Nuclear Factor-κB Activation and Differential Expression of Survivin and Bcl-2 in Human Grade 2–4 Astrocytomas

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BACKGROUND. Antiapoptosis resulting from hyperactivation of the transcription factor NF-κB has been described in several cancer types. It is triggered by the interaction of the tumor necrosis factor (TNF) with its receptors and recruitment of the intermediate factor TNF-receptor associated factor (TRAF) 2. The NF-κB transcriptional activity could amplify the expression of antiapoptotic genes. The authors investigated the activity of NF-κB, and the mRNA expression of TNFα, TNFα receptor, TRAF1, TRAF2, and TRAF-associated NF-κB activator (TANK), and the antiapoptotic genes Bcl-2, c-IAP 1 and 2, and Survivin in human astrocytic tumors.

METHODS. Eight low-grade astrocytomas (LGA), 10 anaplastic astrocytomas (AAs), 10 glioblastoma multiforme (GBM) samples were used; 4 samples of normal brain tissue were used as controls. The NF-κB activation was analyzed by electrophoretic mobility shift assay; TRAF1, TRAF2, TANK/I-TRAF, Bcl-2, c-IAP 1 and 2, and Survivin mRNA expressions were studied using real-time quantitative reverse-transcriptase polymerase chain reaction.

RESULTS. NF-κB hyperactivity was detected in tumor samples. mRNA of antiapoptotic genes, particularly BCL-2 and Survivin, was hyperexpressed in gliomas. Interestingly, BCL-2 was hyperexpressed in LGAs, whereas a very high level of Survivin featured high-grade gliomas. The differential expression of antiapoptotic genes yielded a tight clustering of all LGA and nearly all GBM samples in cluster analysis.

CONCLUSIONS. NF-κB and factors involved in its intracellular activation were up-regulated in gliomas. NF-κB-activated antiapoptotic genes were hyperexpressed in tumor samples, but showed a differential expression with higher levels of Bcl-2 in LGAs and higher levels of Survivin in GBMs. Cancer 2008;112:2258–66. © 2008 American Cancer Society.

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Effects in the regulation of apoptotic cell death contribute to the genesis and progression of tumors. Failure to trigger the cellular suicide program not only predisposes to development of malignancies, but also increases the resistance of tumors to anticancer drugs and irradiation.

Human malignant glioma cells are paradigmatic for their intrinsic resistance to multiple proapoptotic stimuli. However, the molecular bases of this resistance have not been fully elucidated. A pivotal role in apoptosis control is played by the tumor necrosis factor (TNF) family of proteins including TNFα, Fas/CD95 ligand, and TNF-related apoptosis-inducing ligand (TRAIL). These lymphokines could induce apoptosis by binding to a superfamily of TNF
receptors (TNFR), and astrocytoma cells characteristically express many TNFR proteins, including TNFR1, TRAIL-DR4, TRAIL-DR5, and Fas.\textsuperscript{2–4}

On oligomerization of TNF and TNFR, the death domain of the receptor activates the TNFR-associated death domain (TRADD) proteins, which in turn recruit receptor-interacting proteins and lead to apoptosis through pro-caspases activation, forming part of a death-inducing signaling complex (DISC).\textsuperscript{5} Perhaps such activation corresponds to a concurrent and parallel activation of the transcription factor nuclear factor (NF)\textsuperscript{j}-kB.\textsuperscript{6–10} As the cytosolic NF-kB concentration rises the expression of several antiapoptotic genes is amplified.\textsuperscript{11} Candidate antiapoptotic genes for NF-kB induction include, among others, c-IAP (inhibitor of apoptosis) 1 and 2, BCL-2, BCL-X\textsubscript{L}, XIAP, and Survivin.

