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The role of NF-κB in the pathogenesis of glioma

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Abbreviations: EGF, epidermal growth factor; EGFR, epidermal growth factor receptor; EGFRwt, epidermal growth factor receptor wild type; EGFRvIII, epidermal growth factor receptor variant III; GBM, glioblastoma; IκBα, nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, α; NEMO, NF-kappa-B essential modulator; NF-κB, nuclear factor kappa-light-chain-enhancer of activated B cells; PTEN, phosphatase and tensin homolog; RIP1, receptor interacting protein-1; TCGA, The Cancer Genome Atlas; TNFα, tumor necrosis factor α; TRAIL, TNF-related apoptosis-inducing ligand.

Activation of NF-κB affects multiple aspects of cancer biology including cell survival and resistance to treatment. Glioblastoma (GBM) is the most common primary malignant tumor of the brain in adults and is resistant to treatment. Recent studies have reported that NF-κB activation in GBM is widespread and have elucidated the underlying regulatory mechanisms. *EGFR* gene amplification and mutation are among the key genetic alterations in GBM, and aberrant EGFR signaling is a key activator of NF-κB in GBM. In this review we discuss the evidence for activation of NF-κB in GBM and the key signaling pathways involved. Substantial evidence suggests a role for NF-κB in the pathogenesis of GBM and its resistance to treatment, indicating that NF-κB pathways may be useful targets for treatment.

Introduction

Glioblastoma (glioblastoma multiforme; GBM) is the most aggressive primary brain tumor in the adult nervous system and is associated with a poor prognosis.[1] GBM is also the most common type of primary malignant brain tumor in adults. Relative survival estimates for glioblastoma are quite low and only approximately 4.5% of patients survive 5 years after diagnosis.[2] Glioma is grouped into 4 histologic grades based on the degree of differentiation, anaplasia, and aggressiveness as WHO Grade I-IV tumors. Malignant gliomas include anaplastic astrocytoma, anaplastic oligodendroglioma, and anaplastic oligoastrocytoma (Grade III) and GBM (Grade IV).[3]

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The molecular pathogenesis of glioma is thought to involve multiple genetic alterations that result in aberrant activity of pathways involved in proliferation, cell cycle regulation, and apoptosis.[4,5] A series of genetic events have been identified in the clonal evolution of these tumors. The genetic changes detected most frequently in primary GBM include *INK4A* loss, *EGFR* amplification and mutation, *PTEN* loss, and *MDM2* amplification, among other abnormalities.[4,5] More recently, The Cancer Genome Atlas (TCGA) has provided a comprehensive picture of genetic abnormalities in GBM. Based on the molecular signature, GBM has been classified into 4 subclasses: classical, mesenchymal, proneural, and neural. Epidermal growth factor receptor (*EGFR*) gene amplification and mutation is one the most common and striking abnormalities in GBM[4,6] and is usually found in the classical subtype of the disease.[6]

Recent studies suggest an important role for nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) signaling in GBM and implicate NF-κB activation as an important driver of the malignant phenotype that confers a negative prognosis in patients with GBM.[7-9] NF-κB activation is a hallmark of inflammation and has been a focus of intense interest in inflammation-induced cancer.[10] Signs of inflammation in GBMs can be detected in the form of infiltration by macrophages/microglia and lymphocytes, production of inflammatory cytokines, and activation of NF-κB,[11,13] suggesting that inflammation may play a role in gliomagenesis. However, signs of significant inflammation are not prominent in most GBMs and the activation of NF-κB in GBM is likely secondary to genetic changes and aberrant signaling. In this review we discuss recent advances in our understanding of the role of NF-κB signaling in the pathogenesis of GBM.

Activation of NF-κB

NF-κB is a family of transcription factors that bind to the enhancer element of the immunoglobulin kappa light-chain of activated B cells.[14] Structurally, NF-κB is composed of homodimers and heterodimers of the 5 members of the Rel family, namely NF-κB1 (p50/p105), NF-κB2 (p52/100), RelA (p65),

and c-Rel. In unstimulated cells, NF-κB is kept inactive by its interaction with the inhibitor IκBα and the complex is usually located in the cytoplasm. In response to stimuli such as cytokines or DNA damage, IκB kinases IKKα or IKKβ become activated and phosphorylate IκBα leading to its degradation by a K48 ubiquitin-mediated proteasomal mechanism. The free NF-κB now translocates to the nucleus and acts as a transcription factor for various downstream target genes.[15] Cytokines such as TNFα, TRAIL, EGF, and VEGF and DNA damaging agents are able to induce NF-κB by this canonical pathway. Activation of the IKKs involves the participation of a number of upstream components including IKK gamma (also known as NEMO), RIPK1, TAK1, TRAF1/2, and cIAP1/2.[16] In the non-canonical pathway of NF-κB activation, IKKα phosphorylates the p100 precursor leading to the formation of a p52/RelB dimer that translocates to the nucleus and initiates transcription.

NF-κB is activated in GBM

Immunohistochemical staining for the p65 subunit of NF-κB with a p65-specific antibody revealed increased expression of p65 in glioma cells compared to normal brain.[17] The same study found that overexpression of p65 correlated well with the histologic grade of the glioma, being higher in malignant glioma compared to low-grade glioma. The pattern of staining was reported as diffuse cytoplasmic with scattered nuclear staining. Immunohistochemical staining with antibody specific for phospho-p65 revealed increased staining in GBM compared to lower grade glioma.[17] This antibody detects the activated form of the p65 subunit of NF-κB when it is phosphorylated at serine 536. Increased phosphorylation of p65 in GBMs was also confirmed by western blot analysis of frozen tissue derived from tumors.[17] In another study, primary cultures derived from GBMs revealed constitutive NF-κB activation, and increased nuclear localization of the p65 and p50 subunits was detected in GBM but not in normal astrocytes.[18] In a recent study we also showed that NF-κB p65 is frequently phosphorylated in GBM; p65 was phosphorylated in 20 out of the 23 GBM tumors tested.[19] **Figures 1A** and **1D** show increased expression of p65 in one GBM, a moderate level of expression in another GBM (**Fig. 1B and E**), and absent or weak staining for p65 in normal brain (**Fig. 1C**). Nuclear localization of p65 in GBM can be seen in **Fig. 1F**. Increased phosphorylation of the p65 subunit in GBM is shown in **Fig. 2A and C**, whereas a low level of phopsho-p65 staining in normal brain is depicted in **Fig. 2B**. Thus, upregulation and activation of NF-κB is common in gliomas, and particularly in GBM. Activation of NF-κB in GBM in response to cytokines and growth factor signaling is depicted in **Fig. 3**.

