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The Role of Hypoxia in Re-Educating Macrophages in the Tumour Environment

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1. Introduction

Monocytes and macrophages are myeloid cells which originate in the bone marrow and are essential in the primary defence against infection by bacteria, viruses and other pathogens. These cells circulate as monocytes in the bloodstream before undergoing extravasation and migration into adjacent tissues, where they differentiate into resident macrophages. Considerable monocyte extravasation occurs at the initial stages of inflammation, wound healing, tumour onset and various other diseases in response to chemotactic signals. In many instances these inflamed and/or diseased tissues have been shown to include areas of extremely low oxygen tension, termed hypoxia, by the measurement of oxygen concentrations using microelectrodes, use of hypoxic cell markers and/or expression of specific hypoxia-upregulated proteins. Such hypoxic areas are evident in the majority of malignant human cancers, including those of the breast, brain, cervix, head/neck, and soft tissue sarcomas (Raleigh et al., 2001; Vaupel et al., 1989), and are caused by an inability of the supporting vasculature to keep up with the oxygen demands of the rapidly increasing tumour mass (Shannon et al., 2003; Vaupel et al., 2005).

As with inflammation, extensive monocyte extravasation is also an early event in cancer development. Infiltrated monocytes differentiate into tumour-associated macrophages (TAMs), a process which is driven by tumour-secreted chemoattractants (Murdoch et al., 2004). Moreover, TAMs accumulate in high numbers within hypoxic areas, which drives a change in their gene expression through the modulation of such transcription factors as hypoxia-inducible factors (HIFs) 1 and 2 (Burke et al., 2003; Talks et al., 2000), activating transcription factor-4 (ATF-4), and early growth response-1 (egr-1) (Elbarghati et al., 2008). Subsequently, a wide panel of protumour genes are upregulated by hypoxic macrophages which could support tumour growth, survival and metastasis (Fang et al., 2009). This is thought to explain the correlation between high numbers of TAMs and poor patient prognosis in many types of human tumours (Fujimoto et al., 2000; Hamada et al., 2002; Hanada et al., 2000; Heidl et al., 1987; Leek et al., 1996; Lissbrant et al., 2000; Salvesen and Akslen, 1999).

2. Hypoxia as an important microenvironmental signal for 'educating' macrophages in tumours

2.1 Monocyte infiltration into tumours

The mechanisms by which immune cells are recruited into tumours have been well studied, revealing crucial roles for several chemokines and cytokines in the extravasation and infiltration of these cells, including monocytes, from the blood vessels and into the tumour. The chemokine-driven migration of leukocytes is followed by regulation of tumour growth, angiogenesis and metastasis, through alterations in the tumour environment (Balkwill, 2003; Strieter et al., 2004; Vicari and Caux, 2002).

Perhaps the most important monocyte chemoattractants upregulated by tumours are the chemokines, CCL2 and CCL5 (also known as MCP-1 and RANTES, respectively), which are synthesised by several cell types including tumour cells, fibroblasts, endothelial cells and TAMs themselves. Correlation between the expression of CCL2 and the accumulation of TAMs within breast (Ueno et al., 2000), ovarian (Negus et al., 1997), esophageal and squamous cell (Ohta et al., 2002), non-small cell lung cancer (Arenberg et al., 2000), and also glioblastoma (Leung et al., 1997), underscore the importance of this chemokine in monocyte recruitment into tumours. In addition, Bottazzi et al. (1992) demonstrated that when the CCL2 gene was transferred to a murine melanoma and subsequently grown *in vivo*, infiltration of monocytes increased, as evidenced by a doubling of TAM numbers. However, the phenotype of these TAMs may have been anti-tumoural since these CCL2-producing tumours exhibited reduced tumour growth and increased overall survival.

The effects of CCL2 and CCL5 on human monocytes are not just limited to their direct chemotactic capabilities; both ligands are also known to support monocytes in the production of additional chemoattractants and tumour-promoting molecules – for example, analysis of CCL5-induced monocyte gene expression by oligonucleotide array revealed that CCL2, CCL3, CCL4, CXCL8, and CCR1 were consistently induced, suggesting a role for CCL5 in leukocyte recruitment into the tumour. This correlates with the finding that CCL3 and CCL4 are expressed in certain human tumours (Scotton et al., 2001), and that CXCL8 drives adhesion of monocytes to vascular endothelium as part of monocyte recruitment (Gerszten et al., 1999).

The cytokines CSF-1 (colony-stimulating factor-1) and VEGF (vascular endothelial growth factor) are also known to be monocyte chemotactic proteins, and are produced by a variety of cell types, including monocytes and macrophages. By crossing CSF-1 knock-out mice with mice which form spontaneous mammary tumours, Lin et al. (2001) demonstrated the importance of this cytokine in the recruitment of monocytes into tumours, since tumours in the daughter mice showed reduced TAM numbers and slower tumour progression. These features could be reversed by the introduction of CSF-1 by targeted gene expression, confirming that this cytokine is important for TAM infiltration and tumour progression.

The growth factor, VEGF, is best characterised as an angiogenic factor which functions as a potent and specific mitogen for endothelial cells. In the majority of tumour types tested, VEGF mRNA expression is upregulated within the tumour (Ferrara and Davis-Smyth, 1997), primarily by tumour cells and TAMs (Lewis et al., 2000), rather than endothelial cells. The

inverse is true for mRNA expression of VEGF receptors, VEGF-R1 and -R2 (Brown et al., 1993; Plate et al., 1994; Plate et al., 1992), consistent with the hypothesis that VEGF predominantly acts as a paracrine factor to induce angiogenesis. Further studies suggested the expression of this growth factor by infiltrating lymphocytes (Freeman et al., 1995), and its role as a chemoattractant for monocytes and macrophages through VEGF-R1 was discovered (Barleon et al., 1996; Sawano et al., 2001), verified by the fact that murine macrophages lacking VEGF-R1 (from a model of embryonic angiogenesis) exhibited reduced migration in Boyden chambers in response to VEGF (Hiratsuka et al., 1998). Immunohistochemistry in surgically resected breast tumour samples showed that increased VEGF within tumours was associated with higher numbers of TAMs (Leek et al., 2000). These findings suggest that VEGF is not only important for angiogenesis, but also for the recruitment of monocytes (Figure 1a) (Toi et al., 1994).

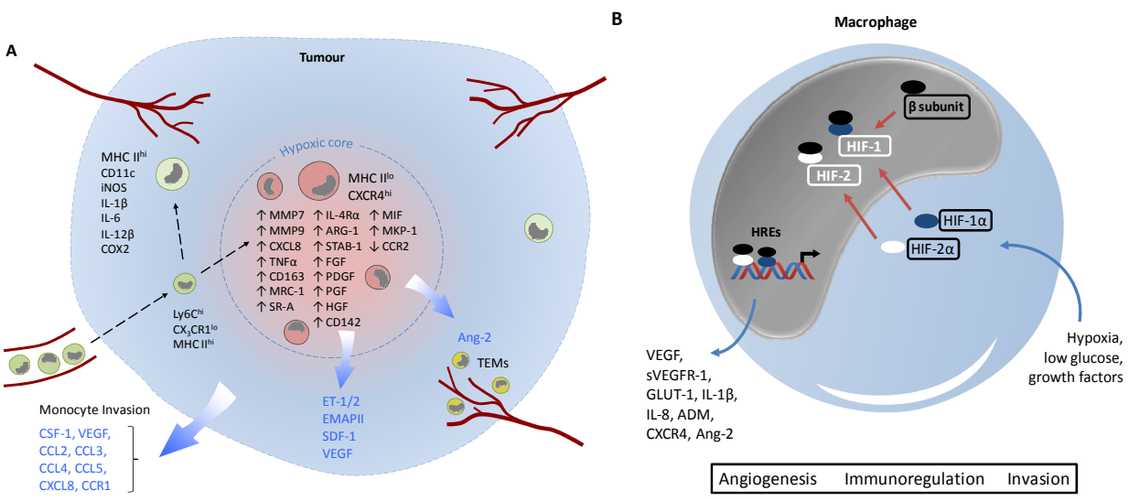

Fig. 1. Tumour hypoxia drives monocyte infiltration, polarization and transcription of hypoxia-regulated genes. a. Tumours and infiltrated macrophages secrete chemoattractants, resulting in the recruitment of monocytes from the blood. Hypoxic conditions commonly found within tumours enhance the polarisation of macrophages toward a protumour phenotype, which leads to the upregulation of a wide array of tumour-supporting genes (such as those shown in the figure) and the downregulation of MHC II. Almost all murine TAMs derive from a population of monocytes defined by $Ly6C^{hi}CX_3CR1^{lo}$ expression, which continuously seed tumours. Two types of murine TAMs, $MHC\ II^{hi}$ and $MHC\ II^{lo}$, have been shown to be located in normoxic and hypoxic areas of tumours, displaying M1 and M2 characteristics, respectively. TIE2-expressing macrophages (TEM) are recruited in response to release of Ang2 (as well as upregulation of Tie-2) by the hypoxic core. TEMs associate with blood vessels and promote tumour angiogenesis. Monocyte/macrophage-derived factors in black, tumour-derived factors in blue. b. Hypoxic conditions result in the stabilisation of HIF-1α and -2α in macrophages, which are then able to bind to a constitutively expressed common β subunit, located in the nucleus. The active transcription factors then bind to HREs in a variety of genes (shown at bottom left) which regulate the immunosuppressive and protumoural functions of macrophages.

