CHAPTER THREE

IKK/Nuclear Factor-kappaB and Oncogenesis: Roles in Tumor-Initiating Cells and in the Tumor Microenvironment

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Contents

Abstract

The IKK/nuclear factor-kappaB pathway (NF-κB) is critical in proper immune function, cell survival, apoptosis, cellular proliferation, synaptic plasticity, and even memory. While NF-κB is crucial for both innate and adaptive immunity, defective regulation of this master transcriptional regulator is seen in a variety of diseases including autoimmune disease, neurodegenerative disease, and, important to this review, cancer. While NF-κB functions in cancer to promote a number of critical oncogenic functions, here we discuss the importance of the NF-κB signaling pathway in contributing to cancer through promotion of the tumor microenvironment and through maintenance/expansion of tumor-initiating cells, processes that appear to be functionally interrelated.

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1. INTRODUCTION

1.1. NF-κB family members

First described almost 30 years ago as a DNA-binding activity involved in the regulation of immunoglobulin κ light-chain gene expression (Sen & Baltimore, 1986), mammalian nuclear factor-kappaB pathway (NF-κB) is a family of five highly conserved transcription factors (RelA/p65, RelB, c-Rel, p50 [NF-κB1/p105 precursor], and p52 [NF-κB2/p100 precursor]) that form different homo- and heterodimers to regulate target gene expression (Baldwin, 2012; Hayden & Ghosh, 2012; Fig. 3.1). These proteins

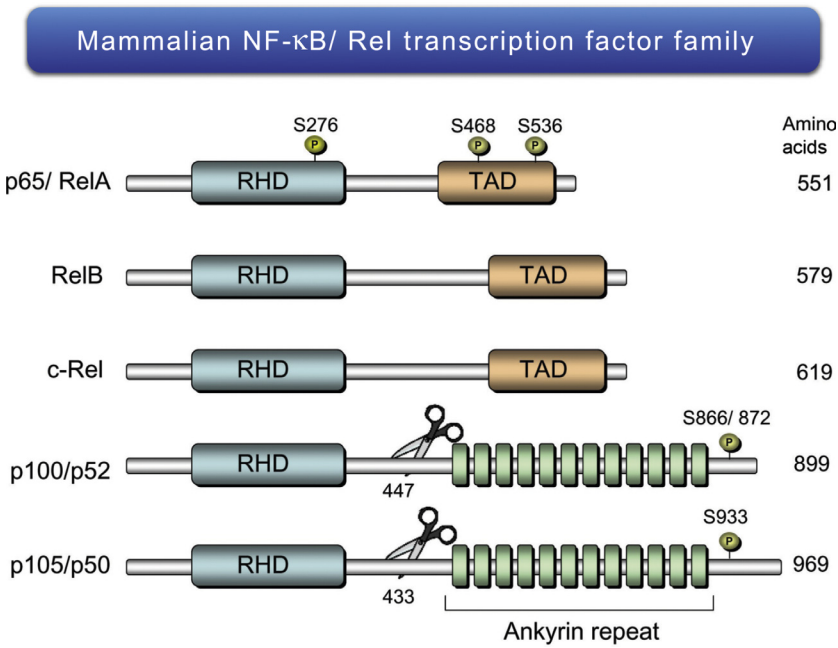

Figure 3.1 NF-κB family members consist of five highly conserved transcription factors including p65/RelA, RelB, c-Rel, p100/p52, and p105/p50. NF-κB members bind as hetero- or homodimers to activate transcription of downstream targets. The rel homology domain (RHD) in the N-terminus mediates DNA binding and binding to other NF-κB family members, while the transcriptional activation domain (TAD) in the C-terminus is critical for transcriptional activity. Phosphorylation occurs where indicated, which are marks of activity. Cleavage of p100 and p105 occur via the ubiquitin–proteasome system and produces the active p52 and p50 proteins, respectively. *Figure used with permission from Wilson (2009).*

contain an approximate 300 amino acid conserved Rel homology domain that contains sequences for nuclear localization, DNA binding, dimerization, and interaction with the inhibitor of kappaB (IκB) proteins (Baldwin, 2012; Hayden & Ghosh, 2012). RelA, RelB, and c-Rel have C-terminal domains that contain transcriptional activation domains, while full-length p100 and p105 contain IκB-like ankyrin repeats (DiDonato, Mercurio, & Karin, 2012). NF-κB proteins, p50 and p52, are produced by proteolytic cleavage of precursors p105 and p100, respectfully. c-Rel is the cellular homologue of v-Rel, the transforming gene of avian reticuloendotheliosis virus. In *Drosophila*, there are three NF-κB family members (Dorsal, Dif, and Relish) that promote dorsoventral patterning in early development and innate immune signaling.

