Abstract

Tumor-associated macrophages (TAM) have been linked with the progression of cancer by favoring tumor angiogenesis, growth, and metastasis. The precise mechanisms that maintain the protumor phenotype of TAM are poorly understood, but recent research has highlighted a number of signaling pathways that are important in TAM phenotype and function. Nuclear factor-κB (NF-κB) is considered the master regulator of inflammatory and immune responses. Recently several genetic studies have indicated NF-κB is an important pathway in TAM for the integration of signals from the tumor microenvironment that promote carcinogenesis. This review will focus on the role of NF-κB in TAM and the potential of targeting this pathway as a novel therapeutic strategy against cancer. Clin Cancer Res; 16(3); 784–9. ©2010 AACR.

Background

There is now strong evidence that mediators and cellular effectors of inflammatory response are significant collaborators in carcinogenesis (1). In particular tumor-associated macrophages (TAM) have emerged as a critical component of the inflammatory microenvironment in tumors linked with tumor progression (2). TAM are recruited into tumors as monocytes from the bloodstream by chemotactic cytokines and growth factors such as: CCL2 (MCP-1), M-CSF (CSF-1), vascular endothelial growth factor (VEGF), angiopoietin-2, and CXCL12 (SDF1), released by both malignant and stromal tumor compartments (1, 3, 4). TAM acquire a specific phenotype that is oriented toward tumor growth, angiogenesis, and immune-suppression (5, 6), and many studies have shown a positive correlation between the number of TAM and poor prognosis in human tumors, including breast, prostate, and bladder cancer (3). Furthermore, blockade of TAM recruitment, for example by the genetic deletion of CSF-1, blocks tumor growth, angiogenesis, and metastasis in experimental models of cancer (7).

Nuclear factor-κB (NF-κB) has been shown as an important transcription factor regulating macrophage activation in response to diverse environmental cues, including stress signals, inflammatory cytokines, and infection (8). NF-κB has recently been shown to be particularly important in driving cancer-related inflammation in mouse models of gastrointestinal and liver cancer; NF-κB activation in myeloid cells was shown to be required for the tumor-promoting action of inflammation in colitis-associated cancer (CAC) and chemically induced hepatocellular carcinoma (HCC; refs. 8–12). We have also shown NF-κB maintains the tumor-promoting phenotype of TAM in a model of ovarian cancer (13).

This review will describe the role of NF-κB in TAM phenotype and function, and we will discuss the potential benefits of targeting this pathway in cancer therapy.

TAM. Macrophages are a very plastic cell lineage and acquire several functionally distinct phenotypes depending on the physiological context (14). In inflammation and cancer two particular macrophage phenotypes have been described: “classically” activated or M1 macrophages are pro-inflammatory and characterized by increased production of pro-inflammatory cytokines, reactive nitrogen and oxygen intermediates (RNI/ROI), and high microbicidal or tumoricidal activity. “Alternatively” activated or M2 macrophages, in contrast, are immunosuppressive and produce anti-inflammatory cytokines including interleukin (IL)-10 and transforming growth factor β (TGFβ); they support angiogenesis, tissue repair, and remodeling (6, 15).