2.2 Monocyte infiltration into areas of hypoxia

Following their infiltration into tumours, macrophages have been shown to accumulate specifically in hypoxic areas, a phenomenon which is thought to be guided by hypoxia-induced chemoattractants and maintained by the suppression of TAM motility in these areas by hypoxia (reviewed by Murdoch et al. (2004)). As would be expected, these oxygen-deprived regions of tumours have been found to have elevated levels of VEGF, produced by both tumour cells and macrophages (Brown et al., 1995; Lee et al., 1998; Lewis et al., 2000). In addition, VEGF expression in a murine model of Lewis lung carcinoma was shown by immunohistochemistry to correlate with pimonidazole stained areas, a marker for hypoxia (Kim et al., 2001). As mentioned previously, this factor is a chemoattractant for monocytes and macrophages and therefore is likely to play a major role in the accumulation of TAM at these sites (Lewis et al., 2000). However, it is worth noting that there is not always a correlation between hypoxia and VEGF expression in human tumours (Janssen et al., 2002; Raleigh et al., 1998). Matschurat and colleagues (2003) found that another monocyte chemotactic, EMAP II, is expressed at high levels in perinecrotic areas of methylcholanthrene fibrosarcomas and B16 murine melanomas in an inactive form, pro-EMAP II. Additionally, they showed that hypoxic tumour cell supernatants *in vitro* demonstrated an increase in EMAP II at the protein level, which was not supported by an induction at the mRNA level. This suggests that the active protein can be induced under hypoxia without the need for transcription, possibly through cleavage of pre-EMAP II to its active form by proteases released from necrotic cells (Zhang and Schwarz, 2002), providing a rapid mechanism for EMAP II upregulation and subsequent macrophage infiltration. This effect explains why macrophages are found at sites positive for EMAP II expression in uveal melanoma (Clarijs et al., 2003).

Also known to be regulated by hypoxia are endothelins, a family of secretory vasoactive peptides involved in vasoconstriction. They also have co-mitogenic functions, enhancing the effects of other such growth factors as PDGF by initiating intracellular signalling through endothelin receptors, ET-RA and ET-RB. Studies of endothelin regulation under hypoxic conditions demonstrated a co-localisation of hypoxia and endothelin ET-2 expression in murine mammary tumours (Grimshaw et al., 2002a). This is significant since ET-2 is thought to bind to ET-RB on macrophages and act as a chemoattractant, explaining the correlation seen between ET-2 expression and ET-RB-positive macrophages in breast tumours (Grimshaw et al., 2004; Grimshaw et al., 2002b). Furthermore, ET-1 (which acts through endothelin-1 receptor A) was recently shown to enhance the invasion and migration of both tumour cells and macrophages. The contribution of these factors to metastasis was supported by the finding that tumour expression of ET-1 and activity of its receptor are required for the development of lung metastases, through a process which is dependent on macrophage infiltration of the lung (Said et al., 2011).

More recently, Wang et al. (2012) identified stromal-derived factor-1 (SDF-1/CXCL12) as a tumour-derived chemoattractant and survival factor for TAMs. In a murine glioma model they showed that SDF-1^{kd} tumours have a different association of TAMs with hypoxia, implying that the secretion of this factor by tumour cells is critical for the accumulation of TAM in hypoxic areas of murine glioma. This factor is known to bind to its receptor, CXC receptor 4 (CXCR4), which is upregulated through a HIF-1-dependent mechanism in

monocytes and macrophages (as well as endothelial cells and tumour cells). Therefore, SDF-1 and its receptor, CXCR4, are very important for the chemotaxis of TAMs to hypoxic tumour sites (Schioppa et al., 2003).

It has been suggested that the accumulation of infiltrating macrophages in tumours, primarily in hypoxic regions, is not just due to chemoattraction, but also to their retention in these areas. Downregulation of chemokine release by tumour cells, and of chemokine receptors by TAMs in hypoxia, effectively dampens TAM motility, thus causing large numbers of macrophages to be trapped in these sites. For example, the expression of CCR2 (the receptor for CCL2/MCP-1) and chemotactic responses to CCL2 in vitro were markedly higher for TAMs isolated from ovarian carcinomas than monocyte-derived macrophages in culture (Negus et al., 1998; Sica et al., 2000).

When cultured with human tumour ascites, the chemotactic response of fresh monocytes to CCL2 was greatly diminished, accompanied by a reduction in CCR2 mRNA levels. Furthermore, inhibition of TNF-α restored CCR2 mRNA expression in monocytes cultured in the presence of ascitic fluid, demonstrating that defective CCR2 expression in TAM may be regulated, at least in part, by this cytokine in tumours (Sica et al., 2000). Therefore, it is possible that macrophage TNF-α production within hypoxic areas of tumours (Guida and Stewart, 1998; Hempel et al., 1996; Scannell et al., 1993) may lead to a downregulation of CCR2 expression on TAMs, decreasing their responsiveness to chemotactic ligands.

An increase in TNF-α expression is also believed to induce mitogen-activated protein kinase phosphatase 1 (MKP-1) (Grimshaw and Balkwill, 2001), a molecule which dephosphorylates extracellular signal-regulated kinase (ERK) 1/2, and p38 mitogen activated protein kinase (p38 MAPK) (Franklin and Kraft, 1997; Sun et al., 1993). Intracellular signalling via p38 MAPK and ERK1/2 is required for the chemotactic response of monocytes and monocytic cell lines to hypoxia-regulated chemokines (Ashida et al., 2001; Wain et al., 2002). Therefore, TNF-α may be an important factor in the hypoxic tumour environment for the suppression of macrophage migration, via a downregulation of CCR2 and an upregulation of MKP-1 (Figure 1a).

3. Hypoxia and its impact on macrophage function

For a long time it has been known that macrophages can be stimulated by environmental signals to exhibit a wide array of phenotypes (Nibbering et al., 1987; Ogle et al., 1994; van Furth, 1980). Two main polarization phenotypes of macrophages have been recognized. These include the classically activated (M1) and alternatively activated (M2) macrophage phenotypes. M1 macrophages are induced by interferon gamma (IFN-γ) and lipopolysaccharide (LPS). These macrophages upregulate pro-inflammatory cytokines (e.g. IL-12, IL-23, TNF, CXCL10), co-stimulatory molecules, produce reactive nitrogen and oxygen intermediates (RNI/ROI), and very little anti-inflammatory cytokines (e.g. IL-10). These cells promote inflammation, apoptosis, and microbicidal activity. Conversely, M2 macrophages are induced by IL-4 and IL-13, promote angiogenesis, cell proliferation and other tissue remodelling and protumoral functions. These cells are characterized in general by an IL-12^{lo}IL-10^{hi} phenotype, upregulate chemokines like CCL17, CCL18 and CCL22, various scavenging receptors and the production of Arginase I. Although the M1-M2 nomenclature is a useful one when assessing the phenotype of macrophages, it is however,

an over-simplification. Not all macrophage fit into these two distinct populations, and so further sub-populations have been defined (Mantovani et al., 2004).

Recently, subsets of differentially polarized TAMs with distinct functions were described by Movahedi et al. (2010) in murine mammary tumours. Their findings showed that almost all TAMs from these tumours were derived from $Ly6C^{hi}CX_3CR1^{lo}$ monocytes, where Ly6C is a monocyte/macrophage differentiation antigen regulated by IFN-γ, and CX_3CR1 is a receptor for CX_3CL1, a chemokine involved in the adhesion and migration of leukocytes. Notably, they found that hypoxic areas had higher numbers of M2-like TAMs, which increased as the tumour progressed (in certain tumours), and were shown to have potent proangiogenic effects *in vivo*. This also correlated with the expression of major histocompatibility complex II (MHC II), whereby MHC II^{hi} macrophages resided in normoxic areas and displayed an M1-like phenotype, and MHC II^{lo} macrophages resided in hypoxic areas and displayed a more M2-like phenotype (Figure 1a). Expression of M1 molecules like Nos2 (iNOS), interleukin (Il)-1β, Il-6, Il-12β and Ptgs2 (or cycloxygenase 2, COX2) were reported in MHC II^{hi} monocytes at the RNA or protein level. By comparison, MHC II^{lo} monocytes expressed such M2-related molecules such as macrophage mannose receptor (MR), scavenger receptor 1 (SR-A), arginase-1 (ARG-1), CD163, stabilin-1 (STAB-1), and interleukin-4Rα (IL-4Rα) (Movahedi et al., 2010). Fitting with their M2-like phenotype and localisation in areas of hypoxia, MHC II^{lo} TAMs were found to have significantly elevated proangiogenic activity *in vivo*. The phenotypic similarity between MHC II^{hi} TAMs and IKKβ-deficient macrophages implies that differences in these MHC II^{hi} and MHC II^{lo} TAM subsets may be driven by NF-κβ activity (Movahedi et al., 2010).