1.2. NF-κB regulation

Two distinct regulatory pathways are known to control NF-κB activation: the canonical and noncanonical pathways (Fig. 3.2). The canonical pathway is controlled through an IKK complex which consists of the catalytic subunits, IKKβ and IKKα, and the regulatory and scaffold subunit, NEMO (IKKγ) (Ghosh, May, & Kopp, 1998; Israel, 2000; Karin & Ben-Neriah, 2000). Under resting conditions, the RelA/p50 heterodimer is held inactive by the IκB proteins. IκB physically blocks the nuclear localization sequence of RelA/p65 which leads to inactivation of the heterodimer. Various stimuli including lipopolysaccharide (LPS) and cytokines such as IL-1β and TNF trigger a signaling cascade through receptor-induced signaling to activate the IKK complex, with IKKβ functioning as the dominant kinase in this cascade (DiDonato et al., 2012). This leads to phosphorylation of IκBα on Ser32/Ser36, resulting in rapid IκBα ubiquitination and proteasome-dependent degradation. Once RelA/p50 is free from IκBα inhibition, it is able to accumulate in the nucleus and bind to kappaB sites within promoters and regulatory regions of genes that regulate apoptosis, the inflammatory response, and cell proliferation (DiDonato et al., 2012; Fig. 3.3). Along with phosphorylation and degradation of IκBα, posttranslational modifications of RelA/p65, including acetylation and methylation, can modulate NF-κB activity (Yang, Tajkhorshid, & Chen, 2010). Additionally, NF-κB activates transcription of the gene encoding its own inhibitor, IκBα, thus providing a negative feedback loop for additional control.

The noncanonical pathway, however, is controlled through an IKKα complex and leads to activation of the p52–RelB heterodimer

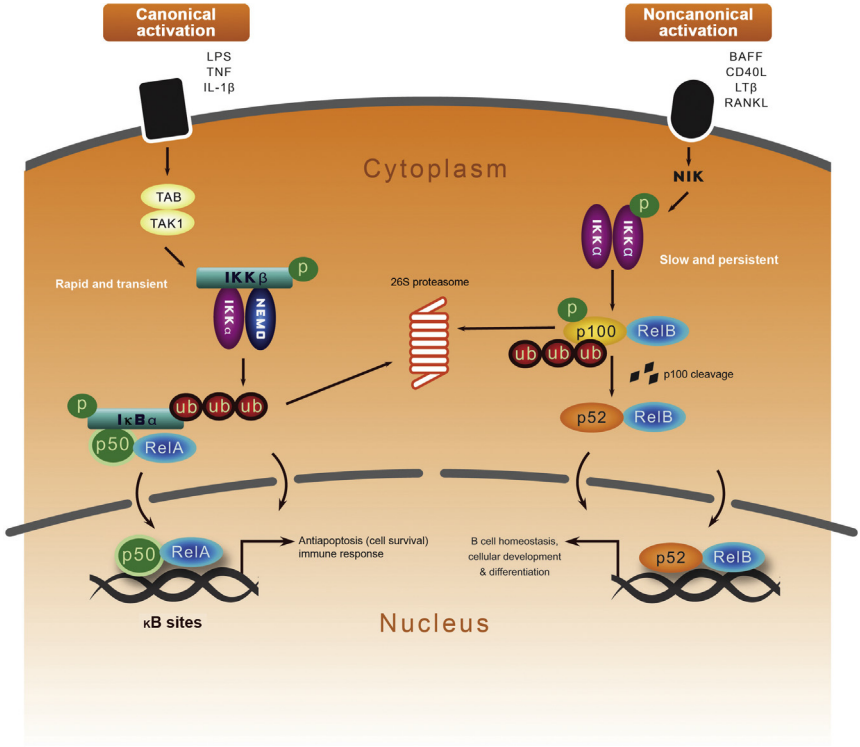

Figure 3.2 The canonical and noncanonical NF-κB pathways. Upon activation of the canonical pathway IKKβ phosphorylates IκBα, which leads to its degradation via the ubiquitin–proteasome system. The RelA/p50 heterodimer is then free to enter the nucleus and promote target gene expression. The noncanonical pathway is activated by LTβ, BAFF, and RANK ligands which causes NIK to phosphorylate the IKKα homodimer. p100 of the p100/RelB heterodimer is then phosphorylated and cleaved to p52. The subsequently formed p52/RelB heterodimer is then free to enter the nucleus to promote target gene expression.

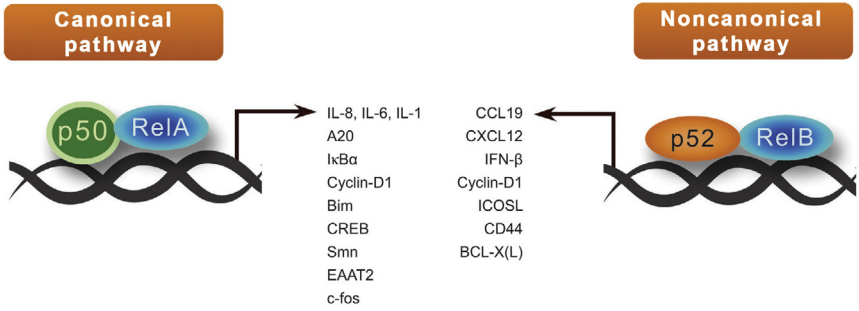

Figure 3.3 Downstream targets activated by NF-κB. Activation of the canonical NF-κB pathway leads to transcriptional activation of genes involved in anti-apoptosis and the immune response, while activation of the noncanonical pathway is involved in B cell homeostasis, cellular development, and differentiation. Note that some factors can be activated by both the canonical and noncanonical pathways such as Cyclin-D1. This is just a shortlist of the downstream targets of NF-κB.