Several studies have shown tumor-associated macrophages (TAM) have a M2-like phenotype; they are poor producers of RNI and ROI related to reduced cytotoxic activity, express low levels of pro-inflammatory cytokines, particularly IL-12, and high levels of IL-10 and TGFβ, they are also poor antigen-presenting cells (6). The M2 phenotype of TAM is associated with increased angiogenesis and metastasis, through expression of VEGF, COX2, epidermal growth factor receptor (EGFR), and matrix metalloproteases (MMP; refs. 2, 3, 5). Clinical studies have shown increased numbers of TAM frequently correlate with angiogenesis, metastasis, and poor prognosis. Elegant work has shown experimentally that macrophage depletion results in a slower rate of progression and fewer pulmonary metastases in a spontaneous mouse model of mammary carcinoma, further studies in this model showed
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NF-κB and TAM. Several studies in mouse models of inflammation-associated cancer show a tumor-promoting role for NF-κB activation in TAM linked to production of pro-inflammatory cytokines including TNFα and IL-6 (Fig. 1). These studies in HCC and CAC suggest a pro-inflammatory role for TAM regulated by NF-κB; this is in contrast to the anti-inflammatory M2 phenotype of TAM described in other models (5, 6, 32). As described previously, NF-κB activation in macrophages has been shown to have anti-inflammatory roles in models of inflammation and infection (27–29, 31). This finding would also conflict with the proposed pro-inflammatory role for NF-κB activation in TAM. It has to be noted that the phenotype of TAM can differ markedly between different cancers (3) and stages of tumor progression (15), it is therefore difficult to extrapolate from one model to another, however it is clear that NF-κB activation in TAM has a role in tumor promotion (20), but is this attributed to pro-or anti-inflammatory roles for NF-κB in macrophages? For example, defective NF-κB function has been shown recently in TAM from chemically induced murine fibrosarcomas associated with a M2 phenotype in this model (32, 33). This defect was attributed to the overexpression of nuclear p50 resulting in formation of p50 homo-dimers responsible for the inhibition of the transcription of pro-inflammatory NF-κB-dependent genes including TNFα and IL-12p40 (Fig. 1). TAM from p50-deficient mice regained a pro-inflammatory (M1) phenotype associated with reduced tumor growth; thought to be attributed to restored canonical NF-κB activity. NF-κB activation has also been associated with tumor regression in a mouse mammary carcinoma model (34). The TLR9 ligand CpG was used to activate NF-κB in combination with an IL-10 receptor antibody and the chemokine CCL16; TAM were redirected toward antitumor functions, acquiring a M1 phenotype. By contrast, we recently showed that targeting IKKβ in TAM isolated from established ovarian cancers increased their tumorigenic activity and switched their tumor-promoting M2 phenotype toward a M1 activation (13). These studies showed the requirement for IKKβ/NF-κB activation to maintain the IL-10high/IL-12low phenotype of TAM in this model. Targeting IKKβ in TAM (13) and macrophages during infection (28) also associated with increased activation of STAT-1, which is critical for IL-12, NOS2, and MHC II expression (Fig. 1), hallmarks of M1 macrophage activation.

These observations were obtained in TAM isolated from tumors in advanced stages and are in contrast to the studies using spontaneous HCC and CAC models described above (9–12, 35). The dynamic changes in the tumor microenvironment during the transition from early to advanced cancer could result in fluctuations in TAM phenotype between M1 and M2 (6).

It is likely both M1 and M2 populations exist within advanced cancers; necrotic areas would be associated
with activation of M1 macrophages, whereas angiogenesis and proliferation may be associated with higher numbers of M2 macrophages (3), the balance between these different macrophage phenotypes can clearly impact tumor progression and the molecular mechanisms involved may represent important therapeutic targets.

Mechanisms regulating NF-κB activation in TAM. The complex role of NF-κB in TAM may arise from the pro- and anti-inflammatory functions of this pathway in macrophages (20). It is generally believed that NF-κB drives pro-inflammatory gene expression, but recent studies have shown NF-κB can also have anti-inflammatory functions in macrophages (27, 29). It is apparent the outcome of NF-κB activation may depend both on the nature of the stimulus and the physiological context.

There is a complicated network of cytokines and other factors released by both malignant cells and TAM that drive tumor progression (6). Notably this network includes pro-inflammatory signals for recruitment and activation of myeloid cells as well as factors that promote the M2 phenotype of TAM. TLR and IL-1 receptor (IL-1R) signaling are important triggers for NF-κB activation. TLR/IL-1R signaling also triggers MAPK and AP-1 activation, coregulating the expression of pro-inflammatory and anti-inflammatory genes. TLR4 also triggers a MyD88-independent pathway, through the adaptor molecule TRIF, to induce the phosphorylation of TBK and the downstream activation of IRF3, which regulates the transcription of type I interferons (IFN). M1 macrophages are characterized by IFN-mediated expression of p50, which negatively regulates NF-κB dependent pro-inflammatory gene transcription. M2 macrophages produce IFN-α/β, which antagonize NF-κB activation, and NF-κB was also recently shown to inhibit STAT1 activity.