Another monocyte population, thought to be distinct from MHC II^{lo} TAMs, are the Tie2-expressing monocytes (TEMs) (Figure 1a). Originally identified in tumour-bearing mice, these monocytes circulate in the mouse blood as $Tie2^{+}CD11b^{+}CD45^{+}$ cells. They comprise a small monocyte subset which migrate towards angiopoietin-2 (Ang-2), a TIE2 ligand that is primarily released by vascular endothelial cells; this is thought to be a possible mechanism by which TEMs are recruited to tumours (Venneri et al., 2007), and more specifically, to highly vascularised areas (De Palma et al., 2003) (Figure 1a). Their role in the promotion of angiogenesis was confirmed by De Palma et al. (2005), who found that selective depletion of TEMs in a murine cancer model resulted in the inhibition of tumour angiogenesis and growth. Furthermore, TEM depletion was found to increase the efficacy of vascular-disrupting agent (VDA) therapy of tumours, suggesting that the action of these cells counteracts the antitumour effects of VDAs (Welford et al., 2011). Understanding more about monocyte subsets uncovers new possibilities for targeting specific subpopulations, which could alter the overall balance of TAM phenotypes. Repolarisation of TAM from an M2- to an M1-like phenotype could restore their antitumour effects, leading to a better patient prognosis.

The implications of macrophage plasticity in cancer biology have gathered increasing interest, both in terms of the phenotypes driven by the tumour microenvironment, and more specifically, by the hypoxic tumour environment. Biswas and colleagues (2008) reviewed the experimental evidence demonstrating that TAMs initially have an M1-like phenotype in areas of chronic inflammation where tumours commonly develop. These, however, respond to secreted cytokines, chemokines, growth factors and stress signals in the (hypoxic) tumour

microenvironment, to express more of an M2-like phenotype in established tumours. This suggests a "re-education" of macrophages, which are recruited by the tumour, initially expressing an M1-like phenotype (thus promoting an inflammatory response); however, their residency within tumours leads to their polarisation and differentiation into M2-skewed TAMs, where their re-educated phenotype is one which promotes angiogenesis, tissue remodelling, immunosuppression and cell proliferation (Biswas et al., 2008).

One important feature of the tumour environment which brings about this phenotypic change in macrophages is hypoxia. Hypoxic regions of tumours commonly form due to the leaky and disorganised nature of tumour blood vessels, meaning that the rapid tumour cell proliferation often surpasses the ability of the poorly-formed vasculature to deliver required oxygen and nutrients (Shannon et al., 2003; Vaupel et al., 2001). Studies with human breast carcinomas (Leek et al., 1999) or animal tumours (Collingridge et al., 2001) have shown that hypoxic tumours contain higher numbers of TAMs. A positive correlation that is also seen between hypoxia and TAM numbers in secondary liver tumours that form as metastases from breast and colorectal tumours (Stessels et al., 2004). There is an inverse relationship between TAM infiltration and patient prognosis seen in many human cancers (Fujimoto et al., 2000; Hamada et al., 2002; Hanada et al., 2000; Heidl et al., 1987; Leek et al., 1996; Lissbrant et al., 2000; Salvesen and Akslen, 1999), which implies that these macrophages adopt a pro-tumoural phenotype, contrasting with their more classic role as pathogen and tumour killing cells and with their ability to initiate an immune response.

4. Molecular pathways mediating the effects of hypoxia on macrophages

4.1 Transcription factors HIFs 1 and 2

The best understood transcription factors mediating the response of macrophages to hypoxia are the hypoxia-inducible factors (HIFs) 1 and 2 (Burke et al., 2003; Fang et al., 2009; Talks et al., 2000) (Figure 1b). Both HIFs are heterodimers consisting of an individual α subunit and a common β subunit which is constitutively expressed. HIF-1α and HIF-2α are tightly controlled, such that, in the presence of oxygen they are quickly degraded by the ubiquitin-proteasome pathway within the cytoplasm. However, hypoxic stress causes an increase in production and stabilisation of these subunits, which are then able to complex with the β subunit within the nucleus and bind to hypoxic response elements (HREs) of certain oxygen-sensitive genes to drive transcription (Jiang et al., 1996; Semenza, 2002). The hypoxia-responsive genes regulated by HIFs are known to be involved in tumour proliferation, metabolism, angiogenesis, apoptosis and metastasis (reviewed by (Harris, 2002)). The data supporting the expression of HIFs by macrophages, especially TAMs, is currently unclear. Talks et al. (2000) showed that hypoxia predominantly upregulates HIF-2α in the pro-monocytic cell line, U937, and the HIF over-expression studies by White et al. (2004), suggested that HIF-2 might be more important for macrophage pro-angiogenic responses to hypoxia. In contrast, human macrophages exposed to tumour-specific levels of hypoxia *in vitro*, as well as those in hypoxic areas of several human tumours *in vivo*, were shown to be capable of inducing high levels of HIF-1 as well as HIF-2 (Burke et al., 2002). Recently, Fang et al. (2009) demonstrated that 18 hour exposure of human macrophages to hypoxia induces expression of VEGF, IL-1β, IL-8, adrenomedullin, CXCR4, and angiopoietin-2. Induction of these genes suggests a potent, pro-tumoural macrophage

phenotype. Using small interfering RNA (siRNA), this gene expression was shown to be mediated via HIF-1 and 2 signalling, thus implicating these transcription factors in the generation of the hypoxia-driven, tumour-promoting macrophage phenotype.

Both HIF-1 and 2 bind to the HRE sequence contained in the promoter region of the VEGF gene and cause its upregulation (Ema et al., 1997; Flamme et al., 1997; Tian et al., 1997). Evidence that TAMs themselves upregulate VEGF in poorly vascularised tumour areas (Lewis et al., 2000) suggests that hypoxia, at least in part, causes TAMs to align with tumour cells in their pro-angiogenic function to increase the supply of oxygen to these areas. Interestingly, the binding of HIF-1 and 2 to the promoter region of *VEGF* in GM-CSF-cultured macrophages is thought to have antagonistic effects on angiogenesis, whereby HIF-1 induces VEGF production and HIF-2 induces the production of the soluble VEGF receptor, sVEGFR-1, in low oxygen conditions. The secretion of sVEGFR-1 is able to neutralise VEGF biologic activity, inhibiting its angiogenic effect; this indicates that the binding of these two transcription factors may have opposing effects on the regulation of angiogenesis (Eubank et al., 2011).

Less is known about the third member of the HIF family, HIF-3α, which shows high similarity to HIFs 1 and 2 and also forms heterodimers with the same β subunit. However, experiments so far show that this factor lacks the C-terminal transactivation domain (CTAD) (Gu et al., 1998), and acts as a dominant-negative regulator of the HIF pathway by antagonising the effects of HIFs 1 and 2 (Makino et al., 2001). HIF-3 was found to be constitutively expressed in monocyte-derived macrophages (MDMs), and was not responsive to hypoxic conditions in either monocytes or MDMs (Elbarghati et al., 2008). However, it was found to be hypoxia-responsive in lung epithelial cells (A549) at both the mRNA and protein level (Li et al., 2006), and so it is clear that further investigation into this transcription factor, with regards to its expression and importance, is needed.

HIFs are not the only hypoxia-responsive transcription factors. Both activating transcription factor-4 (ATF-4) and early growth response-1 (Egr-1) are upregulated in response to hypoxia in several murine and human tumour cell types (Ameri et al., 2004; Yan et al., 1999) and macrophages (Elbarghati et al., 2008), respectively. Both ATF-4 and Egr-1 proteins were found to be transiently upregulated in macrophages following a short hypoxic incubation, but there was no induction seen in monocytes. Interestingly, hypoxic treatment caused Egr-1 protein accumulation in macrophages in both the nucleus and the cytoplasm, in contrast to HIFs 1 and 2, and ATF-4 (Elbarghati et al., 2008), and is thought to play a role in monocyte differentiation into macrophages. Kharbanda et al. (1991) showed that M-CSF-stimulated monocytes demonstrate a dose-dependent increase in EGR-1 mRNA levels, and that inhibition of monocyte differentiation with dexamethasone also abolishes this EGR-1 induction. Therefore, since hypoxia increases the levels of Egr-1 protein, it is possible that hypoxia accelerates the differentiation of monocytes into TAMs (Elbarghati et al., 2008). This is also supported by the work of Oda and colleagues (2006), who demonstrated an increase in the expression of HIF-1α and HIF-1β in differentiating THP-1 cells and human monocytes from peripheral blood. RNA interference studies determined that, although HIF-1α is not essential for macrophage differentiation, it is, however, required for macrophage functional maturation. These findings further suggest that macrophage differentiation may be facilitated by hypoxia.

Various experimental methods have been used to identify HIF targets, including loss of expression in HIF-null cells (Fang et al., 2009; Semenza, 2003), targeting transcribed HIF using siRNA treatment (Fang et al., 2009; Kamlah et al., 2009; Krishnamachary et al., 2003), overexpression of HIFs using expression vectors or induced gene expression in von Hippel-Lindau (*VHL*)-null cells (Wykoff et al., 2000), or by the identification of HREs and HIF binding sites within gene promoter regions (Benita et al., 2009; Hirani et al., 2001; Semenza and Wang, 1992; Zhang et al., 2006). Both HIF1 and 2 were shown to regulate hypoxic MDM induction of VEGFA, GLUT-1, CXCR4, IL-1β, IL-8, and ADM (Fang et al., 2009) (Figure 1b), validating other reports of these as HIF target genes (Benita et al., 2009; Hirani et al., 2001; Semenza, 2003; Zhang et al., 2006).