(Hayden & Ghosh, 2004, 2012). Ligands of BAFF, CD40, and LTβ-R ligands activate the noncanonical pathway by activating NIK which phosphorylates and activates IKKα homodimers, leading to phosphorylation and subsequent proteolytic processing of p100 to p52 (Senftleben et al., 2001). The transcriptionally active RelB–p52 heterodimer can then enter the nucleus to promote transcription of a variety of genes involved in B cell homeostasis and cellular development and differentiation (see Fig. 3.3).

1.3. NF-κB-independent functions for IKK

While IKK is critical for activation of NF-κB complexes downstream of cytokine signaling and through oncoprotein expression, evidence has been presented that IKK can phosphorylate and regulate critical regulatory proteins involved in distinct signaling pathways. For example, it was reported that IKKβ can phosphorylate the tumor suppressor p53 leading to its destabilization (Xia et al., 2009). This finding is consistent with a prosurvival function for IKK signaling, blocking the potential apoptotic functions of p53. IKKβ was shown to phosphorylate the forkhead transcription factor Foxo3a, promoting its ubiquitination and proteolysis (Hu et al., 2004). IKKα phosphorylates the p27/Kip1 cdk inhibitor to promote tumor-initiating cells (TICs) in ErbB2-driven breast cancer (Zhang et al., 2013). Downstream of activated Akt, in a variety of cancers and in response to insulin exposure, IKKα interacts with mTORC1 to drive its activity (Dan, Adli, & Baldwin, 2007). Interestingly, this interaction reciprocally promotes IKK activity to activate NF-κB (Dan et al., 2008). These functions of IKK are clearly relevant to oncogenesis as blocking p53 function, suppressing p27 activity, and driving mTORC1 activity should promote tumorigenic potential of most cancers.

1.4. NF-κB and cancer

NF-κB is activated in a variety of cancers as detected by phosphorylation of IκBα and RelA, elevated levels of nuclear canonical and/or noncanonical forms of NF-κB, phosphorylation of IKK, and elevated expression of NF-κB target genes such as IL-6 and a variety of chemokines/cytokines. Most oncoproteins, such as oncogenic Ras alleles, and growth factor receptors (such as EGFR and ErbB2/Her2) are known to lead to NF-κB signaling (Finco et al., 1997; Merkhofer, Cogswell, & Baldwin, 2010). In fact, IKK/NF-κB is required for efficient cell transformation and tumorigenesis induced by oncogenic Ras (Basseres, Ebbs, Levantini, & Baldwin, 2010; Ling et al.,

2012; Mayo et al., 1997; Meylan et al., 2009; Xia et al., 2012). Loss of tumor suppressors such as PTEN and p53 have been shown to lead to elevated NF-κB activity. Interestingly, loss of p53 was shown to lead to a glucose-driven modification of IKK by O-GlcNac to increase its activity (Kawauchi, Araki, Tobiume, & Tanaka, 2008, 2009). Thus, many oncogenic signaling pathways lead to activation of IKK and NF-κB (see Table 3.1).

In most solid tumors, members of the IKK/NF-κB pathway are rarely mutated, presumably because this pathway functions downstream of oncogenic signaling pathways that are found mutated. These upstream activating mutations typically activate IKK/NF-κB along with additional signaling cascades such as Akt or MEK/Erk. In this regard, Akt signaling can promote IKK/NF-κB signaling. Importantly, both canonical and noncanonical NF-κB have been shown to be important for K-Ras-driven pancreatic cancer cell growth and survival. Downstream of oncogenic K-Ras, GSK-3α coordinates the stability of the TAK1/TAB complex to drive canonical IKK activation and promotes p100 to p52 processing to drive noncanonical NF-κB activation (Bang, Wilson, Ryan, Yeh, & Baldwin, 2013).

Genes encoding NF-κB pathway members have been found to be amplified in a variety of cancers and include *TRAF6*, *IKBKB*, *IKBKG*, *IRAK1*, and *RIPK1* (Beroukhim et al., 2010). IKKβ and IKKγ, members of the important

Table 3.1 Oncogenic signaling pathways that lead to activation of IKK/NF-κB

Oncogenic pathway	NF-κB pathway driven	Cancer type	References
Ras	NF-κB	–	Mayo et al. (1997)
p62, downstream of Ras	IKK/NF-κB	–	Duran et al. (2008)
p53 (loss)	IKK	–	Kawauchi et al. (2009)
Her2/Erb2	NIK/IKK	Breast cancer	Merkhofer et al. (2010)
Her2/Erb2	NIK/IKKα	Breast cancer TICs	Zhang et al. (2013)
Genetic lesions	Noncanonical NF-κB	Mantle cell lymphoma	Rahal et al. (2014)
CARD11	IKK/NF-κB	Diffuse large B lymphoma	Lenz et al. (2008)
Akt, loss of PTEN	IKK/NF-κB	Prostate	Dan et al. (2008)

While members of the NF-κB transcription factor family are rarely mutated in cancer, there are many upstream pathways and other genomic alterations that can lead to downstream activation of IKK and NF-κB in cancer. A few of these oncogenic pathways are listed.