In addition to the p65–p50 heterodimer, other members of the NF-κB family are also reported to mediate NF-κB signaling in glioma. For example, p68 RNA helicase induces an increased nuclear abundance of p50 resulting in NF-κB activation in glioma cells.[20] In mesenchymal glioma the non-canonical pathway of NF-κB, in which RelB has a prominent role, is also involved.[21]

Targets of NF-κB in glioma

NF-κB target genes include cell cycle regulatory genes, antiapoptotic genes, inflammatory cytokines, and cell adhesion molecules that regulate tumor growth and metastasis. The major NF-κB targets include the cell cycle regulatory protein cyclin D1,[22-26] the anti-apoptotic protein XIAP1,[27,28] and inflammatory proteins such as IL-6,[29] IL-8,[30-32] MMP-9,[33-35] MMP-13,[36] and Cox2.[33] The regulation of signal transduction pathways mediating proliferation, release of inflammatory cytokines, and expression of metalloproteinases in the tumor microenvironment by NF-κB activation facilitates tumor growth. It is also important to note that there is extensive crosstalk between NF-κB and oncogenic and tumor suppressor signaling pathways, including those active in GBM.[37-39]

Major Mechanisms of NF-κB Activation in Glioma

Although a large number of stimuli can activate NF-κB in glioma cells, 2 common mechanisms appear to to be particularly important. First, EGFR signaling is known to activate NF-κB.[40,41] Since *EGFR* gene amplification and mutation are common in GBM, aberrant EGFR signaling is likely to be an important mechanism of NF-κB activation in GBM. Second, a genome wide analysis study of 790 clinical glioblastoma samples showed a 23.4% rate of deletion of the *NFKBIA* gene that encodes IκBα.[8] Loss of this key inhibitor of NF-κB activation results in constitutive NF-κB activation. Importantly, deletion of *NFKBIA* was detected in non-classical forms of GBM. Since *EGFR* gene amplification and mutation are detected in the classical subtype of GBM, this suggests a pattern of mutual exclusivity between these 2 major mechanisms of NF-κB activation.[8] NF-κB activation has been reported to promote a mesenchymal phenotype in GBM.[7]

EGFR-mediated NF-κB activation in glioma

EGFR gene amplification and mutations are detected in 40–50% of GBMs and result in increased levels of EGFR wild type (EGFRwt) and mutant forms in tumor cells. EGFRvIII is the most common mutant form found in GBM, being present in approximately 25% of tumors, and has received intense scrutiny because of its increased oncogenic potential compared to EGFRwt.[42-44] EGFRvIII has an in-frame deletion of exons II-VII, resulting in a truncated EGFR that is missing part of the extracellular ligand binding domain and is constitutively active. Both EGFRwt and EGFRvIII have been reported to activate NF-κB but the mechanisms involved appear to be distinct. EGFRwt has been reported to activate NF-κB in glioma cells via a SHP-2- and Gab1-dependent pathway[45] and via a PLC gamma- and PKC epsilon-dependent pathway.[46] At least 2 mechanisms have been described for EGFRvIII-mediated activation of NF-κB, including an mTORC2-dependent pathway.[47]

We recently found that receptor-interacting protein (RIP1, RIPK1) is a key link between EGFR and NF-κB signaling in GBM.[19] RIP1 is known to be an essential component of stress-induced NF-κB activation and is also a central mediator of both apoptotic and necrotic cell death. Thus, depending on the cellular context, RIP1 can induce either cell death through engagement of the cell death machinery or cell survival by activating NF-κB. We have shown that RIP1 is commonly overexpressed in GBMs and confers worse survival.[38] EGFRvIII recruits ubiquitin

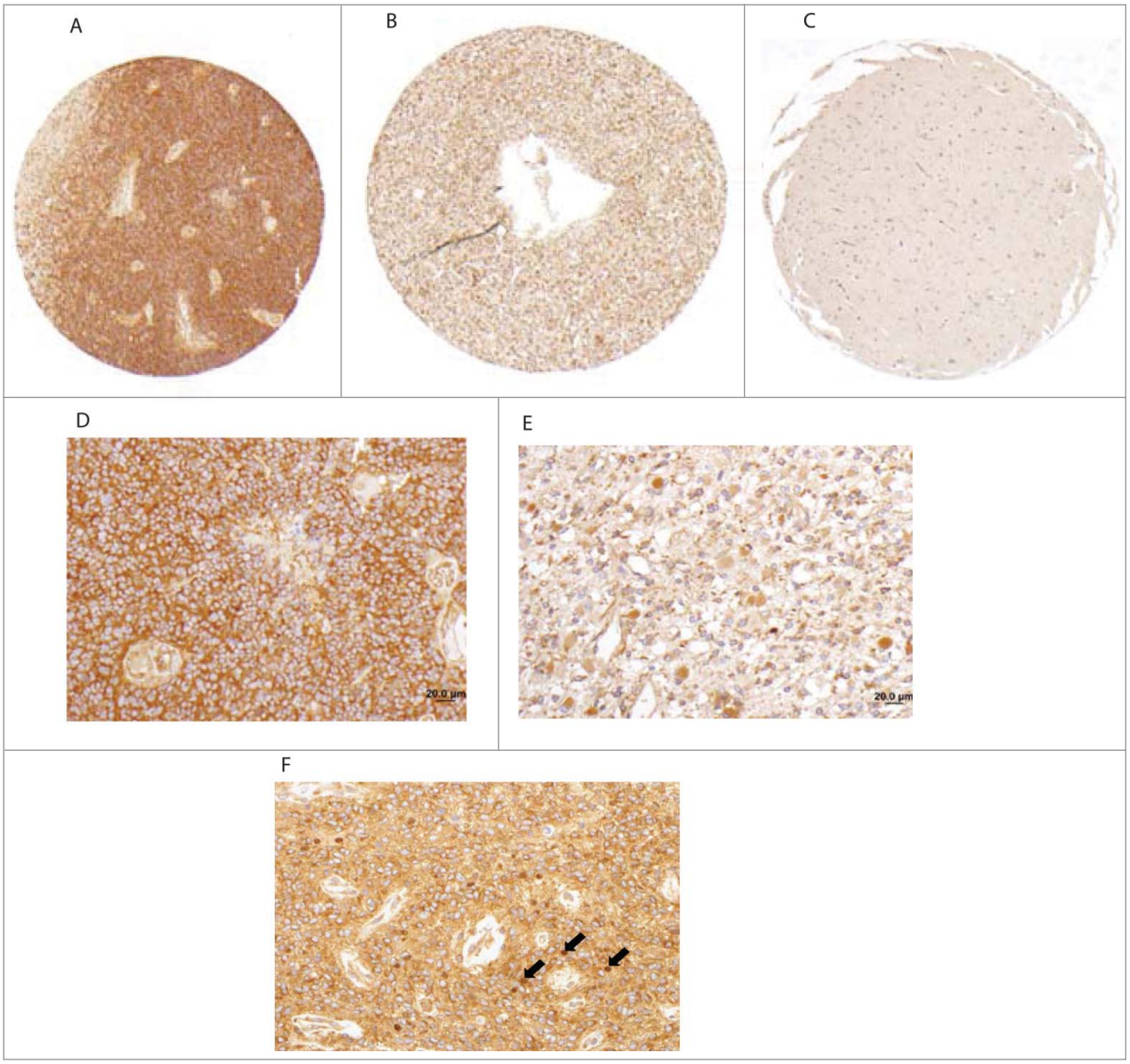

Figure 1. NF-κB is upregulated and activated in GBM. (**A**) and (**D**) Strong staining for the p65 subunit of NF-κB signaling in formalin-fixed paraffin-embedded sections from a GBM tumor. (**B**) and (**E**) Moderate staining for p65 in another GBM. (**C**) Weak staining for p65 in normal brain (cerebral cortex). (**F**) Nuclear localization of the p65 subunit (arrows) in GBM.

