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NF- κ B and tumor-associated macrophages

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Abstract

Tumor-associated macrophages (TAM) have been linked with progression of cancer by favoring tumor angiogenesis, growth and metastasis. The precise mechanisms that maintain the pro-tumor 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- κ B (NF- κ B) is considered the master regulator of inflammatory and immune responses. Recently several genetic studies have indicated NF- κ B is an important pathway in TAM for the integration of signals from tumor microenvironment that promote carcinogenesis. This review will focus on the role of NF- κ B in TAM and the potential of targeting this pathway as novel therapeutic strategy against cancer.

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), 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 towards 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- κ B (NF- κ B) has been demonstrated as an important transcription factor regulating macrophage activation in response to diverse environmental cues, including stress signals, inflammatory cytokines and infection (8). NF- κ B has recently been shown to be particularly important in driving cancer-related inflammation in mouse models of gastrointestinal and liver cancer; NF- κ B activation in myeloid cells was required for the tumor-promoting action of inflammation in colitis-associated cancer (CAC) and chemically-

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induced hepatocellular carcinoma (HCC) (8-12). We have also shown NF- κ B maintains the tumor-promoting phenotype of TAM in a model of ovarian cancer (13).

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

Tumor-associated macrophages (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 IL-10 and TGF β ; 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 TGF β , 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, EGFR and MMPs (2, 3, 5). Clinical studies have shown increased numbers of TAM frequently correlates with angiogenesis, metastasis and poor prognosis. Elegant work has demonstrated 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 TAM have a crucial role in the 'angiogenic switch' when hyperplastic lesions develop into early stage carcinoma (2, 7, 16, 17). Other studies have shown depletion of TAM in mice bearing F9 teratocarcinoma or human A673 rhabdomyosarcoma xenografts resulted in reduced tumor growth and angiogenesis (18). There is also increasing evidence TAM contribute to suppression of anti-tumor immune responses, in particular the M2-phenotype of TAM is associated with increased expression of arginase 1 and indoleamine 2,3-dioxygenase (IDO) that inhibit T cell proliferation, as well as immunosuppressive cytokines IL-10 and TGF β (5, 6). Recent studies have shown macrophage depletion in HPV16-associated cervical cancer reduced tumor growth and restored anti-tumor T cell responses (19), suggesting TAM-mediated immune suppression contributes to tumour progression.

Nuclear Factor- κ B (NF- κ B)

Analysis of the molecular basis of the TAM phenotype has indicated the NF- κ B pathway is an important regulator of TAM transcriptional programs (20). NF- κ B is a generic term for a family of transcription factors that play pivotal roles in inflammation and immunity (21). The family consists of five members: NF- κ B1 (p105/p50), NF- κ B 2 (p100/p52), RelA (p65), RelB and c-Rel (22). Recent studies have described two separate pathways for NF- κ B activation: the 'canonical' pathway is triggered by microbial products and pro-inflammatory cytokines, such as TNF α , IL-1 β and most commonly leads to the activation of RelA-p50

complexes; the ‘alternative’ pathway (23) is activated by lymphotoxin β (LT β) (24), CD40 ligand (CD40L) (23), B cell activating factor (BAFF) (25) and RANK ligand (RANKL) (26) and results in activation of RelB-p52. NF- κ B proteins are usually held inactive in the cytoplasm of resting cells by association with I κ B (Inhibitor of NF- κ B) proteins, which upon stimulation are phosphorylated by I κ B kinase complex (IKK). IKK consists of two active kinase subunits: IKK α (IKK1) and IKK β (IKK2), and a regulatory subunit IKK γ (NEMO). The canonical pathway for NF- κ B activation requires IKK β -mediated I κ B α phosphorylation, whereas the alternative pathway is regulated by IKK α -mediated phosphorylation and processing of p100 (25).

Although NF- κ B is considered a major pro-inflammatory transcription factor, recent studies have shown that NF- κ B activation is not necessarily pro-inflammatory and also regulates anti-inflammatory pathways. We demonstrated the pro- and anti-inflammatory roles of NF- κ B in acute inflammation and bacterial infection by tissue specific deletion of IKK β (27, 28); these studies showed inflammation was inhibited in mice lacking IKK β in lung epithelial cells but deletion of IKK β in myeloid cells increased inflammation. Greten et al. (29) also recently showed an anti-inflammatory role for IKK β in myeloid cells in a model of LPS-induced septic shock. Several other studies have also described anti-inflammatory roles for NF- κ B in inflammation (30, 31).