As seen in other cell systems, TNF-induced NF-kB activation in astrocytoma cells may be mediated by the TNFR-associated factor (TRAF) family, which consists of a group of 6 adapter proteins (TRAF1-TRAF6) that participate in the intracellular signaling activity of several members of the TNFR superfamily. Through appropriate ligand stimulation of TNFRs found on the surface of these cells, TRAF proteins can induce activation of NF-kB, resulting in both cytokine secretion and resistance to apoptosis.\textsuperscript{12–14}

In this study we analyzed the pathway leading to activation of NF-kB, including TNF\textsubscript{α}, TNFR, TRAF2 and its cofactor TRAF-associated NF-kB activator (TANK), and expression of a panel of antiapoptotic genes, including Bcl-2, c-IAP1, c-IAP2, and Survivin in glioma samples obtained in vivo to verify the expression levels in tumors with different grades of malignancy.

\textbf{MATERIALS AND METHODS}

\textbf{Patient Population}

This study included tumor samples histologically verified as grades 2 to 4 astrocytomas obtained in adult patients who had undergone craniotomy for microsurgical tumor removal. All tumors were located in the supratentorial compartment. Only patients who had undergone extensive gross-total resection of the neoplasm (95\% of the tumor volume) were eligible to participate in the study. We excluded oligodendrogial tumors, recurrent tumors, and patients who had undergone neoadjuvant therapy (radio- and/or chemotherapy) before surgery. Furthermore, 4 samples of normal brain tissue were used as controls.

The study included 16 men and 12 women whose mean age was 55.4 ± 16.5 years at surgery (range, 27–76 years). The Karnofsky Performance Status (KPS) scores in these patients were 91.1 ± 12.6 (range, 60–100). According to the revised World Health Organization (WHO) classification,\textsuperscript{15} tumors were diagnosed as low-grade astrocytoma (LGA) (8 patients), anaplastic astrocytoma (AA) (10 patients), and glioblastoma multiforme (GBM) (10 patients). Patients with LGAs had a mean age of 37.1 ± 6 years, a KPS score of 100, and a survival period longer than 208 weeks. Patients with AAs had a mean age of 62.1 ± 15.4 years, a mean KPS score of 86 ± 16.5, and a mean survival period of 109.3 ± 17.9 weeks (median 112 weeks). Patients with GBMs had a mean age of 51.4 ± 12.2 years, a mean KPS score of 89 ± 9.9, and a mean survival period of 51.4 ± 9.9 weeks (median 54 weeks). The total duration of follow-up was 208 weeks postsurgery.

\textbf{Tissue Samples}

All tumor tissue samples were obtained from the neoplastic tissue within 15 minutes of surgical removal. Tissue samples were taken from viable areas of tumor, while trying to avoid areas of gross necrosis. Three anatomic regions were separately analyzed. Tissue samples for analysis were placed in cryovials and immediately flash-frozen in liquid nitrogen in the operating room and stored at −80°C. The tissue adjacent to the frozen samples as well as additional tissue obtained from the resection specimens were formalin-fixed, paraffin-embedded, cut and stained with hematoxylin and eosin, and used for histologic typing and grading on basis of the WHO criteria.

\textbf{Isolation of Nuclear Extracts From Tumor Specimens}

Frozen tumor tissues (≈50 mg) were harvested through homogenization by using a Potter homogenizer (Braun, Melsungen, Germany) in ice-cold hypotonic lysis buffer (10 mM HEPES [pH 7.9], 0.1 mM ethylene glycol-bis-(b-aminoethyl ether)-N,N,N,N-tetraacetic acid [EGTA], 0.1 mM ethylenediamine tetraacetic acid [EDTA], 1 mM dithiothreitol [DTT]; protease inhibitors: 0.5 mM phenyl methylsulfonyl fluoride, aprotonin, pepstatin, leupeptin [10 mg/mL each]; and phosphatase inhibitors: 50 mM NaF; 30 mM b-glycerophosphate, 1 mM Na3VO4, and 20 mM p-nitrophenyl phosphate).

