NF-κB Is Required for Smac Mimetic-Mediated Sensitization of Glioblastoma Cells for γ-Irradiation–Induced Apoptosis

Rebecca Berger1, Claudia Jennewein2, Viola Marschall3, Sabine Karl2, Silvia Cristofanon3, Liane Wagner2, Sri HariKrishna Vellanki2, Stephanie Hehlgans4, Franz Rödel4, Klaus-Michael Debatin2, Albert C. Ludolph1, and Simone Fulda2,3

Abstract

Evasion of apoptosis contributes to radioresistance of glioblastoma, calling for novel strategies to overcome apoptosis resistance. In this study, we investigated the potential of the small molecule Smac mimic BV6 to modulate radiosensitivity of glioblastoma cells. Here, we identify a novel proapoptotic function of NF-κB in γ-irradiation–induced apoptosis of glioblastoma cells by showing, for the first time, that NF-κB is critically required for Smac mimic–mediated radiosensitization. BV6 significantly increases γ-irradiation–triggered apoptosis in several glioblastoma cell lines in a dose- and time-dependent manner. Calculation of combination index (CI) reveals that the interaction of BV6 and γ-irradiation is highly synergistic (CI < 0.3). Molecular studies show that BV6 stimulates NF-κB activation, which is critical for radiosensitization, because genetic inhibition of NF-κB by overexpression of the dominant-negative superrepressor IκBα-SR significantly decreases BV6- and γ-irradiation–induced apoptosis. Also, the BV6-mediated enhancement of γ-irradiation–triggered caspase activation, drop of mitochondrial membrane potential, and cytochrome c release is abolished in cells overexpressing IκBα-SR. Similarly, NF-κB inhibition by ectopic expression of a kinase dead mutant of IKKβ prevents the BV6-mediated sensitization for γ-irradiation. The clinical relevance is underscored by experiments with primary tumor samples showing that BV6 sensitizes primary cultured glioma cells as well as glioblastoma-initiating cancer stem cells derived from surgical specimens for γ-irradiation. In conclusion, we identify NF-κB as a critical mediator of Smac mimic–conferred radiosensitization of glioblastoma cells. These results have important implications for the development of Smac mimic–based combination protocols for radiosensitization of glioblastoma. Mol Cancer Ther; 10(10); 1867–75. ©2011 AACR.
preceding BIR2 domain (9). cIAP1 and cIAP2 function as E3 ubiquitin ligases via their RING finger domain (9). Smac mimetics have been shown to trigger autoubiquitination and proteosomal degradation of cIAPs by stimulating their E3 ubiquitin ligase activity, thereby promoting NF-κB activation and TNFα-dependent cell death, besides neutralizing the inhibitory function of XIAP (10–12). Therefore, therapeutic targeting of IAP proteins by Smac mimetics may offer new possibilities to bypass resistance, for example, resistance to radiation-induced cell death.

The transcription factor NF-κB acts as a dimer consisting of proteins of the NF-κB/Rel family (13). In the canonical pathway, the subunits p65/p50 form the classical dimer that usually mediates transcriptional activation of target genes (13). Upon stimulation, the IκB kinase complex (IKK) becomes activated, which initiates the proteosomal degradation of IκBα, freeing NF-κB to translocate to the nucleus (13). Under many circumstances, NF-κB negatively regulates apoptosis via transcriptional activation of antiapoptotic proteins (13).

We previously reported that Smac peptides, which antagonize XIAP, sensitize glioblastoma cells for TRAIL-induced apoptosis in vitro and in vivo (14). In addition, we showed that genetic or pharmacologic inactivation of XIAP increases radiation-induced apoptosis in glioblastoma, neuroblastoma, and pancreatic carcinoma cells (15–17). To translate the concept of targeting IAP proteins for radiosensitization into a clinically applicable approach to improve the efficacy of radiotherapy in glioblastoma, we investigated in this study the therapeutic potential of BV6 for radiosensitization of glioblastoma.