Clinical-Translational Advances

NF- κ B and TAM

Several studies in mouse models of inflammation-associated cancer show a tumor-promoting role for NF- κ B activation in TAM linked to production of pro-inflammatory cytokines including TNF α and IL-6 (Figure 1). These studies in HCC and CAC suggest a pro-inflammatory role for TAM regulated by NF- κ B; this is in contrast to the anti-inflammatory M2 phenotype of TAM described in other models (5, 6, 32). As described previously, NF- κ B activation in macrophages has been shown to have anti-inflammatory roles in models of inflammation and infection (27-29, 31). This would also conflict with the proposed pro-inflammatory role for NF- κ B 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 studies from one model to another, however it is clear that NF- κ B activation in TAM has a role in tumor promotion (20), but is this attributed to pro- or anti-inflammatory roles for NF- κ B in macrophages? For example, defective NF- κ B function has been demonstrated recently in TAM from chemically-induced murine fibrosarcomas associated with a M2 phenotype in this model (32, 33). This defect was attributed to the over-expression of nuclear p50 resulting in formation of p50 homo-dimers responsible for the inhibition of the transcription of pro-inflammatory NF- κ B-dependent genes including TNF α and IL-12p40 (Figure 1). TAM from p50 deficient mice re-gained a pro-inflammatory (M1) phenotype associated with reduced tumor growth; thought to be attributed to restored canonical NF- κ B activity. NF- κ B activation has also been associated with tumor regression in a mouse mammary carcinoma model (34). The TLR9 ligand CpG was used to activate NF- κ B in combination with an IL-10 receptor antibody and the chemokine CCL16, TAM were redirected towards anti-tumor functions, acquiring a M1

phenotype. By contrast, we recently showed that targeting IKK β in TAM isolated from established ovarian cancers increased their tumoricidal activity and switched their tumor-promoting M2 phenotype towards a M1 activation (13). These studies demonstrated the requirement for IKK β /NF- κ B activation to maintain the IL-10^{high}/IL-12^{low} phenotype of TAM in this model. Targeting IKK β 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 (Figure 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 tumour progression and the molecular mechanisms involved may represent important therapeutic targets.

Mechanisms regulating NF- κ B activation in TAM—The complex role of NF- κ B in TAM may arise from the pro- and anti-inflammatory functions of this pathway in macrophages (20). It is generally believed that NF- κ B drives pro-inflammatory gene expression, but recent studies have shown NF- κ B can also have anti-inflammatory functions in macrophages (27, 29). It is apparent the outcome of NF- κ B 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 but also factors that promote the M2 phenotype of TAM. TLR and IL-1R signaling are important triggers for NF- κ B activation in 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 et al. (37) showed IL-1 β was involved in the production of pro-tumor factors, including VEGF, IL-6 and TNF α , in co-cultures 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-signalling appeared 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 TNF α 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 (LLC), by inducing TNF production in TAM through TLR2 (41). Activation of TLRs on TAM by other ligands, including hyaluronan (HA) fragments (39) or heat shock proteins (HSP) (40), can render them immunosuppressive which may also contribute to tumor progression.

Another facet of the tumor microenvironment linked to NF- κ B signaling in TAM is hypoxia (Figure 1). It has been shown that TAM preferentially localize to poorly vascularised 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- κ B was reported over a decade ago (46), and recently new evidence has confirmed NF- κ B activity is modulated by hypoxia and contributes to cell adaptation to hypoxic conditions (47, 48). Cummins et al. (49) reported in the THP-1 monocytic cell line that hypoxia stimulated NF- κ B activity by increasing IKK β levels. More recently, it was shown that NF- κ B-mediated HIF-1 α mRNA expression, in response to hypoxia, was blocked in IKK β deficient macrophages (50). Activation of NF- κ B by pro-inflammatory stimuli has also been shown to induce HIF activation; Frede et al. (51) showed the TLR4-agonist LPS induced HIF-1 α in human monocytes and macrophages under normoxic conditions, through p44/42 MAPK signaling and NF- κ B activation. Moreover, a synergistic induction of HIF-1 α 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 pro-tumor phenotype of TAM and several of these can also be linked to NF- κ B 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 anti-tumor 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 anti-tumor activity of TAM and fight the cancer from within.

