22] carcinoma due to the hypoxic release of such macrophage che-

moattractants as EMAP-II, endothelin 2, and VEGF.[3] Because macrophages are phagocytes, they may also be attracted to hypoxic, perinecrotic areas along a trail of necrotic debris emanating from dead cells. Hypoxia also entraps TAMs by decreasing their mobility in a number of ways. One such approach involves the hypoxic up-regulation by macrophages of the enzyme mitogen-activated protein kinase phosphatase (MKP-1).[23] This is important because various chemoattractant receptors, including those for CCL2, VEGF, and endothelin 2, stimulate cell migration by phosphorylating the signaling enzymes MEK, ERK1/2, and p38 MAPK. Up-regulated MKP-1 rapidly dephosphorylates these molecules in TAMs, thus terminating the chemotactic response of TAMs to these chemokines.[3,24,25] Hypoxia also inhibits macrophage expression of the chemokine receptors CCR2[26] and CCR5,[27] further helping to immobilize TAMs.

Such accumulation of TAMs in hypoxic/necrotic areas has itself been linked to tumor aggressiveness, with high TAM numbers in such areas correlating with increased lymph node involvement (ie, metastasis) and/or poor prognosis in breast[10] and endometrial[20] cancers. Moreover, high TAM numbers in these areas correlate with increased overall levels of angiogenesis in breast carcinoma,[5,10] suggesting that TAM activity in hypoxic tumor areas may specifically promote tumor angiogenesis and metastasis.

Hypoxic Phenotype of TAMs

Hypoxia induces a profound change in the phenotype of macrophages, promoting increased expression of a wide range of genes (outlined in detail in later sections). This is brought about by the hypoxic up-regulation of such transcription factors as hypoxia-inducible factor (HIF)-1 and HIF-2, consisting of a hypoxia-inducible α subunit (HIF-1α and HIF-2α) and a constitutively expressed β subunit that is common to both HIFs. In the presence of oxygen, HIF-1α and HIF-2α are rapidly degraded in the cytoplasm. In hypoxia, however, they accumulate and translocate to the nucleus where they bind first to the β subunit and then to hypoxic response elements in or near the promoters of oxygen-sensitive genes.[28]

HIF-1α and HIF-2α are up-regulated by human macrophages exposed to hypoxia *in vitro* and by TAMs in hypoxic/necrotic areas of human tumors.[29,30] We have shown that hypoxic macrophages accumulate more HIF-1 than HIF-2,[29] suggesting that HIF-1 may be the major hypoxia-inducible transcription factor in hypoxic TAMs. This corresponds well with previous studies showing that HIF-1α-deficient mouse embryonic stem cells and embryonic fibroblasts are unable to exhibit their normal pattern of gene up-regulation in hypoxia, suggesting an essential role for HIF-1, at least in these cells.[31] Another recent study has shown HIF-2α expression by TAMs to positively correlate with angiogenesis in breast[32] and bladder[33] carcinoma (with poor prognosis in the latter condition;[34]) however, no such analysis for HIF-1 was included in these studies, so no direct comparisons can be made. White and co-workers[35] used adenoviral infection to overexpress HIF-1 and -2 in human macrophages and also found HIF-2 to be the primary inducer of genes encoding angiogenic proteins in these cells. However, the repertoire of genes activated by overexpression of each HIF may differ from that in cells responding to hypoxia, in which HIF levels may naturally be lower. Thus, studies are warranted to examine gene expression in macrophages treated with siRNA to selectively block expression of HIF-1α versus HIF-2α.

Interestingly, recent reports suggest that HIF-1 may play an important role in regulating macrophage recruitment into inflammatory lesions. Mice with a conditional knockout for HIF-1α in myeloid cells were used to examine the role of this factor in regulating macrophage uptake into inflammatory lesions, which, like tumors, are often oxygen-depleted. Loss of HIF-1 reduced macrophage adhesion to and infiltration through an artificial matrix (growth factor-reduced Matrigel) *in vitro*. This was likely a contributing factor in their finding that HIF-1 knockout mice exhibit a markedly diminished inflammatory response in inflamed skin and artificially-induced arthritic joints.[36] Furthermore, hypoxia has been shown to stimulate macrophage adhesion to endothelial cells, in part, by HIF-1 induction of CD18, the β subunit of all β2 integrins.[37] It remains to be seen whether such HIF-1-driven mechanisms play any part in recruiting monocytes into tumors and/or TAM migration into hypoxic tumor areas.

