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Nuclear factor-κB in glioblastoma: insights into regulators and targeted therapy

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Nuclear factor-κB (NF-κB) is a ubiquitous transcription factor that regulates multiple aspects of cancer formation, growth, and treatment response. Glioblastoma (GBM), the most common primary malignant tumor of the central nervous system, is characterized by molecular heterogeneity, resistance to therapy, and high NF-κB activity. In this review, we examine the mechanisms by which oncogenic pathways active in GBM impinge on the NF-κB system, discuss the role of NF-κB signaling in regulating the phenotypic properties that promote GBM and, finally, review the components of the NF-κB pathway that have been targeted for treatment in both preclinical studies and clinical trials. While a direct role for NF-κB in gliomagenesis has not been reported, the importance of this transcription factor in the overall malignant phenotype suggests that more rational and specific targeting of NF-κB-dependent pathways can make a significant contribution to the management of GBM.

Keywords: glioblastoma, glioma, inhibitor, NF-κB.

Glioblastoma (GBM), the most common primary glial neoplasm, is also one of the most aggressive cancers in humans. Among the signaling pathways active in GBM, the nuclear factor-κB (NF-κB) response plays an important role in promoting tumor pathobiology and response to therapy. Although this multi-subunit transcription factor was originally characterized within the context of the immune system, it was subsequently identified as a critical factor in cancer.[1] Like other malignancies, GBM demonstrates high constitutive NF-κB activity,[2–4] and many of the central oncogenic pathways active in GBM converge on the NF-κB system.

Nuclear Factor-κB Activation Pathways

The mammalian NF-κB family is made up of 5 subunits: p50 (NF-κB1, p105), p52 (NF-κB2, p100), p65 (RelA), RelB, and c-Rel (Fig. 1). These proteins exist as homo- and heterodimers with the most abundant form being p50/p65.[5] While each subunit contains an N-terminal Rel homology domain (RHD) that is necessary for DNA binding and subunit dimerization, only p65, RelB, and c-Rel contain a C-terminal transactivation domain (TAD).[6] In general, NF-κB dimers are maintained in the cytoplasm bound to inhibitor-κB (IκB) proteins (Fig. 1). Activation of NF-κB occurs by multiple interrelated pathways that converge on the cytoplasmic IκB kinase (IκK) complex made up of 2 catalytic subunits, IκKα and IκKβ, and a noncatalytic regulatory subunit, IκKγ (NEMO). Following stimulation, phosphorylation of IκB proteins by IκK results in IκB degradation and NF-κB nuclear translocation (Fig. 2). In the nucleus, NF-κB dimers bind to consensus elements (κB-sites) in the regulatory regions of genes involved in a wide range of cellular processes including inflammation, cell survival, and apoptosis.

In the canonical activation response, stimulation of surface receptors such as tumor necrosis factor alpha receptor 1 (TNFR1) results in a signaling cascade that induces phosphorylation of IκBα by IκKβ (Fig. 2).[5] In this pathway, ubiquitination of receptor-interacting protein 1 (RIP1) forms a scaffold for recruitment of the IκK complex via the ubiquitin-binding domain of NEMO.[7] The E3 ligases cIAP1/2 and TRAF2/5 are involved in RIP1 ubiquitination facilitating TAK1 binding and IκK activation. Phosphorylation of IκBα by IκKβ leads to IκBα's ubiquitination and proteosomal degradation.

A second evolutionarily conserved noncanonical or alternate activation pathway has also been described that involves NF-κB inducing kinase (NIK) and p52-containing complexes (Fig. 2).[8] The alternate pathway is induced by a different set of cell surface receptors and is primarily involved in lymphoid development and osteoclastogenesis.[9] In this pathway, phosphorylation of

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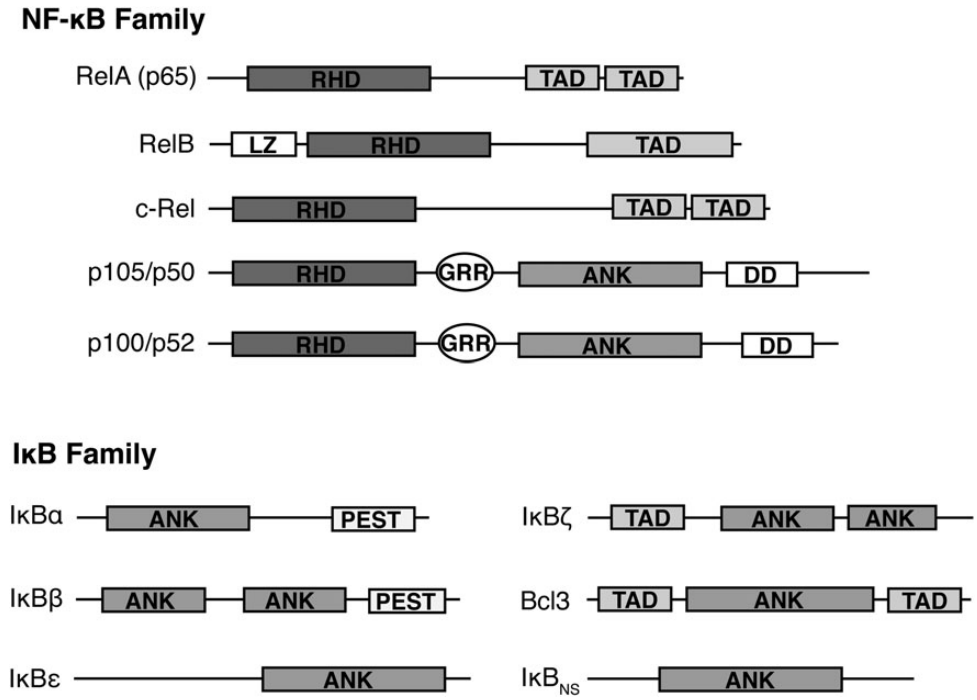

Fig. 1. NF-κB pathway proteins. There are 5 NF-κB subunits that include the 3 Rel proteins (RelA, RelB, and c-Rel) and the 2 non-Rel, precursor proteins (p100 and p105) that give rise to p52 and p50 following proteosomal processing, respectively. The IκB proteins are recognizable by their multiple ankyrin repeats (ANK), and include the classical members (IκBα, IκBβ, and IκBε that sequester NF-κB dimers in the cytoplasm) and the atypical members (IκBζ, Bcl-3 and IκB$_{NS}$) that act to modify nuclear NF-κB activity. The primary structural motifs of each protein are indicated: Rel homology domain (RHD), transactivation domain (TAD); leucine zipper (LZ); glycine-rich region (GRR); death domain (DD); and proline-rich, glutamic acid-rich, serine-rich, and threonine-rich (PEST).

p100 by IκKα leads to p100 proteolytic cleavage and nuclear translocation of p52/RelB dimers.

NF-κB is also activated by an atypical response induced by genotoxic stressors such as DNA double-strand breaks (DSBs), replication stress (RS) and reactive oxygen species.[5,10] Atypical signaling, which actually involves a series of interrelated pathways, is fundamentally different from canonical and alternative pathways in that it involves nuclear-to-cytoplasmic signaling and direct nuclear NF-κB modification. In the setting of DNA DSBs, NF-κB is activated by a mechanism involving ataxia and telangectasia mutated (ATM) and nuclear NEMO (Fig. 2).[11] Sequential phosphorylation and mono-ubiquitination of sumoylated nuclear NEMO result in its nuclear export.[12] In the cytoplasm, NEMO interacts with a protein rich in glutamate-E, leucine-L, lysine-K, and serine-S (ELKS), ATM, and TAK1 to activate IκK and induce NF-κB.

While nuclear translocation is the primary method of regulating NF-κB activity, the overall NF-κB response is determined by the cooperative action of multiple promoter-specific factors. Individual subunit post-translational modification (PTM) plays a central role in regulating NF-κB activity by a variety of mechanisms including modulating dimer/κB-site interactions,[13] restricting transcriptional co-regulator recruitment,[13,14] and directly altering subunit transactivity.[15] In contrast to the response to DSBs, NF-κB signaling downstream of RS and ATM-and-Rad3-related protein (ATR) occurs in a more complex, promoter-specific manner that is regulated to a large extent by subunit PTM. Specifically, ATR induces Chk1-mediated phosphorylation of p65 Thr505 and p50 Ser329,[16,17] a pathway that acts to promote cell death.[10,18] The ATR-mediated NF-κB response is particularly important for the treatment of GBM because temozolomide (TMZ), the primary chemotherapeutic used in the treatment of GBM, induces RS and activates ATR by forming cytotoxic O^6-methylguanine (O^6-meG) lesions.[17,19]

The activation pathways outlined above promote NF-κB signaling; however, given that the NF-κB response is primarily a stimulus-induced system, multiple auto-regulatory mechanisms concomitantly replenish the pools of latent NF-κB dimers to enable repeat stimulation. In this regard, NF-κB induces expression of its own inhibitor, IκBα, which removes DNA-bound dimers and translocates them back to the cytoplasm.[20] Also, NF-κB signaling induces expression of various de-ubiquitinating (DUB) enzymes that degrade the ubiquitin scaffolds necessary for IκK activation. In sum, the overall NF-κB response represents the composite action of a series of complementary pathways that act in a stimulus, subunit, and cell-type specific manner to mediate the eventual downstream effect.