ligases to RIP1, resulting in K63-linked ubiquitination of RIP1. Polyubiquitinated RIP1 binds to TAK1 and NEMO forming a EGFRvIII-associated signaling platform that activates NF-κB. RIP1 is essential for EGFRvIII-mediated NF-κB activation and oncogenicity in an orthotopic model and correlates with NF-κB activation in GBM.[19] Intriguingly, activation of EGFRwt by EGF results in novel negative regulation of EGFRvIII with rapid dissociation of the EGFRvIII-RIP1 signalosome, loss of NF-κB activation, and subsequent formation of a complex of RIP1 with the death adaptor FADD and caspase-8 that results in EGF-driven cell death that requires the kinase activity of RIP1.[19] Thus, RIP1 is also a key life/death switch in a major receptor tyrosine kinase (RTK) signaling system that turns a normally trophic signal into a death signal.

Other activators of NF-κB

In addition to the 2 major mechanisms of NF-κB activation in glioma described above (aberrant EGFR signaling and *NFKBI* deletion), a number of other mechanisms that can activate NF-κB in glioma cells have been identified. For example, we reported that TRADD, a key adaptor in TNFα-mediated activation of NF-κB, is commonly expressed at high levels in GBM and confers a worse prognosis.[48] TRADD is required for TNFα-mediated NF-κB activation in glioma cells. Additionally, GBMs have a high frequency of deletion of chromosome 10, which contains the *TNAIP3* (*A20*) gene locus encoding a negative regulator of NF-κB.[49] Constitutive STAT3 activation in tumors maintains NF-κB activation by sustained acetylation of p65.[50] STAT3 was shown to physically interact with the p65 subunit of NF-κB.[51]

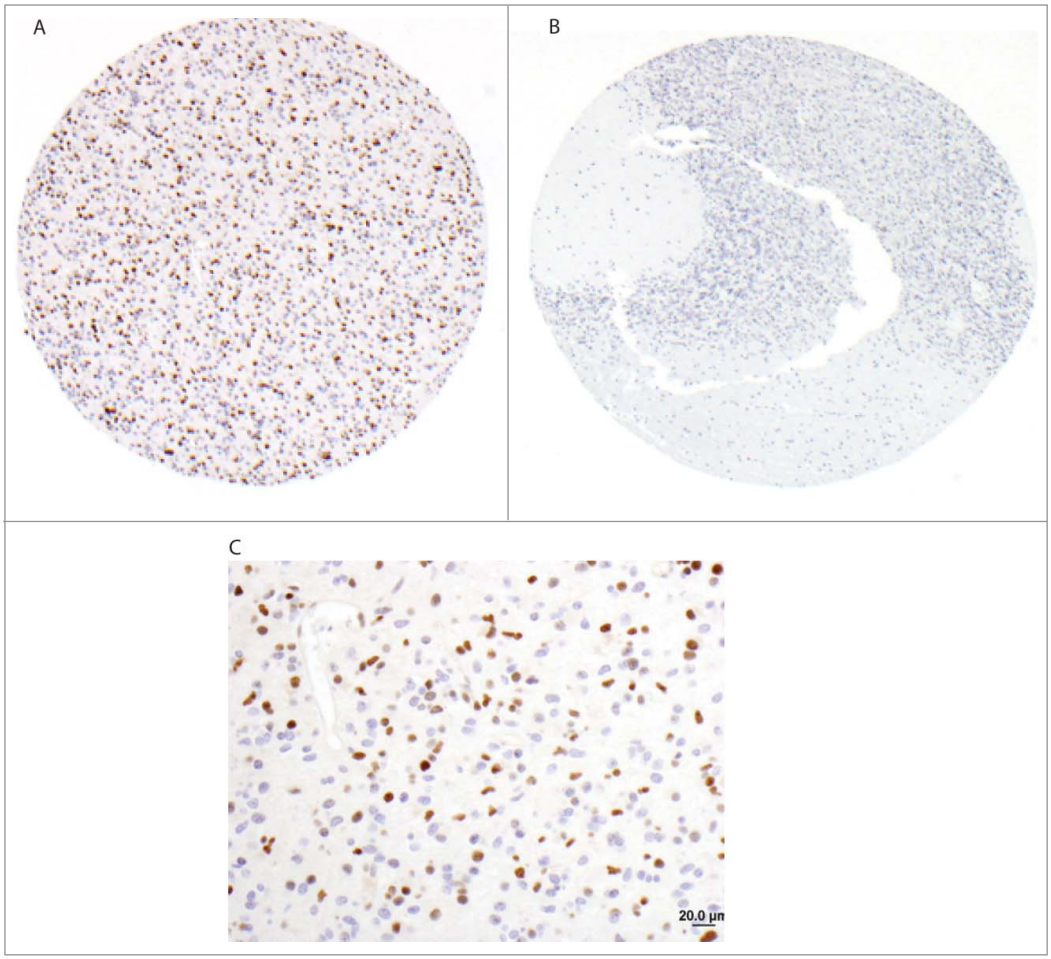

Figure 2. NF-κB is activated in GBM as determined by phosphorylation of the p65 subunit of NF-κB. (**A**) and (**C**). Strong staining for phospho-p65 in GBM. (**B**) Weak staining for phospho-p65 in normal brain (cerebellum).

Interestingly, one study reports that the NF-κB downstream target IL-6 is able to activate STAT3,[29] which suggests a feed-forward loop in glioma. Astrocyte elevated gene 1 (AEG-1) has also been reported to activate NF-κB in glioma cells.[52] Interleukin-8, NIP3 like protein X (NIX),[53] Inhibitor Of Growth Family Member 4 (ING4),[54] and PH domain and Leucine rich repeat Protein Phosphatases (PHPLPS)[55] are among other stimuli reported to influence NF-κB activation in glioma cells. **Table 1** lists known activators of NF-κB in GBM.