The homogenate was centrifuged for 1 minute at 2000 rpm at 4°C to eliminate any unbroken tissues. After 20 minutes on ice, 10\% Nonidet P-40 (Sigma Chemical, St. Louis, Mo) was added to the homogenate for a final concentration of 3.125\%, and the mixture was vortexed and microfuged (10,000 rpm) for 1 minute at 4°C. The nuclear pellet was resuspended in ice-cold hypertonic nuclear extraction buffer containing (in mM): 0.65 KCl, 0.12 Tris base, and 100 KAc, pH 7.5 (the final ionic strength adjusted to 0.4 M KCl). The mixture was vortexed and microfuged (10,000 rpm) for 1 minute at 4°C. The nuclear pellet was resuspended in ice-cold hypertonic nuclear extraction buffer containing (in mM): 0.65 KCl, 0.12 Tris base, and 100 KAc, pH 7.5 (the final ionic strength adjusted to 0.4 M KCl). The mixture was vortexed and microfuged (10,000 rpm) for 1 minute at 4°C. The nuclear pellet was resuspended in ice-cold hypertonic nuclear extraction buffer containing (in mM): 0.65 KCl, 0.12 Tris base, and 100 KAc, pH 7.5 (the final ionic strength adjusted to 0.4 M KCl).
buffer (20 mM HEPES [pH 7.9], 420 mM NaCl, 1 mM EDTA, 1 mM EGTA, 1 mM DTT, protease inhibitors, and phosphatase inhibitors), incubated on ice for 30 minutes with intermittent vortexing, and microfuged (10,000 rpm) for 10 minutes at 4°C. The supernatant was collected as nuclear extract and stored at −20°C. The concentration of total proteins in the samples was determined using the Lowry method. To estimate possible contamination of the nuclear extracts with cytoplasmic extract, lactate dehydrogenase activity was assessed using quantitative kinetic determination.

**Detection of NF-κB DNA-Binding Activity by Electrophoresis Mobility Shift Assay (EMSA)**

Twenty μg of nuclear extract was incubated for 30 minutes at room temperature with 50 fmol of biotin-end labeled 45 mer double-strand NF-κB oligonucleotide from the HIV-LTR, 5'-TTGTACAGAGGA CTTTCCG CTGGGACATTCCGG AGGCCGTGGG-3'. Binding reaction was performed for each patient and controls using the Light-Shift Chemiluminescent EMSA kit (Pierce, Milan, Italy) according to the manufacturer's instructions. Bound complexes were separated on 7.5% nondenaturing polyacrylamide gels, blotted onto nylon membrane, and observed on x-ray film (Kodak, Milan, Italy) by using autoradiography.16 Competitive assays were performed by addition of 50-fold excess of unlabeled probe to the nuclear extract at room temperature for 10 minutes before the addition of the labeled probe.

The results are expressed as relative integrated intensity compared with normal controls and internal positive controls, considering exposure time, background levels, and known protein concentration of an Epstein-Barr virus nuclear antigen-1 extract, with its consensus sequence provided with the Light-Shift Chemiluminescent kit (Pierce), which was used as EMSA control. Relative bands were quantified by densitometric scanning using an EDAS 290 support Kodak 1D Image Analysis Software.

**RNA Isolation and cDNA Reaction**

Total cellular RNA was extracted from specimens of glioma tissue previously frozen in liquid nitrogen and stored at −80°C using the Trizol reagent (Invitrogen, Milan, Italy). Specimens were then treated with the DNase and purified using a Qiagen's kit (Qiagen, Valencia, Calif). The concentration of total RNA and the purity ratio were determined spectrophotometrically, whereas integrity was checked with the Bioanalyzer instrument (Agilent Technologies, Milan, Italy).

### Real-Time Quantitative Reverse-Transcriptase Polymerase Chain Reaction (RT-PCR)

Three μg of total RNA from each sample was reverse-transcribed with the Archive kit (Applied Biosystems, Milan, Italy). Generated cDNA was used as template for real-time quantitative PCR analysis using gene expression products according to the manufacturer's recommendations (Applied Biosystems). Briefly, the reaction mixture contained unlabeled PCR primers and TaqMan MGB probe (FAM dye-labeled) in which the target sequence is located within the primer-generated amplicon. The reporter probe has a fluorescent dye on the 5'-end (FAM) and the quencher on the 3'end (MGB). During the extension phase of the PCR amplification the 5'exonuclease activity of Taq DNA polymerase cleaves the probe releasing the fluorescent dye from reporter. At each cycle of the PCR process, the increase in fluorescence was monitored by the 7300 Real-Time PCR system (Applied Biosystems).