NF-κB Activation in Glioblastoma

Over the past decade, expression profiling has enabled the classification of adult GBM into several well-defined

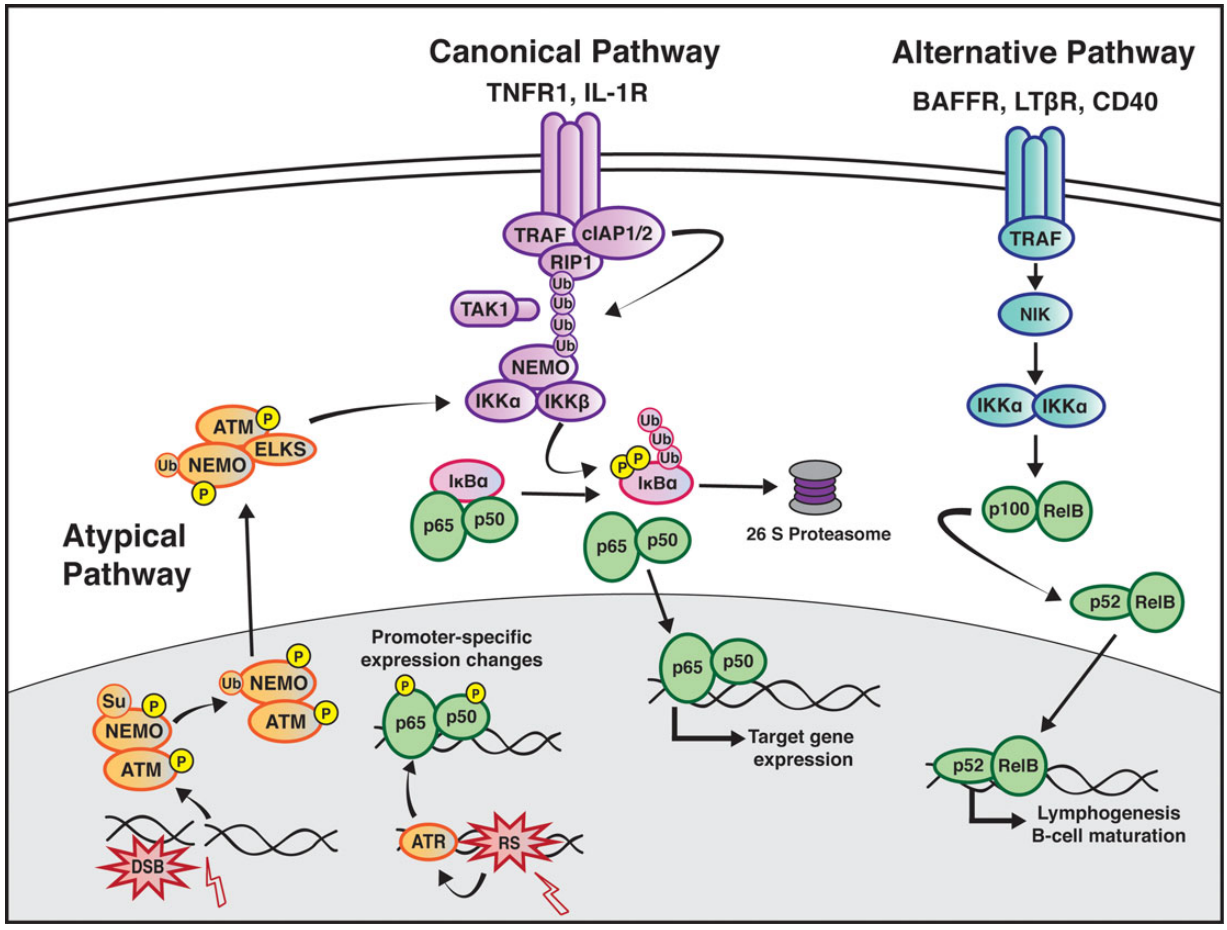

Fig. 2. NF-κB activation pathways. In the canonical pathway, stimulation of receptors such as TNFR1 activates the IκK complex, which comprises IκKα/β and γ (NEMO) and results in phosphorylation of IκBα. Subsequently, ubiquitin-dependent degradation of IκBα releases NF-κB dimers that translocate to the nucleus and modulate target gene expression. The alternate pathway is triggered by surface receptors such as B-cell-activating factor receptor (BAFFR), which stimulates a cascade involving NF-κB-inducing kinase (NIK) and IκK-α. In this response, proteosomal processing of p100 leads to formation of p52-containing dimers that modulate the genes regulating lymphoid development and B-cell maturation. The atypical response comprises a number of pathways that are induced by genotoxic stressors such as DNA double-strand breaks (DSBs) and replication stress (RS). In response to DSBs, ATM interacts with sumoylated nuclear NEMO, resulting in sequential phosphorylation and mono-ubiquitination of NEMO. Subsequently, cytoplasmic translocation of ATM/NEMO leads to stimulation of IκK activity. RS activates ATR, which induces Chk1-mediated phosphorylation of both p50 and p65 and leads to promoter-specific modulation of NF-κB target genes that act to promote cytotoxicity.

subtypes.[21,22] More recently, epigenetic analysis has further modified classification of GBM and also enabled incorporation of pediatric tumors.[23] This molecular analysis has led to better understanding of the role of NF-κB in GBM. In general, GBMs with mesenchymal features demonstrate elevated levels of NF-κB pathway genes such as *TRADD*, *RELB* and *TNFRSF1A*.[22] Activation of NF-κB signaling has also been found to promote mesenchymal differentiation of GBM by modulating downstream transcriptional signaling.[24] Consistent with this observation, the noncanonical NF-κB subunit, RelB, was shown to drive mesenchymal differentiation in GBM.[25] Despite the importance of NF-κB in GBM, mutation or amplification of individual NF-κB subunits is rare in these tumors. The above findings suggest that the elevated NF-κB activity in GBM is likely the result of deregulation of the pathways that modulate the NF-κB response.

Oncogenes and NF-κB in Glioblastoma

Receptor tyrosine kinases (RTKs) are important oncogenic drivers in GBM. Epidermal growth factor receptor (EGFR), the first member of the RTK family, is deregulated in more than 50% of GBMs.[26] EGFR signaling is aberrantly induced in GBM, primarily as a result of gene amplification or mutation, and activation of NF-κB by EGFR in GBM involves both Akt-dependent and -independent mechanisms (Fig. 3).[27,28] EGFR-mediated NF-κB activation promotes both GBM formation and chemoresistance.[29,30] EGFRvIII is one of the best-characterized *EGFR* mutations found in about 30% of all GBMs and more than 60% of tumors with amplified *EGFR*.[31] Despite the inability of EGFRvIII to bind ligands, it is constitutively active and induces NF-κB by a mechanism involving the adaptor protein, RIP1,[32,33] a factor whose expression level has been linked with GBM prognosis.[22,34]

