Intrinsic Anticancer Drug Resistance of Malignant Melanoma Cells Is Abrogated by IFN-β and Valproic Acid

Wynand P. Roos, Eva Jöst, Christina Belohlavek, Georg Nagel, Gerhard Fritz, and Bernd Kaina

Abstract

Malignant melanoma, once metastasized, has a dismal prognosis because of intrinsic resistance to anticancer drugs. First-line therapy includes the methylating agents dacarbazine and temozolomide. Although DNA mismatch repair and O6-methylguanine (O6MeG)-DNA methyltransferase (MGMT) are key determinants of cell toleration to these drugs, there is no correlation between these markers and the therapeutic response in melanoma, indicating as yet unknown mechanisms of drug resistance. We show that in malignant melanoma cells with wild-type p53, the temozolomide-induced DNA damage O6MeG triggers upregulation of the Fas/CD95/Apo-1 receptor without activating the apoptosis cascade. This is due to silencing of procaspase-8. A single treatment with IFN-β reactivated procaspase-8 and sensitized melanoma cells to temozolomide. The key role of procaspase-8 in melanoma cell sensitization was verified by experiments in which the death receptor pathway was blocked by expression of dominant-negative FADD, siRNA knockdown of procaspase-8, or stimulation with Fas/CD95/Apo-1 activating antibody. The expression of procaspase-8 could further be enhanced by additional pretreatment with the histone deacetylase inhibitor valproic acid (VPA), which together with IFN-β caused significant sensitization of melanoma cells in vitro. Sensitization of melanoma cells to temozolomide by IFN-β and VPA was also shown in a xenograft mouse model. The data provide a plausible explanation why therapy of malignant melanomas with alkylating anticancer drugs failed even in trials where the repair of the critical toxic lesion O6MeG was blocked by MGMT inhibitors and suggest approaches to abrogate intrinsic drug resistance by IFN and VPA-mediated reactivation of the death receptor pathway. Cancer Res; 71(12): 4150–60. ©2011 AACR.

Introduction

First-line chemotherapy of malignant melanoma includes the alkylating drugs dacarbazine (DTIC), temozolomide (TMZ), and/or fotemustine (FM; ref. 1). DTIC is the most commonly used drug. TMZ has the same efficacy and molecular mode of action as the active metabolite of DTIC. It can be taken orally, does not need metabolic activation to form the active metabolite 3-methyl-(triazen-1-yl)imidazole-4-carboxamide (MTIC), is able to cross the blood–brain barrier, and can therefore also be used for the treatment of melanoma brain metastases (2). Still, the response rate of the patients suffering from melanoma treated with DTIC or TMZ remains poor, with median survival durations between 5.3 and 6.7 months (3, 4). Treatment combining TMZ with IFN-α can increase median survival to 11.8 months (5). There is, however, no clear rationale for concomitant treatment with IFN and TMZ except the finding that IFNs may cause immunosensitization of the tumor (6).

Agents like TMZ methylate DNA at 13 positions (7). Prominent methylation lesions are N3-methyladenine, N3-methylguanine, and N7-methylguanine, which are repaired by base excision repair (BER; ref. 8). The minor lesion O6-methylguanine (O6MeG) is repaired by O6MeG-DNA methyltransferase (MGMT; ref. 9). MGMT is a key node in resistance of tumor cells to methylating agents (10); thus, MGMT overexpression protects (11) and MGMT inhibition greatly sensitizes toward alkylation-induced cell kill (12). Killing by TMZ of cancer cells is executed by apoptosis, and it has been shown that both the Fas/CD95/Apo-1 extrinsic and the mitochondrial intrinsic pathway can be triggered (13). O6MeG does not trigger apoptosis directly; it requires DNA mismatch repair (MMR; ref. 14). A current model states that during DNA replication, O6MeG erroneously pairs with thymine (15). The O6MeG/T mismatch is recognized by the heterodimer MSH2/MSH6 that recruits PMS2/MLH1 (16). Therefore, loss of MMR or change in MMR expression leads to changes in resistance of tumor cells to methylating agents (17, 18). During DNA replication, MMR causes persistent single-stranded gaps in the DNA (19) that give rise to DNA double-strand breaks (DSB; ref. 20). The cell tolerates these DSBs by homologous recombination,
whereas nonhomologous end-joining only plays a minor role in protection (21).