Materials and Methods

Cell culture and reagents
Glioblastoma cell lines U87MG, A172, and T98G were obtained from the American Type Culture Collection that carries out cell line authentication by short tandem repeat analysis. Cells were cultured in Dulbecco’s Modified Eagle’s Medium (DMEM; Life Technologies Inc.) supplemented with 10% fetal calf serum (Biochrom), 1 mmol/L glutamine (Biochrom), 1% penicillin/streptomycin (Biochrom), and 25 mmol/L HEPES (Biochrom) as described (18). U87MG, A172, and T98G cell lines harbor PTEN mutation and are either p53 wildtype (U87MG, A172) or p53 mutant (T98G; ref. 19). Primary cultured glioblastoma cells and glioblastoma-initiating cancer stem cells obtained from fresh primary glioma specimens were cultured as described using serum-free medium containing 20 μg/mL epidermal growth factor and 10 μg/mL basic fibroblast growth factor for glioblastoma-initiating cancer stem cells (17, 18, 20). The study was approved by the Ethics Committee, Medical Faculty, University of Ulm. The bivalent Smac mimic BV6 has previously been characterized and the structure of the compound has previously been published (Supple-mentary Fig. S1; ref. 11). BV6 was kindly provided by Genentech Inc. All chemicals were purchased from Sigma unless indicated otherwise. N-benzoyloxycarbonyl-Val-Ala-Asp-fluoromethylketone (zVAD.fmk) was obtained from Bachem, Enbrel from Pfizer.

Determination of apoptosis
Cells were treated with γ-irradiation (Nuclear Data, CS-137, 44Tbq, 2 Gy/min) at indicated doses and incubated for indicated times in the presence of BV6 or dimethyl sulfoxide (DMSO) as solvent. The radiation was delivered at the start of the experiment at the beginning of the BV6 exposure time. Apoptosis was determined by fluorescence-activated cell sorting analysis (FACScan; BD Biosciences) of DNA fragmentation of propidium iodide–stained nuclei as described (21).

Clonogenic survival assay
The clonogenic colony formation assay was done on single-cell suspension as described previously (22). Briefly, cells were plated in complete DMEM medium into 6-well plates (BD Biosciences) and 24 hours later, were treated with increasing concentrations of BV6 (0.1; 0.5; 1.0; 1.5 μmol/L), with DMSO or left untreated (mock). After an additional 4 hours, cells were irradiated at room temperature with single doses of X-ray (0, 2, 6, and 10 Gy) using a linear accelerator (SL 75/5; Elekta) with 6 megaelectron volt photons/100-cm focus-surface distance with a dose rate of 4.0 Gy/min. After 11 to 14 days, colonies were stained with methylene blue solution for 30 minutes and counted. Calculation of survival fractions (SF) was done using the equation SF = colonies counted/cells seeded × (PE/100), taking into consideration the individual plating efficiency (PE). Survival variables α and β were fitted according to the linear quadratic equation SF = exp [−α × D − β × D2] with D = dose using EXCEL software (Microsoft). All experiments were repeated at least 3 times. Radiation enhancement ratios at 50% and 10% survival were calculated by transforming the above mentioned equation using α and β values of the individual survival curves.

Determination of mitochondrial membrane potential and cytochrome c release
Tetramethylrhodamine methylester perchlorate (TMRE; 1 μmol/L; Sigma) was used to measure the mitochondrial transmembrane potential. Cells were incubated for 30 minutes at 37°C in the presence of the fluorochrome and immediately analyzed by flow cytometry. Cytochrome c release was determined in permeabilized cells using mouse anticytochrome c monoclonal antibody (BD Biosciences) as described (23).