Like malignant cells, macrophages can switch from aerobic to anaerobic glycolysis by increasing levels of various glycolytic enzymes,[38,39] including phosphoglycerate kinase-1.[35] They also up-regulate their expression of the glucose-transporting receptor Glut-1[36,39] to increase the supply of extracellular glucose for ATP generation via this route. Hypoxic up-regulation of both genes is HIF-1-dependent and is absent in macrophages derived from HIF-1 knockout mice.[36]

It should be noted that HIFs are not the only transcription factors up-regulated by macrophages in response to hypoxia. Others include nuclear factor-κB,[40] Ets-1,[41] Egr-1,[42] and activating transcription factor-4 (ATF-4) (Elberghati L, Murdoch C, and Lewis CE, unpublished observations). However, a role for these transcription factors in regulating gene expression and contributing to the hypoxic phenotype of TAMs has yet to be demonstrated.

Protumor Effects of Macrophages

Recent studies have indicated that TAMs may play an important part in promoting tumor growth and progression. For example, an elegant study by Li et al [43] used an M-CSF knockout mouse model to demonstrate the central role of TAMs in the progression of spontaneous mammary tumors. M-CSF, a growth factor that promotes the survival and differentiation of macrophages,[44] is overexpressed in some human tumors, and elevated M-CSF levels correlate with high TAM numbers and poor prognosis.[45] Li and colleagues[43] showed that mammary tumors grown in M-CSF-knockout mice recruited fewer

