Combined Effect of Proteasome and Calpain Inhibition on Cisplatin-Resistant Human Melanoma Cells


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Abstract

Resistance of tumor cells to cisplatin is a common feature frequently encountered during chemotherapy against melanoma caused by various known and unknown mechanisms. To overcome drug resistance toward cisplatin, a targeted treatment using alternative agents, such as proteasome inhibitors, has been investigated. This combination could offer a new therapeutic approach. Here, we report the biological effects of proteasome inhibitors on the parental cisplatin-sensitive MeWo human melanoma cell line and its cisplatin-resistant MeWo cis1 variant. Our experiments show that proteasome inhibitor treatment of both cell lines impairs cell viability at concentrations that are not toxic to primary human fibroblasts in vitro. However, compared with the parental MeWo cell line, significantly higher concentrations of proteasome inhibitor are required to reduce cell viability of MeWo cis1 cells. Moreover, whereas proteasome activity was inhibited to the same extent in both cell lines, IκBα degradation and nuclear factor-κB (NF-κB) activation in MeWo cis1 cells was proteasome inhibitor independent but essentially calpain inhibitor sensitive. In support, a calpain-specific inhibitor impaired NF-κB activation in MeWo cis1 cells. Here, we show that cisplatin resistance in MeWo cis1 is accompanied by a change in the NF-κB activation pathway in favor of calpain-mediated IκBα degradation. Furthermore, combined exposure to proteasome and calpain inhibitor resulted in additive effects and a strongly reduced cell viability of MeWo cis1 cells. Thus, combined strategies targeting distinct proteolytic pathways may help to overcome mechanisms of drug resistance in tumor cells. (Cancer Res 2006; 66(15): 7598-7605)

Introduction

Melanoma is a malignant neoplasia of melanoblastic origin that is the leading cause of death from cutaneous malignant disease (1). Thus far, in metastatic melanoma, single-agent chemotherapy, including dacarbazine, nitrosoureas, Vinca alkaloids, cisplatin, paclitaxel, and bleomycin, have not been proven to be beneficial, with response rates well below 25% and no demonstration of prolonged survival (2). Cisplatin is frequently part of polychemotherapy regimens in melanoma treatment based on the feature that cells deficient in DNA repair are hypersensitive to cisplatin (3). However, the presence or development of cisplatin resistance is an important clinical limitation. Mechanisms of cisplatin resistance are usually multifactorial and include enhanced efflux, oncogene overexpression, defects in apoptosis pathways, and can be affected by cisplatin-mediated induction of endoplasmic reticulum stress (4, 5). Alternative approaches are urgently needed. Thus, combination of conventional chemotherapeutics with novel biological agents, such as proteasome inhibitors, need to be studied carefully. In this context, proteasome inhibitors have been described that induce cell cycle arrest and apoptosis, and that could sensitize malignant cells to proapoptotic effects of conventional chemotherapeutics (6, 7). The target for proteasome inhibitors is the multicatalytic protease complex called proteasome, which degrades mostly ubiquitinated proteins in the cytosol and nucleus. Therefore, the proteasome system is involved in various pathways, such as regulation of transcription, apoptosis, and cell cycle. In transcriptional regulation, the proteasome also activates nuclear factor-κB (NF-κB) by degrading its inhibitory proteins (IκB). Stimulation of cells by tumor necrosis factor (TNF) results in the phosphorylation and subsequent proteasomal degradation of IκBα, which allows NF-κB to enter the nucleus. There, NF-κB regulates the expression of its target genes. In many tumor cells, prolonged NF-κB activation can lead to inhibition of apoptosis and therapeutic failure. Previous studies indicate that in this context, NF-κB could also be activated in a proteasome-ubiquitin-independent pathway due to calpain-dependent IκBα degradation (8). Calpains are nonlysosomal, calcium-dependent proteases that cleave a specific subset of cellular proteins, including cytoskeletal proteins, membrane receptors, and many transcription factors (9).

The aim of this study was to analyze the biological effects of proteasome inhibition in cisplatin-resistant and cisplatin-sensitive human melanoma cells. In particular, we examined cellular effects of a new proteasome inhibitor, BSc2118 (10). Our data show that proteasome inhibitor treatment of cisplatin-sensitive and cisplatin-resistant melanoma cells induces cell death at concentrations that are not toxic to primary human fibroblasts. Moreover, our data provide evidence that cisplatin-resistant cells are considerably more resistant to proteasome inhibition than parental...
cisplatin-sensitive melanoma cells. However, combined proteasome and calpain inhibition dramatically reduces the cell viability of MeWo
out melanoma cells and helps to overcome the relative proteasome inhibitor resistance of these cells.

Materials and Methods

**Materials.** The proteasome inhibitors MG-132 (Calbiochem, Darmstadt, Germany), PS-341 (Millenium Pharmaceuticals, Ltd., Chiswick, United Kingdom), and BSc2118 (10) were dissolved in DMSO at a concentration of 10 mmol/L. Stock solutions of calpain inhibitors PD150606 (Calbiochem) and E64 (Sigma, St. Louis, MO) were prepared in DMSO at 100 mmol/L. BAY 11-7082 (Calbiochem), an inhibitor of TNF-induced IκBα degradation, was prepared in DMSO at 100 mmol/L. Cisplatin (Medac, Hamburg, Germany) was dissolved in FCS-free RPMI at 1 mg/mL and kept at −20°C. Recombinant mouse TNF-α (hereafter named as TNF; Roche, Mannheim, Germany) was stored at −20°C before use. The specific activity of TNF is 4 × 10^7 units/mg and this TNF is also effective on human cells.