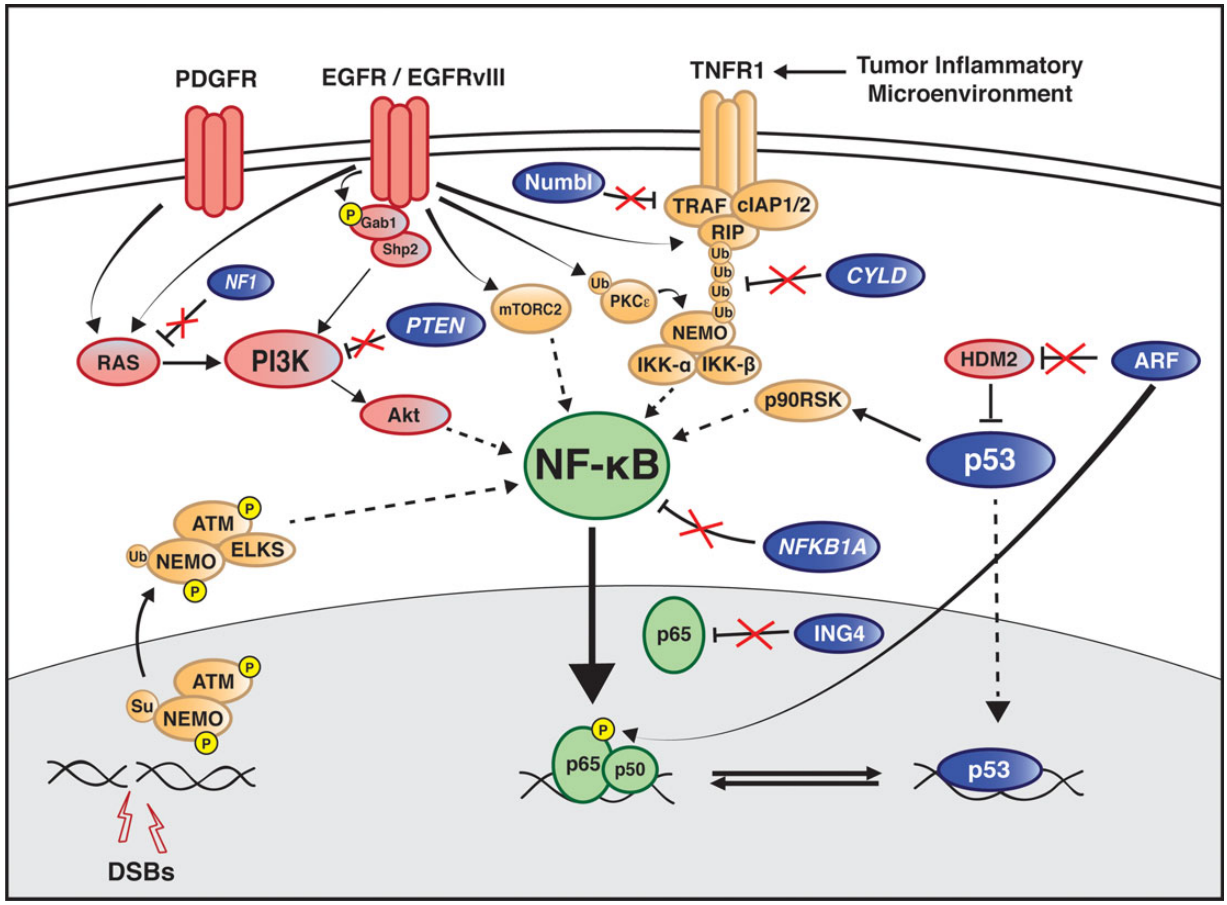

Fig. 3. Modulation of NF-κB signaling by oncogenic pathways in GBM. The link between the primary oncogenic pathways active in GBM and the NF-κB system are illustrated. Oncogenic pathways are shaded in red and tumor suppressor pathways in blue. The RTKs, EGFR and PDGFR, induce NF-κB activation via a number of interrelated pathways, and a reciprocal relationship between *EGFR* amplification and *NFKB1A* deletion is seen. Loss of tumor suppressors such as *PTEN*, *NF1*, and *ARF* promote NF-κB activity because of their physiological role in attenuating the NF-κB response. The p53 pathway is a tumor suppressor response modified in virtually all GBMs. A reciprocal association between p53 and NF-κB signaling is evident at multiple pre- and postnuclear translocational points. In addition to cell-intrinsic responses, the inflammatory GBM microenvironment contributes to high NF-κB activity by triggering canonical and noncanonical signaling.

The connection between NF-κB and EGFR in GBM is also supported at the genomic level by a report demonstrating the mutual exclusivity of *EGFR* amplification and deletion of *NFKBIA*, the gene encoding for IκBα.[35] In this study, it was reported that heterozygous deletion of *NFKBIA* is present in about 25% of GBMs, and patients with either *EGFR* amplification or *NFKBIA* deletion have a shorter median survival than those with normal *NFKBIA* and *EGFR*. Of note, a more recent report found that the incidence of *NFKBIA* deletion in GBM might be much lower than initially reported.[36]

Platelet-derived growth factor receptor (*PDGFR*) is another RTK frequently amplified in GBM.[22] Glial tumors often co-express PDGF and its receptor, setting up an autocrine activation loop.[37] PDGF has been shown to activate NF-κB via Akt, although the mechanistic association between NF-κB and the PDGFR pathway is not as well elucidated as the link to EGFR.[38] NF-κB has also been shown to promote glioma progression in a PDGFβ-driven transgenic mouse glioma model.[39] Moreover, dominant negative PDGFβ, or anti-PDGF neutralizing antibodies, decrease NF-κB activity in glioma cells.[40]

A robust link exists between the Ras oncogene and NF-κB; while *RAS* mutation is rare in GBM, high levels of active Ras are found in GBM due to hyperactive RTKs and loss of tumor suppressors such as *NF1*.[26] Ras signaling activates NF-κB via p65 by a pathway that is independent of classical IκK signaling.[41] Importantly, NF-κB is required for the oncogenic effect of Ras in both cells and animals.[42,43] Despite the numerous studies linking NF-κB and Ras in peripheral tumors, the importance of this pathway in GBM is not known.

Tumor Suppressors, NF-κB, and Glioblastoma

As with oncogenes, many tumor suppressors that promote GBM formation also modulate NF-κB signaling. The p53 pathway is almost universally affected in GBM. In tumors with wild-type *TP53*, p53 signaling is often functionally abrogated by *MDM2* amplification or *INK4a*^{ARF} (*CDKN2A*) mutation.[26] The p53 response intersects with the NF-κB pathway at multiple points (Fig. 3).[5,44] While wild-type p53 was initially reported to activate NF-κB by a mechanism involving the serine/threonine

kinase, p90^{rsk},[45] mutant p53 can also activate NF-κB.[46] *CDKN2A*, a tumor suppressor that modulates p53-mediated cell cycle arrest, is mutated in almost 50% of GBMs,[26] and its protein product can directly inhibit p65 transactivity.[15] These studies and multiple others[44] illustrate the complex manner in which the p53 and NF-κB pathways combine to regulate the downstream response.

Loss of the long arm of chromosome 10 is another frequent finding in GBM, and the tumor suppressor PTEN that is inactivated in 30% of GBM is found in this region.[26] PTEN is a phosphatase that negatively regulates PI3-kinase activity and blocks Akt signaling. Given the overwhelming propensity of Akt to activate NF-κB,[38] the frequent loss of PTEN in GBM plays an important role in activating NF-κB in these tumors.

In addition to these common GBM-associated changes, other tumor suppressors also have prognostic value in GBM and have been linked to NF-κB. For example, CYLD, a DUB that blocks NF-κB activity by removing ubiquitin chains from NF-κB intermediates, is downregulated in glioma, and its expression is inversely associated with patient prognosis.[47] Similarly, expression of ING4, a protein that physically interacts with p65 and blocks angiogenesis by inhibiting NF-κB activity, was inversely correlated with glioma grade.[48]

Together, the above observations indicate that the important oncogenic pathways active in GBM impinge on the NF-κB system and, moreover, act in concert to enhance NF-κB activity.

NF-κB and Glioblastoma Pathobiology

Although NF-κB activity is high in GBM, there is no definitive evidence that NF-κB actually plays a causal role in glioma formation as it does in other malignancies.[1] Nevertheless, NF-κB signaling modulates many of the central hallmarks of cancer including: cellular proliferation, angiogenesis, invasion, and resistance to apoptosis (Fig. 4).[49] In addition, the critical role of NF-κB in promoting inflammation further contributes to the malignant phenotype.

NF-κB Promotes Glioblastoma Cell Survival and Resistance to Therapy

NF-κB is classically considered to be a pro-survival factor that induces the expression of genes promoting cell survival and proliferation. Proteins regulated by NF-κB in GBM that act in this manner include Bcl2, Bclxl, survivin, Cox2, and the inhibitor of apoptosis proteins (IAPs).[50] Cyclin D1 (*CCND1*), another NF-κB target gene involved in survival and proliferation,[51] has also been linked to poor prognosis in GBM patients,[52] and depletion of cyclin D1 in glioma cells blocks proliferation and invasion.[53] Interestingly, while the pro-survival effects of NF-κB play an important role in mediating resistance to therapy in GBM,[24,54] Chk1-mediated inhibition of p50-induced antiapoptotic genes demonstrates a mechanism by which NF-κB signaling also promotes cytotoxicity by chemotherapeutics such as TMZ.[17]