Western blot analysis
Western blot analysis was done as described previously (18) using the following antibodies: mouse anti-caspase-8 (ApoTech Corporation), rabbit anti-caspase-3 (Cell Signaling), mouse anti-XIAP from BD
Biosciences, goat anti–cIAP1 from R&D Systems, Inc., mouse anti–β-actin (Sigma), or mouse anti-glyceraldehyde-3-phosphate dehydrogenase (GAPDH; HyTest) followed by goat–antimouse or goat–antirabbit IgG conjugated to horseradish peroxidase (Santa Cruz Biotechnology). Enhanced chemiluminescence was used for detection (Amersham Bioscience).

**Retroviral transduction**

Retroviral transduction of dominant-negative IkBα superrepressor (IkBα-SR) or kinase dead IKKβ (IKKβ–KD) was done as previously described (24) using the pCFG5-IEGZ retroviral vector system. In brief, PT67 producer cells (Clontech) were transfected with pCFG5-IEGZ vectors using Lipofectamine 2000 (Invitrogen) according to the manufacturer’s recommendation and selected with 0.25 mg/mL Zeocin (Invivogen). Stably transduced glioblastoma cell lines overexpressing IkBα-SR (S32; 36A) or IKKβ–KD were obtained by retroviral spin transduction and subsequent selection with Zeocin.

**Nuclear protein extraction and electrophoretic mobility shift assay**

Nuclear extracts were prepared as previously described (25). In brief, cells were washed, scraped, and collected by centrifugation at 1,000 × g for 5 minutes at 4°C. Cells were resuspended in low salt buffer, allowed to swell on ice for 12 minutes, followed by addition of a 10% Igepal CA-650 solution and centrifugation. The pelleted nuclei were resuspended in high salt buffer and nuclear supernatants were obtained by centrifugation. Protein concentrations were determined using the BCA Protein Assay Kit (Pierce). For electrophoretic mobility shift assay (EMSA), the following oligomers were used: NF-κB 5′-AGTTGAGGGACCTTTCACGGC-3′ (sense), Sp1 5′-ATTCGATCGGGGC-GGGCGAG-3′ (antisense), and ε-Bighead-3-phosphate dehydrogenase (GAPDH; HyTest) according to the manufacturer’s recommendation and purified on Sephadex columns (Micro Bio-Spin P30; Biorad Laboratories). Binding reactions containing 5 μg nuclear extract, 1 μg Poly(dIdC; Sigma), labeled oligonucleotide (10,000 cpm) and 5× binding buffer were incubated on ice and resolved by electrophoresis in nondenaturing 6% polyacrylamide gels and assessed by autoradiography. For supershift experiments, nuclear extracts were preincubated on ice with the following antibodies: p-50 (sc-7178X), p-65 (sc-372X), and cRel (sc-70X), all from Santa Cruz Biotechnology. Representative EMSAs are shown.

**Statistical analysis**

Statistical significance was assessed by Student’s t test using Winstat software (R. Fitch Software). Drug interactions were analyzed by the combination index (CI) method based on that described by Chou (26) using CalcuSyn software (Biosoft). CI < 0.9 indicates synergism, <0.3 high synergism, 0.9 to 1.1 additivity, and >1.1 antagonism.