Evidence that hypoxia induces a protumour phenotype in TAMs is not just limited to observed changes in RNA and protein expression; functional studies with hypoxic or HIF-expressing TAMs have also confirmed this phenotypic shift in macrophages. TAM-induced endothelial cell migration and tubule formation, reported by Chen et al. (2011), confirms the angiogenesis-promoting actions of TAM suggested by RNA and protein expression. More specifically, HIF-1α has been implicated in these protumour functional effects of TAMs. Doedens et al. (2010) report a dose-dependent suppression of T-cell proliferation by macrophages, demonstrating this immunosuppressive effect to be enhanced under hypoxia in a HIF-1α-dependent manner.

4.2 HIF relation with other pathways

It is clear that hypoxia drives a tumour-promoting phenotype in macrophages – it does this through the activation of hypoxia-responsive transcription factors, predominantly HIF-1 and 2, and their crosstalk with other signalling pathways. One such example is Toll-like receptor (TLR) signalling; TLR receptors are known to activate the innate immune system upon recognition of various pathogen-associated molecular patterns (PAMPs), including Lipopolysaccharide (LPS), bacterial DNA, and double-stranded RNA (Kaisho and Akira, 2006). In humans there are 10 functional members of the TLR family (TLR1-TLR10), of which, TLR4 is possibly the most involved in macrophage hypoxic response. This particular receptor recognises LPS, but has more recently been shown to be a receptor for certain endogenous molecules associated with damaged cells and tissues (Zhang and Mosser, 2008).

In their study of the relationship between hypoxic stress and TLR activity of macrophages, Kim et al. (2010) showed that hypoxia (and the hypoxia mimetic, $CoCl_2$) increased TLR4 messenger RNA and protein expression in the murine macrophage cell line, RAW264.7. This was unique to TLR4 and not seen with any of the other TLRs. Through the manipulation of macrophage HIF-1α gene expression, they demonstrated that hypoxic upregulation of TLR4 was dependent upon HIF-1 signalling, as well as showing that overexpression of HIF-1α enhanced TLR4 expression. Using chromatin immunoprecipitation (ChIP) they discovered that HIF-1α binds to the TLR4 promoter under hypoxic conditions, and the resultant induction of TLR4 in these macrophages increased the expression of interleukin-6 (IL-6), cyclooxygenase-2 (COX-2) and interferon-inducible protein-10 (IP-10) (Kim et al., 2010). Therefore, it is likely that, at sites which are challenged by hypoxic stress, macrophages upregulate TLR4 and become more sensitive to infection and inflammatory signals.

In addition to HIF-1α regulating TLR4, Sumbayev (2008) demonstrated that the inverse is also true. In human myeloid cells, TLR4 signalling (induced by the gram-negative bacterial ligand, LPS), activates crosstalk of HIF-1α and apoptosis signal-regulating kinase 1 (ASK1) pathways. Through the activation of p38 mitogen-activated protein kinase (p38 MAPK), ASK1 was found to stabilise HIF-1α, and knockdown of HIF-1α led to a reduced TLR4-dependent induction of pro-inflammatory cytokines. Similarly, TLR7 and 8 (involved in the recognition of viral single-stranded RNA) were also found to induce HIF-1α, although ASK1 was not found to be involved (Nicholas and Sumbayev, 2009).

Evidence has been given for the role of HIFs 1 and 2 in these pro-tumour actions of TAM in hypoxic areas, but more recently it has emerged that HIF-1 may also be responsive under normoxic conditions. Such stimuli as LPS, cytokines (e.g. TNFα), growth factors, insulin, thrombin and vasoactive peptides cause HIF-1α stabilisation in normoxia, via nuclear factor-kappa B (NF-κB) signalling. This key transcription factor was shown by Rius et al. (2008) to be upregulated following a 2-4h exposure of murine bone marrow-derived macrophages (BMDMs) to low oxygen. They also demonstrated that basal levels of NF-κB were required for the accumulation of HIF-1α protein in hypoxic cells, using macrophages from an IKK-beta knock-out ($IKK\beta^{-/-}$) mouse. This implies that IKKβ, an important activator of NF-κB through phosphorylation-induced degradation of IkB inhibitors, has important contributions to macrophage response to hypoxia. Since NF-κB has a crucial and well characterised role in inflammation, IKKβ represents a significant molecule which may link the hypoxic response to innate immunity and infection (Rius et al., 2008).

5. Hypoxia, macrophage function and tumour progression

By contributing to angiogenesis, metastasis, invasion, immunosuppression, chemo- and radio-resistance, and altering metabolism, macrophages are known to greatly influence the survival and progression of cancer (see Biswas et al. (2008)). This phenotype of TAMs is also known to be influenced by hypoxia, which induces a distinct protumour phenotype. Expression of various growth factors, including fibroblast growth factor 2 (FGF2), platelet-derived growth factor (PDGF), placental growth factor (PGF), and hepatocyte growth factor (HGF), have been found to be upregulated *in vitro* by macrophages under hypoxia (White et al., 2004). These factors, in addition to VEGF, function as tumour cell mitogens and support tumour growth in hypoxic regions (Fang et al., 2009; Lewis et al., 2000).

Another key process in tumour progression is angiogenesis. The expression of VEGF (a potent mitogen and well characterised pro-angiogenic factor) by hypoxic macrophages has been discussed previously. However, other key proteins reported by White and colleagues (2004) include CXCL8, angiopoietin, cyclooxygenase-2 (COX-2) and inducible nitric oxide synthase (iNOS), all of which were identified in cDNA arrays as genes that are transcriptionally upregulated in primary macrophages under hypoxia. Induction of these genes by macrophages is likely to be crucial for tumour angiogenesis.

Additionally, hypoxic TAMs also release tissue factor (CD142) (Compeau et al., 1994) and macrophage inhibitory factor (MIF) (Schmeisser et al., 2005), which are thought to be involved in invasion and metastasis. Tissue factor expression by hypoxic TAMs (as well as tumour cells, endothelial cells and fibroblasts), induces the production of thrombin, which in turn promotes tumour cell metastasis (Versteeg et al., 2004). Furthermore, MIF has been

shown to promote tumour cell motility in a murine colon cancer cell line *in vitro* and *in vivo* (Sun et al., 2005). These factors may act indirectly through matrix metalloproteases (MMPs), such as MMP-9, which is stimulated by MIF and degrades the basement membrane and extracellular matrix (ECM) (Hagemann et al., 2004). This weakens the attachment of tumour cells to these structural supports and enables their subsequent invasion and metastasis. Further evidence of MMP induction comes from a co-culture of macrophages with tumour cells in hypoxia, which revealed an upregulation of macrophage MMP-7 production in low oxygen conditions *in vitro* and human tumours (Burke et al., 2003).

Finally, the hypoxic phenotype of TAMs also includes various immunosuppressive functions which are achieved through several mechanisms; these include the expression of immunosuppressive factors prostaglandin E_2 (PGE2) and IL-10 (Ertel et al., 1993; Murata et al., 2002), whose presence within the tumour microenvironment can downregulate the tumouricidal abilities of TAMs. In addition, PGE2 and IL-10 inhibit the functions of T cells and other effector cells of the immune system (Elgert et al., 1998), which combined with hypoxic inhibition of macrophage phagocytosis and presentation of antigens (Leeper-Woodford and Mills, 1992; Murata et al., 2002), suppresses the triggering of an adaptive immune response directed toward the tumour. Doedens et al. (2010) recently reported hypoxia- and HIF-1α-dependent suppression of T-cell proliferation by macrophages. Hypoxia, therefore, drives the macrophage towards a protumour phenotype which regulates tumour growth, angiogenesis, invasion, metastasis and immunosuppression.

6. Targeting tumour hypoxia for therapy

Under the stresses associated with hypoxia in areas of tumour ischemia (predominantly low oxygen and low glucose concentrations), tumour cells are forced to respire anaerobically and reduce their proliferation. This challenges many conventional cancer therapies such as chemotherapy, since their mechanism of action relies on the rapidly replication of tumour cells. In addition to this, the poorly developed tumour vasculature, which contributes to the development of hypoxia in the first place, also impedes the delivery of drugs to these areas of the tumour.

In light of this, antiangiogenic "vessel normalizing" strategies are being developed which aim to improve tumour vasculature for better anticancer treatment and reduced metastasis. Rolny and colleagues (2011) demonstrated that histidine-rich glycoprotein (HRG), a host-derived factor, is able to significantly reduce hypoxia and to polarise TAMs away from a protumour phenotype.