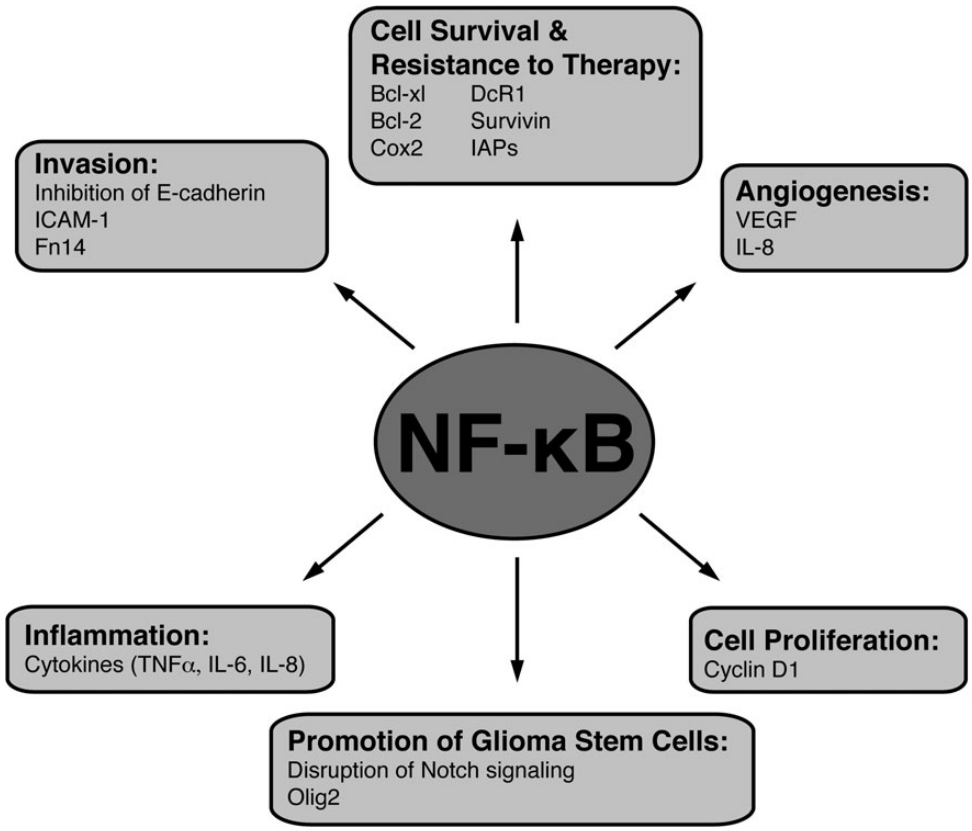

Fig. 4. NF-κB-regulated processes and factors central to GBM pathobiology. The NF-κB family regulates the expression of numerous proteins that are critical for the survival and progression of GBM. The various responses modulated by NF-κB signaling are highlighted.

NF-κB and Angiogenesis

New blood vessel formation is critical for the maintenance of an expanding tumor, and vascular proliferation is a central tenet in the pathological diagnosis of glioblastoma. Neovascularization in GBM is driven primarily by vascular endothelial growth factor (VEGF). Not only is *VEGF* an NF-κB target gene,[55] it is also induced by NF-κB-regulated factors such as IL-6.[56] Another proangiogenic NF-κB target gene is *IL8*. While IL-8 levels are low in normal tissue, in GBM the IL-8 level is high, a finding related both to loss of the tumor suppressor *ING4*,[48] and to the presence of macrophages and microglia in the GBM microenvironment.[57] These finding not only demonstrate the importance of NF-κB to angiogenesis but also highlight how multiple factors in GBM converge on NF-κB to promote this malignant feature.

NF-κB, Migration, and Invasion

Migration of GBM cells into the surrounding brain parenchyma is one of the primary reasons for tumor recurrence, and NF-κB signaling significantly contributes to this property of GBM cells.[39] Pin1, a prolyl isomerase that stabilizes p65 and promotes its transcriptional activity, is overexpressed in GBM and promotes cellular migration via IL-8.[58] Astrocyte elevated gene-1 (*AEG-1*), an HIV-1-inducible gene that is overexpressed in GBM, is another factor that interacts with p65 to promote glioma cell migration.[59] p65 has also been shown to interact with STAT3 at the *ICAM-1* promoter to stimulate migration of GBM cells in response to ionizing radiation.[60]

To identify novel factors associated with GBM invasion, expression profiling was performed, and fibroblast growth factor-inducible 14 (*FN14*) was identified as a gene significantly upregulated in migrating cells.[61] Fn14 is regulated in an NF-κB-dependent manner via a κB site in its promoter.[62] In addition, high *FN14* mRNA expression correlates with poor GBM survival. In a similar fashion, connective tissue growth factor (*CTGF*) was identified as a gene important in invading GBM cells.[63] CTGF induces NF-κB binding to the *ZEB-1* promoter, a transcriptional repressor of E-cadherin and a pathway necessary for glioma cell invasion.[64] One negative regulator of the NF-κB response that promotes invasion and is downregulated in GBM is Numblike (Numbl), a cytoplasmic protein involved in brain morphogenesis that attenuates NF-κB and invasion by acting on upstream TRAF proteins.[65]

NF-κB, Inflammation, and Glioblastoma

NF-κB signaling has been directly linked to multiple stages of inflammation-associated carcinogenesis.[66] Although no definitive association between inflammation, NF-κB, and gliomagenesis has been described, many inflammatory mediators, cytokines, and immunosuppressive factors are upregulated in the glioma microenvironment.[67] Several of these factors are not only NF-κB-regulated genes but are also activators of NF-κB signaling and act together with intrinsic oncogenic pathways to promote NF-κB activation (Fig. 3). Interestingly, it was recently reported that the elevated NF-κB status in the GBM microenvironment is promoted by inflammatory cells such as macrophages and microglia and acts to augment GBM proliferation and resistance to therapy.[24]

NF-κB and Glioma Stem-like Cells

Accumulating evidence suggests that subpopulations of tumor-initiating or stem-like cells are a major reason for GBM recurrence and resistance to treatment.[68] Notably, GBM stem-like cells have higher levels of nuclear p65 and NF-κB-dependent gene expression than regular glioma cells.[69] Also, NF-κB signaling has been linked to the proliferation, migration, and differentiation of neural stem cells,[70] a potential cell of origin of brain tumors. RelB an NF-κB subunit that is highly expressed in mesenchymal GBM regulates expression of Olig2,[25] a critical factor in normal and tumorigenic stem-like cell proliferation. Finally, it has been suggested that NF-κB signaling may prevent GBM stem-like cells from acquiring a mature postmitotic phenotype; consistent with this, blockade of NF-κB promotes senescence.[71] In general, multiple studies suggest that the NF-κB pathway acts in a similar fashion in stem-like cells as in regular GBM cells to promote malignancy and enhance treatment resistance.

Therapeutic Targeting of the NF-κB Pathway

The importance of NF-κB to glioma pathobiology suggests that targeting it may be a fruitful approach for treating GBM. In this section, we review the numerous strategies that have been used to block NF-κB signaling, both specifically and nonspecifically. While the bulk of the data involve in vitro and preclinical studies, we also discuss the few clinical trials in which the NF-κB pathway has been targeted in GBM.

Specific NF-κB Inhibition

IκK Inhibition

The importance of the IκK complex in regulating NF-κB activation, coupled with the druggable nature of kinase activity, has made IκK a primary target for pharmacotherapy. While many IκK inhibitors have been used in the treatment of peripheral cancers, only a few have been studied in GBM (Fig. 5). In one study, the antiglioma effect of several NF-κB inhibitors was demonstrated in vitro.[72] These agents were found to sensitize GBM cells to cisplatin and doxorubicin and were even effective against chemotherapy-resistant clones. One agent, BAY11-7082, was shown to have an IC_{50} in GBM cells that was over 4-fold lower than normal astrocytes. In another study, a novel glycosylated indolocarbazol that blocks IκKβ activity, EC-70124, was shown to induce senescence in GBM stem-like cells.[71] While these results support the use of IκK inhibition as an antiglioma strategy, even highly selective agents such as BAY11-7082 also modulate other signaling pathways,[73] suggesting that the effects reported may not be solely due to inhibition of NF-κB.