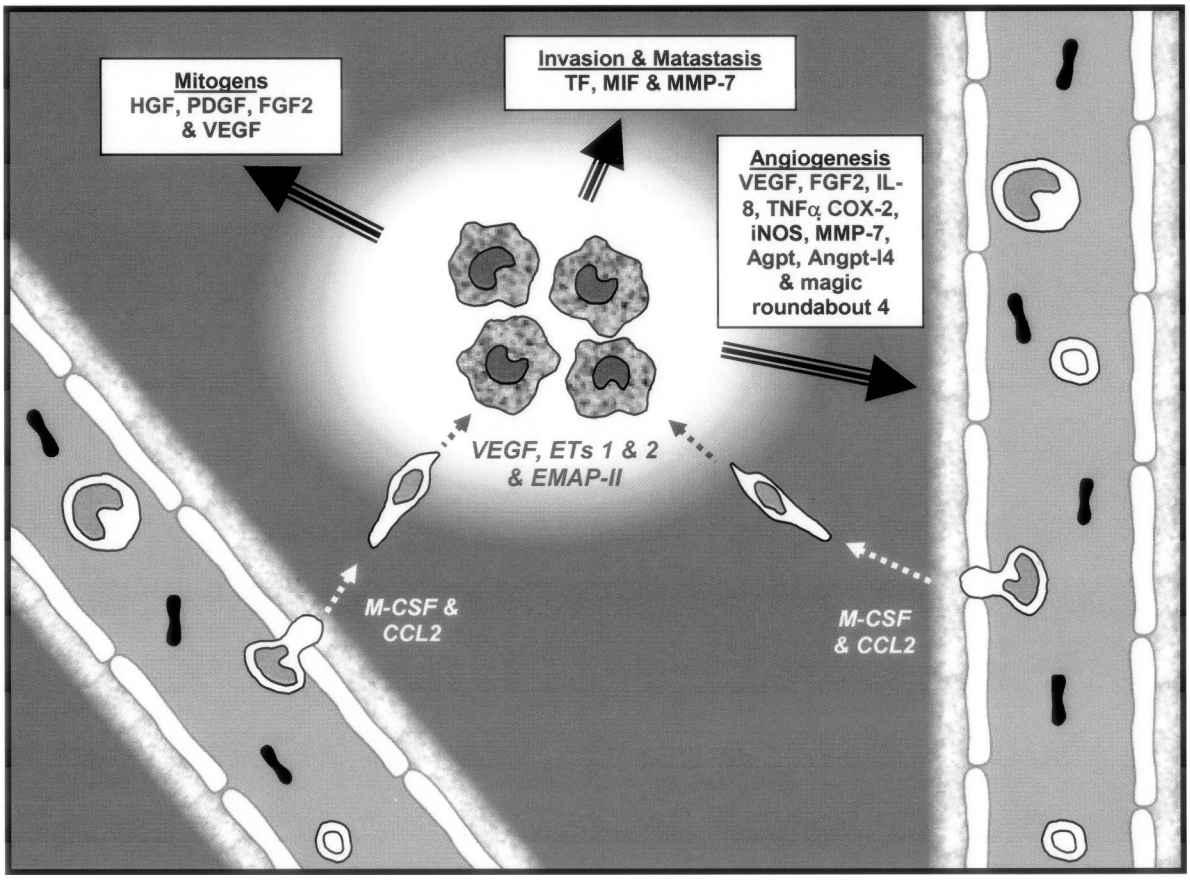

Figure 2. Macrophage functions in a hypoxic tumor area: involvement in tumor growth, invasion, metastasis, and angiogenesis. Monocytes are recruited from the tumor vasculature by such chemoattractants released by tumors as M-CSF and CCL2. They then differentiate into TAMs and migrate into and become immobilized in areas of transient or chronic hypoxia (shown here in white) in response to VEGF, endothelins (ETs) 1 and 2, and EMAP-II, which are up-regulated in hypoxic areas. TAMs then respond to the hypoxia by up-regulating a broad array of genes encoding proteins that promote the proliferation, invasion, and metastasis of tumor cells as well as tumor angiogenesis. See text for abbreviations used.

TAMs, progressed more slowly from preinvasive to malignant lesions, and exhibited reduced metastasis compared to their normal, M-CSF-expressing counterparts. This finding was confirmed in a recent study using siRNA to inhibit M-CSF expression by MCF-7 xenografts, which resulted in a marked reduction in TAM numbers, tumor growth, and angiogenesis.[46]

Under tumor-associated hypoxia macrophages show marked changes in expression patterns of several genes. These are outlined below, where genes have been grouped into categories according to their effect on tumor growth, invasion/metastasis, angiogenesis, and immune evasion (Figure 2).

Growth Factors

Tumor cell mitogens have been shown to be up-regulated by hypoxic macrophages *in vitro*. These include fibroblast growth factor 2 (FGF2), platelet-derived growth factor, placental growth factor, and hepatocyte growth factor,[35] although TAM up-regulation of these factors in hypoxic tumor areas *in vivo* has yet to be demonstrated. TAMs are also an important source of epidermal growth factor (EGF) in human breast tumors.[47] EGF is up-regulated by hypoxia in other cell types but there is no evidence to date of this in macrophages. However, the fact that hypoxia up-regulates and activates receptors for EGF[48] suggests that possible EGF release by TAMs could have potent protective and mitogenic effects on neighboring tumor cells in hypoxic areas. This effect may be supported by the hypoxic macrophage up-regulation of adrenomedullin,[35] a peptide with effects similar to those of EGF on tumor cells *in vitro*.[49]

Various groups have shown that macrophages up-regulate the important endothelial cell mitogen VEGF at both the mRNA and protein levels in response to hypoxia.[50,51] Furthermore, we have shown that TAMs express VEGF almost exclusively in avascular and perinecrotic areas of breast carcinomas.[52] Cramer and colleagues[36] showed that the hypoxic induction of this cytokine is primarily dependent on HIF-1 in murine peritoneal macrophages, although low-level VEGF release occurred in the absence of HIF-1 (suggesting a minor role for HIF-2 or other transcription factors in hypoxic induction of VEGF in this cell type). Tumor cell proliferation, as well as tumor angiogenesis, is likely to be affected by the hypoxic induction of VEGF release, as various tumor cell types express type 1 and 2 receptors for VEGF, and to be responsive to VEGF with growth stimulation *in vitro* and *in vivo*.[53,54]

Invasion and Metastasis

Hypoxic macrophages are also likely to promote the invasive and/or metastatic behavior of tumor cells by releasing such proinvasive factors as macrophage inhibitory factor[55] and tissue factor.[56] Once thought to act mainly on macrophages to inhibit their migration and cause macrophage accumulation at sites of inflammation, macrophage inhibitory factor is now known to modulate the activities of a number of cell types in tumors, including stimulation of tumor cell motility.[57] This may involve indirect effects such as macrophage inhibitory factor-stimulated release of matrix metalloproteinase 9, which in turn degrades components of the basement membrane and extracellular matrix, thereby increasing the motility of tumor cells.[58] Grimshaw and colleagues[59] showed that when macrophages are co-cultured with tumor cells in the presence of endothelins 1 and 2 (cytokines that are themselves up-regulated in hypoxic tumor areas and known to have receptors on both macrophages and tumor cells), the secretion of MMP-2 and -9 by tumor cells was increased, stimulating the invasive behavior of tumor cells. Moreover, macrophages synthesize elevated levels of MMP-7 mRNA and protein when exposed to hypoxia *in vitro* and in avascular areas of human tumors.[29] This multifunctional MMP stimulates tumor invasion through the basement membrane into normal surrounding tissues.[60]