IKK complex, are also amplified in certain cancers as is the IKK-related kinase IKKε (Beroukhim et al., 2010; Boehm et al., 2007). In hematologic malignancies such as multiple myeloma and diffuse large B cell lymphoma, both canonical and noncanonical signaling pathways exhibit activating mutations (Annunziata et al., 2007; Keats et al., 2007; Lenz et al., 2008).

In cancer, NF-κB is proposed to contribute to oncogenesis through the induction of genes encoding proteins involved in suppressing apoptosis, promoting invasion and angiogenesis, and enhancing epithelial–mesenchymal transition (EMT) (Baldwin, 2001; Basseres & Baldwin, 2006). As described earlier, IKK can promote oncogenic phenotypes separate from its ability to promote NF-κB activation. NF-κB can have both tumor suppressive (Perkins & Gilmore, 2006) and oncogenic (Basseres & Baldwin, 2006) properties and is at the forefront of studies on inflammation and cancer (Greten et al., 2004; Pikarsky et al., 2004). As the topic of NF-κB in cancer is very expansive, we will focus on two areas of research in this review that have not been extensively reviewed: NF-κB involvement in the tumor microenvironment and in TICs.

2. TUMOR MICROENVIRONMENT

2.1. Background on the tumor microenvironment

Tumors are heterogenic and are composed of tumor cells, which are heterogenous themselves, and noncancerous cells that are recruited into the tumor. A wide array of noncancerous cells associated with tumors make up the tumor stroma/microenvironment including macrophages, fibroblasts, T cells, B cells, neutrophils, etc. Research on the tumor microenvironment has seen an explosion of interest in recent years as it has become apparent that an environment rich in cells of the immune system such as macrophages and T cells, and nonimmune cells like fibroblasts promote tumor growth, aggressive properties like metastasis, resistance to chemotherapy, and relapse (Sun et al., 2012). The tumor microenvironment is further complicated by the presence of various cytokines, hormones, and growth factors produced by both tumor and stromal cells, many of which work to promote tumor survival, growth, and metastasis. Work on the tumor microenvironment has debunked the classical cell-autonomous view of cancer, and new therapies are aimed at targeting stromal cells in addition to cancer cells. The NF-κB pathway has been implicated in a variety of tumor-promoting roles within the stroma and may be a viable therapeutic target for these cells (Fig. 3.4).

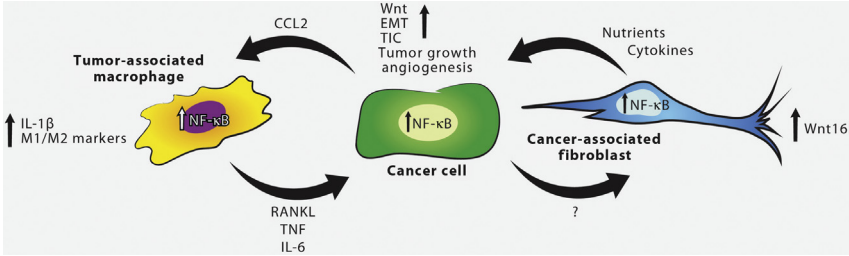

Figure 3.4 NF-κB pathway is involved in stromal communication with cancer cells. Stromal cells like macrophages and fibroblasts are influenced by cancer cells, partially by NF-κB, which promotes altered stromal cell phenotype and function. Through the NF-κB pathway, tumor stroma can drive aggressive properties in cancer cells like EMT, invasion, and TIC expansion.

2.2. NF-κB and tumor-associated macrophages

2.2.1 Macrophage phenotypes

It is becoming recognized that the canonical NF-κB signaling pathway is a central player not only in tumor progression but also in tumor-associated macrophage (TAM) regulation. Macrophages are a heterogeneous population of phagocytic leukocytes and are required for proper immune function, tissue remodeling, and repair. Classical activation of macrophages by bacterial products like LPS and IFNγ results in Toll-like receptor signaling and subsequent NF-κB activation. Classically activated macrophages adopt a proinflammatory, M1 phenotype that is characterized by expression of iNOS, MHC class II molecules, antigen presentation, and inflammatory cytokines like IL-12^{high}, IL-6, and IL-1β. Macrophages should normally target and destroy cancer cells, but macrophages that are recruited to tumors by the release of chemoattractants including CSF-1, CC chemokines, and VEGF (Biswas & Lewis, 2010; Mantovani, Allavena, Sica, & Balkwill, 2008) are often activated to an immunosuppressive (M2) phenotype (Pollard, 2008). Protumorigenic M2 macrophages are characterized by the production of matrix metalloproteinases, anti-inflammatory factors such as IL-10^{high} and IL-12^{low}, are able to promote angiogenesis and tissue remodeling activity, and have poor antigen presenting ability (Gordon, 2003; Lewis & Pollard, 2006). As tumor grade/stage increase, macrophages tend to become more M2 in phenotype, but tumors often contain TAMs with mixed M1/M2 markers (Biswas et al., 2006; Movahedi et al., 2010; Van Ginderachter et al., 2006; Fig. 3.5). TAMs are often present in large numbers in a variety of tumors (composing upwards of half of the total invasive breast cancer tumor mass), which is associated with poor prognosis in both breast