---

**Results**

**Smac mimic sensitizes glioblastoma cells for γ-irradiation–induced apoptosis**

To explore the therapeutic potential of small molecule Smac mimetics for radiosensitization of glioblastoma, we selected BV6, a bivalent Smac mimic that antagonizes XIAP, cIAP1, and cIAP2 (11). For combination experiments with γ-irradiation, we used a concentration of BV6 that triggered minimal apoptosis as single agent (Fig. 1A–C). Importantly, the addition of BV6 significantly enhanced γ-irradiation–induced apoptosis in a dose-dependent manner in several glioblastoma cell lines, as determined by the analysis of DNA fragmentation (Fig. 1A–C). Apoptotic cell death was confirmed by Annexin V staining (Supplementary Fig. S2A). Calculation of CI revealed that BV6 cooperated in a highly synergistic manner with γ-irradiation to trigger apoptosis (Fig. 1D, Supplementary Table S1). Furthermore, the cooperative induction of apoptosis by the combination treatment with BV6 and γ-irradiation occurred in a time-dependent fashion (Fig. 1E). To explore whether BV6 exerts an effect on long-term survival following radiation, we carried out colony assays. We selected 4 concentrations of BV6 for these experiments (0.1–1.5 μmol/L BV6) after determining the plating efficiency following treatment with BV6 (data not shown). BV6 potentiated the suppression of colony formation upon irradiation (Fig. 1F–H) and increased the radiation enhancement ratio (Table 1). This effect was most pronounced in the cell line A172 and gradually decreased in the cell lines T98G and U87MG, however, reaching also a level of significance following irradiation with higher doses in the latter 2 cell lines. This indicates that BV6 markedly increases radiation-induced apoptosis in all 3 glioblastoma cell lines, whereas the BV6-mediated sensitization for suppression of colony formation was most pronounced in A172 cells. Analysis of LC3 lipidation as a marker of autophagy revealed that treatment with BV6 and/or γ-irradiation had little effect on the induction of autophagy (Supplementary Fig. S2B). Together, this set of experiments shows that the Smac mimetic BV6 primes glioblastoma cells for γ-irradiation–induced apoptosis.

**BV6 triggers caspase-dependent apoptosis**

To gain insights into the underlying molecular mechanisms that mediate the synergistic action of BV6 and γ-irradiation, we tested the effect of distinct pharmacologic inhibitors that interfere with individual pathways. The addition of the broad-range caspase inhibitor zVAD.fmk significantly reduced BV6- and γ-irradiation–induced apoptosis (Fig. 2A), showing that caspase activity was required for apoptosis induction. Next, we tested the involvement of TNFα, which has been implicated in BV6-mediated cell death (10–12, 27). The addition of the TNFα antagonistic antibody Enbrel did not confer protection against BV6- and γ-irradiation–induced apoptosis, although it
significantly reduced BV6- and TNFα-induced apoptosis that was used as a positive control (Fig. 2B and C). This indicates that BV6 and γ-irradiation induce apoptosis independently of an autocrine/paracrine TNFα loop.

Identification of NF-κB as a critical mediator of BV6-mediated sensitization for γ-irradiation–induced apoptosis

Next, we investigated whether modulation of NF-κB activity by BV6 is critical for radiosensitization, as IAP proteins are involved in the regulation of NF-κB signaling (9). Treatment with BV6 stimulated NF-κB DNA binding, whereas the combination treatment with BV6 and γ-irradiation resulted in reduced NF-κB DNA binding compared with treatment with BV6 alone (Fig. 3A). Stimulation of NF-κB transcriptional activity by BV6 was confirmed by luciferase reporter assay (Supplementary Fig. S3). To examine the composition of the NF-κB subunits, we conducted supershift analysis. A prominent shift was found upon the addition of p50 antibody, although some reduction of NF-κB DNA binding was observed upon the addition of ReI, cRe, and p52 antibodies (Fig. 3B). This indicates that the DNA-binding complex upon combined treatment with BV6 and γ-irradiation predominately consists of the NF-κB subunit p50.

To investigate the functional role of NF-κB in this model of apoptosis, we used glioblastoma cell lines, in which NF-κB activation is inhibited by overexpression of IκBα superrepressor (IκBα-SR; ref. 24). This dominant-negative
form of IκBα harbors 2 point mutations and therefore cannot be phosphorylated and degraded, thus preventing NF-κB translocation to the nucleus and subsequent NF-κB activation. Importantly, inhibition of NF-κB prevented the BV6-mediated sensitization for γ-irradiation–induced apoptosis in different glioblastoma cell lines (Fig. 3C–E), showing that NF-κB exerts a proapoptotic function in this model of apoptosis.