The concept of delivering a prodrug systemically - for subsequent activation in specific areas of the body - has been applied to cancer biology, but is most often limited by the level of expression of the activating enzyme at the target site. Rather than hypoxia inhibiting cancer therapy, some recent therapeutic strategies have focussed on utilising cellular responses to these harsh conditions, twinned with the prodrug therapeutic design, to creatively activate cytotoxic agents within the hypoxic tumour microenvironment. Griffiths et al. (2000) made use of macrophage accumulation in areas of low oxygen to deliver gene therapy to pathological hypoxia. They genetically modified macrophages to express the enzyme cytochrome p450 under hypoxic conditions, which when expressed, converts the systemically administered pro-drug cyclophosphamide into its active form exclusively in

these areas and causes tumour cell death. This system was then adapted further to deliver an oncolytic adenovirus to these areas by co-transducing macrophages with a hypoxia-regulated E1A/B construct, as well as an E1A-dependent virus which can only proliferate within a prostate-tumour (using a prostate-specific promoter) (Muthana et al., 2011). E1A/B proteins were only synthesised once the host cell (the macrophage) had infiltrated into areas of extreme hypoxia in tumours. This then subsequently activated the proliferation of the oncolytic adenovirus and its release. The virus then infected and killed surrounding prostate tumour cells – in both hypoxic and non-hypoxic areas of tumours. This three-step process (the homing of macrophages to hypoxic sites, hypoxia-responsive proliferation of the adenovirus, and the limiting of viral replication to within prostate tumour cells), makes this system very specific for killing tumour cells within the hypoxic areas prostate tumours (Muthana et al., 2011). This hypoxia-based therapy was seen to eradicate both primary and secondary tumours in mice.

It has been suggested that the best use of this therapy would be in combination with conventional therapies, since this could potentially eliminate both the slower proliferating (hypoxic) and the highly proliferating areas of the tumour. Using a mathematical model, Owen et al. (2011) predict that the use of a macrophage-based, hypoxia-responsive therapy immediately before or during conventional chemotherapy would produce significant antitumour effects.

7. Concluding remarks

Macrophage accumulation in tumours is known to correlate with poor patient prognosis in the majority of cancer types (Fujimoto et al., 2000; Hamada et al., 2002; Hanada et al., 2000; Heidl et al., 1987; Leek et al., 1996; Lissbrant et al., 2000; Salvesen and Akslen, 1999). This can be explained by the tumour-promoting phenotype of TAMs, induced by the tumour microenvironment. Here we have reviewed how hypoxia, a key component of many malignant tumours (Raleigh et al., 2001; Vaupel et al., 1989), is centrally involved in the polarisation of TAMs. The shift in TAM phenotype under hypoxia has been shown not just at the expression level, but also at a functional level as well. In many of these reports, the HIF-1 transcription factor was found to be crucially important. Studies with HIF-1α knockout mice have revealed its role, not just in regulating responses to pathological hypoxia, but also to physiological low oxygen conditions as part of normal oxygen homeostasis. It is possible that HIF-1 signalling (including its activation of other pathways), is a major factor in determining the polarisation and function of macrophages in different environments (Dehne and Brune, 2009).

With considerable assistance from hypoxic, M2-like TAMs, tumour cells in hypoxic areas have the necessary support and drive to migrate and invade into adjacent tissues, evade the immune system and prevent a targeted adaptive immune response, and travel through the vasculature to form metastases at secondary sites. This has large implications in cancer therapy, especially since hypoxic tumour cells are less affected by most conventional therapeutic strategies. Inefficient targeting of such tumour cells is likely to contribute to the well-documented relapse in many chemotherapy- and radiotherapy-treated cancer patients; therefore, more creative and innovative therapeutic methodologies need to be developed based on our continually growing understanding of tumour hypoxia, to enhance patient long-term survival.

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9. References

Ameri, K., Lewis, C. E., Raida, M., Sowter, H., Hai, T., and Harris, A. L. (2004). Anoxic induction of ATF-4 through HIF-1-independent pathways of protein stabilization in human cancer cells. Blood *103*, 1876-1882.

Arenberg, D. A., Keane, M. P., DiGiovine, B., Kunkel, S. L., Strom, S. R., Burdick, M. D., Iannettoni, M. D., and Strieter, R. M. (2000). Macrophage infiltration in human non-small-cell lung cancer: the role of CC chemokines. Cancer Immunol Immunother *49*, 63-70.

Ashida, N., Arai, H., Yamasaki, M., and Kita, T. (2001). Differential signaling for MCP-1-dependent integrin activation and chemotaxis. Ann N Y Acad Sci *947*, 387-389.

Balkwill, F. (2003). Chemokine biology in cancer. Semin Immunol *15*, 49-55.

Barleon, B., Sozzani, S., Zhou, D., Weich, H. A., Mantovani, A., and Marme, D. (1996). Migration of human monocytes in response to vascular endothelial growth factor (VEGF) is mediated via the VEGF receptor flt-1. Blood *87*, 3336-3343.

Benita, Y., Kikuchi, H., Smith, A. D., Zhang, M. Q., Chung, D. C., and Xavier, R. J. (2009). An integrative genomics approach identifies Hypoxia Inducible Factor-1 (HIF-1)-target genes that form the core response to hypoxia. Nucleic Acids Res *37*, 4587-4602.

Biswas, S. K., Sica, A., and Lewis, C. E. (2008). Plasticity of macrophage function during tumor progression: regulation by distinct molecular mechanisms. J Immunol *180*, 2011-2017.

Bottazzi, B., Walter, S., Govoni, D., Colotta, F., and Mantovani, A. (1992). Monocyte chemotactic cytokine gene transfer modulates macrophage infiltration, growth, and susceptibility to IL-2 therapy of a murine melanoma. J Immunol *148*, 1280-1285.

Brown, L. F., Berse, B., Jackman, R. W., Tognazzi, K., Guidi, A. J., Dvorak, H. F., Senger, D. R., Connolly, J. L., and Schnitt, S. J. (1995). Expression of vascular permeability factor (vascular endothelial growth factor) and its receptors in breast cancer. Hum Pathol *26*, 86-91.

Brown, L. F., Berse, B., Jackman, R. W., Tognazzi, K., Manseau, E. J., Senger, D. R., and Dvorak, H. F. (1993). Expression of vascular permeability factor (vascular endothelial growth factor) and its receptors in adenocarcinomas of the gastrointestinal tract. Cancer Res *53*, 4727-4735.

Burke, B., Giannoudis, A., Corke, K. P., Gill, D., Wells, M., Ziegler-Heitbrock, L., and Lewis, C. E. (2003). Hypoxia-induced gene expression in human macrophages: implications for ischemic tissues and hypoxia-regulated gene therapy. Am J Pathol *163*, 1233-1243.

Burke, B., Tang, N., Corke, K. P., Tazzyman, D., Ameri, K., Wells, M., and Lewis, C. E. (2002). Expression of HIF-1alpha by human macrophages: implications for the use of macrophages in hypoxia-regulated cancer gene therapy. J Pathol *196*, 204-212.

Chen, P., Huang, Y., Bong, R., Ding, Y., Song, N., Wang, X., Song, X., and Luo, Y. (2011). Tumor-associated macrophages promote angiogenesis and melanoma growth via adrenomedullin in a paracrine and autocrine manner. Clin Cancer Res *17*, 7230-7239.

Clarijs, R., Schalkwijk, L., Ruiter, D. J., and de Waal, R. M. (2003). EMAP-II expression is associated with macrophage accumulation in primary uveal melanoma. Invest Ophthalmol Vis Sci *44*, 1801-1806.

Collingridge, D. R., Hill, S. A., and Chaplin, D. J. (2001). Proportion of infiltrating IgG-binding immune cells predict for tumour hypoxia. Br J Cancer *84*, 626-630.

Compeau, C. G., Ma, J., DeCampos, K. N., Waddell, T. K., Brisseau, G. F., Slutsky, A. S., and Rotstein, O. D. (1994). In situ ischemia and hypoxia enhance alveolar macrophage tissue factor expression. Am J Respir Cell Mol Biol *11*, 446-455.

De Palma, M., Venneri, M. A., Galli, R., Sergi Sergi, L., Politi, L. S., Sampaolesi, M., and Naldini, L. (2005). Tie2 identifies a hematopoietic lineage of proangiogenic monocytes required for tumor vessel formation and a mesenchymal population of pericyte progenitors. Cancer Cell *8*, 211-226.

De Palma, M., Venneri, M. A., Roca, C., and Naldini, L. (2003). Targeting exogenous genes to tumor angiogenesis by transplantation of genetically modified hematopoietic stem cells. Nat Med *9*, 789-795.

Dehne, N., and Brune, B. (2009). HIF-1 in the inflammatory microenvironment. Exp Cell Res *315*, 1791-1797.

Doedens, A. L., Stockmann, C., Rubinstein, M. P., Liao, D., Zhang, N., DeNardo, D. G., Coussens, L. M., Karin, M., Goldrath, A. W., and Johnson, R. S. (2010). Macrophage expression of hypoxia-inducible factor-1 alpha suppresses T-cell function and promotes tumor progression. Cancer Res *70*, 7465-7475.

Elbarghati, L., Murdoch, C., and Lewis, C. E. (2008). Effects of hypoxia on transcription factor expression in human monocytes and macrophages. Immunobiology *213*, 899-908.

Elgert, K. D., Alleva, D. G., and Mullins, D. W. (1998). Tumor-induced immune dysfunction: the macrophage connection. J Leukoc Biol *64*, 275-290.

Ema, M., Taya, S., Yokotani, N., Sogawa, K., Matsuda, Y., and Fujii-Kuriyama, Y. (1997). A novel bHLH-PAS factor with close sequence similarity to hypoxia-inducible factor 1alpha regulates the VEGF expression and is potentially involved in lung and vascular development. Proc Natl Acad Sci U S A *94*, 4273-4278.

Ertel, W., Singh, G., Morrison, M. H., Ayala, A., and Chaudry, I. H. (1993). Chemically induced hypotension increases PGE2 release and depresses macrophage antigen presentation. Am J Physiol *264*, R655-660.