NF-κB Activation Plays A Role in The Pathogenesis of GBM and in Resistance to Treatment

NF-κB activation is widespread in cancer and there is substantial experimental evidence suggesting its involvement in both cancer development and resistance to treatment.[56-60] NF-κB activation may be linked to the resistance of glioblastoma cells to O^6-alkylating agents.[61-63] Various studies involving glioma-derived cell lines and mouse models also clearly suggest a pathogenic role for NF-κB in the regulation of gliomagenesis. Studies of TNFα-induced NF-κB in a panel of 6 glioma cell lines confirmed the presence of a p50/p65 heterodimer that controls cell cycle progression.[64] NF-κB may influence proliferation or invasion of glioma cells in culture[13,65,66] and NF-κB activation has also been implicated in the maintenance of glioblastoma initiating stem-like cells.[67] As discussed previously, EGFRvIII-mediated activation is an important driver of NF-κB. Several studies have demonstrated that inhibition of NF-κB, either directly by silencing p65 or indirectly by silencing Rictor or RIP1, blocks EGFRvIII-mediated oncogenicity in orthotopic mouse models.[19,32,47] As described below, a number of strategies to inhibit NF-κB are effective in preclinical models of GBM, further supporting a key role for NF-κB in the pathogenesis of GBM. For example, RelB is a driver of NF-κB that is expressed in mesenchymal glioma and RelB knockdown results prevents tumor formation in mice.[21]

Targeting NF-κB in Glioma

Glioblastoma is an intractable tumor that is resistant to current treatment approaches. The main challenges in GBM treatment may be the invasive nature of the tumor, which makes complete resection of the tumor difficult; a dynamic tumor genome; multiple pathways driving the malignant phenotype; and the blood brain barrier, which limits the availability of drugs to the tumor. Since the emergence of NF-κB as a driver of multiple aspects of gliomagenesis and resistance to treatment the NF-κB signaling network has become an attractive target for intervention.[59,68,69] Furthermore, a large number of drugs that target NF-κB are available.[70] Importantly, a number of preclinical studies have documented that inhibition of NF-κB using various strategies, including curcumin, non-steroidal anti-inflammatory drugs, or antibodies, suppresses growth and chemoresistance of human glioma cells.[13,18,61,62,71-76] Sulfasalazine showed promise in a mouse intracranial model, appearing to act via inhibition of NF-κB, but was not effective in a clinical trial.[18,77]

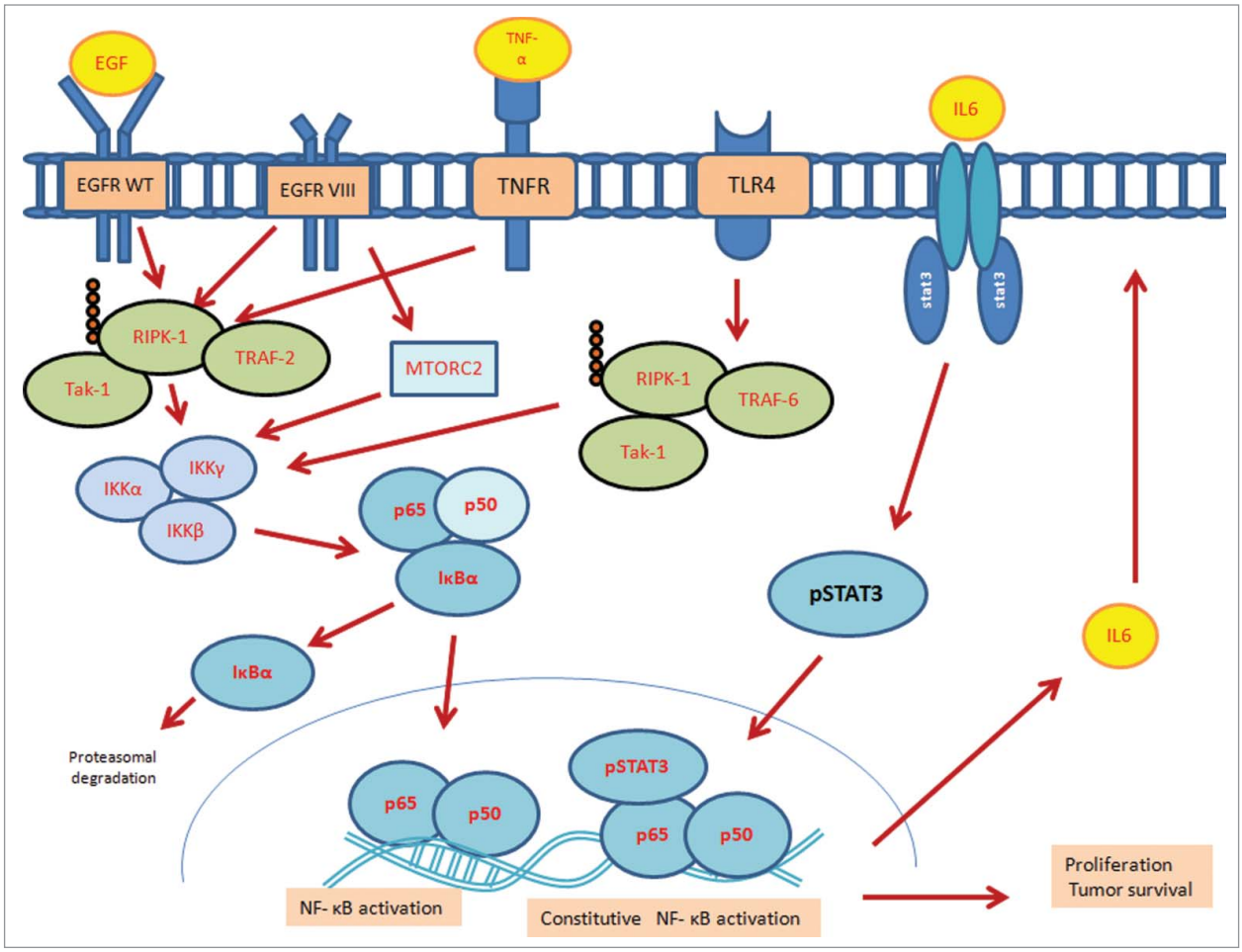

Figure 3. Major cytokine and growth factor receptor signaling pathways that activate NF-κB in GBM, including the EGFR signaling network. Most signaling networks regulate the activation of IKKs, which in turn results in degradation of IKBα and the translocation of NF-κB subunits to the nucleus where they initiate transcription of target genes.

Anti-inflammatory drugs have also been used in combination with other treatments but so far have not shown impressive results[78] although they appear to be safe.[79,80] However, patients were not stratified with respect to NF-κB status, and certain subsets of patients may benefit from targeting NF-κB.

A number of other drugs that target NF-κB have shown promise in preclinical studies either as single agents or in combination with temozolomide. Studies indicate that inhibition of NF-κB may synergize with temozolomide to inhibit glioma cells.

