Expression of Nuclear Factor-κB, Tumor Necrosis Factor Receptor Type 1, and c-Myc in Human Astrocytomas

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Abstract

Tumor necrosis factor receptor type 1 (TNFR1) and c-Myc are important in signal transduction in tumor necrosis factor-α (TNF-α)-induced cytotoxicity, whereas activation of nuclear factor-κB (NF-κB) protects against TNF-α-induced apoptosis. This study investigated the expression of NF-κB, TNFR1, and c-Myc in human astrocytoma tissues by reverse transcriptase-polymerase chain reaction (PCR) and immunohistochemical analysis. TNFR1 messenger ribonucleic acid (mRNA) and c-Myc mRNA were frequently expressed in malignant astrocytomas, especially in glioblastomas, compared with low-grade astrocytomas by PCR analysis. TNFR1 and c-Myc mRNAs were barely detectable in normal brain tissues. NF-κB p50 and p65 subunit mRNAs were detected in various grades of astrocytomas, with frequent expression in malignant astrocytomas. The presence of activated NF-κB was confirmed by nuclear localization in neoplastic astrocytes as determined by immunohistochemistry. Both p50 and p65 subunits were inhomogeneously expressed in neoplastic astrocytes of glioblastoma, but only in a few scattered tumor cells in low-grade astrocytoma, and almost undetectable in normal brain tissues. These results indicate that TNFR1 and C-Myc are overexpressed in malignant astrocytomas, and this may increase the cellular sensitivity to the cytotoxic action of TNF-α. NF-κB p50 and p65 were simultaneously induced and activated in malignant astrocytomas. Our results suggest that the constitutive activation of NF-κB subunits in malignant astrocytoma, especially in glioblastoma, could be associated with the resistance to TNF-α immunotherapy, and indicates new therapeutic strategies for malignant astrocytomas.

Key words: astrocytoma, tumor necrosis factor-α immunotherapy, nuclear factor-κB

Introduction

Tumor necrosis factor-α (TNF-α) is a multifunctional cytokine that binds to specific cell surface receptors (TNFRs) to activate multiple signal transduction pathways, which are important in cell growth regulation, immunomodulation, inflammation, and autoimmune processes, and also exhibits cytotoxic activity, especially against tumor cells. Recombinant TNF-α is cytotoxic for tumor cells and has tumor necrotic activity. Recombinant TNF-α is effective for treating advanced cancer, but is also associated with severe toxicity. Mutant recombinant TNF-α with altered N-terminal regions may avoid these drawbacks of TNF-α. Human mutant recombinant TNF-α (TNF-SAM2) has reduced toxicity in vivo and increased cytotoxicity against tumor cells. To determine whether TNF-SAM2 is useful for the treatment of malignant gliomas, we previously studied the effect of TNF-SAM2 with an alkylating agent on rat C6 experimental glioma, and observed a dramatic antitumor effect in vivo. We also reported early results in patients treated with TNF-SAM2 as part of initial therapy for glioblastoma, but the preliminary results of TNF-SAM2 treatment for malignant astrocytomas, especially for glioblastoma, achieved only a modest level of significance.

TNF-α triggers a biochemical pathway that leads to programmed cell death, i.e., apoptosis, but also activates a key molecule that can block this apoptotic pathway, which reflects the dual nature of TNF-α. Cells treated with drugs that block protein synthesis are more easily killed by TNF-α, indicating that several genes involved in the response to inflammation, infection, and stress are turned on to...
Radiation, oxidants, and cytokines such as TNF-α by various inducers, including viruses, bacteria, and ionizing radiation protects cells from being killed. Since the cell-killing effects of TNF-α and other apoptotic agents are enhanced by the protein synthesis inhibitor cycloheximide, the activation of NF-κB probably up-regulates transcription of a gene or group of genes encoding proteins involved in protection against cell killing. Inhibition of NF-κB nuclear translocation enhances apoptotic killing by these reagents. These findings suggest a possible mechanism of cellular resistance to killing by TNF-α and other chemotherapeutic agents. c-Myc is a transcription factor that regulates cell proliferation and contributes to tumorigenesis when its expression is deregulated. Constitutive, deregulated c-Myc expression maintains deoxyribonucleic acid (DNA) synthesis, prevents growth arrest, and induces apoptosis. Up-regulation of c-Myc induced by TNF-α contributes to the mediation of the cytotoxic action of TNF-α. c-Myc impairs the TNF-α-induced activation of phospholipase A₂, JNK protein kinase, and cell survival-signaling associated NF-κB transcription factor complex. Increased expression of c-Myc oncprotein strongly increases cellular sensitivity to TNF-α cytotoxicity.