To investigate whether NF-κB regulates apoptosis by affecting the Smac mimetic–triggered downregulation of IAP proteins, we determined expression levels of IAP proteins by Western blotting. Treatment with BV6 caused rapid and profound downregulation of cIAP1 both in cells overexpressing IκBα-SR and vector control (Supplementary Fig. S4), consistent with the current model that Smac mimetics stimulate autoubiquitination and proteasomal degradation of IAP proteins with a RING domain and E3 ligase activity (10–12). Also, XIAP protein expression decreased upon treatment with BV6 alone and with the combination of BV6 and γ-irradiation, both in cells overexpressing IκBα-SR and in vector control cells (Supplementary Fig. S1). These findings suggest that NF-κB–conferred radiosensitivity is not mediated by differential modulation of IAP proteins.

To further explore the regulation of apoptosis by NF-κB, we monitored activation of the caspase cascade and mitochondrial perturbations. Strikingly, NF-κB inhibition profoundly reduced activation of caspase-8 and -3 into active fragments (Fig. 4A).

### Table 1. Radiation response of glioblastoma cells upon treatment with BV6 and radiation

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>A172</td>
<td>Mock treated</td>
<td>32.4</td>
<td>0.2274</td>
<td>0.0178</td>
<td>2.54</td>
<td>6.66</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>DMSO treated</td>
<td>28.6</td>
<td>0.1813</td>
<td>0.0232</td>
<td>2.81</td>
<td>6.79</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>BV6 [1.5 µmol/L]</td>
<td>1.1</td>
<td>0.0000</td>
<td>1.1488</td>
<td>0.78</td>
<td>3.62</td>
<td>1.42</td>
<td>4.80</td>
</tr>
<tr>
<td>T98G</td>
<td>Mock treated</td>
<td>15.7</td>
<td>0.2142</td>
<td>0.0029</td>
<td>3.11</td>
<td>9.53</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>DMSO treated</td>
<td>16.0</td>
<td>0.1984</td>
<td>0.0050</td>
<td>3.23</td>
<td>9.39</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>BV6 [1.5 µmol/L]</td>
<td>6.2</td>
<td>0.2738</td>
<td>0.0045</td>
<td>2.43</td>
<td>1.33</td>
<td>7.49</td>
<td>1.25</td>
</tr>
<tr>
<td>U87MG</td>
<td>Mock treated</td>
<td>14.2</td>
<td>0.2371</td>
<td>0.0067</td>
<td>2.72</td>
<td>7.94</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>DMSO treated</td>
<td>13.9</td>
<td>0.2224</td>
<td>0.0080</td>
<td>2.83</td>
<td>8.03</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>BV6 [1.5 µmol/L]</td>
<td>8.0</td>
<td>0.1905</td>
<td>0.0146</td>
<td>2.96</td>
<td>0.95</td>
<td>7.63</td>
<td>1.05</td>
</tr>
</tbody>
</table>

NOTE: Radiation enhancement ratios at 50% (LD50) and 10% survival (LD10) were calculated by transforming the linear quadratic equation \( SF = \exp \left( -\alpha \times D - \beta \times D^2 \right) \) using \( \alpha \) and \( \beta \) values of the individual survival curves.

### Figure 2.

BV6-mediated sensitization to γ-irradiation–induced apoptosis is caspase dependent. A172 cells were treated for 168 hours with 8 Gy γ-irradiation and/or 2 µmol/L BV6 in the presence or absence of 20 µmol/L zVAD.fmk or 100 µg/mL Enbrel. Treatment with 10 ng/mL TNF-α and 2 µmol/L BV6 for 48 hours served as a positive control for Enbrel. Apoptosis was determined by FACS analysis of DNA fragmentation of propidium iodide–stained nuclei. Data are mean ± SEM of 3 (A, B) or 1 (C) independent experiments conducted in triplicate; **, \( P < 0.001 \) comparing BV6 with solvent; n.s., not significant.
BV6- and γ-irradiation-induced loss of mitochondrial membrane potential and cytochrome c release into the cytosol were markedly decreased in cells overexpressing IκBα-SR compared with vector control cells (Fig. 4B and C).