IFNs are cytokines (22) that are produced in response to infection. There are 2 groups of IFNs, type I (IFN-α and -β) and type II (IFN-γ). Type I IFNs bind to the same receptor, which leads to activation of the Janus-activated kinase/STAT signaling pathway and changes in gene expression (23). IFNs on their own have cytotoxic effects in cancer cells, including melanomas. The anticancer effect in melanoma cells has been ascribed to the inhibition of cell growth (24) and the induction of apoptosis (25). In one trial where IFN-α was administered concomitantly with TMZ, the rationale was that IFN would cause immunosensitization of the tumor (6).

In addition to any effects IFN may have on the immune system, it may also have a direct impact on TMZ-induced cell kill, which has to date not been addressed in preclinical studies. Here, we show that p53 wild-type (p53wt) melanoma cells are refractory in death receptor–induced apoptosis despite the induction of Fas/CD95/Apo-1 expression following TMZ, which is due to silencing of procaspase-8 in these cells. A single IFN-β pretreatment could reactivate procaspase-8 expression and greatly ameliorated the response to O6MeG when the cells were treated with TMZ, in vitro and in melanoma xenografts. The expression of procaspase-8 by IFN-β could further be enhanced by the addition of the histone deacetylase (HDAC) inhibitor valproic acid (VPA), which leads to a significant sensitization of melanoma cells in vitro and in melanoma xenografts. The data provide a rational basis for the coadministration of the apoptosis modulators IFN and VPA together with methylating anticancer drugs and suggest new therapeutic interventions.

Materials and Methods

Cell lines and culture conditions

All cell lines (D05, D14, RPMI7951, and A375) were described previously (26, 27). A375 was from American Type Culture Collection (ATCC), RPMI7951 from The German Cell Culture Depository (DSMZ), and D05 and D14 were from C.W. Schmidt (Queensland Institute of Medical Research, Queensland, Australia) and checked there (28) and in the laboratory of Dr. Wölfl (Mainz). All cell lines were checked for mycoplasma contamination before experimental use. Cells were cultivated at 5% CO₂, 37°C in humidified atmosphere. D05 and D14 cells were cultivated in RPMI-1640, whereas RPMI7951 and A375 were cultivated in Dulbecco’s modified Eagle’s medium. In all cases, 10% fetal bovine serum, 100 U/mL penicillin, and 100 μg/mL streptomycin were present. D05 and A375 cells are p53wt, whereas D14 and RPMI7951 cells are p53mt (26).

Drugs and drug treatment

IFN-α (interferon alfa-2a, Roferon-A) was from Hoffmann-La Roche AG. IFN-β (interferon beta-1a, Rebif) was from Merck Serono. VPA (Sigma) stock solution (100 mmol/L) was prepared in distilled sterile H2O. TMZ (Schering-Plough) and FM (Muphoran) were prepared and handled as described (13, 26). A preincubation with 300 IU/mL IFNs for 24 hours followed by medium change to remove IFNs was done before TMZ, FM, or γ-ray (IR) treatment. Pretreatment of cells with VPA (1 mmol/L for 168 hours, replacing the medium and adding fresh VPA approximately every 48 hours) was done followed by medium change to remove VPA before TMZ addition. To deactivate the MGMT protein, O6-benzylguanine (O6BG; ref. 29) was added to the cells (final concentration 10 μmol/L) 1 hour before drug treatment. Ionizing irradiation was done by a Cs-137 source at 3.75 Gy/minute. Fas/CD95/Apo-1 activating antibody (APO-1-3, Alexis Biochemicals, Axxora Deutschland GmbH) was used at a dose of 25 ng/mL along with 25 ng/mL protein A. TNF-related apoptosis-inducing ligand (SuperKillerTRAIL, Alexis Biochemicals, Axxora Deutschland GmbH) was used at a dose of 25 ng/mL.