The NF-κB/Rel family of transcription factors participates in the regulation of a diverse range of genes involved in inflammation, immune response, acute-phase responses, lymphoid differentiation, growth control, and development. NF-κB complexes consist of members of a multigene family of five major proteins: p50, p65 (Rel A), c-Rel, p52, and Rel B. In general, the most abundant dimer of inducible NF-κB is a p50/p65 heterodimer. NF-κB activity is regulated by the binding protein-inhibition molecule IκB in the cytoplasm. NF-κB is quiescent in combination with IκB, but is activated for nuclear entry when dissociated from IκB resulting from stimulation of cells by various inducers, including viruses, bacteria, radiation, oxidants, and cytokines such as TNF-α. The heterodimer translocates to the nucleus and participates in the transcriptional regulation of numerous genes. Activation of NF-κB is also implicated in many pathological conditions. Deregulation of NF-κB activity is thought to be directly associated with cellular transformation and tumorigenicity. NF-κB is a critical regulator of cytokine-inducible gene expression, and has an anti-apoptotic function. Distinct signaling pathways initiated by the engagement of TNF-α with its receptor lead to both apoptosis and the activation of NF-κB, which has a negative effect on the induction of apoptosis.

The inhibition of NF-κB activation by steroid hormones, antioxidants, protease inhibitors, and other compounds may provide a pharmacological basis for interfering with pathological inflammatory conditions and cancer. Disruption of the protective mechanism induced by NF-κB makes cells much more vulnerable to killing by TNF-α and other chemotherapeutic agents. These findings suggest that TNF-α may improve the efficacy of treatment for many malignant tumors.

This study examined possible changes in the expression of NF-κB, TNFR type 1 (TNFR1), and c-Myc in malignant astrocytomas to investigate the mechanism of the resistance of malignant astrocytoma to TNF-α immunotherapy, and to establish a new chemotherapeutic strategy for malignant astrocytoma, especially for glioblastoma.

### Materials and Methods

#### I. Tissue specimens

Fresh human brain tumor tissues and normal brain tissue samples adjacent to tumor were obtained in the operating room from patients who underwent therapeutic removal of brain tumors. Normal brain tissues were available because excision was necessary for gross total removal of the tumors. The samples were flash-frozen in liquid nitrogen immediately after surgical removal and stored at −80°C. The histological diagnosis was confirmed by standard light-microscopic evaluation of sections stained with hematoxylin and eosin. The classification of human brain tumors used in this study is based on the revised World Health Organization criteria for tumors of the central nervous system.

All tumor tissues examined in the present study were from primary resections, and none of the patients had undergone chemotherapy including corticosteroid administration or radiation therapy before resection. Patients with anaplastic astrocytomas (grade III) or glioblastomas (grade IV) were subsequently treated with combination chemotherapy consisting of ranimustine (MCNU) and TNF-SAM in conjunction with radiotherapy. Written informed consent was obtained from each patient. This study was approved by the institutional review board.

#### II. Ribonucleic acid (RNA) extraction and reverse transcriptase-polymerase chain reaction (RT-PCR) analysis

Possible contamination by neoplastic cells infiltrating into surrounding normal brain tissue may occur in the analysis of bulk samples of normal brain tissue adjacent to tumor in genetic and biochemical
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studies. Therefore, we analyzed messenger RNA (mRNA) expression using RT-PCR of frozen tissue sections which were identified histologically as tumor or normal brain.28) Fresh tumor and normal brain tissues were frozen in liquid nitrogen after embedding in OCT compound (Miles Scientific, Elkhart, Ind., U.S.A.). Frozen sections containing normal brain tissues or tumors were selected for RT-PCR analysis following microscopic examination of sequential sections stained with hematoxylin and eosin. mRNA was then extracted from 10 sequential 5-μm thick frozen sections. Polyadenylated mRNA was extracted using the MicroFast Track kit in accordance with the manufacturer’s instructions (Invitrogen, San Diego, Calif., U.S.A.). The RNA was primed with random hexamers and reverse-transcribed into the first-strand complementary DNA (cDNA) using a cDNA cycle kit (Invitrogen).