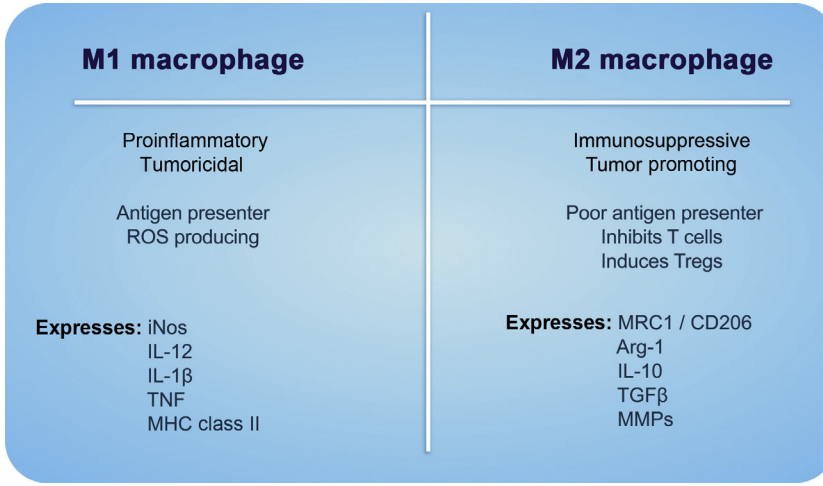

Figure 3.5 M1/M2 macrophage activation. Macrophages are recruited to tumors and are often activated to an either classical (M1) or an alternative (M2) phenotype. M1 macrophages are proinflammatory and tumoricidal in nature, while M2 macrophages are immunosuppressive and promote the tumor.

cancer (Anton & Glod, 2009; Laoui et al., 2011; Mahmoud et al., 2012) and pancreatic cancer (Kurahara et al., 2011).

2.2.2 NF-κB and TAMs

TAMs have the ability to promote cancer cell migration, tumor growth, and angiogenesis, and the NF-κB pathway may play a role in all of these functions. The NF-κB pathway can inhibit normal M1 activation when overexpressed in macrophages and therefore may decrease tumor cell phagocytosis. This is important as TAMs are known to have high levels of the NF-κB member p50 (Saccani et al., 2006). It has been demonstrated for some time now that depletion of macrophages from the tumor site can decrease progression of the tumor. Macrophages can impart invasive properties on breast cancer cells like invasion by secreting TNFα, which activates NF-κB and JNK in breast cancer cells. This crosstalk can be inhibited by the use of a TNFα neutralizing antibody (Hagemann et al., 2005). Alternatively, ovarian cancer cells are capable of activating macrophages *in vitro* to a phenotype similar to what is as seen in ovarian cancer TAMs, which is dependent on TNFα and NF-κB (Hagemann et al., 2006). Studies like these demonstrate the bidirectional impact that NF-κB signaling has on both cancer cells and TAMs. To study TAMs more specifically, a LysMCre/floxed IKKβ mouse model was created where IKKβ is deleted in

the myeloid lineage (Greten et al., 2004). It was found that IKKβ deletion in the myeloid lineage reduced colon tumor incidence and tumor size in a mouse model of colitis-associated cancer (Greten et al., 2004). Francis Balkwill and colleague's important paper on the reeducation of TAMs demonstrated that NF-κB signaling can maintain an immunosuppressive (M2) TAM phenotype. It was found that the NF-κB signaling in TAMs was being driven by malignant epithelial cells and that inhibition of IKKβ in macrophages could increase tumoricidal activity of the macrophages and decrease tumor growth in xenograft models (Hagemann et al., 2008).

Metastasis-associated macrophages (MAMs) are a subpopulation of stromal macrophage that has the ability to promote cancer cell extravasation and metastatic cell growth (Wynn, Chawla, & Pollard, 2013). It was found that pulmonary metastatic tumor cells and the surrounding stroma release the NF-κB target gene CCL2, which attracts CCR2 expressing inflammatory monocytes (Qian et al., 2011). These inflammatory monocytes develop into MAMs and promote metastasis. Inhibiting the CCL2/CCR2 signaling pathway by siRNA or neutralizing antibodies against CCL2 reduced lung colonization in experimental metastasis assays and reduced transendothelial migration of 4173 cells *in vitro*. Importantly, CCL2 blockade after intravenous injection of MDA-MB231 cells reduced lung tumor burden and increased mouse survival (Qian et al., 2011). Another independent study confirmed that lymphoma-derived mesenchymal stromal cells (L-MSCs) were effective at recruiting macrophages via CCR2 and that bone marrow mesenchymal cells treated with TNFα (a potent activator on NF-κB) were able to acquire the same macrophage recruiting properties as L-MSCs (Ren et al., 2012). These studies indicate the importance of the NF-κB target gene CCL2 (and NF-κB itself) in recruitment of macrophages and in inflammatory macrophage seeding and promotion of lung metastases.