Table 1. Regulators of NF-κB in glioblastoma multiforme

Regulator	Mode of action	Reference
EGFR, EGFRVIII	Amplification, Mutation	8,19,20,27,28,52
IkBa	deletion	8
TRADD	Nuclear TRADD	29
A20	Deletion	30
Stat3	p65 acetylation	31
NIX	p65 phosphorylation	35
ING4	p65 phosphorylation	36
PHPLPs	I kappa B beta phosphorylation	37

Temozolomide is a first-line chemotherapy drug in the treatment of GBM. It can cross the blood brain barrier and provides a modest improvement in survival.[81] A preclinical study reported that BV6, a SMAC mimetic, sensitizes glioma cells to temozolamide-induced death in a RIP1- and NF-κB–dependent manner.[82] Niclosamide, a salicylanilide compound that may act in part by inhibition of NF-κB, inhibits the growth of glioma cells; interestingly, results of this study suggested that niclosamide synergizes with temozolomide in glioma cells with *NFKBIA* deletion.[83] Resveratrol, a natural phenolic compound commonly used in other types of cancer, also inhibits NF-κB in glioma cells by inhibiting mir-21,[84] and embellin, a novel XIAP inhibitor, induces apoptosis in glioma cells by inhibiting NF-κB.[85]

Concluding Comments

As in other types of cancers, NF-κB has emerged as an important regulator of the malignant phenotype in malignant glioma, and in particular GBM. Important advances have been made in identifying the genetic alterations that lead to deregulated

NF-κB activation in GBM. There is convincing evidence demonstrating that NF-κB is activated in GBM and a number of studies have elucidated the mechanisms involved in NF-κB activation in GBM. EGFR signaling is an important driver of NF-κB activation in GBM and progress has been made in understanding the mechanisms of NF-κB activation by wild type and mutant EGFR. It has not been possible to determine whether NF-κB activation is an early event in GBM, but it may have a role in the maintenance of glioma-initiating stem-like cells. A large number of preclinical studies suggest that the NF-κB signaling network is a promising target for treatment in GBM. Whether targeting the NF-κB network will prove effective in the treatment of patients with GBM is an important question that may be answered in the near future.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

References

1. Reardon DA, Wen PY. Therapeutic advances in the treatment of glioblastoma: rationale and potential role of targeted agents. Oncologist 2006; 11:152-64; PMID:16476836; http://dx.doi.org/10.1634/theoncologist.11-2-152
2. Ostrom QT, Gittleman H, Farah P, Ondracek A, Chen Y, Wolinsky Y, Stroup NE, Kruchko C, Barnholtz-Sloan JS. CBTRUS statistical report: Primary brain and central nervous system tumors diagnosed in the United States in 2006-2010. Neuro-oncology 2013; 15 Suppl 2:ii1-56; PMID:24137015
3. Omuro A, DeAngelis LM. Glioblastoma and other malignant gliomas: a clinical review. JAMA : the journal of the American Medical Association 2013; 310:1842-50; http://dx.doi.org/10.1001/jama.2013.280319
4. Cancer Genome Atlas Research Network. Comprehensive genomic characterization defines human glioblastoma genes and core pathways. Nature 2008; 455:1061-8; PMID:18772890
5. Furnari FB, Fenton T, Bachoo RM, Mukasa A, Stommel JM, Stegh A, Hahn WC, Ligon KL, Louis DN, Brennan C, et al. Malignant astrocytic glioma: genetics, biology, and paths to treatment. Genes Dev 2007; 21:2683-710; PMID:17974913; http://dx.doi.org/10.1101/gad.1596707
6. Verhaak RG, Hoadley KA, Purdom E, Wang V, Qi Y, Wilkerson MD, Miller CR, Ding L, Golub T, Mesirov JP, et al. Integrated genomic analysis identifies clinically relevant subtypes of glioblastoma characterized by abnormalities in PDGFRA, IDH1, EGFR, and NF1. Cancer Cell 2010; 17:98-110; PMID:20129251; http://dx.doi.org/10.1016/j.ccr.2009.12.020
7. Bhat KP, Balasubramaniyan V, Vaillant B, Ezhilarasan R, Hummelink K, Hollingsworth F, Wani K, Heathcock L, James JD, Goodman LD, et al. Mesenchymal Differentiation Mediated by NF-kappaB Promotes Radiation Resistance in Glioblastoma. Cancer Cell 2013; 24:331-46; PMID:23993863; http://dx.doi.org/10.1016/j.ccr.2013.08.001
8. Bredel M, Scholtens DM, Yadav AK, Alvarez AA, Renfrow JJ, Chandler JP, Yu IL, Carro MS, Dai F, Tagge MJ, et al. NFKBIA deletion in glioblastomas. N Engl J Med 2011; 364:627-37; PMID:21175304; http://dx.doi.org/10.1056/NEJMoa1006312
9. Korkolopoulou P, Levidou G, Saetta AA, El-Habr E, Eftichiadis C, Demenagas P, Thymara I, Xiromeritis K, Boviatsis E, Thomas-Tsagli E, et al. Expression of nuclear factor-kappaB in human astrocytomas: relation to pI kappa Ba, vascular endothelial growth factor, Cox-2, microvascular characteristics, and survival. Human pathology 2008; 39:1143-52; PMID:18495209; http://dx.doi.org/10.1016/j.humpath.2008.01.020
10. Karin M, Cao Y, Greten FR, Li ZW. NF-kappaB in cancer: from innocent bystander to major culprit. Nat Rev Cancer 2002; 2:301-10; PMID:12001991; http://dx.doi.org/10.1038/nrc780
11. Conti A, Ageunnouz M, La Torre D, Cardali S, Angileri FF, Buemi C, Tomasello C, Iacopino DG, D'Avella D, Vita G, et al. Expression of the tumor necrosis factor receptor-associated factors 1 and 2 and regulation of the nuclear factor-kappaB antiapoptotic activity in human gliomas. J Neurosurg 2005; 103:873-81; PMID:16304992; http://dx.doi.org/10.3171/jns.2005.103.5.0873
12. Roggendorf W, Strupp S, Paulus W. Distribution and characterization of microglia/macrophages in human brain tumors. Acta Neuropathol (Berl) 1996; 92:288-93; http://dx.doi.org/10.1007/s004010050520