The assays used were: TNFα (Hs 00174128-m1), TNFα receptor (Hs 01042313-m1), TANK (Hs 00370305-m1), TRAF1 (Hs 01090170-m1), TRAF2 (Hs 00184186-m1), Bcl-2 (Hs 00153350-m1), c-IAP1 (Hs 00244967-m1), c-IAP2 (Hs 00236911-m1), Survivin (Hs 00978503-m1). To normalize the expression of cDNA we used the expression level of actin (4326315 E.) simultaneously detected in each assay in the same reaction as an endogen target. In 25 μL PCR reactions containing 2.5 μL of cDNA and diluted assay gene 1:20 the reaction is started with the following program: stage 1: 50°C at 2 minutes; Stage 2: 95°C at 10 minutes; Stage 3: 95°C at 15 seconds; and Stage 4: 60°C at 1 minute.

The comparative cycle threshold (Ct) method (Applera Genomics, Foster City, Calif) was used to analyze the data by generating relative values of the amount of target cDNA. Relative quantification (RQ) for these genes is expressed as fold variation over control and was calculated by the ΔΔCt method using control samples as calibrators.

### Statistical Analysis

Statistical analysis was accomplished using the unpaired Student t-test to compare the expression levels of mRNA of TNFα, TNFR, TRAF1, TRAF2, TANK/I-TRAF, Bcl2, c-IAP1, c-IAP2, and Survivin as quantified on real-time RT-PCR and NF-κB activity on EMSA in LGAs, AAs, and GBMs. The Spearman nonparametric correlation test was used to assess the correlation among mRNA expression levels and the nominal variables (WHO grade and KPS score). The Pearson correlation test was used to obtain correlation values among numerical variables. Data
analysis was performed with INSTAT, v. 3.0, and PRISM, v. 4.0 (GraphPad, San Diego, Calif).

Unsupervised hierarchical cluster analysis was performed on the log-transformed real-time PCR data, choosing unfiltered and average linkage clustering (correlation uncentered) calculations using Gene Cluster (http://rana.stanford.edu/software). We viewed the results by using the Treeview software (http://rana.stanford.edu/software) to generate a dendrogram depicting the similarity of expression between samples with supplementary correlation coefficients for samples within a cluster.

A probability value less than .05 was considered statistically significant. All values are expressed as the means ± SD.

RESULTS

NF-κB DNA-Binding Activity
The NF-κB DNA-binding activity was detected in nuclear extracts obtained from all tumor samples (Fig. 1). Most tumors showed a variation in NF-κB/DNA-binding activity levels in 1 to 2 increments across the 3 regions analyzed per tumor. Lower NF-κB levels could be attributed to large representation of necrotic tissue, according to histologic assessment of the formalin-fixed tissue sections. In other instances lower NF-κB activity levels was attributable to the small number of tumor cells. NF-κB activity levels from 1 to 3 areas of histologically pure tumor tissue from each specimen were considered for quantitative analysis; we averaged data when multiple samples were selected for measurements.17

Definitive quantitative data obtained using densitometric analysis of bands revealed that the DNA-binding was 8.7 ± 2.9% in normal brain tissue, 18.6% ± 11% in LGAs, 22.9% ± 8.4% in AAs, and 35.9% ± 8.7% in GBMs (Fig. 1A). The levels of NF-κB/DNA-binding activity were correlated with the WHO tumor grade (P < .001). Figure 1B summarizes results of statistical analysis among groups.

Differential Expression of Genes in Gliomas
Relative quantification for the studied genes was expressed as fold changes over normal brain tissue. A summary of the relevant data is displayed in Figure 2.