To amplify the cDNA, 2-μl aliquots of the reverse-transcribed cDNA for frozen tissue samples were subjected to 30 cycles of PCR in 50 μl of 1 × buffer (10 mM Tris-HCl, pH 8.3, 1.5 mM MgCl2, 50 mM KCl, 1% gelatin, and 5% dimethyl sulfoxide) containing 0.2 mmol/l each of deoxyadenosine triphosphate, deoxythymidine triphosphate, deoxycytidine triphosphate, and deoxythymidine triphosphate, 2.0 U of Taq DNA polymerase (Boehringer Mannheim, Indianapolis, Ind., U.S.A.), and c-Myc-specific oligonucleotide primers (50 pmol of the sense primer 5′-CTGGACAGAGGAGACAATT-3′, corresponding to nt 238–257, and 50 pmol of the antisense primer 5′-GTGGTGGCGGTGGAGGAG-3′, complementary to nt 5087–5106).31) TNFR1 p55-specific oligonucleotide primers (50 pmol of the sense primer 5′-ATTGGTGGATCAGTCCG-3′, corresponding to nt 355–374, and 50 pmol of the antisense primer 5′-CACAAACACATTGAGAG-3′, complementary to nt 893–912).11) NF-κB p50-specific oligonucleotide primers (50 pmol of the sense primer 5′-TGCTAGGTCGATGGAG-3′, corresponding to nt 1561–1580, and 50 pmol of the antisense primer 5′-GTCTGTGCTTTTGTGGT-3′, complementary to nt 2283–2302).10) and NF-κB p65-specific oligonucleotide primers (50 pmol of the sense primer 5′-AGAAAGAGGACATTGAGG-3′, corresponding to nt 658–677, and 50 pmol of the antisense primer 5′-CCCCACGATCTCTCACTA-3′, complementary to nt 1315–1334).23) Each cycle consisted of denaturation at 94°C for 60 seconds, primer annealing at 62°C for c-Myc, 55°C for TNFR1 p55, 59°C for NF-κB p50, and 58°C for NF-κB p65 for 60 second each, extension at 72°C for 60 seconds, and a final extension at 72°C for 7 minutes in a Robo-Cycler 96 Temperature Cycler (Stratagene, La Jolla, Calif., U.S.A.). The efficiency of cDNA synthesis from each tissue sample was estimated by PCR with glyceraldehyde-3-phosphate dehydrogenase (GAPDH)-specific primers. GAPDH cDNA was amplified with primers corresponding to nt 27–46 (5′-ACGGATTGTCGTGATGGG-3′) and complementary to nt 238–257 (5′-TGAATTTGAGGATCTGGC-3′) under the same conditions used for TNFR1 p55. Samples of each PCR product were electrophoresed in 1.5% agarose gel and photographed as ethidium bromide-fluorescent bands. The PCR procedure was performed at least three times for each sample. After amplification, 40 μl of the PCR products of c-Myc, TNFR1 p55, and NF-κB (p50 and p65) were electrophoresed on 3% agarose gel. The amplified bands were cut out, eluted, and subjected to direct sequencing to confirm the identity of the transcripts using an automated DNA sequencer (ABI377; Perkin-Elmer, Norwalk, Conn., U.S.A.).

III. Immunohistochemistry for NF-κB p50 and p65 in human brain tumor tissues

The identity of cells which expressed NF-κB p50 and p65, and the subcellular distribution of NF-κB subunits were determined by an indirect avidin-biotin complex immunohistochemical analysis of frozen sections that were sequential to the sections used for the RT-PCR analysis. The nuclear localization of p50 and p65 subunits correlates with an activated state of these subunits. The samples were obtained from different patients and consisted of six normal brain tissues, seven low-grade astrocytomas (fibrillary astrocytomas, grade II), six anaplastic astrocytomas (grade III), and 12 glioblastomas (grade IV). The appropriate concentration of the primary antibody against the human NF-κB p50: sc-114 and NF-κB p65: sc-109 (a rabbit polyclonal antibody; Santa Cruz Biotechnology, Santa Cruz, Calif., U.S.A.) was determined by titration. Cryostat sections from frozen tissues embedded in OCT were cut at 5 μm onto silane-coated glass slides and then fixed in 100% acetone for 15 minutes at –20°C. The sections were then blocked with 1% non-fat milk for 1 hour at room temperature and incubated with primary antibody diluted to a protein concentration of 0.1 μg/ml in 2.5% bovine serum albumin in Tris-buffered saline (TBS: 50 mM Tris-HCl, pH 7.6, 137 mM NaCl) overnight in a humidity chamber at 4°C. After the sections were washed three times for 20 minutes in TBS with 0.1% Tween-20, the tissue samples were incubated with biotinylated horse anti-rabbit secondary antibody (Vector Laboratories, Burlingame, Calif., U.S.A.), and then with streptavidin-alkaline phosphatase (Dako Corp., Carpinteria, Calif., U.S.A.). Alkaline phosphatase activity
was visualized using a substrate solution consisting of naphthol AS-BI phosphate, levamisole, and New fuchsin (Sigma Chemical Co., St. Louis, Mo., U.S.A.). Sections were then counterstained in hematoxylin, dehydrated, and mounted for examination. Positive immunoreactivity was indicated by a pink precipitate in the cell cytoplasm and nucleus. Negative control studies were performed using a non-specific normal rabbit serum as a primary antibody. For control sections for NF-κB, primary antibodies were preabsorbed with an equal amount of the corresponding peptide and incubated.