Tissue factor, a transmembrane protein primarily involved in the coagulation cascade, also plays an important role in the metastasis of solid tumors. Within tumors tissue factor is overexpressed by tumor cells, endothelial cells, fibroblasts, and macrophages,[61] and it promotes the generation of thrombin in tumors. This, in turn, activates the metastatic activity of tumor cells via the thrombin receptors PAR-1 and/or -2.[61]

Angiogenesis

Many of the above products of hypoxic macrophages are also likely to influence tumor angiogenesis. A recent study used restricted cDNA arrays (ie, limited number of genes analyzed) to show that the mRNA species for more than 30 proangiogenic genes were up-regulated by hypoxia in primary macrophages.[35] The best characterized of these, apart from VEGF, were the cytokines FGF2, CXCL8 (IL-8), and angiopoietin; the type I receptor for VEGF; and the proangiogenic enzymes cyclooxygenase-2 (COX-2) and inducible form of nitric oxide synthase (iNOS).

Other less well known proangiogenic factors produced by hypoxic macrophages include the 16-kd pleiotropic cytokine leptin. This protein is primarily expressed in adipose tissue, induces endothelial cells to express VEGF, and is highly angiogenic *in vivo*. The type I receptor for VEGF,[60] the receptor Magic roundabout,[62] and the cytokine angiopoietin-like 4 (angptl4)[63] are also expressed. Moreover, expression of the latter two genes by endothelial cells is known to be stimulated by hypoxia and is reported to be found mainly on newly formed blood vessels and in ischemic, perinecrotic areas in human tumors.[63,64] However, to date, neither the expression of these two molecules by TAMs in such tumor sites nor the significance of this to the regulation of tumor angiogenesis has been investigated.

Immune Evasion

Exposure of TAMs to hypoxia may also inhibit their participation in anti-tumor immune mechanisms as hypoxia stimulates secretion of such potent immunosuppressive factors as prostaglandin E_2[65] and IL-10.[66] These molecules inhibit immune effector cell (eg, T cell) function, impair the development of immune cells by acting on the early stages of immunopoiesis, and reduce the cytotoxicity of TAMs toward tumor cells.[67,68] Hypoxia has also been shown to inhibit the ability of macrophages to phagocytose dead or dying cells[69] and to present antigens to T cells.[66,68] It also reduces the surface expression of CD80,[70] a co-stimulatory molecule on macrophages that is needed for the full activation of T-cell responses to antigenic peptides. By contrast, hypoxia enhances the direct cytotoxicity of macrophages toward some forms of tumor cells (ie, TNF-sensitive targets) by up-regulating their release of TNF-α.[71]

Beyond Tumor-Associated Hypoxia

Taken together, the responses of macrophages to hypoxia suggest that TAM accumulation in hypoxic/necrotic areas is highly likely to promote the growth and spread of malignant tumors via a diverse array of hypoxia-driven mechanisms. However, when assessing the importance of these effects on tumor progression, it should be remembered that virtually all of the above effects have been demonstrated using macrophages or monocyte/macrophage cell lines *in vitro*, rather than macrophages isolated from tumors. This is primarily because macrophages are difficult to isolate from tissues like tumors without altering the way they have been conditioned by the tumor environment. TAMs may show different responses after such conditioning, so ideally these studies should be repeated using macrophages isolated from tumors. However, important hypoxia-regulated molecules like HIF-1 and -2, VEGF, and MMP-7 are up-regulated by both macrophages exposed to hypoxia *in vitro* and TAMs in hypoxic areas of human tumors,[29,30,50–52] suggesting that close correlations may exist between the *in vitro* and *in vivo* responses of macrophages. Further studies should be performed to determine whether such correlations exist for the other hypoxia-induced genes/proteins reviewed here. Furthermore, because many of these genes are up-regulated by both TAMs and tumor cells under hypoxia, conditional knockout experiments (eg, to ablate HIF expression in macrophages versus tumor cells) are now warranted to distinguish the relative contribution to tumor progression of hypoxia-induced (or at least HIF-driven) gene expression by TAMs versus tumor cells.