Eubank, T. D., Roda, J. M., Liu, H., O'Neil, T., and Marsh, C. B. (2011). Opposing roles for HIF-1alpha and HIF-2alpha in the regulation of angiogenesis by mononuclear phagocytes. Blood *117*, 323-332.

Fang, H. Y., Hughes, R., Murdoch, C., Coffelt, S. B., Biswas, S. K., Harris, A. L., Johnson, R. S., Imityaz, H. Z., Simon, M. C., Fredlund, E., *et al.* (2009). Hypoxia-inducible factors 1 and 2 are important transcriptional effectors in primary macrophages experiencing hypoxia. Blood *114*, 844-859.

Ferrara, N., and Davis-Smyth, T. (1997). The biology of vascular endothelial growth factor. Endocr Rev *18*, 4-25.

Flamme, I., Frohlich, T., von Reutern, M., Kappel, A., Damert, A., and Risau, W. (1997). HRF, a putative basic helix-loop-helix-PAS-domain transcription factor is closely related to hypoxia-inducible factor-1 alpha and developmentally expressed in blood vessels. Mech Dev *63*, 51-60.

Franklin, C. C., and Kraft, A. S. (1997). Conditional expression of the mitogen-activated protein kinase (MAPK) phosphatase MKP-1 preferentially inhibits p38 MAPK and stress-activated protein kinase in U937 cells. J Biol Chem *272*, 16917-16923.

Freeman, M. R., Schneck, F. X., Gagnon, M. L., Corless, C., Soker, S., Niknejad, K., Peoples, G. E., and Klagsbrun, M. (1995). Peripheral blood T lymphocytes and lymphocytes infiltrating human cancers express vascular endothelial growth factor: a potential role for T cells in angiogenesis. Cancer Res *55*, 4140-4145.

Fujimoto, J., Sakaguchi, H., Aoki, I., and Tamaya, T. (2000). Clinical implications of expression of interleukin 8 related to angiogenesis in uterine cervical cancers. Cancer Res *60*, 2632-2635.

Gerszten, R. E., Garcia-Zepeda, E. A., Lim, Y. C., Yoshida, M., Ding, H. A., Gimbrone, M. A., Jr., Luster, A. D., Luscinskas, F. W., and Rosenzweig, A. (1999). MCP-1 and IL-8 trigger firm adhesion of monocytes to vascular endothelium under flow conditions. Nature *398*, 718-723.

Griffiths, L., Binley, K., Iqball, S., Kan, O., Maxwell, P., Ratcliffe, P., Lewis, C., Harris, A., Kingsman, S., and Naylor, S. (2000). The macrophage - a novel system to deliver gene therapy to pathological hypoxia. Gene Ther *7*, 255-262.

Grimshaw, M. J., and Balkwill, F. R. (2001). Inhibition of monocyte and macrophage chemotaxis by hypoxia and inflammation--a potential mechanism. Eur J Immunol *31*, 480-489.

Grimshaw, M. J., Hagemann, T., Ayhan, A., Gillett, C. E., Binder, C., and Balkwill, F. R. (2004). A role for endothelin-2 and its receptors in breast tumor cell invasion. Cancer Res *64*, 2461-2468.

Grimshaw, M. J., Naylor, S., and Balkwill, F. R. (2002a). Endothelin-2 is a hypoxia-induced autocrine survival factor for breast tumor cells. Mol Cancer Ther *1*, 1273-1281.

Grimshaw, M. J., Wilson, J. L., and Balkwill, F. R. (2002b). Endothelin-2 is a macrophage chemoattractant: implications for macrophage distribution in tumors. Eur J Immunol *32*, 2393-2400.

Gu, Y. Z., Moran, S. M., Hogenesch, J. B., Wartman, L., and Bradfield, C. A. (1998). Molecular characterization and chromosomal localization of a third alpha-class hypoxia inducible factor subunit, HIF3alpha. Gene Expr *7*, 205-213.

Guida, E., and Stewart, A. (1998). Influence of hypoxia and glucose deprivation on tumour necrosis factor-alpha and granulocyte-macrophage colony-stimulating factor expression in human cultured monocytes. Cell Physiol Biochem *8*, 75-88.

Hagemann, T., Robinson, S. C., Schulz, M., Trumper, L., Balkwill, F. R., and Binder, C. (2004). Enhanced invasiveness of breast cancer cell lines upon co-cultivation with macrophages is due to TNF-alpha dependent up-regulation of matrix metalloproteases. Carcinogenesis *25*, 1543-1549.

Hamada, I., Kato, M., Yamasaki, T., Iwabuchi, K., Watanabe, T., Yamada, T., Itoyama, S., Ito, H., and Okada, K. (2002). Clinical effects of tumor-associated macrophages and dendritic cells on renal cell carcinoma. Anticancer Res *22*, 4281-4284.

Hanada, T., Nakagawa, M., Emoto, A., Nomura, T., Nasu, N., and Nomura, Y. (2000). Prognostic value of tumor-associated macrophage count in human bladder cancer. Int J Urol *7*, 263-269.

Harris, A. L. (2002). Hypoxia--a key regulatory factor in tumour growth. Nat Rev Cancer *2*, 38-47.

Heidl, G., Davaris, P., Zwadlo, G., Jagoda, M. S., Duchting, S., Bierhoff, E., Gruter, T., Krieg, V., and Sorg, C. (1987). Association of macrophages detected with monoclonal antibody 25 F 9 with progression and pathobiological classification of gastric carcinoma. J Cancer Res Clin Oncol *113*, 567-572.

Hempel, S. L., Monick, M. M., and Hunninghake, G. W. (1996). Effect of hypoxia on release of IL-1 and TNF by human alveolar macrophages. Am J Respir Cell Mol Biol *14*, 170-176.

Hirani, N., Antonicelli, F., Strieter, R. M., Wiesener, M. S., Ratcliffe, P. J., Haslett, C., and Donnelly, S. C. (2001). The regulation of interleukin-8 by hypoxia in human macrophages--a potential role in the pathogenesis of the acute respiratory distress syndrome (ARDS). Mol Med *7*, 685-697.

Hiratsuka, S., Minowa, O., Kuno, J., Noda, T., and Shibuya, M. (1998). Flt-1 lacking the tyrosine kinase domain is sufficient for normal development and angiogenesis in mice. Proc Natl Acad Sci U S A *95*, 9349-9354.

Janssen, H. L., Haustermans, K. M., Sprong, D., Blommestijn, G., Hofland, I., Hoebers, F. J., Blijweert, E., Raleigh, J. A., Semenza, G. L., Varia, M. A., *et al.* (2002). HIF-1A, pimonidazole, and iododeoxyuridine to estimate hypoxia and perfusion in human head-and-neck tumors. Int J Radiat Oncol Biol Phys *54*, 1537-1549.

Jiang, B. H., Semenza, G. L., Bauer, C., and Marti, H. H. (1996). Hypoxia-inducible factor 1 levels vary exponentially over a physiologically relevant range of O2 tension. Am J Physiol *271*, C1172-1180.

Kaisho, T., and Akira, S. (2006). Toll-like receptor function and signaling. J Allergy Clin Immunol *117*, 979-987; quiz 988.

Kamlah, F., Eul, B. G., Li, S., Lang, N., Marsh, L. M., Seeger, W., Grimminger, F., Rose, F., and Hanze, J. (2009). Intravenous injection of siRNA directed against hypoxia-inducible factors prolongs survival in a Lewis lung carcinoma cancer model. Cancer Gene Ther *16*, 195-205.

Kharbanda, S., Nakamura, T., Stone, R., Hass, R., Bernstein, S., Datta, R., Sukhatme, V. P., and Kufe, D. (1991). Expression of the early growth response 1 and 2 zinc finger genes during induction of monocytic differentiation. J Clin Invest *88*, 571-577.

Kim, M. S., Kwon, H. J., Lee, Y. M., Baek, J. H., Jang, J. E., Lee, S. W., Moon, E. J., Kim, H. S., Lee, S. K., Chung, H. Y., *et al.* (2001). Histone deacetylases induce angiogenesis by negative regulation of tumor suppressor genes. Nat Med *7*, 437-443.

Kim, S. Y., Choi, Y. J., Joung, S. M., Lee, B. H., Jung, Y. S., and Lee, J. Y. (2010). Hypoxic stress up-regulates the expression of Toll-like receptor 4 in macrophages via hypoxia-inducible factor. Immunology *129*, 516-524.

Krishnamachary, B., Berg-Dixon, S., Kelly, B., Agani, F., Feldser, D., Ferreira, G., Iyer, N., LaRusch, J., Pak, B., Taghavi, P., and Semenza, G. L. (2003). Regulation of colon carcinoma cell invasion by hypoxia-inducible factor 1. Cancer Res *63*, 1138-1143.

Lee, A. H., Dublin, E. A., Bobrow, L. G., and Poulsom, R. (1998). Invasive lobular and invasive ductal carcinoma of the breast show distinct patterns of vascular endothelial growth factor expression and angiogenesis. J Pathol *185*, 394-401.

Leek, R. D., Hunt, N. C., Landers, R. J., Lewis, C. E., Royds, J. A., and Harris, A. L. (2000). Macrophage infiltration is associated with VEGF and EGFR expression in breast cancer. J Pathol *190*, 430-436.