13. Nagai S, Washiyama K, Kurimoto M, Takaku A, Endo S, Kumanishi T. Aberrant nuclear factor-kappaB activity and its participation in the growth of human malignant astrocytoma. J Neurosurg 2002; 96:909-17; PMID:12005399; http://dx.doi.org/10.3171/jns.2002.96.5.0909
14. Sen R, Baltimore D. Multiple nuclear factors interact with the immunoglobulin enhancer sequences. Cell 1986; 46:705-16; PMID:3091258; http://dx.doi.org/10.1016/0092-8674(86)90346-6
15. Hayden MS, Ghosh S. Signaling to NF-kappaB. Genes Dev 2004; 18:2195-224; PMID:15371334; http://dx.doi.org/10.1101/gad.1228704
16. Napetschnig J, Wu H. Molecular basis of NF-kappaB signaling. Annual Review of Biophysics 2013; 42:443-68; PMID:23495970; http://dx.doi.org/10.1146/annurev-biophys-083012-130338
17. 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; 84:941-51; PMID:15184909; http://dx.doi.org/10.1038/labinvest.3700123
18. Robe PA, Bentires-Alj M, Bonif M, Rogister B, Deprez M, Haddada H, Khac MT, Jolois O, Erkmen K, Merville MP, et al. In vitro and in vivo activity of the nuclear factor-kappaB inhibitor sulfasalazine in human glioblastomas. Clin Cancer Res 2004; 10:5595-603; PMID:15328202; http://dx.doi.org/10.1158/1078-0432.CCR-03-0392
19. Puliyappadamba VT, Chakraborty S, Chauncey SS, Li L, Hatanpaa KJ, Mickey B, Noorani S, Shu HK, Burma S, Boothman DA, et al. Opposing Effect of EGFRWT on EGFRvIII-Mediated NF-kappaB Activation with RIP1 as a Cell Death Switch. Cell Rep 2013; 4:764-75; PMID:23972990; http://dx.doi.org/10.1016/j.celrep.2013.07.025
20. Wang R, Jiao Z, Li R, Yue H, Chen L. p68 RNA helicase promotes glioma cell proliferation in vitro and in vivo via direct regulation of NF-kappaB transcription factor p50. Neuro-oncology 2012; 14:1116-24; PMID:22810421; http://dx.doi.org/10.1093/neuonc/nos131
21. 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:e57489; PMID:23451236; http://dx.doi.org/10.1371/journal.pone.0057489
22. Arato-Ohshima T, Sawa H. Over-expression of cyclin D1 induces glioma invasion by increasing matrix metalloproteinase activity and cell motility. International Journal of Cancer 1999; 83:387-92; PMID:10495432; http://dx.doi.org/10.1002/(SICI)1097-0215(19991029)83:3%3c387::AID-IJC15%3e3.0.CO;2-O
23. Cavalla P, Dutto A, Piva R, Richiardi P, Grosso R, Schiffer D. Cyclin D1 expression in gliomas. Acta Neuropathologica 1998; 95:131-5; PMID:9498046; http://dx.doi.org/10.1007/s004010050776
24. Pykett M, Azzam E, Little J. Differential regulation of cdk2 and cyclin D1 in irradiated human glioma cells. International Journal of Oncology 1997; 10:93-9; PMID:21533350
25. Maxwell M, Galanopoulos T, Antoniades H. Cell-cycle regulator cyclin D1 mRNA and protein overexpression occurs in primary malignant gliomas. International Journal of Oncology 1996; 9:493-7; PMID:21541540
26. Ueki K, Ono Y, Henson JW, Efird JT, von Deimling A, Louis DN. CDKN2/p16 or RB alterations occur in the majority of glioblastomas and are inversely correlated. Cancer Research 1996; 56:150-3; PMID:8548755
27. Naumann U, Bahr O, Wolburg H, Altenberend S, Wick W, Liston P, Ashkenazi A, Weller M. Adenoviral expression of XIAP antisense RNA induces apoptosis in glioma cells and suppresses the growth of xenografts in nude mice. Gene Therapy 2007; 14:147-61; PMID:16957768
28. Wagenknecht B, Glaser T, Naumann U, Kugler S, Isenmann S, Bahr M, Korneluk R, Liston P, Weller M. Expression and biological activity of X-linked inhibitor of apoptosis (XIAP) in human malignant glioma. Cell Death and Differentiation 1999; 6:370-6; PMID:10381630; http://dx.doi.org/10.1038/sj.cdd.4400503
29. McFarland BC, Hong SW, Rajbhandari R, Twitty GB, Jr., Gray GK, Yu H, Benveniste EN, Nozell SE. NF-kappaB-induced IL-6 ensures STAT3 activation and tumor aggressiveness in glioblastoma. PloS One 2013; 8:e78728; PMID:24244348; http://dx.doi.org/10.1371/journal.pone.0078728
30. Raychaudhuri B, Vogelbaum MA. IL-8 is a mediator of NF-kappaB induced invasion by gliomas. J Neurooncol 2011; 101:227-35; PMID:20577780; http://dx.doi.org/10.1007/s11060-010-0261-2
31. Brat DJ, Bellail AC, Van Meir EG. The role of interleukin-8 and its receptors in gliomagenesis and tumoral angiogenesis. Neuro-oncology 2005; 7:122-33; PMID:15831231; http://dx.doi.org/10.1215/S1152851704001061
32. Bonavia R, Inda MM, Vandenberg S, Cheng SY, Nagane M, Hadwiger P, Tan P, Sah DW, Cavenee WK, Furnari FB. EGFRvIII promotes glioma angiogenesis and growth through the NF-kappaB, interleukin-8 pathway. Oncogene 2012; 31:4054-66; PMID:22139077; http://dx.doi.org/10.1038/onc.2011.563
33. Jiang L, Wu J, Yang Y, Liu L, Song L, Li J, Li M. Bmi-1 promotes the aggressiveness of glioma via activating the NF-kappaB/MMP-9 signaling pathway. BMC Cancer 2012; 12:406; PMID:22967049; http://dx.doi.org/10.1186/1471-2407-12-406
34. Ryu HH, Jung S, Jung TY, Moon KS, Kim IY, Jeong YI, Jin SG, Pei J, Wen M, Jang WY. Role of metallothionein 1E in the migration and invasion of human glioma cell lines. International Journal of Oncology 2012; 41:1305-13; PMID:22843066
35. Jiang L, Lin C, Song L, Wu J, Chen B, Ying Z, Fang L, Yan X, He M, Li J, et al. MicroRNA-30e* promotes human glioma cell invasiveness in an orthotopic xenotransplantation model by disrupting the NF-kappaB/IkappaBalpha negative feedback loop. The Journal of Clinical Investigation 2012; 122:33-47; PMID:22156201; http://dx.doi.org/10.1172/JCI58849
36. Yeh WL, Lu DY, Lee MJ, Fu WM. Leptin induces migration and invasion of glioma cells through MMP-13