Isolation and stimulation of primary human lymphocytes

The isolation of lymphocytes from buffy coat blood and stimulation with anti-CD3 plus anti-CD28 (BD PharMingen) have been described previously (30).

Quantification of apoptosis

The analysis of the sub-G₁ fraction and the analysis of Annexin V/propidium iodide (PI) double-stained cells by flow cytometry have been described previously (13, 31).

Transfection of melanoma cells with MGMT, DN-FADD, and siRNA against procaspase-8

Stable MGMT transfectants were generated by cotransfection of D05 cells with the mammalian expression vector (pSV2MGMT) harboring the MGMT gene as described previously (11) and the pSV2neo plasmid for selection. Stable dominant-negative FADD (DN-FADD) transfectants were generated in D05 and A375 cells by using the pcdNA3-FADD-DN plasmid, as described previously (32). Stable transfectants with siRNA targeted toward procaspase-8 were generated by transfecting the previously described plasmid (33) in A375. Following selection, surviving clones were tested for protein expression by Western blot.

Preparation of protein extracts and MGMT activity assay

Protein extracts were prepared for Western blot analysis and MGMT activity testing as described (13). MGMT activity was determined as described (34).

Immunoblotting

SDS-PAGE was done as described (13, 31). Antibodies used were anti-caspase-3 (Cell Signaling), anti-caspase-7 (Cell Signaling), anti-caspase-8 (Cell Signaling), anti-caspase-9 (Cell Signaling), anti-FADD (Calbiochem, Merck), anti-Fas (Santa Cruz Biotechnology), anti-MLH1 (Oncogene), anti-MSH2 (Oncogene), anti-MSH6 (BD Pharmingen), anti-MLH1 (Santa Cruz), anti-PMS2 (BD Pharmingen), anti-MGMT (Chemicon International, Inc.), and anti-ERK2 (extracellular signal regulated kinase 2; Santa Cruz).

Immunofluorescence labeling and microscopy

Immunofluorescence labeling of γH2AX foci was done as described (13). The antibodies used were anti-γH2AX...
(Upstate) and Alexa Fluor 546 (Molecular Probes). Foci were not scored in apoptotic cells.

**Animal experiments**

Immunodeficient mice (NOD.CB17-Prkdcscid/J) were housed in a sterile environment and allowed free access to food and water. A375 human melanoma xenografts were initiated by injecting $8 \times 10^6$ cells in the right and left flank and the treatments began when tumor volumes reached a suitable value. Four animals each were assigned to the different groups and tumor volume was standardized across the groups. The mice received 1 dose intraperitoneally (i.p.) of TMZ (150 mg/kg). One hour prior to TMZ injection, all mice received O6BG, 30 mg/kg i.p. Animal weights and tumor volumes were measured at indicated times following TMZ injection. A total of 2 groups that received IFN-β and VPA pretreatment were treated as follows. Six days preceding O6BG/TMZ injection mice were injected i.p. once daily with 500 mg/kg VPA. One day preceding O6BG/TMZ injection mice were injected i.p. with $2 \times 10^5$ IU IFN-β. The *in vivo* experiments were done twice, once with female mice and once with male mice.