Results

I. RT-PCR for c-Myc, TNFR1 (p55), and NF-κB (p50 and p65) in various grades of astrocytoma

No gene products of interest were amplified using PCR when total RNA extracts from the cell lines were incubated in RT reactions without RT (data not shown). Direct sequencing analyses of RT-PCR products showed that the c-Myc, TNFR1 (p55), and NF-κB (p50 and p65) transcripts were identical. cDNA prepared from normal brain tissue, low-grade astrocytoma (grade II), anaplastic astrocytoma (grade III), and glioblastoma (grade IV) was subjected to PCR amplification for c-Myc, TNFR1 (p55), and NF-κB (p50 and p65) transcripts (Table 1). Five representative cases for each histological group are shown in Fig. 1. Amplified 534-bp bands, corresponding to the size of amplified c-Myc, was obtained in all cases, whereas TNFR1 and both NF-κB p50 and p65 were not detected in one case of glioblastoma. No factors (c-Myc, TNFR1, and both NF-κB p50 and p65) were detected in one case of anaplastic astrocytoma, and both NF-κB p50 and p65 were negative whereas c-Myc and TNFR1 were positive in another case. All factors were positive in two cases of low-grade astrocytoma, whereas all were negative in four other cases. Only one sample of normal brain tissues showed positive amplified bands of NF-κB (p50 and p65). The inability to detect c-Myc, TNFR1 (p55), and NF-κB (p50 and p65) amplification product in these samples was not related to the absence or quality of mRNA, since the presence of intact mRNA in adjacent normal brain specimens was demonstrated by amplification of the constitutively expressed GAPDH gene (Fig. 1). Adjacent normal brain tissues showed strong amplification to levels similar to those generated by the brain tumor samples.

We conclude that TNFR1 p55 mRNA expression increased significantly as tumor grade increased from low-grade astrocytoma to glioblastoma, and was correlated significantly with the presence of c-Myc and NF-κB (p50 and p65).

II. Immunohistochemical localization of NF-κB p50 and p65 in human astrocytomas and normal brain tissue

Glioblastoma (grade IV) samples from 11 patients in which the expression of NF-κB p50 and p65 mRNA was observed by PCR displayed moderate heterogeneous nuclear and cytoplasmic immunostaining with the NF-κB p50 antibody (sc-114) (Fig. 2A, B) and the NF-κB p65 antibody (sc-109) (Fig. 2C, D), which was restricted to neoplastic astrocytes. This representative sample displayed expression of NF-κB p50 and p65 mRNA by PCR (Fig. 1A, lane 3). There was no detectable staining of the tumor in the absorption of the primary antibody, or a non-specific normal rabbit serum as a primary antibody (data not shown).

Tumor cells showed focal moderate to weak positivity in anaplastic astrocytoma (grade III) (Fig. 2E, F for p50; Fig. 2G, H for p65). In this representative case, expression of NF-κB p50 and p65 mRNA was observed by PCR (Fig. 1B, lane 2). In contrast to these specimens, there was no detectable staining of the tumor in samples that were negative for NF-κB p50 and p65 mRNA expression by PCR (data not shown).