The TNFα mRNA expression was 1.8 ± 2.9 in LGAs, 2.7 ± 0.7 in AAs, and 3.1 ± 1.8 in GBMs. A correlation was found between expressions of TNFα and Bcl-2 (P = .01) and between those of TNFα and c-IAP1 (P = .03).

The TNFR mRNA expression was 0.9 ± 0.7 in LGAs, 0.74 ± 0.78 in AAs, and 0.85 ± 0.56 in GBMs. A correlation was found between expressions of TNFR and TRAF2 (P = .06).

The TRAF1 mRNA expression was 4.6 ± 4.7 in LGAs, 24.6 ± 15.3 in AAs, and 11.7 ± 13.9 in GBMs. The expression levels in AAs were statistically higher as compared with LGAs (P = .003). A correlation was found between expressions of TRAF1 and TRAF2 (P < .001), TRAF1 and TANK (P = .03), and TRAF1 and c-IAP2 (P = .01).

The TRAF2 mRNA expression was 7.8 ± 5.3 in LGAs, 12.4 ± 6.8 in AAs, and 7.6 ± 4.6 in GBMs. A correlation was found between expression levels of TRAF2 and TANK (P < .001) and TRAF2 and c-IAP2 (P = .002).

The TANK mRNA expression was 6.5 ± 6.5 in LGAs, 7.6 ± 7.1 in AAs, and 1.7 ± 1.9 in GBMs. The expression levels in LGAs and AAs were statistically higher as compared with the levels in GBMs (P = .04 and P = .02, respectively). A correlation was found between expression levels of TANK and those of Bcl2 (P = .01), c-IAP1 (P = .003), c-IAP2 (P = .02), and Survivin (P = .04).
The mRNA levels of Bcl2 were 132.9 ± 116.4 in LGA; 2.5 ± 2.3 in AAs, and 0.25 ± 0.34 in GBMs. The expression levels in LGA samples were statistically higher as compared with the levels in AAs and GBMs, (P = .003 and P = .002, respectively). The levels in AAs were statistically different from that in GBMs (P = .006). A correlation was found between expression levels of Bcl2 and those of TANK (P = .003), TNF (P = .02), and Survivin (P = .05).

The mRNA levels of c-IAP1 were 4.4 ± 3.8 in LGA; 1.9 ± 1.9 in AAs, and 2.9 ± 1.8 in GBMs. A correlation was found between expression levels of c-IAP1 and those of TRAF1 (P = .01), TRAF2 (P = .002), and c-IAP1 (P = .04).

The mRNA levels of Survivin were 231.5 ± 188.6 in LGA; 533.8 ± 303.3 in AAs, and 1615 ± 279 in GBMs. The expression levels in LGA samples differed from those in AAs (P = .03) and those in GBMs (P < .001). Also, levels in AAs were statistically different from those in GBMs (P < .001). An inverse correlation was found between expression levels of Survivin and those of c-IAP1 (P = .01), TANK (P = .04); a correlation was also found between Survivin and WHO grade (P < .001) and NF-κB nuclear activity (P = .001).

To investigate whether glioma histotypes could be distinguished with a set of apoptosis-regulating genes we performed an unsupervised hierarchical cluster analysis (Fig. 3). Cluster analysis with c-IAP1, c-IAP2, TRAF1, TRAF2, Survivin, and Bcl2 yielded a tight clustering of all LGA samples and nearly all AA and GBM samples. The control samples also clustered into a group.

DISCUSSION
Our results suggest that higher mRNA levels of antiapoptotic proteins such as Bcl2, TRAF1, and members of the IAP family of genes, particularly Survivin, can be found in human astrocytic tumors when compared with normal brain tissue. This was associated with an aberrant transcriptional activity of NF-κB recruited through TNFR-TRAF2-TANK activation. The expression of antiapoptotic factors, particularly those of Bcl2 and Survivin, changed significantly in relation to tumor grading.