It has been shown that Wnt signaling and promotion of colon cancer cells can also be promoted by macrophages through the NF-κB pathway. More specifically, TAMs and the NF-κB-dependent target gene IL-1β can activate NF-κB-dependent PDK1/AKT signaling in colon cancer cells, thus inactivating GSK3β, which enhances Wnt signaling and tumor promotion (Kaler, Godasi, Augenlicht, & Klampfer, 2009).

2.2.3 Noncanonical NF-κB signaling in TAM promotion of cancer

While noncanonical NF-κB signaling in TAM regulation has not received the same attention as the canonical pathway, several studies do reveal a role

for this pathway in the ability of macrophages to drive cancer. The first indication that the noncanonical pathway may been involved is that macrophages express many noncanonical NF-κB activation ligands, such as LTβ, LTα, and RANKL, and also many of the receptors for these ligands (Biswas & Lewis, 2010; Bonizzi & Karin, 2004). In a model of prostate cancer, an inactivating IKKα mutation slows prostate cancer progression and inhibits metastasis in TRAMP mice, which express a prostate specific SV40 T antigen (Luo et al., 2007). Gene analysis revealed that IKKα was promoting metastasis by inhibiting the *Maspin* gene. IKKα and the noncanonical pathway is proposed to be driven in these prostate cancer cells by RANKL-expressing inflammatory cells (Luo et al., 2007).

2.2.4 Tumor inhibiting role for NF-κB signaling in the tumor microenvironment?

All of these studies mentioned earlier demonstrate how macrophages promote cancer through the NF-κB pathway. However, it has also been found that activation of NF-κB may be beneficial in a cancer setting, which leads to the question of whether NF-κB expression during a specific window of tumor progression is beneficial in inhibiting tumorigenesis. In a chemically induced (diethylnitrosamine) mouse model of liver cancer, mice that lacked IKKβ in hepatocytes exhibited increased hepatocarcinogenesis which correlated with increased ROS production and JNK activation (Maeda, Kamata, Luo, Leffert, & Karin, 2005). However, in mice that lacked IKKβ in both hepatocytes and Kupffer cells (liver macrophages), hepatocarcinogenesis was fourfold lower than in control mice (Maeda et al., 2005). These cellular differences could be due to the important roll Kupffer cells play in providing essential NF-κB dependent mitogens to the hepatocellular carcinoma (Qian & Pollard, 2010), which is blocked by NF-κB signaling ablation.

In a more recent study, Tet-inducible mice were produced to express either IKKβ or dominant negative IκBα in macrophages to either activate or inactivate NF-κB signaling, respectively (Connelly et al., 2011). Using these mice, it was found that activation of NF-κB in macrophages during the seeding period reduced mammary tumor metastasis to the lung (Connelly et al., 2011). Whether these observations are specific to certain mouse models of cancer remains to be seen but should be further investigated as NF-κB inhibition as part of cancer therapy could have aversive outcomes during certain stages of disease.

2.3. NF-κB and tumor-associated T lymphocytes

T cells are vital members of the adaptive immune system and are responsible for cell-mediated actions including activating macrophages via Th1 and Th2 cytokines, helping B cells produce antibodies, and killing cells infected with viruses and other pathogens. The presence of T cells within a tumor setting is associated with improved patient survival (Fridman et al., 2011; Zhang et al., 2003), and as a result, immunotherapy to increase T cell function in cancer patients is currently showing promise. A recent article by Amer Beg's group demonstrates an important tumor suppressive role for NF-κB by enhancing T cell recruitment, T cell-mediated immune surveillance, and antitumor responses in lung cancer (Hopewell et al., 2013). This was demonstrated in a Lewis lung carcinoma mouse model that constitutively expressed IKKβ and had an induced OVA-specific CD8 T cell response. In this model, lung tumors initially grew but were later rejected, while control tumors (LLC model with CD8 T cell response but no constitutively active IKKβ) grew without restriction (Hopewell et al., 2013). NF-κB activity was also found to be strongly associated with T cell infiltration in human lung tumors. Their results indicate that NF-κB is required for a full antitumor CD8 T cell response in murine and human lung tumors and may be partially due to NF-κB enhanced expression of the T cell chemokine CCL2 (Hopewell et al., 2013). This result contrasts with the previously mentioned tumor-promoting role CCL2 plays in recruiting TAMs and MAMs, which promotes metastasis.

2.4. NF-κB promotes recruitment of regulatory T lymphocytes in lung cancer

Regulatory T lymphocytes (Tregs) are a subpopulation of T cell ($FoxP3^+CD4^+CD25^+$) that help control immune responses and prevent autoimmunity by regulating immune system homeostasis. The positive functions of Tregs in preventing autoimmunity can quickly be negated, however, if they continue to suppress normal T cell function. Constitutive NF-κB expression in lung epithelial cells dramatically increased Treg numbers in IKTA mice (express the constitutively active form of IKKβ) (Zaynagetdinov et al., 2012). This study found that NF-κB overexpression was sufficient to cause lung tumors in a urethane mouse model and that NF-κB overexpression during the promotion of tumorigenesis is the critical timeframe for cancer promotion (Zaynagetdinov et al., 2012). Depletion of

Tregs in the IKTA mouse model resulted in improved tumor burden, implicating the importance of functional T cells for tumor cell clearance.