Leek, R. D., Landers, R. J., Harris, A. L., and Lewis, C. E. (1999). Necrosis correlates with high vascular density and focal macrophage infiltration in invasive carcinoma of the breast. Br J Cancer *79*, 991-995.

Leek, R. D., Lewis, C. E., Whitehouse, R., Greenall, M., Clarke, J., and Harris, A. L. (1996). Association of macrophage infiltration with angiogenesis and prognosis in invasive breast carcinoma. Cancer Res *56*, 4625-4629.

Leeper-Woodford, S. K., and Mills, J. W. (1992). Phagocytosis and ATP levels in alveolar macrophages during acute hypoxia. Am J Respir Cell Mol Biol *6*, 326-334.

Leung, S. Y., Wong, M. P., Chung, L. P., Chan, A. S., and Yuen, S. T. (1997). Monocyte chemoattractant protein-1 expression and macrophage infiltration in gliomas. Acta Neuropathol *93*, 518-527.

Lewis, J. S., Landers, R. J., Underwood, J. C., Harris, A. L., and Lewis, C. E. (2000). Expression of vascular endothelial growth factor by macrophages is up-regulated in poorly vascularized areas of breast carcinomas. J Pathol *192*, 150-158.

Li, Q. F., Wang, X. R., Yang, Y. W., and Lin, H. (2006). Hypoxia upregulates hypoxia inducible factor (HIF)-3alpha expression in lung epithelial cells: characterization and comparison with HIF-1alpha. Cell Res *16*, 548-558.

Lin, E. Y., Nguyen, A. V., Russell, R. G., and Pollard, J. W. (2001). Colony-stimulating factor 1 promotes progression of mammary tumors to malignancy. J Exp Med *193*, 727-740.

Lissbrant, I. F., Stattin, P., Wikstrom, P., Damber, J. E., Egevad, L., and Bergh, A. (2000). Tumor associated macrophages in human prostate cancer: relation to clinicopathological variables and survival. Int J Oncol *17*, 445-451.

Makino, Y., Cao, R., Svensson, K., Bertilsson, G., Asman, M., Tanaka, H., Cao, Y., Berkenstam, A., and Poellinger, L. (2001). Inhibitory PAS domain protein is a negative regulator of hypoxia-inducible gene expression. Nature *414*, 550-554.

Mantovani, A., Sica, A., Sozzani, S., Allavena, P., Vecchi, A., and Locati, M. (2004). The chemokine system in diverse forms of macrophage activation and polarization. Trends Immunol *25*, 677-686.

Matschurat, S., Knies, U. E., Person, V., Fink, L., Stoelcker, B., Ebenebe, C., Behrensdorf, H. A., Schaper, J., and Clauss, M. (2003). Regulation of EMAP II by hypoxia. Am J Pathol *162*, 93-103.

Movahedi, K., Laoui, D., Gysemans, C., Baeten, M., Stange, G., Van den Bossche, J., Mack, M., Pipeleers, D., In't Veld, P., De Baetselier, P., and Van Ginderachter, J. A. (2010). Different tumor microenvironments contain functionally distinct subsets of macrophages derived from Ly6C(high) monocytes. Cancer Res *70*, 5728-5739.

Murata, Y., Ohteki, T., Koyasu, S., and Hamuro, J. (2002). IFN-gamma and pro-inflammatory cytokine production by antigen-presenting cells is dictated by intracellular thiol redox status regulated by oxygen tension. Eur J Immunol *32*, 2866-2873.

Murdoch, C., Giannoudis, A., and Lewis, C. E. (2004). Mechanisms regulating the recruitment of macrophages into hypoxic areas of tumors and other ischemic tissues. Blood *104*, 2224-2234.

Muthana, M., Giannoudis, A., Scott, S. D., Fang, H. Y., Coffelt, S. B., Morrow, F. J., Murdoch, C., Burton, J., Cross, N., Burke, B., *et al.* (2011). Use of macrophages to target therapeutic adenovirus to human prostate tumors. Cancer Res *71*, 1805-1815.

Negus, R. P., Stamp, G. W., Hadley, J., and Balkwill, F. R. (1997). Quantitative assessment of the leukocyte infiltrate in ovarian cancer and its relationship to the expression of C-C chemokines. Am J Pathol *150*, 1723-1734.

Negus, R. P., Turner, L., Burke, F., and Balkwill, F. R. (1998). Hypoxia down-regulates MCP-1 expression: implications for macrophage distribution in tumors. J Leukoc Biol *63*, 758-765.

Nibbering, P. H., Leijh, P. C., and van Furth, R. (1987). Quantitative immunocytochemical characterization of mononuclear phagocytes. II. Monocytes and tissue macrophages. Immunology *62*, 171-176.

Nicholas, S. A., and Sumbayev, V. V. (2009). The involvement of hypoxia-inducible factor 1 alpha in Toll-like receptor 7/8-mediated inflammatory response. Cell Res *19*, 973-983.

Oda, T., Hirota, K., Nishi, K., Takabuchi, S., Oda, S., Yamada, H., Arai, T., Fukuda, K., Kita, T., Adachi, T., *et al.* (2006). Activation of hypoxia-inducible factor 1 during macrophage differentiation. Am J Physiol Cell Physiol *291*, C104-113.

Ogle, C. K., Wu, J. Z., Mao, X., Szczur, K., Alexander, J. W., and Ogle, J. D. (1994). Heterogeneity of Kupffer cells and splenic, alveolar, and peritoneal macrophages for the production of TNF, IL-1, and IL-6. Inflammation *18*, 511-523.

Ohta, M., Kitadai, Y., Tanaka, S., Yoshihara, M., Yasui, W., Mukaida, N., Haruma, K., and Chayama, K. (2002). Monocyte chemoattractant protein-1 expression correlates with macrophage infiltration and tumor vascularity in human esophageal squamous cell carcinomas. Int J Cancer *102*, 220-224.

Owen, M. R., Stamper, I. J., Muthana, M., Richardson, G. W., Dobson, J., Lewis, C. E., and Byrne, H. M. (2011). Mathematical modeling predicts synergistic antitumor effects of combining a macrophage-based, hypoxia-targeted gene therapy with chemotherapy. Cancer Res *71*, 2826-2837.

Plate, K. H., Breier, G., Weich, H. A., Mennel, H. D., and Risau, W. (1994). Vascular endothelial growth factor and glioma angiogenesis: coordinate induction of VEGF

receptors, distribution of VEGF protein and possible in vivo regulatory mechanisms. Int J Cancer *59*, 520-529.

Plate, K. H., Breier, G., Weich, H. A., and Risau, W. (1992). Vascular endothelial growth factor is a potential tumour angiogenesis factor in human gliomas in vivo. Nature *359*, 845-848.

Raleigh, J. A., Calkins-Adams, D. P., Rinker, L. H., Ballenger, C. A., Weissler, M. C., Fowler, W. C., Jr., Novotny, D. B., and Varia, M. A. (1998). Hypoxia and vascular endothelial growth factor expression in human squamous cell carcinomas using pimonidazole as a hypoxia marker. Cancer Res *58*, 3765-3768.

Raleigh, J. A., Chou, S. C., Bono, E. L., Thrall, D. E., and Varia, M. A. (2001). Semiquantitative immunohistochemical analysis for hypoxia in human tumors. Int J Radiat Oncol Biol Phys *49*, 569-574.

Rius, J., Guma, M., Schachtrup, C., Akassoglou, K., Zinkernagel, A. S., Nizet, V., Johnson, R. S., Haddad, G. G., and Karin, M. (2008). NF-kappaB links innate immunity to the hypoxic response through transcriptional regulation of HIF-1alpha. Nature *453*, 807-811.

Rolny, C., Mazzone, M., Tugues, S., Laoui, D., Johansson, I., Coulon, C., Squadrito, M. L., Segura, I., Li, X., Knevels, E., *et al.* (2011). HRG inhibits tumor growth and metastasis by inducing macrophage polarization and vessel normalization through downregulation of PlGF. Cancer Cell *19*, 31-44.

Said, N., Smith, S., Sanchez-Carbayo, M., and Theodorescu, D. (2011). Tumor endothelin-1 enhances metastatic colonization of the lung in mouse xenograft models of bladder cancer. J Clin Invest *121*, 132-147.

Salvesen, H. B., and Akslen, L. A. (1999). Significance of tumour-associated macrophages, vascular endothelial growth factor and thrombospondin-1 expression for tumour angiogenesis and prognosis in endometrial carcinomas. Int J Cancer *84*, 538-543.

Sawano, A., Iwai, S., Sakurai, Y., Ito, M., Shitara, K., Nakahata, T., and Shibuya, M. (2001). Flt-1, vascular endothelial growth factor receptor 1, is a novel cell surface marker for the lineage of monocyte-macrophages in humans. Blood *97*, 785-791.

Scannell, G., Waxman, K., Kaml, G. J., Ioli, G., Gatanaga, T., Yamamoto, R., and Granger, G. A. (1993). Hypoxia induces a human macrophage cell line to release tumor necrosis factor-alpha and its soluble receptors in vitro. J Surg Res *54*, 281-285.

Schioppa, T., Uranchimeg, B., Saccani, A., Biswas, S. K., Doni, A., Rapisarda, A., Bernasconi, S., Saccani, S., Nebuloni, M., Vago, L., *et al.* (2003). Regulation of the chemokine receptor CXCR4 by hypoxia. J Exp Med *198*, 1391-1402.