NF-κB is a dimeric transcription factor controlling the expression of several regulators of immune, inflammatory, and acute phase responses. A role of NF-κB in the genesis and progression of cancer has been demonstrated as well, and over the past few years a constitutive NF-κB activation has been described in a variety of epithelial and lymphoid cancers. Recently, Nagai et al.25 and Gill et al.26 provided evidence for a role of this transcription factor in the proliferation and survival of glioblastoma cell lines. Weaver et al.27 recently reported that NF-κB activation in response to chemotherapeutic agents protected U87 and U251 glioblastoma cells in vitro. We recently observed the presence of aberrant NF-κB DNA-binding activity in WHO Grades 2 to 4 astrocytomas surgically removed from humans compared with normal brain tissue.17
The aberrant hyperactivation of NF-κB in neoplasm seems to be mediated, at least in part, by TNF-related proteins stimulation, as shown by the experimental evidence that TNF or soluble CD40L induce nuclear translocation of NF-κB and by the evidence that the inhibition of NF-κB activation renders the cells sensitive to TNF killing. The signal transduction mechanism emanating from the TNFR is thought to be mediated by TRAF2, a signaling intermediate that has been shown to be recruited to the cytoplasmic tail of TNFR through a TNFR/TRADD/TRAF2 interaction. On the basis of this hypothesis, TNF can either induce apoptosis through FADD (FAS associating protein with death domain) and caspase recruitment or promote survival through TRAF2 recruitment and NF-κB induction. Accordingly, it is reasonable to speculate that constitutive activation of NF-κB in malignant gliomas may be mediated, at least in part, by TNFR/TRAF2-driven mechanisms.

For the first time it has been demonstrated that cells from the majority of tumor specimens express moderate to strong levels of TRAF2 mRNA (Fig. 3), confirming results of a previous study showing an intense protein expression in gliomas. Similar findings have been reported in other cancer cells, such as Hodgkin and Reed-Sternberg cells and hepatoma cells. We studied also, for the first time in gliomas, another member of this family, TRAF1. TRAF1 is an NF-κB-inducible protein. It appears that it works in conjunction with TRAF2 and c-IAPs to suppress fully TNF-induced apoptosis. This action may be achieved through the direct suppression of caspase activation in the TNFR signaling complex by c-IAPs, which are specifically recruited through TRAF1 and TRAF2. TRAF1 mRNA was hyperexpressed in tumor specimens, particularly in AAs.

To develop a comprehensive understanding of the molecular mechanism of TRAF-mediated signals, some proteins that associate with TRAF proteins with a regulatory function were included in our study. TANK and I-TRAF1 were recently identified; they are identical and bind all members of TRAF except TRAF4 through the TRAF-C domain. The function of TANK is not clear because of conflicting results. Data in 1 report show that TANK and TRAF2 activate NF-κB synergistically, whereas those from another study show that TANK apparently inhibits TRAF2-mediated NF-κB activation. We found a correlation between the expression levels of TNFR and that of TRAF 1 and 2 and TANK. This could suggest that all those proteins are interwoven in the activatory network of NF-κB.

The mechanism by which apoptosis can be blocked by the nuclear translocation of NF-κB seems to be related to the expression of a series of antiapoptotic genes. In fact, TNF-induced death-resistant phenotype of some glioma cells can be converted into sensitivity by treatment with protein synthesis inhibitors. This suggests the presence of short-lived antiapoptotic genes expression in gliomas/Angileri et al. 2263
apoptosis inhibitors. On the basis of their NF-κB-dependent expression and antiapoptotic function, genes encoding for c-IAP1, c-IAP2, TRAF1, Bcl-2, Bcl-Xl, X-IAP, and Survivin, among others, have been proposed as target genes in such a mechanism. The quantitative analysis of mRNA of antiapoptotic factors showed that Survivin was intensely hyperexpressed and the levels of this expression were correlated with the grading of tumors. Conversely, Bcl-2 and c-IAP1 had higher expression levels in LGAs and Bcl-2 expression was inversely correlated with the WHO grading. Furthermore, we also found that the mRNA level of TRAF1 was higher in AAs as compared with LGAs.