**Results and Discussion**

**IFN-β sensitizes melanoma cells to TMZ-induced apoptosis**

The studies were initiated with cell lines well characterized as to their p53 status (26). In the p53wt cell line D05, IFN-α and IFN-β caused significant sensitization to TMZ ($P = 0.03$ and $P < 0.0001$ for IFN-α and IFN-β, respectively) when MGMT was depleted with the specific MGMT inhibitor O6BG (Fig. 1A). When O6BG was omitted, D05 cells showed sensitization toward TMZ only following IFN-β pretreatment ($P = 0.0012$). In p53mt D14 cells, IFN-α and IFN-β had a very weak sensitization effect on TMZ, which was significant for IFN-β ($P = 0.002$) when depleting MGMT with O6BG. Activation of caspase-3 and -7, the apoptosis executing caspases, was also enhanced following IFN-β pretreatment in D05 cells (Fig. 1B). For the chloroethylating anticancer drug FM, a significant increase in sensitivity was observed only in p53wt D05, but not in p53mt D14 cells pretreated with IFN-β and O6BG ($P = 0.024$; Fig. 2A). Interestingly, no sensitization was found following treatment with IR (4 or 10 Gy) in D05 and D14 cells (Fig. 2B). A comparison of IFN-α and IFN-β revealed that IFN-β is more...
malignant cell type known to undergo O6MeG-triggered apoptosis in primary melanoma cells, we repeated the experiments with a non-genotoxic agent (Fig. 1A and B).

Therefore, is subject to regulation by p53 (35). Thus, in glioma cells, the apoptotic response on methylating agents is clearly p53-dependent (13, 36). In contrast, in melanoma cells, TMZ-induced apoptosis was not stimulated by p53 (26). Because the most significant sensitization of IFN-β to TMZ was observed in the p53wt melanoma cell line, we determined whether p53 is required for this effect. To this end, we compared the apoptotic response of a panel of melanoma cell lines differing in their p53 status treated with TMZ, with or without IFN-β pretreatment. The p53wt cell lines were D05 and A375, whereas the p53mt cell lines were D14 and RPMI7951 (27, 37). Data in Fig. 3A show the x-fold increase in TMZ-induced apoptosis with IFN-β pretreatment compared with TMZ without IFN-β. A significant increase in apoptosis level in the p53wt cells (up to 4-fold) was observed for D05 and A375, but not for the p53mt cells D14 and RPMI7951 (Fig. 3A). The data are taken to indicate that the sensitization effect of IFN-β in TMZ-treated melanoma cells is dependent on p53, which is of interest as most malignant melanomas express p53wt (38–40).

**IFN-β sensitzes p53wt melanoma cells to the apoptosis-inducing TMZ lesion O6MeG**

In our previous study with brain tumor cells, we have shown that p53 has a significant impact on the cytotoxic response of glioma cells to TMZ by upregulating the Fas/CD95/Apo-1 receptor (13). The gene encoding Fas/CD95/Apo-1 harbors a p53 binding site in its promoter, and therefore, is subject to regulation by p53 (35). Thus, in glioma cells, the apoptotic response on methylating agents is clearly p53-dependent (13, 36). In contrast, in melanoma cells, TMZ-induced apoptosis was not stimulated by p53 (26). Because the most significant sensitization of IFN-β to TMZ was observed in the p53wt melanoma cell line, we determined whether p53 is required for this effect. To this end, we compared the apoptotic response of a panel of melanoma cell lines differing in their p53 status treated with TMZ, with or without IFN-β pretreatment. The p53wt cell lines were D05 and A375, whereas the p53mt cell lines were D14 and RPMI7951 (27, 37). Data in Fig. 3A show the x-fold increase in TMZ-induced apoptosis with IFN-β pretreatment compared with TMZ without IFN-β. A significant increase in apoptosis level in the p53wt cells (up to 4-fold) was observed for D05 and A375, but not for the p53mt cells D14 and RPMI7951 (Fig. 3A). The data are taken to indicate that the sensitization effect of IFN-β in TMZ-treated melanoma cells is dependent on p53, which is of interest as most malignant melanomas express p53wt (38–40).