2.5. NF-κB and cancer-associated fibroblasts

Like immune cells, fibroblasts are also recruited to tumors and can promote aggressive properties in the cancer cells. In fact, cancer-associated fibroblasts (CAFs) are metabolically (Chaudhri et al., 2013) and phenotypically (Bhowmick, Neilson, & Moses, 2004) different from normal fibroblasts and provide epithelial cancer cells with vital nutrients. This is especially important in supporting a growing tumor before a blood supply is established (Martinez-Outschoorn et al., 2013). The NF-κB pathway has also been implicated in CAF promotion of a variety of cancers including pancreatic, mammary, skin, and prostate cancers. Doug Hanahan's group found that NF-κB was driving an inflammatory signature in CAFs from mouse and human skin, mammary, and pancreatic tumors. CAFs from these tumors mediated tumor-promoting inflammation, macrophage recruitment, neovascularization, and tumor growth, all of which was abolished when NF-κB was inhibited (Erez, Truitt, Olson, Arron, & Hanahan, 2010). Another more recent study found that NF-κB activates Wnt16B in prostate stromal fibroblasts, which promoted EMT in neoplastic prostate cells (Sun et al., 2012). Even more interesting is the finding of WNT16B promoted cancer cell survival following cytotoxic therapy in a cell nonautonomous manner (Sun et al., 2012).

3. TICs/CANCER STEM CELLS

Solid tumors are typically heterogeneous, being comprised of cells with different phenotypic and signaling properties. One subset of cells, known as tumor-initiating cells (TICs) or cancer stem cells, exhibit properties of self-renewal and tumorigenic potential when delivered to immunodeficient mice recipients (Charafe-Jauffret, Ginestier, & Birnbaum, 2009; D'Angelo & Wicha, 2010; Ginestier et al., 2010; Fig. 3.6). Additionally, these cells exhibit an EMT phenotype, are chemo/radioresistant, and are invasive. In certain characteristics, TICs share similarities with embryonic and adult stem cells. While it is proposed that TICs give rise to the more differentiated cancer cells within a tumor, evidence has been presented that these differentiated cancer cells can also give rise to TICs. Given the importance of IKK and NF-κB in cancer, it is not surprising that research from several different groups has converged on a concept that these signaling modules could regulate the so-called TIC phenotype.

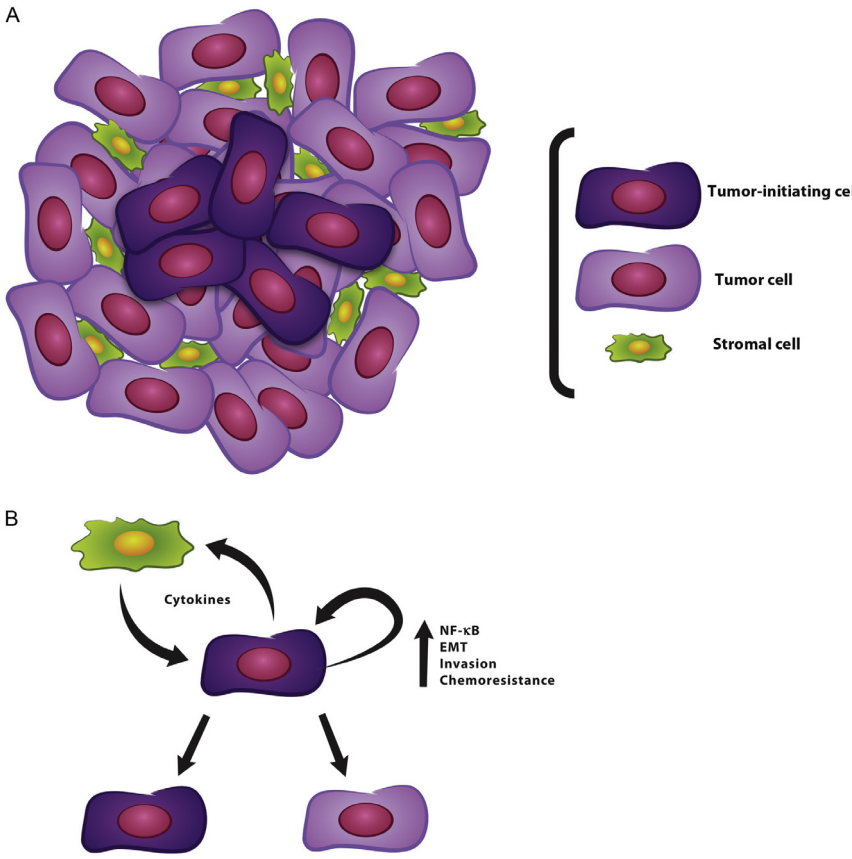

Figure 3.6 Tumor-initiating cells are a subpopulation of chemoresistant cancer cell. Tumor-initiating cells (TICs) are a subpopulation of cancer cell that are in part driven by canonical and noncanonical NF-κB signaling, and are known to be resistant to chemotherapies. (A) TICs are able to self-renew, can also produce non-TIC cancer cells, and are influenced by tumor stroma (B).