RNA interference is an alternative and potentially more specific method for targeting IκK. MiR-218, a microRNA that targets the 3′-UTR region of IκKβ mRNA, is downregulated in patient-derived GBM tissue.[74,75] Ectopic delivery of miR-218 inhibits NF-κB activity and invasion of GBM cells.[74] While RNA interference can reduce the off-target effects seen with chemical inhibitors, microRNAs can also have multiple targets. In fact, miR-218 was shown to inhibit NF-κB by an alternative mechanism involving EGFR–co-amplified and overexpressed protein.[75]

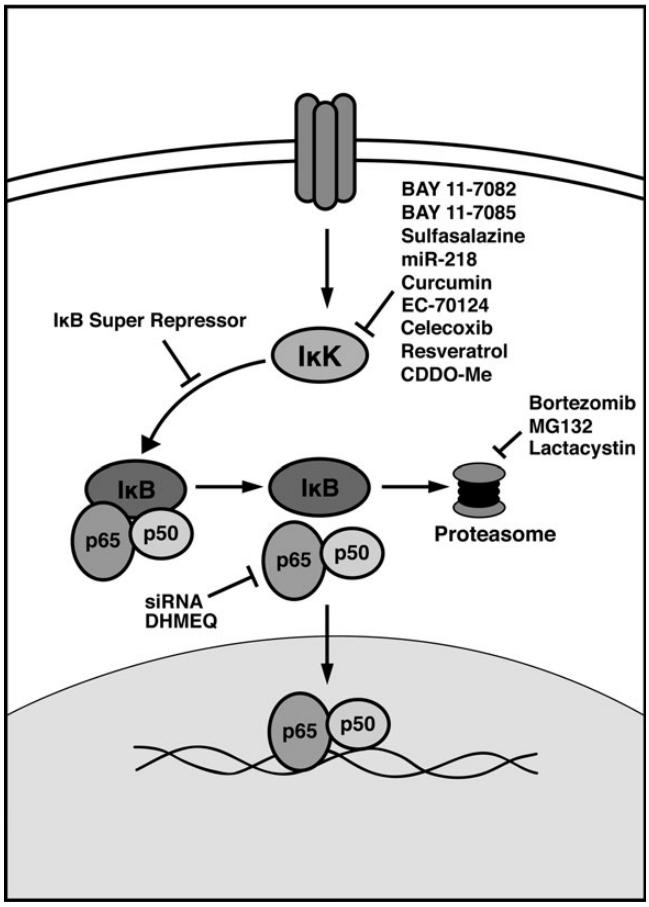

Fig. 5. Target sites and inhibitors of the NF-κB pathway used in GBM. NF-κB signaling can be targeted at multiple points to block activity, and a wide range of both specific and nonspecific agents have been used for NF-κB inhibition in GBM. The IκK complex is the primary regulation point of NF-κB activity and has been targeted by the largest number of agents. Downstream of IκK, IκBα degradation has been blocked in GBM using either the IκB super repressor or, more nonspecifically, with proteasome inhibitors. Depletion of individual NF-κB subunits has also been examined using RNA interference.

Despite the importance of IκK in the NF-κB response, this kinase has multiple non–NF-κB-related functions.[76] While IκKβ can phosphorylate p53, tuberous sclerosis 1, and FOXO3a, IκKα can enter the nucleus and interact with TGFβ-regulated Smad2/3.[77] These observations suggest that targeting IκK can lead to numerous NF-κB–independent actions that are not related to the off-target effects of the specific inhibitors. The potentially unpredictable nature of targeting the IκK complex emphasizes the importance of critically examining each agent individually. Nevertheless, given the heterogeneous nature of GBM, targeting a factor like IκK that modulates more than one signaling response might have a greater chance of achieving a therapeutic effect in this tumor.

IκBα Super Repressor

In contrast to the promiscuous nature of IκK, IκB proteins regulate NF-κB signaling in a more selective manner by specifically interacting with NF-κB dimers. Mutation of serines 32 and 36 in IκBα gives rise to a super repressor (IκBαSR), which is a protein that cannot be phosphorylated or degraded (Fig. 5). Whether used alone or in combination with DNA-damaging agents or cytokines, IκBαSR invariably improves the antiglioma effect.[24,71,78] Similarly, the combination of herpes simplex virus thymidine kinase (HSV-tk) and IκBαSR augments HSV-tk/ganciclovir suicide-gene therapy in GBM.[79] These studies highlight the potential of sequestering NF-κB subunits in the cytoplasm to enhance the therapeutic efficacy of antiglioma agents.

Targeting NF-κB Subunits

Perhaps the most specific method for blocking NF-κB is to directly target the subunits themselves. Given the importance of p65 in mediating NF-κB signaling, most groups have focused on this subunit. Knockdown of p65 induces cytotoxicity in GBM cells,[72] and expression of a p65 shRNA leads to a decrease in GBM xenograft growth and vascular density.[33] Another method to specifically block protein expression involves the use of intracellular antibodies (intrabodies). Expression of a single-chain intrabody against p65 in GBM cells was found to downregulate NF-κB–dependent gene expression and attenuate intracranial xenograft growth compared with control.[80] Pharmacological targeting of NF-κB at the subunit level has also been performed in GBM. Dehydroxymethylepoxyquinomicin (DHMEQ) is a small molecule that binds specific cysteine residues in p65 and other Rel homology proteins to block NF-κB nuclear translocation and DNA binding.[81] In GBM, DHMEQ inhibits NF-κB activity, decreases cell proliferation, and increases the survival of animals bearing intracranial xenografts.[82]

In contrast to experiments targeting p65, it was reported that loss of the p50 subunit in GBM cells actually has the opposite effect in that it attenuates the cytotoxicity of chemotherapeutics such as TMZ.[17] This effect of NF-κB is consistent with the paradoxical requirement of NF-κB for apoptosis in response to certain types of DNA damage.[10,83] Taken together, these findings illustrate the subunit-specific effects of NF-κB in modulating the response to DNA damaging agents.

Nonspecific NF-κB Inhibition

Proteasome Inhibitors

The NF-κB response is regulated to a large extent by ubiquitination, and proteasomal degradation of IκB proteins is an important step in NF-κB activation. Proteasome inhibition is a common strategy to attenuate NF-κB activity, and several proteasome inhibitors have been used in the treatment of experimental GBM including bortezomib, lactacystin, and MG132.[84,85] Bortezomib (Velcade, PS-341), a peptide that blocks the 20S subunit of the proteasome, downregulates antiapoptotic genes to induce cytotoxicity in GBM cells,[86] and enhances the anti-GBM effect of the chemotherapeutic vorinostat.[85]

Other Nonspecific Inhibitors

Given the central role of NF-κB in the inflammatory response, many natural and synthetic anti-inflammatory agents target the NF-κB pathway as part of their mechanism of action.

Although these agents have multiple non–NF-κB-related effects, their antitumor response is often linked to their action on NF-κB. One group of compounds that blocks NF-κB activity is the nonsteroidal anti-inflammatory drug (NSAID) family. These agents have antiproliferative effects in cancer cells that are at least partially attributed to inhibition of IκK. NSAIDs have differing NF-κB–attenuating and cytotoxic potencies, and celecoxib, one of the more potent NF-κB inhibitors,[87] has been shown to decrease proliferation and increase apoptosis in GBM cells.[88]

Resveratrol (trans-3,4′,5-trihydroxystilbene), a naturally occurring anti-inflammatory compound found in red wine, is another nonspecific NF-κB inhibitor that has shown promise in the treatment of GBM. Resveratrol inhibits NF-κB by targeting several points along the activation pathway including IκK and RIP1.[89,90] Although resveratrol induces cell death in U251 GBM cells[91] and increases survival of rats bearing intracranial gliomas,[92] it has not yet been used in humans. Triterpenoids are a group of naturally occurring compounds that have been used as anti-inflammatory and anticancer agents. The synthetic triterpenoid, 2-cyano-3,12-dioxooleana-1,9(11)-dien-28-oic acid (CDDO), has antitumor efficacy, and its methyl ester CDDO-Me inhibits IκK activity[93] and induces cytotoxicity in a number of GBM cell lines.[94] Another noteworthy agent is curcumin (diferuloylmethane) a biomolecule found in turmeric that inhibits nuclear p65 translocation via inhibition of IκK and Akt.[95] The efficacy of curcumin, both alone and in combination with chemotherapeutics, has been demonstrated in experimental GBM models.[96]

NF-κB Inhibition in Clinical Studies

Despite the success of targeting NF-κB in preclinical GBM models, only nonspecific agents have been used in the clinical setting. Sulfasalazine is an agent that inhibits IκK and is often used for the management of rheumatoid arthritis. In a phase 1/2 study involving 10 patients with progressive malignant glioma, subjects were assigned to one of 4 doses of oral sulfasalazine.[97] Unfortunately, no clinical responses were observed with a median progression-free survival (PFS) of 32 days. Four patients developed grade 4 toxicity, and 2 patients died, leading to early termination of the study.

The promising preclinical results obtained with bortezomib led to its use in patients with newly diagnosed and recurrent GBM.[98–100] In a phase 2 study, vorinostat and bortezomib were used in 37 patients with recurrent GBM.[98] However, as the median overall survival (OS) was only 3.2 months, the authors did not recommend continued evaluation at the dosing schedule used. Also, while a phase 1 study combining bortezomib with TMZ/IR was reported to be well tolerated and safe,[99] no phase 2 data have been presented.