This could suggest that antiapoptosis is present in grade 2 to 4 gliomas but is controlled by different proteins in tumors with different histologic features. By a simultaneous analysis of the panel of antiapoptotic genes we found that many of the investigated samples showed a remarkable similarity in expression, which resulted in clustering of the individual samples in the cluster analysis.

Higher Bcl-2 expression levels determined clustering of LGAs. In contrast to the expectation that the expression of the antiapoptotic protein Bcl-2 increased with malignancy, we observed higher mRNA levels in LGAs as compared with AAs and GBMs. This result agrees with similar observations obtained in glioma by using immunohistochemistry. Furthermore, similar results were found in basal cell carcinoma and a surprising association between good prognostic features and the improved survival of patients with tumors that are Bcl-2-positive compared with those that are negative was also found in breast cancer.

Glioblastoma is characterized by an apoptosis expression signature too, but members of the IAP family, particularly Survivin, rather than Bcl-2, could define the GBM-specific profile. Survivin and c-IAP2 are members of a relatively novel family of factors acting on the apoptosis control termed inhibitor of apoptosis proteins (c-IAPs). Humans possess 8 IAP family members: NAIP, c-IAP1, c-IAP2, XIAP, Ts-XIAP, ML-IAP, Apollon, and Survivin. The central mechanisms of IAP apoptotic suppression appear to be through direct caspase and pro-caspase inhibition (primarily caspases 3 and 7) and modulation of and by the transcription factor NF-κB. Many studies have revealed a circumstantial association of IAPs and neoplasia and several reports have shown that suppression of c-IAP protein levels can sensitize cancer cells to chemotherapeutic drugs. An analysis of 3.5 million human transcriptomes identified Survivin as among the top 4 transcripts uniformly up-regulated in cancers, but not in normal tissues. Thus, Survivin expression is 1 of the most tumor-specific of all human gene products. Recently, several studies using RT-PCR, immunohistochemistry, and Western blot have shown that the mRNA and protein of Survivin are overexpressed in gliomas. Moreover, overexpression of Survivin is significantly associated with tumorogenesis and progression of gliomas, as well as the poor prognosis of patients with gliomas.

According to these findings and on the bases of our data, the expression of Survivin or Bcl-2 may have some value in discriminating different glial tumors. The meaning of this differential expression is not clear. Recently, it was demonstrated that cells can be classified in 2 types concerning the apoptotic pathway. In type 1 cells TNF triggering leads to strong caspase-8 activation at the DISC, which bypasses mitochondria directly, leading to activation of other caspases such as caspase-3 and subsequently to apoptosis. In contrast, in type 2 cells death depends on amplification of death-receptor signals via the mitochondrial pathway controlled by Bcl-2 family members. In this scenario, LGAs and primary GBMs may have different mitochondria-dependent/independent apoptotic mechanisms prevalent.

Nevertheless, findings similar to ours have been obtained in B-cell lymphomas. NF-κB activation is a common phenomenon in non-Hodgkin lymphomas resulting in the expression of distinct sets of NF-κB target genes. Bcl-2 and Survivin were identified as key NF-κB targets and their expression distinguished small and aggressive B-cell lymphomas, respectively. In that context Survivin expression, in contrast to Bcl-2, was associated with a signature of cell proliferation demonstrated by microarray evidence of a contextual expression of protein devoted to the control of cell cycle. This may contribute to the poor prognosis and phenotype of aggressive B-cell lymphomas as well as GBMs. Note also that as a chromosomal passenger protein, Survivin is capable, per se, of maintaining normal cell mitosis and promoting cell proliferation. In addition, Survivin also plays an important role in the angiogenesis process.

Conversely, LGAs are characterized by a low rate of proliferation and prolonged life span, which suggests that defective apoptosis pathways, rather than aberrant cell cycle progression, may play a prominent role in the pathogenesis of such tumors. Finally, it has been demonstrated that steady-state levels of mRNA of Bcl-2 can be significantly modified posttranscriptionally. Accordingly, posttranscriptional stability changes of the Bcl-2 mRNA may play a role in this difference of mRNA levels between Bcl-2 and Survivin and further studies in this direction are warranted.
REFERENCES