Immunoreactivity to NF-κB p50 and p65 was also detectable in four low-grade astrocytoma (grade II) samples, with a few scattered tumor cells (Fig. 2I, J for p50; Fig. 2K, L for p65) in the sample that dis-

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Table 1  Comparative expression of c-Myc, tumor necrosis factor receptor type 1 (TNFR1), and nuclear factor-κB (NF-κB) messenger ribonucleic acids by reverse transcriptase-polymerase chain reaction

<table>
<thead>
<tr>
<th>Histological diagnosis</th>
<th>Total cases</th>
<th>Positive cases (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>c-Myc</td>
</tr>
<tr>
<td>Glioblastoma</td>
<td>12</td>
<td>12 (100%)</td>
</tr>
<tr>
<td>Anaplastic astrocytoma</td>
<td>6</td>
<td>5 (83.3%)</td>
</tr>
<tr>
<td>Low-grade astrocytoma</td>
<td>7</td>
<td>2 (28.6%)</td>
</tr>
<tr>
<td>Normal brain</td>
<td>6</td>
<td>0</td>
</tr>
</tbody>
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played positive expression of NF-κB p50 and p65 by PCR (Fig. 1C, lane 5). The remaining three low-grade astrocytomas showed no detectable NF-κB p50 or p65 immunoreactivity (data not shown).

Frozen sections derived from normal brain tissue identified histologically as white matter, including NF-κB p50- and p65-positive samples (Fig. 1D, lane 2), displayed no immunoreactivity (Fig. 2M, N for p50; Fig. 2O, P for p65), which suggests that astrocytes in normal cerebral white matter express low levels of NF-κB p50 and p65 under normal conditions.

Discussion

TNFR regulates cell viability, differentiation, and cell death via a common set of signal molecules. Biological activities of TNF-α are initiated through interaction with two specific TNF receptors, TNFR1 (p55) and TNFR2 (p75). TNF-α activates a cytotoxic signal as well as a protective signal against cell death. The induction of cell death by TNF-α occurs independently of protein synthesis, which implies that TNFRs can activate latent machinery through a protein-interaction cascade. Induction of cell death by TNF-α involves a group of proteases, the caspases (or ICE/CED3 proteases), which are also central in apoptotic processes. c-Myc is a transcriptional activator implicated in the control of cell proliferation, differentiation, and transformation, but is also involved in the regulation of apoptosis. The ICE/CED3-family protease, CPP32, may be critical in c-Myc-induced apoptosis, and c-Myc induces cellular susceptibility to the cytotoxic action of TNF-α which involves impaired NF-κB activation. Signals mediated by c-Myc could render cells more sensitive to the cytotoxic action of TNF-α. Oncogenic activation of c-Myc occurs in glioblastoma, resulting from constitutive protein expression due to genetic alterations such as chro-

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mosomal translocation or gene amplification.31) The present study found that TNFR1 and c-Myc were frequently expressed in malignant astrocytomas in vivo, especially in glioblastomas, the most aggressive and phenotypically transformed of the gliomas, as compared with low-grade astrocytomas by PCR analysis. TNFR1 and c-Myc mRNA were barely detectable in normal brain tissues. Therefore, simultaneous expression of TNFR1 and c-Myc may increase cellular sensitivity to the cytotoxic action of...
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TNF-\(\alpha\) in malignant astrocytoma, especially in glioblastoma.

The cytotoxic effects of TNF-\(\alpha\) on most cells are only evident if RNA or protein synthesis is inhibited, suggesting that de novo RNA or protein synthesis protects cells from TNF-\(\alpha\) cytotoxicity, probably through the induction of protective genes.\(^4\) TNF-\(\alpha\) activates NF-\(\kappa\)B through a pathway separate from apoptosis, and the activation of NF-\(\kappa\)B protects against TNF-\(\alpha\)-induced apoptosis.\(^2^{1}\) TNF-\(\alpha\) transmits one signal that elicits cell death and another, dependent on the NF-\(\kappa\)B p65 subunit, that protects against death by inducing gene expression.\(^4\) The finding that cells with a block in NF-\(\kappa\)B signaling are more susceptible to TNF-\(\alpha\)-induced apoptosis is consistent with the observation that TNF-\(\alpha\) cytotoxicity can be greatly enhanced by the addition of inhibitors of protein and RNA synthesis (for example, cycloheximide and actinomycin D).\(^3^{36}\) Therefore, activation of NF-\(\kappa\)B by TNF-\(\alpha\) is a major route for induction of the protective mechanism against TNF-\(\alpha\)-induced cytotoxicity, and this balance between the induced cytotoxic and protective effects might account for the ability of TNF-\(\alpha\) to act selectively.\(^3^{4}\)