A number of clinical trials using celecoxib in malignant glioma have been completed.[101–103] Reardon et al. conducted a phase 2 trial using CPT-11 (irinotecan) and celecoxib in 37 patients with grade III and IV glioma who had progressive disease. Median PFS and OS were 11 and 31.5 weeks, respectively.[101] This 17% objective response was similar to the 15% observed using CPT-11 alone.[104] In another study, 50 patients with newly diagnosed GBM and residual disease following radiotherapy and no prior chemotherapy were treated with TMZ, thalidomide, and celecoxib. Median PFS and OS were 5.9 and 12.6 months, respectively, with 9 partial responses and 22 cases of stable disease.[102] These results were also similar to a previous study using only TMZ and thalidomide, indicating that the addition of celecoxib was unlikely to have provided any significant benefit.

In summary, although the use of nonspecific NF-κB inhibitors in malignant glioma patients has been safe and generally well tolerated, these agents have not demonstrated any significant advantage over more conventional therapies. However, interpretation of these studies is somewhat difficult as most of them lack measurement of intratumoral NF-κB inhibition or drug accumulation.

Summary

The NF-κB response indeed contributes to the pathogenicity of GBM by modulating many of the pathways central to the malignant phenotype and promoting mesenchymal transition. Moreover, despite its multifaceted role in the response to DNA damage, in general NF-κB signaling attenuates the efficacy of cytotoxic agents. To date, studies manipulating NF-κB as a potential therapeutic approach have focused on nonspecific agents or on targets such as IκK that impact multiple signaling pathways. However, given the broad range of responses regulated by NF-κB signaling, it is likely that strategies that more rationally manipulate specific subsets of the NF-κB response will be the most successful for treating GBM. In this regard, it has been shown that inhibition of specific downstream NF-κB-regulated targets can significantly enhance the antiglioma effect.[105]

In conclusion, the importance of the NF-κB pathway to GBM growth and treatment resistance suggests that improved understanding of the mechanism by which this transcription factor is regulated in GBM is a strategy that can make a significant impact in the successful management of these tumors.

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References

1. Rayet B, Gelinas C. Aberrant rel/nfkb genes and activity in human cancer. *Oncogene*. 1999;18(49):6938–6947.
2. Raychaudhuri B, Han Y, Lu T, Vogelbaum MA. Aberrant constitutive activation of nuclear factor kappaB in glioblastoma multiforme drives invasive phenotype. *J Neurooncol*. 2007;85(1):39–47.
3. Wang H, Wang H, Zhang W, Huang HJ, Liao WS, Fuller GN. Analysis of the activation status of Akt, NFkappaB, and Stat3 in human diffuse gliomas. *Lab Invest*. 2004;84(8):941–951.
4. Nagai S, Washiyama K, Kurimoto M, Takaku A, Endo S, Kumanishi T. Aberrant nuclear factor-κB activity and its participation in the

growth of human malignant astrocytoma. *J Neurosurg*. 2002; 96(5):909–917.

5. Perkins ND. Integrating cell-signalling pathways with NF-kappaB and IKK function. *Nat Rev Mol Cell Biol*. 2007;8(1):49–62.
6. Hayden MS, Ghosh S. NF-kappaB, the first quarter-century: remarkable progress and outstanding questions. *Genes Dev*. 2012;26(3):203–234.
7. Wertz IE, Dixit VM. Signaling to NF-kappaB: regulation by ubiquitination. *Cold Spring Harb Perspect Biol*. 2010;2(3): a003350.
8. Senftleben U, Cao Y, Xiao G, et al. Activation by IKKalpha of a second, evolutionary conserved, NF-kappa B signaling pathway. *Science*. 2001;293(5534):1495–1499.
9. Sun SC. Non-canonical NF-kappaB signaling pathway. *Cell Res*. 2011;21(1):71–85.
10. Crawley CD, Kang S, Bernal GM, et al. S-phase-dependent p50/ NF-kB1 phosphorylation in response to ATR and replication stress acts to maintain genomic stability. *Cell Cycle*. 2015;14(4): 566–760.
11. Wu ZH, Miyamoto S. Many faces of NF-kappaB signaling induced by genotoxic stress. *J Mol Med*. 2007;85(11):1187–1202.
12. Huang TT, Wuerzberger-Davis SM, Wu ZH, Miyamoto S. Sequential modification of NEMO/IKKgamma by SUMO-1 and ubiquitin mediates NF-kappaB activation by genotoxic stress. *Cell*. 2003;115(5):565–576.
13. Crawley CD, Raleigh DR, Kang S, et al. DNA damage-induced cytotoxicity is mediated by the cooperative interaction of phospho-NF-kappaB p50 and a single nucleotide in the kappaB-site. *Nucleic Acids Res*. 2013;41(2):764–774.
14. Leung TH, Hoffmann A, Baltimore D. One nucleotide in a kappaB site can determine cofactor specificity for NF-kappaB dimers. *Cell*. 2004;118(4):453–464.
15. Rocha S, Garrett MD, Campbell KJ, Schumm K, Perkins ND. Regulation of NF-kappaB and p53 through activation of ATR and Chk1 by the ARF tumour suppressor. *EMBO J*. 2005;24(6): 1157–1169.
16. Rocha S, Perkins ND. ARF the integrator: linking NF-kappaB, p53 and checkpoint kinases. *Cell Cycle*. 2005;4(6):756–759.
17. Schmitt AM, Crawley CD, Kang S, et al. p50 (NF-kappaB1) is an effector protein in the cytotoxic response to DNA methylation damage. *Mol Cell*. 2011;44(5):785–796.
18. Wu ZH, Miyamoto S. Induction of a pro-apoptotic ATM-NF-kappaB pathway and its repression by ATR in response to replication stress. *EMBO J*. 2008;27(14):1963–1973.
19. Stojic L, Mojas N, Cejka P, et al. Mismatch repair-dependent G2 checkpoint induced by low doses of SN1 type methylating agents requires the ATR kinase. *Genes Devel*. 2004;18(11):1331–1344.
20. Ruland J. Return to homeostasis: downregulation of NF-kappaB responses. *Nat Immunol*. 2011;12(8):709–714.
21. Phillips HS, Kharbanda S, Chen R, et al. Molecular subclasses of high-grade glioma predict prognosis, delineate a pattern of disease progression, and resemble stages in neurogenesis. *Cancer Cell*. 2006;9(3):157–173.
22. Verhaak RG, Hoadley KA, Purdom E, et al. Integrated genomic analysis identifies clinically relevant subtypes of glioblastoma characterized by abnormalities in PDGFRA, IDH1, EGFR, and NF1. *Cancer Cell*. 2010;17(1):98–110.
23. Sturm D, Bender S, Jones DT, et al. Paediatric and adult glioblastoma: multiform (epi)genomic culprits emerge. *Nat Rev Cancer*. 2014;14(2):92–107.
24. Bhat KP, Balasubramaniyan V, Vaillant B, et al. Mesenchymal differentiation mediated by NF-kappaB promotes radiation resistance in glioblastoma. *Cancer Cell*. 2013;24(3):331–346.
25. Lee DW, Ramakrishnan D, Valenta J, Parney IF, Bayless KJ, Sitcheran R. The NF-kappaB RelB protein is an oncogenic driver of mesenchymal glioma. *PLoS One*. 2013;8(2):e57489.
26. Cancer Genome Atlas Research N. Comprehensive genomic characterization defines human glioblastoma genes and core pathways. *Nature*. 2008;455(7216):1061–1068.
27. Kapoor GS, Zhan Y, Johnson GR, O'Rourke DM. Distinct domains in the SHP-2 phosphatase differentially regulate epidermal growth factor receptor/NF-kappaB activation through Gab1 in glioblastoma cells. *Mol Cell Biol* 2004;24(2):823–836.
28. Sethi G, Ahn KS, Chaturvedi MM, Aggarwal BB. Epidermal growth factor (EGF) activates nuclear factor-kappaB through IkappaBalpha kinase-independent but EGF receptor-kinase dependent tyrosine 42 phosphorylation of IkappaBalpha. *Oncogene*. 2007;26(52):7324–7332.
29. Tanaka K, Babic I, Nathanson D, et al. Oncogenic EGFR signaling activates an mTORC2-NF-kappaB pathway that promotes chemotherapy resistance. *Cancer Discov*. 2011;1(6):524–538.
30. Yang W, Xia Y, Cao Y, et al. EGFR-induced and PKCepsilon monoubiquitylation-dependent NF-kappaB activation upregulates PKM2 expression and promotes tumorigenesis. *Mol Cell*. 2012; 48(5):771–784.
31. Aldape KD, Ballman K, Furth A, et al. Immunohistochemical detection of EGFRvIII in high malignancy grade astrocytomas and evaluation of prognostic significance. *J Neuropathol Exp Neurol*. 2004;63(7):700–707.
32. Puliyappadamba VT, Chakraborty S, Chauncey SS, et al. Opposing effect of EGFRWT on EGFRvIII-mediated NF-kappaB activation with RIP1 as a cell death switch. *Cell Rep*. 2013;4(4):764–775.
33. Bonavia R, Inda MM, Vandenberg S, et al. EGFRvIII promotes glioma angiogenesis and growth through the NF-kappaB, interleukin-8 pathway. *Oncogene*. 2012;31(36):4054–4066.
34. Park S, Hatanpaa KJ, Xie Y, et al. The receptor interacting protein 1 inhibits p53 induction through NF-kappaB activation and confers a worse prognosis in glioblastoma. *Cancer Res*. 2009;69(7): 2809–2816.
35. Bredel M, Scholtens DM, Yadav AK, et al. NFKBIA deletion in glioblastomas. *N Engl J Med*. 2011;364(7):627–637.
36. Patane M, Porrati P, Bottega E, et al. Frequency of NFKBIA deletions is low in glioblastomas and skewed in glioblastoma neurospheres. *Mol Cancer*. 2013;12:160.
37. Shih AH, Holland EC. Platelet-derived growth factor (PDGF) and glial tumorigenesis. *Cancer Lett*. 2006;232(2):139–147.
38. Romashkova JA, Makarov SS. NF-kappaB is a target of AKT in anti-apoptotic PDGF signalling. *Nature*. 1999;401(6748):86–90.
39. Holmes KM, Annala M, Chua CY, et al. Insulin-like growth factor-binding protein 2-driven glioma progression is prevented by blocking a clinically significant integrin, integrin-linked kinase, and NF-kappaB network. *Proc Natl Acad Sci USA*. 2012; 109(9):3475–3480.
40. Smith D, Shimamura T, Barbera S, Bejcek BE. NF-kappaB controls growth of glioblastomas/astrocytomas. *Mol Cell Biochem*. 2008; 307(1-2):141–147.
41. Hanson JL, Anest V, Reuther-Madrid J, Baldwin AS. Oncoprotein suppression of tumor necrosis factor-induced NF kappa B activation is independent of Raf-controlled pathways. *J Biol Chem*. 2003;278(37):34910–34917.