production. Glia 2009; 57:454-64; PMID:18814267; http://dx.doi.org/10.1002/glia.20773
37. Perkins ND. Integrating cell-signalling pathways with NF-kappaB and IKK function. Nat Rev Mol Cell Biol 2007; 8:49-62; PMID:17183360; http://dx.doi.org/10.1038/nrm2083
38. Park S, Hatanpaa KJ, Xie Y, Mickey BE, Madden CJ, Raisanen JM, Ramnarain DB, Xiao G, Saha D, Boothman DA, et al. The receptor interacting protein 1 inhibits p53 induction through NF-kappaB activation and confers a worse prognosis in glioblastoma. Cancer Res 2009; 69:2809-16; PMID:19339267; http://dx.doi.org/10.1158/0008-5472.CAN-08-4079
39. Park S, Zhao D, Hatanpaa KJ, Mickey BE, Saha D, Boothman DA, Story MD, Wong ET, Burma S, Georgescu MM, et al. RIP1 activates PI3K-Akt via a dual mechanism involving NF-kappaB-mediated inhibition of the mTOR-S6K-IRS1 negative feedback loop and down-regulation of PTEN. Cancer Res 2009; 69:4107-11; PMID:19435890; http://dx.doi.org/10.1158/0008-5472.CAN-09-0474
40. Habib AA, Chatterjee S, Park SK, Ratan RR, Lefebvre S, Vartanian T. The epidermal growth factor receptor engages receptor interacting protein and nuclear factor-kappa B (NF-kappa B)-inducing kinase to activate NF-kappa B. Identification of a novel receptor-tyrosine kinase signalosome. J Biol Chem 2001; 276:8865-74; PMID:11116146; http://dx.doi.org/10.1074/jbc.M008458200
41. Sun L, Carpenter G. Epidermal growth factor activation of NF-kappaB is mediated through IkappaBalpha degradation and intracellular free calcium. Oncogene 1998; 16:2095-102; PMID:9572490; http://dx.doi.org/10.1038/sj.onc.1201731
42. Hatanpaa KJ, Burma S, Zhao D, Habib AA. Epidermal growth factor receptor (EGFR) in glioma: Signal transduction, neuropathology, imaging and radioresistance Neoplasia 2010; 12:675-84; PMID:20824044
43. Huang PH, Xu AM, White FM. Oncogenic EGFR signaling networks in glioma. Sci Signal 2009; 2:re6; PMID:19738203
44. Nishikawa R, Ji XD, Harmon RC, Lazar CS, Gill GN, Cavenee WK, Huang HJ. A mutant epidermal growth factor receptor common in human glioma confers enhanced tumorigenicity. Proc Natl Acad Sci U S A 1994; 91:7727-31; PMID:8052651; http://dx.doi.org/10.1073/pnas.91.16.7727
45. 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; 24:823-36; PMID:14701753; http://dx.doi.org/10.1128/MCB.24.2.823-836.2004
46. Yang W, Xia Y, Cao Y, Zheng Y, Bu W, Zhang L, You MJ, Koh MY, Cote G, Aldape K, et al. EGFR-Induced and PKCepsilon Monoubiquitylation-Dependent NF-kappaB Activation Upregulates PKM2 Expression and Promotes Tumorigenesis. Mol Cell 2012; 48:771-84; PMID:23123196; http://dx.doi.org/10.1016/j.molcel.2012.09.028
47. Tanaka K, Babic I, Nathanson D, Akhavan D, Guo D, Gini B, Dang J, Zhu S, Yang H, De Jesus J, et al. Oncogenic EGFR signaling activates an mTORC2-NF-kappaB pathway that promotes chemotherapy resistance. Cancer Discov 2011; 1:524-38; PMID:22145100; http://dx.doi.org/10.1158/2159-8290.CD-11-0124
48. Chakraborty S, Li L, Tang H, Xie Y, Puliyappadamba VT, Raisanen J, Burma S, Boothman DA, Cochran B, Wu J, et al. Cytoplasmic TRADD Confers a Worse Prognosis in Glioblastoma. Neoplasia 2013; 15:888-97; PMID:23908590
49. Yadav AK, Renfrow JJ, Scholtens DM, Xie H, Duran GE, Bredel C, Vogel H, Chandler JP, Chakravarti A, Robe PA, et al. Monosomy of chromosome 10 associated with dysregulation of epidermal growth factor signaling in glioblastomas. JAMA: The Journal of the American Medical Association 2009; 302:276-89.; PMID:19602687; http://dx.doi.org/10.1001/jama.\2009.1022
50. Lee H, Herrmann A, Deng JH, Kujawski M, Niu G, Li Z, Forman S, Jove R, Pardoll DM, Yu H. Persistently activated Stat3 maintains constitutive NF-kappaB activity in tumors. Cancer Cell 2009; 15:283-93; PMID:19345327; http://dx.doi.org/10.1016/j.ccr.2009.02.015
51. 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; 32:5144-55; PMID:23178493; http://dx.doi.org/10.1038/onc.2012.546
52. 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; 68:1478-84; PMID:18316612; http://dx.doi.org/10.1158/0008-5472.CAN-07-6164
53. Lu Y, Wang L, He M, Huang W, Li H, Wang Y, Kong J, Qi S, Ouyang J, Qiu X. Nix protein positively regulates NF-kappaB activation in gliomas. PLoS One 2012; 7:e44559; PMID:22984526; http://dx.doi.org/10.1371/journal.pone.0044559
54. Nozell S, Laver T, Moseley D, Nowoslawski L, De Vos M, Atkinson GP, Harrison K, Nabors LB, Benveniste EN. The ING4 tumor suppressor attenuates NF-kappaB activity at the promoters of target genes. Mol Cell Biol 2008; 28:6632-45; PMID:18779315; http://dx.doi.org/10.1128/MCB.00697-08
55. Agarwal NK, Zhu X, Gagea M, White CL, 3rd, Cote G, Georgescu MM. PHLPP2 suppresses the NF-kappaB pathway by inactivating IKKbeta kinase. Oncotarget 2014; 5:815-23; PMID:24553260
56. Munshi A, Kurland JF, Nishikawa T, Chiao PJ, Andreeff M, Meyn RE. Inhibition of constitutively activated nuclear factor-kappaB radiosensitizes human melanoma cells. Mol Cancer Ther 2004; 3:985-92; PMID:15299081
57. Kim BY, Kim KA, Kwon O, Kim SO, Kim MS, Kim BS, Oh WK, Kim GD, Jung M, Ahn JS. NF-kappaB inhibition radiosensitizes Ki-Ras-transformed cells to ionizing radiation. Carcinogenesis 2005; 26:1395-403; PMID:15802300; http://dx.doi.org/10.1093/carcin/bgi081
58. Wang CY, Cusack JC, Jr., Liu R, Baldwin AS, Jr. Control of inducible chemoresistance: enhanced anti-tumor therapy through increased apoptosis by inhibition of NF-kappaB. Nature Medicine 1999; 5:412-7; PMID:10202930; http://dx.doi.org/10.1038/10577
59. Nakanishi C, Toi M. Nuclear factor-kappaB inhibitors as sensitizers to anticancer drugs. Nat Rev Cancer 2005; 5:297-309; PMID:15803156; http://dx.doi.org/10.1038/nrc1588
60. Kunigal S, Lakka SS, Joseph P, Estes N, Rao JS. Matrix metalloproteinase-9 Inhibition Down-Regulates Radiation-Induced Nuclear Factor-{kappa}B Activity Leading to Apoptosis in Breast Tumors. Clin Cancer Res 2008; 14:3617-26; PMID:18519796; http://dx.doi.org/10.1158/1078-0432.CCR-07-2060
61. Weaver KD, Yeyeodu S, Cusack JC, Jr., Baldwin AS, Jr., Ewend MG. Potentiation of chemotherapeutic agents following antagonism of nuclear factor kappa B in human gliomas. J Neurooncol 2003; 61:187-96; PMID:12675310; http://dx.doi.org/10.1023/A:1022554824129
62. Bredel M, Bredel C, Juric D, Duran GE, Yu RX, Harsh GR, Vogel H, Recht LD, Scheck AC, Sikic BI. Tumor necrosis factor-α-induced protein 3 as a putative regulator of nuclear factor-kappaB-mediated resistance to O6-alkylating agents in human glioblastomas. J Clin Oncol 2006; 24:274-87; PMID:16365179; http://dx.doi.org/10.1200/JCO.2005.02.9405
63. Lavon I, Fuchs D, Zrihan D, Efroni G, Zelikovitch B, Fellig Y, Siegal T. Novel mechanism whereby nuclear factor kappaB mediates DNA damage repair through regulation of O(6)-methylguanine-DNA-methyltransferase. Cancer Res 2007; 67:8952-9; PMID:17875738; http://dx.doi.org/10.1158/0008-5472.CAN-06-3820
64. Otsuka G, Nagaya T, Saito K, Mizuno M, Yoshida J, Seo H. Inhibition of nuclear factor-kappaB activation confers sensitivity to tumor necrosis factor-α by impairment of cell cycle progression in human glioma cells. Cancer Res 1999; 59:4446-52; PMID:10485496
65. Raychaudhuri B, Han Y, Lu T, Vogelbaum MA. Aberrant constitutive activation of nuclear factor kappaB in glioblastoma multiforme drives invasive phenotype. J Neurooncol 2007; 85:39-47; PMID:17479228; http://dx.doi.org/10.1007/s11060-007-9390-7
66. Westhoff MA, Zhou S, Nonnenmacher L, Karpel-Massler G, Jennewein C, Schneider M, Halatsch ME, Carragher NO, Baumann B, Krause A, et al. Inhibition of NF-kappaB signaling ablates the invasive phenotype of glioblastoma. Molecular Cancer Research: MCR 2013; 11:1611-23; PMID:24145173; http://dx.doi.org/10.1158/1541-7786.MCR-13-0435-T
67. Nogueira L, Ruiz-Ontanon P, Vazquez-Barquero A, Lafarga M, Berciano MT, Aldaz B, Grande L, Casafont I, Segura V, Robles EF, et al. Blockade of the NFkappaB pathway drives differentiating glioblastoma-initiating cells into senescence both in vitro and in vivo. Oncogene 2011; 30:3537-48; PMID:21423202; http://dx.doi.org/10.1038/onc.2011.74
68. Nogueira L, Ruiz-Ontanon P, Vazquez-Barquero A, Moris F, Fernandez-Luna JL. The NFkappaB pathway: a therapeutic target in glioblastoma. Oncotarget 2011; 2:646-53; PMID:21896960
69. Atkinson GP, Nozell SE, Benveniste ET. NF-kappaB and STAT3 signaling in glioma: targets for future therapies. Expert review of neurotherapeutics 2010; 10:575-86; PMID:20367209; http://dx.doi.org/10.1586/ern.10.21
70. Karin M, Yamamoto Y, Wang QM. The IKK NF-kappa B system: a treasure trove for drug development. Nat Rev Drug Discov 2004; 3:17-26; PMID:14708018; http://dx.doi.org/10.1038/nrd1279
71. Joki T, Heese O, Nikas DC, Bello L, Zhang J, Kraeft SK, Seyfried NT, Abe T, Chen LB, Carroll RS, et al. Expression of cyclooxygenase 2 (COX-2) in human glioma and in vitro inhibition by a specific COX-2 inhibitor, NS-398. Cancer Res 2000; 60:4926-31; PMID:10987308
72. King JG Jr, Khalili K. Inhibition of human brain tumor cell growth by the anti-inflammatory drug, flurbiprofen. Oncogene 2001; 20:6864-70; PMID:11687965; http://dx.doi.org/10.1038/sj.onc.1204907
73. 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; 102:522-38; PMID:17596214; http://dx.doi.org/10.1111/j.1471-4159.2007.04633.x
74. Kang KB, Wang TT, Woon CT, Cheah ES, Moore XL, Zhu C, Wong MC. Enhancement of glioblastoma radioresponse by a selective COX-2 inhibitor celecoxib: inhibition of tumor angiogenesis with extensive tumor necrosis. Int J Radiat Oncol Biol Phys 2007; 67:888-96; PMID:17293239; http://dx.doi.org/10.1016/j.ijrobp.2006.09.055
75. Karmakar S, Banik NL, Ray SK. Curcumin suppressed anti-apoptotic signals and activated cysteine proteases for apoptosis in human malignant glioblastoma U87MG cells. Neurochem Res 2007; 32:2103-13; PMID:17562168; http://dx.doi.org/10.1007/s11064-007-9376-z
76. 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; 13:2178-90; PMID:17404102; http://dx.doi.org/10.1158/1078-0432.CCR-06-1711
77. Robe PA, Martin DH, Nguyen-Khac MT, Artesi M, Deprez M, Albert A, Vanbelle S, Califice S, Bredel M, Bours V. 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; PMID:19840379; http://dx.doi.org/10.1186/1471-2407-9-372