Our present results demonstrate that activated NF-\(\kappa\)B is induced in various grades of astrocytomas, with increased expression in glioblastoma. The presence of the activated form of NF-\(\kappa\)B was confirmed by nuclear localization in neoplastic astrocytes, as determined by the well-characterized anti-NF-\(\kappa\)B p50 and p65 antibodies.\(^2^{25}\) NF-\(\kappa\)B immunoreactivity, as visualized by immunohistochemistry, was restricted to neoplastic astrocytes. Simultaneous expression of the activated form of NF-\(\kappa\)B with TNFR1 and c-Myc in malignant astrocytomas may allow neoplastic astrocytes to escape the death signals of TNF-\(\alpha\). c-Myc-induced sensitivity is inhibited by overexpression of the p65 subunit of NF-\(\kappa\)B, which also increases NF-\(\kappa\)B activity. The level of TNF-\(\alpha\) cytotoxicity towards cells with active c-Myc is dependent on cellular NF-\(\kappa\)B activity.\(^1^{7}\)

Based on the results of this study and the clinical results of TNF-SAM\(_2\) treatment,\(^7\) the activation of NF-\(\kappa\)B in neoplastic astrocytes may contribute to the resistance of malignant astrocytomas to TNF-\(\alpha\). Etoposide and vincristine, chemotherapeutic agents that induce apoptotic cell death, also activate NF-\(\kappa\)B, although binding to a distinct receptor.\(^2^{9},^{36}\) These anticancer agents may be less effective at inducing apoptotic cell death because of the concomitant activation of NF-\(\kappa\)B.\(^3^{6}\) Disruption of the protective mechanism induced by NF-\(\kappa\)B makes cells much more vulnerable to killing by TNF-\(\alpha\) and other chemotherapeutic agents and ionizing radiation.\(^4^{,25,2^{35},3^{6}}\) Glucocorticoids, salicylates, and antioxidants inhibit NF-\(\kappa\)B activation.\(^3^{,1^{0},2^{6},2^{7}}\) If tumor cell sensitivity to TNF-\(\alpha\) and other chemotherapeutic agents is controlled by NF-\(\kappa\)B, the combination of NF-\(\kappa\)B inhibitors and TNF-\(\alpha\) may dramatically enhance the antitumor activity of TNF-\(\alpha\). Therefore, combined therapy that inhibits NF-\(\kappa\)B function in the presence of apoptotic stimuli may lower the anti-apoptotic threshold of tumors and provide more effective treatment against resistant forms of malignant tumors. Alternative approaches that inhibit the nuclear translocation of NF-\(\kappa\)B, including gene therapy delivery of IkB\(\alpha\) or the local administration of antisense oligonucleotides to NF-\(\kappa\)B,\(^3^{6}\) may be very beneficial for treating tumors when combined with standard anticancer therapies.

Our study also showed that NF-\(\kappa\)B was induced and activated in the early stage of astrocytoma. NF-\(\kappa\)B is implicated in the transformation and tumorigenicity of various cancers.\(^6^{,1^{3}–1^{5}}\) NF-\(\kappa\)B can stimulate the transcription of genes that may be essential for malignant transformation. An important consequence of the activation of NF-\(\kappa\)B might be increased genomic instability, which appears to accelerate neoplastic progression. Thus, the induction and activation of NF-\(\kappa\)B appear to be involved in both formation of low-grade astrocytoma and progression towards glioblastoma.

In conclusion, our study demonstrated that NF-\(\kappa\)B is constitutively active in malignant astrocytomas, and may be important in the antiapoptotic pathway against the TNF-\(\alpha\)-induced apoptotic signal. This may be the mechanism for the resistance of malignant astrocytomas to TNF-\(\alpha\) therapy, and offers a way to establish new therapeutic strategies for malignant astrocytomas, especially glioblastoma. Further studies are needed on the specific role of NF-\(\kappa\)B in malignant astrocytomas.

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**Commentary**

As the authors pointed out, the effect of therapy with TNFα in patients with glioblastoma is unsatisfactory despite the dramatic antitumor effect of TNFα in vitro and in vivo. The authors posit that the constitutive activation of NF-κB subunits which protect against TNFα-induced apoptosis may be associated with the resistance of glioblastoma patients to TNFα immunotherapy. Besides being implicated in transformation and tumorigenicity, NF-κB may be involved in glioma cell attachment via the increased expression of integrin.1) Otsuka et al. (ref. 25 of this article) previously reported that inhibition of NF-κB activation confers sensitivity of TNFα in human glioma cells. This is in agreement with this manuscript. Furthermore, in their un-referenced earlier publication,2) the authors reported that there was no significant correlation between the efficacy of TNF-SAM2 treatment and the expression of NF-κB.

**References**


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