42. Finco TS, Westwick JK, Norris JL, Beg AA, Der CJ, Baldwin AS Jr. Oncogenic Ha-Ras-induced signaling activates NF-kappaB transcriptional activity, which is required for cellular transformation. *J Biol Chem*. 1997;272(39):24113–24116.
43. Meylan E, Dooley AL, Feldser DM, et al. Requirement for NF-kappaB signalling in a mouse model of lung adenocarcinoma. *Nature*. 2009;462(7269):104–107.
44. Schneider G, Kramer OH. NFkappaB/p53 crosstalk-a promising new therapeutic target. *Biochim Biophys Acta*. 2011;1815(1): 90–103.
45. Ryan KM, Ernst MK, Rice NR, Vousden KH. Role of NF-kappaB in p53-mediated programmed cell death. *Nature*. 2000; 404(6780):892–897.
46. Weisz L, Damalas A, Liontos M, et al. Mutant p53 enhances nuclear factor kappaB activation by tumor necrosis factor alpha in cancer cells. *Cancer Res*. 2007;67(6):2396–2401.
47. Song L, Liu L, Wu Z, et al. TGF-beta induces miR-182 to sustain NF-kappaB activation in glioma subsets. *J Clin Invest*. 2012; 122(10):3563–3578.
48. Garkavtsev I, Kozin SV, Chernova O, et al. The candidate tumour suppressor protein ING4 regulates brain tumour growth and angiogenesis. *Nature*. 2004;428(6980):328–332.
49. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. *Cell*. 2011;144(5):646–674.
50. Koul D, Takada Y, Shen R, Aggarwal BB, Yung WK. PTEN enhances TNF-induced apoptosis through modulation of nuclear factor-kappaB signaling pathway in human glioma cells. *Biochem Biophys Res Commun*. 2006;350(2):463–471.
51. Witzel II, Koh LF, Perkins ND. Regulation of cyclin D1 gene expression. *Biochem Soc Trans*. 2010;38(Pt 1):217–222.
52. Sallinen SL, Sallinen PK, Kononen JT, et al. Cyclin D1 expression in astrocytomas is associated with cell proliferation activity and patient prognosis. *J Pathol*. 1999;188(3):289–293.
53. Wang J, Wang Q, Cui Y, et al. Knockdown of cyclin D1 inhibits proliferation, induces apoptosis, and attenuates the invasive capacity of human glioblastoma cells. *J Neurooncol*. 2012; 106(3):473–484.
54. Bredel M, Bredel C, Juric D, et al. Tumor necrosis factor-alpha-induced protein 3 as a putative regulator of nuclear factor-kappaB-mediated resistance to O6-alkylating agents in human glioblastomas. *J Clin Oncol*. 2006;24(2):274–287.
55. Chilov D, Kukk E, Taira S, et al. Genomic organization of human and mouse genes for vascular endothelial growth factor C. *J Biol Chem*. 1997;272(40):25176–25183.
56. Loeffler S, Fayard B, Weis J, Weissenberger J. Interleukin-6 induces transcriptional activation of vascular endothelial growth factor (VEGF) in astrocytes in vivo and regulates VEGF promoter activity in glioblastoma cells via direct interaction between STAT3 and Sp1. *Int J Cancer*. 2005;115(2):202–213.
57. Brat DJ, Bellail AC, Van Meir EG. The role of interleukin-8 and its receptors in gliomagenesis and tumoral angiogenesis. *Neuro Oncol*. 2005;7(2):122–133.
58. Atkinson GP, Nozell SE, Harrison DK, Stonecypher MS, Chen D, Benveniste EN. The prolyl isomerase Pin1 regulates the NF-kappaB signaling pathway and interleukin-8 expression in glioblastoma. *Oncogene*. 2009;28(42):3735–3745.
59. Sarkar D, Park ES, Emdad L, Lee SG, Su ZZ, Fisher PB. Molecular basis of nuclear factor-kappaB activation by astrocyte elevated gene-1. *Cancer Res*. 2008;68(5):1478–1484.
60. Kesanakurti D, Chetty C, Rajasekhar Maddirela D, Gujrati M, Rao JS. Essential role of cooperative NF-kappaB and Stat3 recruitment to ICAM-1 intronic consensus elements in the regulation of radiation-induced invasion and migration in glioma. *Oncogene*. 2013;32(43):5144–5155.
61. Tran NL, McDonough WS, Donohue PJ, et al. The human Fn14 receptor gene is up-regulated in migrating glioma cells in vitro and overexpressed in advanced glial tumors. *Am J Pathol*. 2003;162(4):1313–1321.
62. Tran NL, McDonough WS, Savitch BA, et al. Increased fibroblast growth factor-inducible 14 expression levels promote glioma cell invasion via Rac1 and nuclear factor-kappaB and correlate with poor patient outcome. *Cancer Res*. 2006;66(19): 9535–9542.
63. Demuth T, Rennert JL, Hoelzinger DB, et al. Glioma cells on the run - the migratory transcriptome of 10 human glioma cell lines. *BMC Genomics*. 2008;9:54.
64. Edwards LA, Woolard K, Son MJ, et al. Effect of brain- and tumor-derived connective tissue growth factor on glioma invasion. *J Natl Cancer Inst*. 2011;103(15):1162–1178.
65. Tao T, Cheng C, Ji Y, et al. Numbl inhibits glioma cell migration and invasion by suppressing TRAF5-mediated NF-kappaB activation. *Mol Biol Cell*. 2012;23(14):2635–2644.
66. Karin M. NF-kappaB as a critical link between inflammation and cancer. *Cold Spring Harb Perspect Biol*. 2009;1(5):a000141.
67. Sen E. Targeting inflammation-induced transcription factor activation: an open frontier for glioma therapy. *Drug Discov Today*. 2011;16(23–24):1044–1051.
68. Bao S, Wu Q, McLendon RE, et al. Glioma stem cells promote radioresistance by preferential activation of the DNA damage response. *Nature*. 2006;444(7120):756–760.
69. Garner JM, Fan M, Yang CH, et al. Constitutive activation of signal transducer and activator of transcription 3 (STAT3) and nuclear factor kappaB signaling in glioblastoma cancer stem cells regulates the Notch pathway. *J Biol Chem*. 2013;288(36): 26167–26176.
70. Widera D, Mikenberg I, Kaltschmidt B, Kaltschmidt C. Potential role of NF-kappaB in adult neural stem cells: the underrated steersman? *Int J Dev Neurosci*. 2006;24(2–3):91–102.
71. Nogueira L, Ruiz-Ontanon P, Vazquez-Barquero A, et al. Blockade of the NFkappaB pathway drives differentiating glioblastoma-initiating cells into senescence both in vitro and in vivo. *Oncogene*. 2011;30(32):3537–3548.
72. Zanotto-Filho A, Braganhol E, Schroder R, et al. NFkappaB inhibitors induce cell death in glioblastomas. *Biochem Pharmacol*. 2011;81(3):412–424.
73. Lee J, Rhee MH, Kim E, Cho JY. BAY 11-7082 is a broad-spectrum inhibitor with anti-inflammatory activity against multiple targets. *Mediators Inflamm*. 2012;2012:416036.
74. Song L, Huang Q, Chen K, et al. miR-218 inhibits the invasive ability of glioma cells by direct downregulation of IKK-beta. *Biochem Biophys Res Commun*. 2010;402(1):135–140.
75. Xia H, Yan Y, Hu M, et al. MiR-218 sensitizes glioma cells to apoptosis and inhibits tumorigenicity by regulating ECOP-mediated suppression of NF-kappaB activity. *Neuro Oncol*. 2013;15(4):413–422.
76. Chariot A. The NF-kappaB-independent functions of IKK subunits in immunity and cancer. *Trends Cell Biol*. 2009;19(8): 404–413.