NF-κB inhibition by kinase dead IKKβ abolishes BV6-mediated radiosensitization

To confirm the proapoptotic role of NF-κB in this model of apoptosis, we used an alternative approach to inhibit NF-κB activity by overexpression of a kinase dead version of IKKβ (IKKKD). Expression levels of IKKKD were controlled by Western blot analysis (Fig. 5A). EMSA confirmed that IKKKD inhibits NF-κB activation upon TNFα stimulation that was used as a positive control for NF-κB activation (Fig. 5B). Importantly, NF-κB inhibition by IKKKD significantly reduced apoptosis by the combination treatment with BV6 and γ-irradiation (Fig. 5C). These data confirm by an independent genetic approach that NF-κB promotes BV6- and γ-irradiation–induced apoptosis.

BV6 sensitizes primary cultured glioma cells and glioblastoma-initiating cancer stem cells for γ-irradiation

Finally, we extended our experiments to primary glioma samples obtained from surgical specimens to investigate the potential clinical relevance of BV6 for radiosensitization. Of note, BV6 significantly increased γ-irradiation–induced apoptosis in primary cultured glioma cells (Fig. 6A). Furthermore, we explored the effect of BV6 on the sensitivity of glioblastoma-initiating cancer stem cells toward γ-irradiation because they have been implicated in radioresistance (28). Glioblastoma-initiating cancer stem cells were isolated from clinical samples and were characterized as we previously described (17, 20) by CD133 and Nestin staining and by their ability to differentiate into the 3 different neuronal lineages (data not shown). Importantly, BV6 significantly increased...
γ-irradiation–induced apoptosis of glioblastoma-initiating cancer stem cells obtained from several specimens (Fig. 6B–D). These findings show that BV6 sensitizes primary cultured glioma cells as well as glioblastoma-initiating cancer stem cells for γ-irradiation.

Discussion

Because defects in apoptosis programs, for example, high expression of antiapoptotic molecules, can cause resistance to treatment regimens including radiotherapy (8), current attempts to improve the outcome of glioblastoma patients depend on strategies to increase apoptosis sensitivity. In this study, we identify a new proapoptotic role of NF-κB in γ-irradiation-mediated apoptosis of glioblastoma cells by showing, for the first time, that NF-κB is critically required for Smac mimetic–triggered radiosensitization. This conclusion is supported by several independent pieces of evidence. First, BV6 and γ-irradiation cooperate to trigger apoptosis in glioblastoma cells. This interaction is highly synergistic in A172 glioblastoma cells (CI < 0.3). Second, BV6 stimulates NF-κB activation, which is required for the potentiation of γ-irradiation–induced apoptosis because genetic inhibition of NF-κB by overexpression of the dominant-negative superrepressor IκBα–SR significantly reduces BV6- and γ-irradiation–induced apoptosis. Also, the BV6–conferred increase in γ-irradiation–induced caspase activation, loss of mitochondrial membrane potential, and cytochrome c release is abolished in cells overexpressing IκBα–SR. This observation suggests that Smac may be crucial for BV6–mediated radiosensitization through NF-κB activation.

Figure 4. NF-κB inhibition by IκBα–SR prevents BV6–mediated sensitization to γ-irradiation–induced caspase activation and mitochondrial outer membrane permeabilization. A172 cells stably transduced with a vector containing IκBα–SR or empty control vector were treated with 2 μM BV6 and/or 8 Gy γ-irradiation for indicated times. In A, cleavage of caspase-8 and -3 was assessed by Western blotting; cleavage fragments are indicated by arrowhead. GAPDH served as loading control. One representative of 3 experiments is shown. In B and C, mitochondrial transmembrane potential (B) and cytochrome c release (C) were assessed by FACS analysis. Data are mean ± SEM of 3 independent experiments conducted in triplicate; **, P < 0.001 comparing IκBα–SR overexpressing with control vector cells following γ-irradiation.