Schmeisser, A., Marquetant, R., Illmer, T., Graffy, C., Garlichs, C. D., Bockler, D., Menschikowski, D., Braun-Dullaeus, R., Daniel, W. G., and Strasser, R. H. (2005). The expression of macrophage migration inhibitory factor 1alpha (MIF 1alpha) in human atherosclerotic plaques is induced by different proatherogenic stimuli and associated with plaque instability. Atherosclerosis *178*, 83-94.

Scotton, C., Milliken, D., Wilson, J., Raju, S., and Balkwill, F. (2001). Analysis of CC chemokine and chemokine receptor expression in solid ovarian tumours. Br J Cancer *85*, 891-897.

Semenza, G. (2002). Signal transduction to hypoxia-inducible factor 1. Biochem Pharmacol *64*, 993-998.

Semenza, G. L. (2003). Targeting HIF-1 for cancer therapy. Nat Rev Cancer *3*, 721-732.

Semenza, G. L., and Wang, G. L. (1992). A nuclear factor induced by hypoxia via de novo protein synthesis binds to the human erythropoietin gene enhancer at a site required for transcriptional activation. Mol Cell Biol *12*, 5447-5454.

Shannon, A. M., Bouchier-Hayes, D. J., Condron, C. M., and Toomey, D. (2003). Tumour hypoxia, chemotherapeutic resistance and hypoxia-related therapies. Cancer Treat Rev *29*, 297-307.

Sica, A., Saccani, A., Bottazzi, B., Bernasconi, S., Allavena, P., Gaetano, B., Fei, F., LaRosa, G., Scotton, C., Balkwill, F., and Mantovani, A. (2000). Defective expression of the monocyte chemotactic protein-1 receptor CCR2 in macrophages associated with human ovarian carcinoma. J Immunol *164*, 733-738.

Stessels, F., Van den Eynden, G., Van der Auwera, I., Salgado, R., Van den Heuvel, E., Harris, A. L., Jackson, D. G., Colpaert, C. G., van Marck, E. A., Dirix, L. Y., and Vermeulen, P. B. (2004). Breast adenocarcinoma liver metastases, in contrast to colorectal cancer liver metastases, display a non-angiogenic growth pattern that preserves the stroma and lacks hypoxia. Br J Cancer *90*, 1429-1436.

Strieter, R. M., Belperio, J. A., Phillips, R. J., and Keane, M. P. (2004). Chemokines: angiogenesis and metastases in lung cancer. Novartis Found Symp *256*, 173-184; discussion 184-178, 259-169.

Sumbayev, V. V. (2008). LPS-induced Toll-like receptor 4 signalling triggers cross-talk of apoptosis signal-regulating kinase 1 (ASK1) and HIF-1alpha protein. FEBS Lett *582*, 319-326.

Sun, B., Nishihira, J., Yoshiki, T., Kondo, M., Sato, Y., Sasaki, F., and Todo, S. (2005). Macrophage migration inhibitory factor promotes tumor invasion and metastasis via the Rho-dependent pathway. Clin Cancer Res *11*, 1050-1058.

Sun, H., Charles, C. H., Lau, L. F., and Tonks, N. K. (1993). MKP-1 (3CH134), an immediate early gene product, is a dual specificity phosphatase that dephosphorylates MAP kinase in vivo. Cell *75*, 487-493.

Talks, K. L., Turley, H., Gatter, K. C., Maxwell, P. H., Pugh, C. W., Ratcliffe, P. J., and Harris, A. L. (2000). The expression and distribution of the hypoxia-inducible factors HIF-1alpha and HIF-2alpha in normal human tissues, cancers, and tumor-associated macrophages. Am J Pathol *157*, 411-421.

Tian, H., McKnight, S. L., and Russell, D. W. (1997). Endothelial PAS domain protein 1 (EPAS1), a transcription factor selectively expressed in endothelial cells. Genes Dev *11*, 72-82.

Toi, M., Hoshina, S., Takayanagi, T., and Tominaga, T. (1994). Association of vascular endothelial growth factor expression with tumor angiogenesis and with early relapse in primary breast cancer. Jpn J Cancer Res *85*, 1045-1049.

Ueno, T., Toi, M., Saji, H., Muta, M., Bando, H., Kuroi, K., Koike, M., Inadera, H., and Matsushima, K. (2000). Significance of macrophage chemoattractant protein-1 in macrophage recruitment, angiogenesis, and survival in human breast cancer. Clin Cancer Res *6*, 3282-3289.

van Furth, R. (1980). Monocyte origin of Kupffer cells. Blood Cells *6*, 87-92.

Vaupel, P., Kallinowski, F., and Okunieff, P. (1989). Blood flow, oxygen and nutrient supply, and metabolic microenvironment of human tumors: a review. Cancer Res *49*, 6449-6465.

Vaupel, P., Kelleher, D. K., and Hockel, M. (2001). Oxygen status of malignant tumors: pathogenesis of hypoxia and significance for tumor therapy. Semin Oncol *28*, 29-35.

Vaupel, P., Mayer, A., Briest, S., and Hockel, M. (2005). Hypoxia in breast cancer: role of blood flow, oxygen diffusion distances, and anemia in the development of oxygen depletion. Adv Exp Med Biol *566*, 333-342.

Venneri, M. A., De Palma, M., Ponzoni, M., Pucci, F., Scielzo, C., Zonari, E., Mazzieri, R., Doglioni, C., and Naldini, L. (2007). Identification of proangiogenic TIE2-expressing monocytes (TEMs) in human peripheral blood and cancer. Blood *109*, 5276-5285.

Versteeg, H. H., Spek, C. A., Peppelenbosch, M. P., and Richel, D. J. (2004). Tissue factor and cancer metastasis: the role of intracellular and extracellular signaling pathways. Mol Med *10*, 6-11.

Vicari, A. P., and Caux, C. (2002). Chemokines in cancer. Cytokine Growth Factor Rev *13*, 143-154.

Wain, J. H., Kirby, J. A., and Ali, S. (2002). Leucocyte chemotaxis: Examination of mitogen-activated protein kinase and phosphoinositide 3-kinase activation by Monocyte Chemoattractant Proteins-1, -2, -3 and -4. Clin Exp Immunol *127*, 436-444.

Wang, S. C., Hong, J. H., Hsueh, C., and Chiang, C. S. (2012). Tumor-secreted SDF-1 promotes glioma invasiveness and TAM tropism toward hypoxia in a murine astrocytoma model. Lab Invest *92*, 151-162.

Welford, A. F., Biziato, D., Coffelt, S. B., Nucera, S., Fisher, M., Pucci, F., Di Serio, C., Naldini, L., De Palma, M., Tozer, G. M., and Lewis, C. E. (2011). TIE2-expressing macrophages limit the therapeutic efficacy of the vascular-disrupting agent combretastatin A4 phosphate in mice. J Clin Invest *121*, 1969-1973.

White, J. R., Harris, R. A., Lee, S. R., Craigon, M. H., Binley, K., Price, T., Beard, G. L., Mundy, C. R., and Naylor, S. (2004). Genetic amplification of the transcriptional response to hypoxia as a novel means of identifying regulators of angiogenesis. Genomics *83*, 1-8.

Wykoff, C. C., Pugh, C. W., Maxwell, P. H., Harris, A. L., and Ratcliffe, P. J. (2000). Identification of novel hypoxia dependent and independent target genes of the von Hippel-Lindau (VHL) tumour suppressor by mRNA differential expression profiling. Oncogene *19*, 6297-6305.

Yan, S. F., Lu, J., Zou, Y. S., Soh-Won, J., Cohen, D. M., Buttrick, P. M., Cooper, D. R., Steinberg, S. F., Mackman, N., Pinsky, D. J., and Stern, D. M. (1999). Hypoxia-associated induction of early growth response-1 gene expression. J Biol Chem *274*, 15030-15040.

Zhang, A., Meng, L., Wang, Q., Xi, L., Chen, G., Wang, S., Zhou, J., Lu, Y., and Ma, D. (2006). Enhanced in vitro invasiveness of ovarian cancer cells through up-regulation of VEGF and induction of MMP-2. Oncol Rep *15*, 831-836.

Zhang, F. R., and Schwarz, M. A. (2002). Pro-EMAP II is not primarily cleaved by caspase-3 and -7. Am J Physiol Lung Cell Mol Physiol *282*, L1239-1244.
Zhang, X., and Mosser, D. M. (2008). Macrophage activation by endogenous danger signals. J Pathol *214*, 161-178.

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Tumor microenvironment represents an extremely dynamic niche shaped by the interplay of different cell types (e.g. tumor cells, stromal cells), their soluble products (e.g.cytokines, chemokines and growth factors) and varied physico-chemical conditions (e.g low oxygen concentration or hypoxia). Recent studies have identified myelomonocytic cells as key players in regulating the tumor microenvironment and hence, tumor progression in a variety of cancers. In view of these findings, the present book attemps to provide a comprehensive account of the diversity of tumor microenvironment across different cancers and how myelomonocytic cells have taken the center-stage in regulating this niche to direct cancer progression. A better understanding of the myelomonocytic cells and the mechanisms by which they regulate cancer progression will open new vistas in cancer therapeutics.

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