**TMZ, similar to DTIC, is a genotoxic anticancer drug that induces a broad spectrum of DNA alkylation lesions. Theoretically, IFN-β may sensitize melanoma cells to different DNA methylation lesions. To determine whether sensitization to TMZ by IFN-β is related to the specific adduct O6MeG, we**
Apoptosis was assayed by the sub-G1 method 144 hours after TMZ greatly sensitized cells to the combination treatment with RPMI7951 cell lines treated with O6BG and 50 cells. A, sensitization of the p53wt D05 and A375 and the p53mt D14 and C, induced apoptosis in D05 MGMT transfectants, see the blot in this figure). Under MGMT 800 fmol/mg protein (Fig. 3B; for MGMT protein in the stable MGMT following transfection, that is, approximately 200 up to compared isogenic D05 cells by expressing different levels of MGMT following transfection, that is, approximately 200 up to 800 fmol/mg protein (Fig. 3B; for MGMT protein in the stable MGMT transfectants, see the blot in this figure). Under MGMT expressing conditions, the sensitization of D05 cells by IFN-β was completely abrogated, whereas inactivation of MGMT with OBG in both parental and MGMT-transfected cells greatly sensitized cells to the combination treatment with IFN-β and TMZ (Fig. 3C). These results obtained with MGMT-transfected cells clearly show that sensitization of melanoma cells to TMZ by IFN-β is due to the TMZ-induced DNA damage O6MeG.

**IFN-β does not influence MGMT and MMR protein expression or DSB formation in melanoma cells following TMZ treatment**

The DNA adduct O6MeG is dependent on MMR for conversion to toxic DSBs and, therefore, MGMT, MMR, and DSBs are determinants of O6MeG-induced cell kill (10, 41). Due to this, we ascertained whether IFN-β has an influence on these parameters by determining MGMT and MMR protein expression and DSBs arising from the processing of O6MeG. Treatment of D05 and D14 cells with 300 μL/mL IFN-β neither had an influence on the expression of MGMT nor the MMR proteins MSH2, MSH6, PMS2, and MLH1 (Fig. 4A). Since the processing of O6MeG gives rise to the formation of DSBs (20, 30), which was also recently shown for melanoma cells (26), we determined whether sensitization of melanoma cells by IFN-β is related to γH2AX foci formation. TMZ induced γH2AX foci, which was shown 24 up to 72 hours following treatment, and IFN-β had no significant effect on the γH2AX foci level (for representative example Fig. 4B, and for quantification Fig. 4C). We infer that IFN-β neither has an impact on the repair of O6MeG lesions by MGMT nor the conversion of O6MeG/T lesions into DSBs.

**IFN-β induces procaspase-8 expression and thereby reactivates the Fas/CD95/Apo-1 apoptotic pathway**

Since no difference in repair and O6MeG processing could be detected in the presence or absence of IFN-β, we reasoned that IFN-β might increase the effectiveness of triggering the apoptotic pathway following O6MeG induction. In p53wt glioma cells, it is known that TMZ can activate the Fas/CD95/Apo-1–dependent death receptor along with the mitochondrial apoptosis pathway, whereas in p53mt cells, only the mitochondrial pathway is activated (13). As shown in Fig. 5A, in the p53wt melanoma cell lines D05 and A375, TMZ caused upregulation of the Fas/CD95/Apo-1 receptor, which is expected as the gene encoding Fas/CD95/Apo-1 harbors a p53 binding site in its promoter and is subject to regulation by p53 (35). In the p53mt cell lines D14 and RPMI7951, no increase in Fas/CD95/Apo-1 was detected. We also observed that cells exhibiting both p53wt (D05) and p53mt (D14) status activated the mitochondrial apoptosis marker caspase-9 following TMZ treatment, as shown by the increase in the activated fragments of this caspase (Fig. 5A).

As the effect of IFN-β was different in p53wt and mutant cells, we surmised that the death receptor-associated procaspase-8 could be involved. Therefore, we checked its expression following IFN-β treatment in both cell types. In the D05 and A375 cells, IFN-β caused a clear upregulation of procaspase-8, whereas in the p53mt lines D14 and RPMI7951, no procaspase-8 upregulation was observed (Fig. 5B). We should note that D14 and RPMI7951 cells already express procaspase-8 that could not be further enhanced by IFN-β. Obviously, the basal procaspase-8 level