It is also important to note that TAMs in avascular and necrotic tumor areas are not exposed to hypoxia alone

but to a range of ischemic stresses resulting from the poor local vascular supply. These include low glucose and pH, high lactate, the presence of dead or dying cells (and their necrotic debris), and possibly exposure to various ischemia-induced cytokines and growth factors released by TAMs and other cell types. Their effects on TAMs may contribute to their function in these areas. For example, exposure to low pH has been shown to activate expression of such genes as iNOS by increasing nuclear factor-κB activity in the nucleus of cultured rat peritoneal macrophages.[72] Low pH also has multiple effects on the production of TNF-α by alveolar macrophages by increasing TNF-α mRNA expression as well as TNF-α protein retention, so less protein is actually secreted.[73] The latter effect contrasts with the ability of hypoxia to stimulate TNF-α release by macrophages as mentioned earlier,[71] so it would be interesting to see what effect combined exposure has on such macrophage functions. Interestingly, hypoxia fails to elicit this effect in the presence of low extracellular glucose; a combination that results in TNF-α inhibition.[74] Exposure to high lactate levels increases VEGF mRNA and protein production by macrophages *in vitro*.[75] The fact that VEGF mRNA and protein are up-regulated by TAMs in ischemic areas of breast carcinomas[52] suggests that hypoxia up-regulates this factor even when TAMs are exposed to the other complement of ischemic stresses present in hypoxic/necrotic sites.

Interestingly, hypoxia is found not only in malignant tumors but also in such inflamed tissues as the synovia of arthritic (rheumatoid) joints and in physiological wound healing. As in tumors, macrophages are recruited to these sites[3] and express various hypoxia-induced factors central to such disease-associated processes as angiogenesis.[75,76] However, the resolution of wounds, for example, results in restoration of tissue integrity and perfusion, and macrophage presence is then reduced to preinjury levels. This contrasts with the situation in malignant tumors, in which high numbers of macrophages persist, prompting some to suggest that tumors are wounds that do not heal. The reasons for this have been reviewed by us previously.[76]

Therapeutic Implications

The hypoxia-driven, protumor functions of macrophages described in this review may impinge on the recovery of tumors after treatment with agents known to induce hypoxia. These include radiotherapy,[77] photodynamic therapy,[78] anti-angiogenic agents such as endostatin[79] or vascular damaging agents such as combretastatin-A-4,[80] ZD6126[81] or alphastatin.[82] Macrophages infiltrate large areas of hypoxia and cell destruction that result from such therapies;[77,78] however, on exposure to hypoxia macrophages are likely to switch to the protumor phenotype described previously. In this way, TAMs may promote the revascularization, reoxygenation, and regrowth of the tumor once the therapy concludes. It would be interesting to see if blocking this post-therapy infiltration slowed or halted tumor recovery.

Attempts have been made to reduce the overall numbers of TAMs in tumors using such pharmacological agents as linomide, pentoxifylline, and genistein. In a rat model of prostate cancer, these drugs markedly reduced TAM numbers (<50% of controls), along with tumor growth and angiogenesis.[83] However, it may prove more effective to specifically target TAMs in hypoxic areas because a number of studies have inferred that TAMs in well-vascularized areas of tumors may perform beneficial functions for the host—TAM numbers in these areas are linked to a favorable prognosis.[84] This could be accomplished by targeting macrophage-specific cytotoxins or cytotoxic gene therapies to proteins induced by hypoxia on the surface of macrophages (eg, GLUT-1, Magic roundabout, or neuromedin B receptor[29,35,36]).

Efforts are also being made to use macrophages to carry genes into poorly vascularized tumor areas.[85] Recently, the success of this approach was demonstrated *in vitro* using macrophages infected with an adenovirus expressing a HIF-activated gene encoding a prodrug that activates cytochrome P450. When breast tumor spheroids (small, three-dimensional tumor masses) were infiltrated by such macrophages and then exposed to cyclophosphamide, the cytotoxic metabolite released by macrophages caused gross morphological damage to tumor cells in spheroids.[86] *In vivo* studies are being conducted to test the efficacy of this new cell-based gene therapy in tumor-bearing mice.

Concluding Remarks

Inflammation and cancer have been causally linked with a central inflammatory cell type, the macrophage, which is present in malignant tumors in large numbers linked to enhanced tumor progression. As described here, hypoxia has a profound effect on macrophage functions in tumors, eliciting a distinct protumor phenotype in which they produce a wide array of growth factors, cytokines, and enzymes to stimulate the growth, invasion, metastasis, angiogenesis, and immune evasion of tumors. Current studies to unearth the molecular mechanisms that attract macrophages to these hypoxic tumor sites and/or the signaling pathways that mediate the effects of hypoxia on macrophages should highlight new targets for new anti-cancer strategies.

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