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Fig. 1. Signaling pathways regulating TAM phenotype and function. Various factors have been suggested to activate the protumor phenotype of TAM; ECM components, such as hyaluronan and versican, pro-inflammatory cytokines IL-1 and TNFα, and tumor hypoxia activate both HIF-1 and NF-κB. Collectively these signaling pathways favor the transcription of genes promoting angiogenesis, malignant cell survival, invasion, and metastasis. Engagement of TLRs or IL-1R triggers expression of pro-inflammatory cytokines and anti-apoptotic genes through the adaptor protein MyD88 and classical NF-κB activation. TLR/IL-1R signaling also triggers MAPK and AP-1 activation, coregulating the expression of pro-inflammatory and anti-inflammatory genes. TLR4 also triggers a MyD88-independent pathway, through the adaptor molecule TRIF, to induce the phosphorylation of TBK and the downstream activation of IRF3, which regulates the transcription of type I interferons (IFN). M1 macrophages are characterized by IFN-mediated expression of p50, which negatively regulates NF-κB dependent pro-inflammatory gene transcription. M2 macrophages produce IFN-α/β, which antagonize NF-κB activation, and NF-κB was also recently shown to inhibit STAT1 activity.
macrophages in the context of infection and inflammation, and several tumor-derived signals have also been shown to activate these pathways in TAM (36). Voronov and colleagues (37) showed IL-1β was involved in the production of protumor factors, including VEGF, IL-6, and TNFα, in cocultures of macrophages and B16 melanoma cells. Our studies have also shown macrophages from IL-1R-deficient mice failed to promote tumor growth in a model of ovarian cancer, and IL-1-signaling seemed to be important to maintain the M2 phenotype in this model (13). As described above, MyD88 signaling (a critical adaptor for TLR/IL-1R) is also linked with IL-6 and TNFα production in HCC and CAC (35, 38). Moreover, a recent study from Karin and colleagues showed the extracellular matrix (ECM) proteoglycan versican increased metastatic growth of Lewis lung carcinoma, by inducing TNF production in TAM through TLR2 (39). Activation of TLRs on TAM by other ligands, including hyaluronan fragments (40) or heat shock proteins (41), can render them immunosuppressive, which may also contribute to tumor progression.

Another facet of the tumor microenvironment linked to NF-κB signaling in TAM is hypoxia (Fig. 1). It has been shown that TAM preferentially localize to poorly vascularized regions of tumors (3, 42), and high TAM density in these sites correlates with enhanced tumor angiogenesis and poor prognosis in breast cancer patients (42). Hypoxia induces a profound change in the phenotype of TAM through the activation of hypoxia-inducible factor (HIF) 1 and 2 (43, 44). Macrophages respond to hypoxic conditions by up-regulating an array of genes that promote proliferation, invasion, and metastasis of malignant cells and tumor angiogenesis, these include: FGF-2, PDGF, HGF, EGF, VEGF, and MMP9 (45). Hypoxia-mediated induction of NF-κB was reported more than a decade ago (46), and recently new evidence has confirmed NF-κB activity is modulated by hypoxia and contributes to cell adaptation to hypoxic conditions (47, 48). Cummins and colleagues (49) reported in the THP-1 monocytic cell line that hypoxia stimulated NF-κB activity by increasing IKKβ levels. More recently, it was shown that NF-κB-mediated HIF-1α mRNA expression, in response to hypoxia, was blocked in IKKβ-deficient macrophages (50). Activation of NF-κB by pro-inflammatory stimuli has also been shown to induce HIF activation; Frede and colleagues (51) showed the TLR4 agonist LPS induced HIF-1α in human monocytes and macrophages under normoxic conditions, through p44/42 mitogen-activated protein kinase (MAPK) signaling and NF-κB activation. Moreover, a synergistic induction of HIF-1α protein and transcriptional activity by hypoxia and LPS in macrophages has been described (52).

Many different factors have been suggested to be important in maintaining the protumor phenotype of TAM and several of these can also be linked to NF-κB activation. But further studies are required to determine a hierarchy for these factors and what the critical mediators or pathways are that can be targeted to block the tumor-promoting functions of these cells and possibly restore antitumor activity.

**TAM as targets in cancer therapy.** Given the strong association between macrophages and cancer progression TAM have emerged as a target in the battle against cancer, and in particular the concept to restore the antitumor activity of TAM and fight the cancer from within.