Figure 5. NF-κB inhibition by kinase dead IκBα–KD inhibits BV6–mediated radiosensitization. A, A172 (left) and U87MG (right) cells were stably transduced with a vector containing kinase dead IκBα–KD (IκBα–KD) or empty control vector (co). Expression of IκBα–KD was determined by Western blotting. In B, NF-κB activation in control and IκBα–KD overexpressing cells was determined by EMSA after stimulation with 10 ng/mL TNFα for 1 hour. In C, IκBα–KD overexpressing and control cells were treated with 2 μM BV6 and/or 8 Gy (A172) or 6 Gy (U87MG) γ-irradiation for 18 hours. Apoptosis was determined by FACS analysis of DNA fragmentation of propidium iodide–stained nuclei. Data are mean ± SEM of 3 independent experiments conducted in triplicate; **, P < 0.001 comparing IκBα–KD overexpressing with control vector cells.
IκB-κ. Similarly, NF-κB inhibition by ectopic expression of a kinase-dead form of IKKκ abolishes the BV6-mediated radiosensitization. Together, these findings provide clear evidence that NF-κB promotes apoptosis upon treatment with BV6 and γ-irradiation.

The novelty of this article, in particular, resides in the identification of NF-κB as a critical mediator of BV6-mediated sensitization for radiosensitization of glioblastoma, which warrants further investigation. Although inhibition of NF-κB decreases Smac mimetic–mediated cell death in ovarian carcinoma and rhabdomyosarcoma cells (10), NF-κB inhibition has been reported to enhance Smac mimetic–triggered cytotoxicity in lung or prostate carcinoma cells (37, 38). These findings point to a differential pro- or antiapoptotic role of NF-κB in Smac mimetic-induced cell death, possibly dependent on the cell type and/or cell line. Additional studies are required to unravel the underlying molecular mechanisms of NF-κB-mediated regulation of Smac mimetic–induced cell death.

Our results have important implications for the development of combination protocols with radiotherapy and Smac mimetic in the treatment of glioblastoma. Of note, BV6 also sensitizes primary cultured glioma cells derived from surgical specimens for γ-irradiation, underscoring the clinical relevance of our findings. Furthermore, BV6 significantly increased γ-irradiation–induced apoptosis in glioblastoma-initiating cancer stem cells, which have recently been accused of conferring radioresistance (28). In conclusion, Smac mimetics such as BV6 present a promising strategy for radiosensitization of glioblastoma, which warrants further investigation.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

We thank L. Nonnenmacher for providing primary cultured glioma cells and glioblastoma-initiating cancer stem cells and C. Hugenberg for expert secretarial assistance.
Requirement of NF-κB for Radiosensitization by Smac

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Received March 24, 2011; revised August 9, 2011; accepted August 15, 2011; published OnlineFirst August 22, 2011.

Grant Support

This work has been partially supported by grants from the Deutsche Forschungsgemeinschaft, the European Community (ApopTrain, APOSYS), and IAPv/18 (S. Fulda).

References

Induced Apoptosis - Irradiation γ for Glioblastoma Cells is Required for Smac Mimetic-Mediated Sensitization of NF-κB.

Rebecca Berger, Claudia Jennewein, Viola Marschall, et al.


Updated version
Access the most recent version of this article at:
doi:10.1158/1535-7163.MCT-11-0218

Supplementary Material
Access the most recent supplemental material at:
http://mct.aacrjournals.org/content/suppl/2011/08/18/1535-7163.MCT-11-0218.DC1

Cited articles
This article cites 37 articles, 9 of which you can access for free at:
http://mct.aacrjournals.org/content/10/10/1867.full#ref-list-1

Citing articles
This article has been cited by 3 HighWire-hosted articles. Access the articles at:
http://mct.aacrjournals.org/content/10/10/1867.full#related-urls

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.