77. Espinosa L, Margalef P, Bigas A. Non-conventional functions for NF-kappaB members: the dark side of NF-kappaB. *Oncogene*. 2014;34(18):2279–2287.
78. Weaver K, Yeyeodu S, Cusack J Jr, Baldwin A Jr, Ewend M. Potentiation of chemotherapeutic agents following antagonism of nuclear factor kappa B in human gliomas. *J Neurooncol*. 2003;61(3):187–196.
79. Moriuchi S, Glorioso JC, Maruno M, et al. Combination gene therapy for glioblastoma involving herpes simplex virus vector-mediated codelivery of mutant IkappaBalpha and HSV thymidine kinase. *Cancer Gene Ther*. 2005;12(5):487–496.
80. Li L, Gondi CS, Dinh DH, Olivero WC, Gujrati M, Rao JS. Transfection with anti-p65 intrabody suppresses invasion and angiogenesis in glioma cells by blocking nuclear factor-kappaB transcriptional activity. *Clin Cancer Res*. 2007;13(7):2178–2190.
81. Yamamoto M, Horie R, Takeiri M, Kozawa I, Umezawa K. Inactivation of NF-κB components by covalent binding of (−)-dehydroxymethylepoxyquinomicin to specific cysteine residues. *J Med Chem*. 2008;51(18):5780–5788.
82. Fukushima T, Kawaguchi M, Yorita K, et al. Antitumor effect of dehydroxymethylepoxyquinomicin, a small molecule inhibitor of nuclear factor-kappaB, on glioblastoma. *Neuro Oncol*. 2012; 14(1):19–28.
83. Campbell KJ, Rocha S, Perkins ND. Active repression of antiapoptotic gene expression by RelA(p65) NF-kappa B. *Mol Cell*. 2004;13(6):853–865.
84. Wagenknecht B, Hermisson M, Groscurth P, Liston P, Krammer PH, Weller M. Proteasome inhibitor-induced apoptosis of glioma cells involves the processing of multiple caspases and cytochrome c release. *J Neurochem*. 2000;75(6):2288–2297.
85. Premkumar DR, Jane EP, Agostino NR, DiDomenico JD, Pollack IF. Bortezomib-induced sensitization of malignant human glioma cells to vorinostat-induced apoptosis depends on reactive oxygen species production, mitochondrial dysfunction, Noxa upregulation, Mcl-1 cleavage, and DNA damage. *Mol Carcinog*. 2013;52(2):118–133.
86. Yin D, Zhou H, Kumagai T, et al. Proteasome inhibitor PS-341 causes cell growth arrest and apoptosis in human glioblastoma multiforme (GBM). *Oncogene*. 2005;24(3):344–354.
87. Takada Y, Bhardwaj A, Potdar P, Aggarwal BB. Nonsteroidal anti-inflammatory agents differ in their ability to suppress NF-kappaB activation, inhibition of expression of cyclooxygenase-2 and cyclin D1, and abrogation of tumor cell proliferation. *Oncogene*. 2004;23(57):9247–9258.
88. Sareddy GR, Geeviman K, Ramulu C, Babu PP. The nonsteroidal anti-inflammatory drug celecoxib suppresses the growth and induces apoptosis of human glioblastoma cells via the NF-kappaB pathway. *J Neurooncol*. 2012;106(1):99–109.
89. Kundu JK, Shin YK, Kim SH, Surh YJ. Resveratrol inhibits phorbol ester-induced expression of COX-2 and activation of NF-kappaB in mouse skin by blocking IkappaB kinase activity. *Carcinogenesis*. 2006;27(7):1465–1474.
90. Youn HS, Lee JY, Fitzgerald KA, Young HA, Akira S, Hwang DH. Specific inhibition of MyD88-independent signaling pathways of TLR3 and TLR4 by resveratrol: molecular targets are TBK1 and RIP1 in TRIF complex. *J Immunol*. 2005;175(5):3339–3346.
91. Jiang H, Zhang L, Kuo J, et al. Resveratrol-induced apoptotic death in human U251 glioma cells. *Mol Cancer Ther*. 2005;4(4): 554–561.
92. Tseng S-H, Lin S-M, Chen J-C, et al. Resveratrol suppresses the angiogenesis and tumor growth of gliomas in rats. *Clin Cancer Res*. 2004;10(6):2190–2202.
93. Ahmad R, Raina D, Meyer C, Kharbanda S, Kufe D. Triterpenoid CDDO-Me blocks the NF-kappaB pathway by direct inhibition of IKKbeta on Cys-179. *J Biol Chem*. 2006;281(47):35764–35769.
94. Gao X, Deeb D, Jiang H, Liu Y, Dulchavsky SA, Gautam SC. Synthetic triterpenoids inhibit growth and induce apoptosis in human glioblastoma and neuroblastoma cells through inhibition of prosurvival Akt, NF-kappaB and Notch1 signaling. *J Neurooncol*. 2007;84(2):147–157.
95. Bava SV, Puliappadamba VT, Deepti A, Nair A, Karunagaran D, Anto RJ. Sensitization of taxol-induced apoptosis by curcumin involves down-regulation of nuclear factor-kappaB and the serine/threonine kinase Akt and is independent of tubulin polymerization. *J Biol Chem*. 2005;280(8):6301–6308.
96. Dhandapani KM, Mahesh VB, Brann DW. Curcumin suppresses growth and chemoresistance of human glioblastoma cells via AP-1 and NFkappaB transcription factors. *J Neurochem*. 2007; 102(2):522–538.
97. Robe PA, Martin DH, Nguyen-Khac MT, et al. Early termination of ISRCTN45828668, a phase 1/2 prospective, randomized study of sulfasalazine for the treatment of progressing malignant gliomas in adults. *BMC Cancer*. 2009;9:372.
98. Friday BB, Anderson SK, Buckner J, et al. Phase II trial of vorinostat in combination with bortezomib in recurrent glioblastoma: a north central cancer treatment group study. *Neuro Oncol*. 2012;14(2):215–221.
99. Kubicek GJ, Werner-Wasik M, Machtay M, et al. Phase I trial using proteasome inhibitor bortezomib and concurrent temozolomide and radiotherapy for central nervous system malignancies. *Int J Radiat Oncol Biol Phys*. 2009;74(2):433–439.
100. Phuphanich S, Supko JG, Carson KA, et al. Phase 1 clinical trial of bortezomib in adults with recurrent malignant glioma. *J Neurooncol*. 2010;100(1):95–103.
101. Reardon DA, Quinn JA, Vredenburgh J, et al. Phase II trial of irinotecan plus celecoxib in adults with recurrent malignant glioma. *Cancer*. 2005;103(2):329–338.
102. Kesari S, Schiff D, Henson JW, et al. Phase II study of temozolomide, thalidomide, and celecoxib for newly diagnosed glioblastoma in adults. *Neuro Oncol*. 2008;10(3): 300–308.
103. Levin VA, Giglio P, Puduvalli VK, et al. Combination chemotherapy with 13-cis-retinoic acid and celecoxib in the treatment of glioblastoma multiforme. *J Neurooncol*. 2006;78(1):85–90.
104. Friedman HS, Petros WP, Friedman AH, et al. Irinotecan therapy in adults with recurrent or progressive malignant glioma. *J Clin Oncol*. 1999;17(5):1516.
105. Mansour NM, Bernal GM, Wu L, et al. Decoy Receptor DcR1 Is Induced in a p50/Bcl3-Dependent Manner and Attenuates the Efficacy of Temozolomide. *Cancer Res*. 2015;75(10):2039–2048.