The role of NF-κB in the pathogenesis of glioma

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Abstract

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. EGF 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

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.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 of the most common and striking abnormalities in GBM4,6 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.7,8 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. 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. 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. 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. 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. 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. 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. 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 phospho-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. In mesenchymal glioma the non-canonical pathway of NF-κB, in which RelB has a prominent role, is also involved.

**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, the anti-apoptotic protein XIAP, and inflammatory proteins such as IL-6, IL-8, MMP-9, MMP-13, and Cox2. 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.

**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 be particularly important. First, EGFR signaling is known to activate NF-κB. 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. 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. NF-κB activation has been reported to promote a mesenchymal phenotype in GBM.

**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 and via a PLC gamma- and PKC epsilon-dependent pathway. At least 2 mechanisms have been described for EGFRvIII-mediated activation of NF-κB, including an mTORC2-dependent pathway.

We recently found that receptor-interacting protein (RIP1, RIPK1) is a key link between EGFR and NF-κB signaling in GBM. 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. EGFRvIII recruits ubiquitin...
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. 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. 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 NFKB1 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. TRADD is required for TNFα-mediated activation of NF-κB. Constitutive STAT3 activation in tumors maintains NF-κB activation by sustained acetylation of p65. STAT3 was shown to physically interact with the p65 subunit of NF-κB.
Interestingly, one study reports that the NF-κB downstream target IL-6 is able to activate STAT3, 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. Interleukin-8, NIP3 like protein X (NIX), Inhibitor Of Growth Family Member 4 (ING4), and PH domain and Leucine rich repeat Protein Phosphatases (PHPLPS) 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. NF-κB activation may be linked to the resistance of glioblastoma cells to O6-alkylating agents. 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. NF-κB may influence proliferation or invasion of glioma cells in culture and NF-κB activation has also been implicated in the maintenance of glioblastoma initiating stem-like cells. 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. 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, ReB is a driver of NF-κB that is expressed in mesenchymal glioma and ReB knockdown results prevents tumor formation in mice.

**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. Furthermore, a large number of drugs that target NF-κB are available. 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. Sulfasalazine showed promise in a mouse intracranial model, appearing to act via inhibition of NF-κB, but was not effective in a clinical trial.
Anti-inflammatory drugs have also been used in combination with other treatments but so far have not shown impressive results, although they appear to be safe. 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, EGFRVIII | Mode of action | Reference |
|---------------------|---------------|-----------|
| EGFR, EGFRVIII      | Amplification, Mutation | 8,19,20,27,28,52 |
| IκBα                | deletion      | 8         |
| TRADD               | Deletion      | 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. A preclinical study reported that BV6, a SMAC mimic, sensitizes glioma cells to temozolomide-induced death in a RIP1- and NF-κB–dependent manner. 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. Resveratrol, a natural phenolic compound commonly used in other types of cancer, also inhibits NF-κB in glioma cells by inhibiting mir-21, and embelin, a novel XIAP inhibitor, induces apoptosis in glioma cells by inhibiting NF-κB.

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.

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