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**Figure 3.** Influence of p53 and O6MeG on IFN-β–sensitized melanoma cells. A, sensitization of the p53wt D05 and A375 and the p53mt D14 and RPMI7951 cell lines treated with O6BG and 50 μmol/L TMZ by IFN-β. Apoptosis was assayed by the sub-G1 method 144 hours after TMZ addition. Experiments were done at least 3 times. B, MGMT activity in D05, D14, and D05 MGMT–transfected cells. HeLa MR and HeLa S3 were used as negative and positive controls, respectively. Right, Western blot analysis of D05 MGMT–transfected cells (the clones D05MGMT4 and D05MGMT30). ERK2 was used as loading control. C, induced apoptosis in D05 MGMT–transfected cells by 50 μmol/L TMZ in the absence or presence of O6BG pretreatment. Experiments were done at least 3 times.
in these cells has no impact on TMZ-induced killing, which is explained by the lack of basal expression and upregulation of the death receptor in these p53mt cells (see Fig. 5A). Epigenetic downregulation of procaspase-8 gene expression has been shown in several tumors, which is presumably due to promoter methylation (42, 43). IFN-β may be effective in demethylating the procaspase-8 promoter or stimulate its expression. Thus, it has been shown that IFN-α (44) and IFN-γ stimulate procaspase-8 expression and the promoter of procaspase-8 contains an IFN-response element (45).

If procaspase-8 is the limiting factor in preventing the effective activation of the Fas/CD95/Apo-1 apoptosis pathway, inactivating the pathway as a whole or knocking down procaspase-8 should abolish the sensitization to TMZ following IFN-β pretreatment. Both options were tested: Fas/CD95/Apo-1-dependent activation of the death receptor pathway was prevented by stable transfection of D05 with DN-FADD and procaspase-8 was knocked down in A375 cells. DN-FADD clearly decreased the effectiveness of IFN-β in sensitizing D05 cells to TMZ (Fig. 5C), whereas knockdown of procaspase-8 completely abolished the sensitization of A375 cells by IFN-β to TMZ (Fig. 5D). Collectively, the data provide evidence that IFN-β sensitizes melanoma cells to TMZ due to the upregulation of procaspase-8, thereby reactivating the cells ability to utilize the death receptor apoptosis pathway following O6MeG induction in MGMT deficient/depleted cells.

**Both the induction of Fas/CD95/Apo-1 by TMZ and the upregulation of procaspase-8 by IFN-β are required for the sensitization observed during combination treatment**

Having shown that the silenced death receptor pathway can be reactivated in p53wt melanoma cells by IFN-β, we asked whether stimulation of the Fas/CD95/Apo-1 receptor by an external ligand would be able to ameliorate the TMZ response in the presence or absence of IFN-β. We made use of non-transfected and DN-FADD–transfected D05 cells to substantiate the killing effects that were brought about by the death receptor. Treatment with Fas/CD95/Apo-1 activating antibody (AB) did not induce apoptosis significantly, whereas treatment with AB plus IFN-β or AB plus TMZ was effective (Fig. 6A). The strongest response was observed when the AB was administered after TMZ in IFN-β pretreated cells. Similar results were obtained with A375 cells (Fig. 6C). We should note that in A375 cells, treatment with IFN-β plus AB did not induce apoptosis significantly, whereas the inclusion of TMZ caused a dramatic apoptotic response. This is likely due to the low basal Fas/CD95/Apo-1 receptor status of A375 cells.
(Fig. 5A), which needs to be upregulated by TMZ. In p53mt D14 cells, sensitization by the activating antibody did not occur, either in the presence or the absence of TMZ and IFN-β (Fig. 6B), showing that the slight sensitization of D14 cells by IFN-β (see Fig. 1A) does not rely on Fas/CD95/Apo-1. Induction of procaspase-8 by IFN-β should also influence Fas/CD95/Apo-1-independent apoptosis, for example, by administering TRAIL, which activates a different group of receptors, DR4 and DR5. This was, in fact, observed: Pretreatment with IFN-β sensitized all melanoma cell lines tested to TRAIL (Fig. 6D). Collectively, these findings show that IFN-β greatly sensitizes p53wt melanoma cells to TMZ due to reactivation of the Fas/CD95/Apo-1 pathway by upregulation of procaspase-8.