As mentioned above, NF- κ B activation in macrophages has been shown to contribute to carcinogenesis in several models of inflammation-associated cancer (8-12). Maeda et al. (11) demonstrated, in DEN-induced hepatocellular carcinoma (HCC), that targeted deletion of IKK β 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- κ B activation through over-expression of I κ B α reduced tumor formation that was attributed to a tumor-promoting role for TNF α . (12). Naugler and colleagues (35) demonstrated the TLR adaptor protein MyD88, is required upstream of NF- κ B 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 et al. (11) and Luedde et al. (10) have demonstrated that inhibition of NF- κ B by targeted deletion of IKK β or NEMO in hepatocytes triggered apoptosis that was linked to TNF α and IL-6 production by KC. However, hepatocyte apoptosis was shown to increase tumor-growth through compensatory

proliferation, possibly driven by TNF α and IL-6 produced by KC activation. These studies illustrate the delicate balance between the cytoprotective roles of NF- κ B in malignant cells and the activation of NF- κ B in tumor-associated stromal cells including TAM. IKK β deletion in myeloid cells has also been shown to reduce growth of tumors in colitis-associated colorectal cancer (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- κ B activation in myeloid cells in this model (9). However, these studies did demonstrate the anti-apoptotic role of NF- κ B in malignant epithelial cells was linked to initiation of carcinogenesis.

Several existing anti-cancer 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 TNF α , IL-1 β and NOS2-derived nitric oxide (NO), by activating NF- κ B. However, as with all current anti-cancer treatments, due to a lack of specificity there are serious side-effects associated with these agents (56). Conversely, strategies to target NF- κ B activation in TAM are also likely to show success based on evidence from experimental models (9-11, 13), indeed targeting NF- κ B activation in both malignant cells and TAM may be of added benefit due to the role of NF- κ B 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- κ B (10, 35), suggesting at least in this model that specific targeting of NF- κ B in TAM may be more beneficial. Systemically targeting NF- κ B may also have adverse effects given its fundamental role in innate immunity (22), but specific targeting of NF- κ B in macrophages has been shown to increase immunity to infection associated with activation of M1 macrophages (27), again suggesting the specific targeting of NF- κ B in TAM would avoid these adverse effects.

In summary, NF- κ B 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- κ B in TAM: NF- κ B activation can drive a pro-inflammatory phenotype of TAM during early stages of tumor initiation and growth. IKK β deletion in TAM reduced growth of liver (9, 10), colon (9) and ovarian cancer (13) in mice. While in a model of fibrosarcoma defective NF- κ B activity in TAM was associated with tumor progression (32, 33). Notably, while NF- κ B activation in TAM was associated with a pro-inflammatory phenotype in HCC and CAC (9, 35), in ovarian cancer NF- κ B 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- κ B in macrophage biology have generated a somewhat muddled picture of the specific role NF- κ B plays in inflammation-associated cancer. Nevertheless, there is clearly great potential in targeting NF- κ B to block the tumor-promoting roles of TAM in cancer and possibly restoring their intrinsic anti-tumor 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- κ B may have an impact in the clinic.

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Figure 1. Signaling pathways regulating TAM phenotype and function

Various factors have been suggested to activate the pro-tumor phenotype of TAM; extracellular matrix (ECM) components, such as hyaluronan (HA) and versican, pro-inflammatory cytokines IL-1 and TNF α , and tumor hypoxia activates both HIF-1 and NF- κ B. Collectively these signaling pathways favor the transcription of genes promoting angiogenesis, malignant cell survival, invasion and metastasis. Engagement of TLRs or the IL-1 receptor (IL-1R) triggers expression of pro-inflammatory cytokines and anti-apoptotic genes through the adaptor protein MyD88 and classical NF- κ B activation. TLR/IL-1R signaling also triggers MAPK and AP-1 activation, co-regulating 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 STAT1/2-dependent genes including NOS2, IL-12 and MHC II. Whereas, the M2 phenotype is associated with IL-10, IL-4 and IL-13 signaling through STAT3 and STAT6. There are several levels of cross-talk between these pathways that regulate macrophage activation; over-expression of p50 negatively regulates NF- κ B dependent pro-inflammatory gene transcription, STAT3/6 antagonize STAT1 activation, and NF- κ B was also recently shown to inhibit STAT1 activity.