78. Levin VA, Giglio P, Puduvalli VK, Jochec J, Groves MD, Yung WK, Hess K. Combination chemotherapy with 13-cis-retinoic acid and celecoxib in the treatment of glioblastoma multiforme. J Neurooncol 2006; 78:85-90; PMID:16391896; http://dx.doi.org/10.1007/s11060-005-9062-4
79. Tuettenberg J, Grobholz R, Korn T, Wenz F, Erber R, Vajkoczy P. Continuous low-dose chemotherapy plus inhibition of cyclooxygenase-2 as an antiangiogenic therapy of glioblastoma multiforme. J Cancer Res Clin Oncol 2005; 131:31-40; PMID:15565458; http://dx.doi.org/10.1007/s00432-004-0620-5
80. Reardon DA, Quinn JA, Vredenburgh J, Rich JN, Gururangan S, Badruddoja M, Herndon JE, 2nd, Dowell JM, Friedman AH, Friedman HS. Phase II trial of irinotecan plus celecoxib in adults with recurrent malignant glioma. Cancer 2005; 103:329-38; PMID:15558802; http://dx.doi.org/10.1002/cncr.20776
81. Stupp R, Mason WP, van den Bent MJ, Weller M, Fisher B, Taphoorn MJ, Belanger K, Brandes AA, Marosi C, Bogdahn U, et al. Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma. N Engl J Med 2005; 352:987-96; PMID:15758009; http://dx.doi.org/10.1056/NEJMoa043330
82. Wagner L, Marschall V, Karl S, Cristofanon S, Zobel K, Deshayes K, Vucic D, Debatin KM, Fulda S. Smac mimetic sensitizes glioblastoma cells to Temozolomide-induced apoptosis in a RIP1- and NF-kappaB-dependent manner. Oncogene 2013; 32:988-97; PMID:22469979; http://dx.doi.org/10.1038/onc.2012.108
83. Wieland A, Trageser D, Gogolok S, Reinartz R, Hofer H, Keller M, Leinhaas A, Schelle R, Normann S, Klaas L, et al. Anticancer effects of niclosamide in human glioblastoma. Clin Cancer Res 2013; 19:4124-36; PMID:23908450; http://dx.doi.org/10.1158/1078-0432.CCR-12-2895
84. Li H, Jia Z, Li A, Jenkins G, Yang X, Hu J, Guo W. Resveratrol repressed viability of U251 cells by miR-21 inhibiting of NF-kappaB pathway. Molecular and Cellular Biochemistry 2013; 382:137-43; PMID:23793554; http://dx.doi.org/10.1007/s11010-013-1728-1
85. Park SY, Lim SL, Jang HJ, Lee JH, Um JY, Kim SH, Ahn KS, Lee SG. Embelin induces apoptosis in human glioma cells through inactivating NF-kappaB. Journal of Pharmacological Sciences 2013; 121:192-9; PMID:23514760; http://dx.doi.org/10.1254/jphs.12137FP