3.1. TICs and NF-κB

Early evidence for the involvement of IKK/NF-κB in TICs was shown by Karin and colleagues (Cao, Luo, & Karin, 2007), who showed that IKKα is required for the self-renewal of TICs from carcinogen-induced and Her2-driven breast cancers. Subsequently, Pestell and colleagues (Liu et al., 2010) found that inducible suppression of NF-κB (via expression of the super-repressor form of IκBα) in the adult mammary epithelium delayed the onset and number of Her2-driven breast tumors. Gene expression analysis showed that inhibition of NF-κB blocked expression of genes associated with stem cells, including Nanog and Sox2, and blocked TIC expansion *in vitro*.

Recently, Zhang et al. (2013) showed that IKKα promotes ErbB2/Her2-driven TICs via phosphorylation and induction of nuclear export of the cdk inhibitor p27 (Zhang et al., 2013). Our group showed that both IKKα and IKKβ promote TICs from basal-like and claudin-low breast cancer cells (Kendellen, Bradford, Lawrence, Clark, & Baldwin, 2013). In this regard, canonical and noncanonical NF-κB subunits drive TIC maintenance, and TICs (characterized by the CD44+ group) exhibited elevated NF-κB and IKK activity as compared to the CD44− subset. Additionally, we showed that NF-κB promotes EMT in breast cancer cells, a process known to be involved in TIC maintenance/expansion. Evidence was presented that inflammatory cytokines regulated by NF-κB and working in autocrine/paracrine pathways promotes TIC properties.

Rajasekhar, Studer, Gerald, Socci, and Scher (2011) found that NF-κB activation is elevated in a subset of prostate tumor cells with tumor-initiating properties (Rajasekhar et al., 2011). These cells exhibit low PSA and AR levels. The use of certain inhibitors that can affect NF-κB activation blocked secondary-sphere formation of these cells. Schwitalla et al. (2013) showed that NF-κB modulates Wnt signaling and that ablation of RelA/p65 in intestinal epithelium retards crypt stem cell expansion (Schwitalla et al., 2013). Elevated NF-κB signaling enhances Wnt activation and induces dedifferentiation of non-stem cells that acquire tumor-initiating capacity. We showed that RelA is important in maintaining hematopoietic stem cells (HSCs), as knockout of RelA in this compartment blocks levels of early progenitors (Stein & Baldwin, 2013). Evidence was presented that NF-κB controls the expression of genes associated with HSCs. For example, loss of RelA leads to downregulation of expression of Mpl which is the receptor for thrombopoietin, a factor involved in promoting HSCs. Consistent with the involvement of RelA in driving early stemness, knockout of RelA led to expression of monocyte differentiation markers.

3.2. TICs, cytokines, and tumor microenvironment

It is well established that hematopoietic stem cells are influenced by their microenvironment; thus, it is important to consider that tumor microenvironment could have effects on TICs. As with normal stem cells, TICs are regulated by both intrinsic and extrinsic signals. Evidence indicates that tumor development and cellular hierarchy coevolve with the tumor microenvironment during the process of tumorigenesis (Polyak, Haviv, & Campbell, 2009). Other evidence supports the concept that paracrine signaling coordinates tumorigenic progression, including maintenance of TICs.

As described earlier, tumor cells produce and secrete factors that attract and regulate many of the cells that constitute the tumor microenvironment. In this regard, immunomodulatory cells provide both stimulatory and inhibitory effects on tumor development and progression (Mantovani, 2009; Yu, Pardoll, & Jove, 2009). Understanding mechanisms derived from the microenvironment that promote TICs could have significant potential relative to new therapeutic approaches for cancer.

Both IL-6 and IL-1 were shown to regulate breast TIC self-renewal, and the genes encoding these cytokines are known to be regulated by NF-κB (Ginestier et al., 2010; Iliopoulos, Hirsch, Wang, & Struhl, 2011). IL-6 is known to link inflammation and malignant transformation in a pathway involving NF-κB, Lin28, and the Let-7 miRNA (Iliopoulos, Hirsch, & Struhl, 2009). Korkaya, Liu, and Wicha (2011) showed that IL-6 controls resistance to trastuzumab in Her2+ breast cancer by expanding the TIC population. Our data showed that inflammatory cytokines controlled by NF-κB promote/expand the TIC phenotype of basal-like and claudin-low breast cancer cells (Kendellen et al., 2013). In that study, IL-1 and IL-6, but not IL-8, promoted TIC expansion. Iliopoulos et al. (2011) showed that inducible formation of breast cancer TICs occurred by IL-6 secretion (Iliopoulos et al., 2011). Additionally, evidence has been presented that STAT3 (e.g., activated downstream of IL-6) functions in inflammation-driven cancer (Iliopoulos, Jaeger, Hirsch, Bulyk, & Struhl, 2010), and it is known that STAT3 and NF-κB can function to promote expression of certain pro-oncogenic genes (Lee et al., 2009; Yu et al., 2009).

4. CONCLUSIONS

The NF-κB pathway plays a dynamic role in various cancers and is becoming more understood as a key player in stromal interactions and in TIC function within the tumor. The influence of NF-κB can be seen in the form of tumor progression and other aggressive properties like TIC expansion and invasion. This highlights the fact that NF-κB is a viable target for therapeutic options for both cancer and stromal cells.

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