As mentioned above, NF-κB activation in macrophages has been shown to contribute to carcinogenesis in several models of inflammation-associated cancer (8–12). Maeda and colleagues (11) showed, in DEN-induced HCC, that targeted deletion of IKKα in myeloid cells resulted in a marked reduction in tumor onset and burden. It was also shown in a genetic model of HCC, that inhibition of NF-κB activation through overexpression of IκBα reduced tumor formation that was attributed to a tumor-promoting role for TNFα (12). Naugler and colleagues (35) showed the TLR adaptor protein MyD88 is required upstream of NF-κB activation in DEN-HCC for IL-6 expression by TAM, and IL-6 production was linked to tumor growth. These studies also suggested that hepatocyte necrosis stimulated pro-inflammatory cytokine production by liver macrophages [Kupffer cells (KC)] during tumor development. Maeda and colleagues (11) and Luedde and colleagues (10) have shown that inhibition of NF-κB by targeted deletion of IKKβ or NEMO in hepatocytes triggered apoptosis that was linked to TNFα and IL-6 production by KC. However, hepatocyte apoptosis was shown to increase tumor-growth through compensatory proliferation, possibly driven by TNFα and IL-6 produced by KC activation. These studies illustrate the delicate balance between the cytoprotective roles of NF-κB in malignant cells and the activation of NF-κB in tumor-associated stromal cells including TAM. IKKβ deletion in myeloid cells has also been shown to reduce growth of tumors in CAC (9). Significantly, the initiation of carcinogenesis and mutation of the tumor-suppressor β-catenin in intestinal epithelial cells was not affected, indicating the tumor-promoting role for NF-κB activation in myeloid cells in this model (9). However, these studies did show the anti-apoptotic role of NF-κB in malignant epithelial cells was linked to initiation of carcinogenesis.

Several existing anticancer agents, such as paclitaxel, have been proposed to act at least in part to inhibit TAM recruitment or function (53–55). Interestingly, most of these agents activate TAM to produce pro-inflammatory mediators such as TNFα, IL-1β, and NOS2-derived nitric oxide (NO), by activating NF-κB. However, as with all current anticancer treatments, due to a lack of specificity, there are serious side effects associated with these agents (56). Conversely, strategies to target NF-κB activation in TAM are also likely to show success on the basis of evidence from experimental models (9–11, 13), indeed targeting NF-κB activation in both malignant cells and TAM may be of added benefit due to the role of NF-κB in malignant cell survival (9–11). However, studies in mouse DEN-HCC also showed increased hepatocyte apoptosis provided a tumor-promoting signal to liver macrophages through NF-κB (10, 35), suggesting at least in this model that specific targeting of NF-κB in TAM may be more beneficial.
Systemically targeting NF-κB may also have adverse effects given its fundamental role in innate immunity (22), but specific targeting of NF-κB in macrophages has been shown to increase immunity to infection associated with activation of M1 macrophages (27), again suggesting the specific targeting of NF-κB in TAM would avoid these adverse effects.

In summary, NF-κB is reported to be constitutively activated in transformed cells in many tumors and contribute to cell survival (57), however, a multifaceted role has been described for NF-κB in TAM. NF-κB activation can drive a pro-inflammatory phenotype of TAM during early stages of tumor initiation and growth. IKKβ deletion in TAM reduced growth of liver (9, 10), colon (9), and ovarian cancer (13) in mice, although in a model of fibrosarcoma, defective NF-κB activity in TAM was associated with tumor progression (32, 33). Notably, although NF-κB activation in TAM was associated with a pro-inflammatory phenotype in ICC and CAC (9, 35), in ovarian cancer NF-κB activity was linked with an anti-inflammatory M2 phenotype (13), which was paralleled in models of infection (27). The plasticity of TAM phenotype and the complex role of NF-κB in macrophage biology have generated a somewhat muddled picture of the specific role NF-κB plays in inflammation-associated cancer. Nevertheless, there is clearly great potential in targeting NF-κB to block the tumor-promoting roles of TAM in cancer and possibly restoring their intrinsic antitumor activity. However, it is highly unlikely such a strategy will have a significant impact on advanced cancer alone, but in combination with conventional therapeutic approaches “re-educating” TAM by targeting NF-κB may have an impact in the clinic.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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Alessandra Mancino and Toby Lawrence


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