**VPA greatly ameliorates the sensitization of melanoma cells by IFN-β in vitro**

Is it possible to further increase the procaspase-8 level and TMZ sensitivity of melanoma cells? To address this question, we used the HDAC inhibitor VPA, which is known to cause...
gene reactivation (46), and D05 cells were pretreated with VPA, IFN-β, or both. As shown in Fig. 7, IFN-β on its own caused increase of procaspase-8 protein expression, whereas VPA on its own did not. When D05 cells were pretreated with IFN-β and VPA, a very strong increase in procaspase-8 expression was observed (Fig. 7A; for quantification see the induction factor). The combinations of IFN-β and VPA pretreatment also greatly sensitized D05 cells to low-dose TMZ (30 μmol/L; Fig. 7B). The data revealed that combining IFN-β and VPA was more effective than the single treatments in enhancing the killing effect of TMZ in melanoma cells.

**IFN-β and VPA ameliorate the anticancer effect of TMZ in vivo**

To ascertain whether the findings can be translated to the melanoma cell growth in vivo, we determined the effect of A375 cells grown s.c. in nonobese diabetic/severe combined immunodeficient (NOD/SCID) mice. First, we analyzed whether the enhanced sensitivity of IFN-β pretreated cells would have an influence on melanoma cell growth treated with TMZ in vivo. Tumor sizes were determined 20 days after TMZ treatment. IFN-β on its own had a slight effect on tumor growth. A single treatment with TMZ (150 mg/kg) delayed tumor growth, whereas TMZ in combination with a single...
IFN-β pretreatment showed improved tumor growth inhibition (Fig. 7C, left). Thus, IFN-β sensitizes melanoma cells both in vitro and in vivo. Second, we determined the effect of combined treatment. IFN-β and VPA pretreatment on its own had no effect on tumor growth, whereas TMZ (150 mg/kg) in combination with VPA and IFN-β pretreatment
showed significant tumor growth inhibition (Fig. 7C, right). At the end of the investigation period, IFN-β improved the reduction of tumor size by approximately 25% compared with animals treated with TMZ/O6BG only, whereas IFN-β plus VPA caused a significant improvement by approximately 45%. Similar to what was observed in vitro, the treatments were most effective when VPA and IFN-β were applied concomitantly prior to TMZ compared with pretreatment with IFN-β alone. A model is shown in Fig. 7D. We are aware that the pathways evoked by combining VPA and IFN-β are complex, and therefore we cannot exclude that additional mechanisms become activated by concomitant treatment with the drugs, but it is reasonable to posit that reactivation of the Fas/CD95/Apo-1 pathway is most critical for eliciting a killing response triggered by the TMZ/DTIC–induced DNA adduct O6MeG.

For TMZ and DTIC, MGMT is a key factor in drug resistance (10). MGMT can be inactivated nearly to completion by O6BG and O6-(4-bromothenyl)guanine (O6BTG), both currently included in clinical trials (47). A recently published trial, however, showed no benefit for melanoma patients receiving TMZ concomitantly with O6BTG (48). In light of our current data, it is pertinent to conclude that melanomas cannot respond to TMZ/dacarbazine-based chemotherapy because the death receptor pathway is silenced due to lack of proapoptotic signaling induced by alkylating agents.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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References


7. Beranek DT. Distribution of methyl and ethyl adducts following alkyla


12. Mangison GP, Santibanez-Koref MF. O6-alkylguanine-DNA alkyl


15. Loechler EL, Green CL, Essigmann JM. In vivo mutagenesis by O6-

16. Stojic L, Brun R, Jiricny J.Mismatch repair and DNA damage signal-


19. Mejas N, Lopes M, Jiricny J. Mismatch repair-deficient process-

20. Ochs K, Kaina B. Apoptosis induced by DNA damage O6-methylgua
nine is Bcl-2 and caspase-9 regulated and Fas/caspase-8 indepen


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