**Cell culture.** MeWo and MeWo out cells were cultured in RPMI 1640 (Biochrom, Berlin, Germany) supplemented with 10% FCS, penicillin (100 units/mL), and streptomycin (100 μg/mL) as described (11). MeWo out cells were cultured with additional cisplatin at 1 μg/mL. Primary human fibroblasts (PromoCell GmbH, Heidelberg, Germany) were grown in fibroblast growth medium supplied by the manufacturer.

**Cell proliferation assays.** The cytotoxic/cytostatic effects of proteasome inhibitors on melanoma cells or human primary fibroblasts were examined in vitro using the crystal violet assay, as previously described (12). Briefly, 5 × 10^3 cells per well were seeded in 96-well microtiter plates in a total volume of 100 μL/well (Greiner) and proteasome inhibitor exposure was done the following day. Serial dilutions of MG-132, PS-341, and BSc2118 (final concentrations 0-1,000 nmol/L) were added in quadruplicates. After an incubation period of 24, 48, or 72 hours with proteasome inhibitors, cells were stained with 0.1% crystal violet (Sigma). Cytotoxic/cytostatic effect was expressed as relative viability of treated cells (percentage of control cells incubated with medium only) and was calculated as follows: relative viability = (A - A0) / (A - A0), where A0 is background absorbance, A is experimental absorbance, and A is the absorbance of untreated controls.

To assess drug sensitivity in melanoma cells, we also did colony growth assay using 96-well plates. Cells were plated at 20 per well and BSc2118 was used at the same concentrations as before. After 15 days of incubation, colonies were stained with crystal violet and were counted. BSc2118 was used at the same concentrations as before. After 15 days of incubation, colonies were stained with crystal violet and were counted. BSc2118 was used at the same concentrations as before. After 15 days of incubation, colonies were stained with crystal violet and were counted.

**Protease activity assays in cell extracts and purified 20S proteasomes.** Cells were seeded in 96-well plates at a density of 1 × 10^4 per well. After incubation with proteasome inhibitors (MG-132, PS-341, and BSc2118) from 0 to 1,000 nmol/L, cells were washed with PBS and lysed [20 mmol/L Tris-HCl (pH 6.8), 50 mmol/L NaCl, 2 mmol/L MgCl2, 0.1% NP40, and protease inhibitors Complete, Roche]. Protein content was estimated by the crystal violet assay, as previously described (12). Briefly, 5 × 10^3 cells per well were seeded in 96-well microtiter plates in a total volume of 100 μL/well (Greiner) and proteasome inhibitor exposure was done the following day. Serial dilutions of MG-132, PS-341, and BSc2118 (final concentrations 0-1,000 nmol/L) were added in quadruplicates. After an incubation period of 24, 48, or 72 hours with proteasome inhibitors, cells were stained with 0.1% crystal violet (Sigma). Cytotoxic/cytostatic effect was expressed as relative viability of treated cells (percentage of control cells incubated with medium only) and was calculated as follows: relative viability = (A - A0) / (A - A0), where A0 is background absorbance, A is experimental absorbance, and A is the absorbance of untreated controls.

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**Apoptosis measurement using flow cytometry.** Apoptosis was analyzed after 24 and 48 hours of exposure of melanoma cells to BSc2118 (10-500 nmol/L), PD150606 (25-300 μmol/L), or BAY 11-7082 (5-10 μmol/L). Apoptotic cells were labeled by Annexin V–FITC, necrotic cells were marked by the uptake of propidium iodide according to the instructions from the manufacturer (BioSource, Camarillo, CA). Living cells are defined as negative for both Annexin V and propidium iodide, early apoptotic cells are positive for Annexin V only, and late apoptotic and necrotic cells are positive for both dyes. The samples were analyzed on a FACSCalibur flow cytometer (Becton Dickinson) and evaluated using CellQuest software.

**Immunoblot analysis.** Protein expression in melanoma cells was analyzed as shown before (17). Briefly, samples containing 50 μg of protein were separated on a 10% SDS-PAGE, transferred to nitrocellulose membranes, and probed with rabbit polyclonal anti-IκBα antibody and mouse anti-β-tubulin antibody (Santa Cruz Biotechnology, Santa Cruz, CA).

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The viability of the cells was determined by crystal violet assay. Pertinent results are illustrated in Fig. 1A and 1B for MeWo and MeWo_{cis1} cells, respectively, in the presence or absence of BSc2118. In MeWo cells, BSc2118 concentrations >500 nmol/L were required to affect their viability. In contrast to melanoma cells, primary human fibroblasts were strikingly insensitive to proteasome inhibition and extremely high BSc2118 concentrations (>500 nmol/L) were required to affect their viability.

To investigate the effect of proteasome inhibition on tumor cell outgrowth, we did cell colony formation assay. MeWo and MeWo_{cis1} cells were exposed to serial concentrations of BSc2118 for 15 days and colonies formed were counted. As revealed in Fig. 1B, <10 nmol/L of BSc2118 were needed to achieve a 50% reduction in outgrowth of MeWo cells. An inhibitor concentration of 20 nmol/L was sufficient to stop MeWo cell colony formation completely. In contrast, MeWo_{cis1} cells turned out to be considerably more resistant to proteasome inhibition. Nevertheless, a BSc2118 concentration of 30 nmol/L was sufficient to completely impair colony formation.

To test the potency and selectivity of BSc2118, we compared its antiproliferative effect on melanoma cells with that of the commonly used proteasome inhibitor MG-132, which displays structural similarity to BSc2118, and the inhibitor PS-341, which was recently approved for clinical application in relapsed multiple myeloma (19, 20). For this purpose, melanoma cells were incubated with increasing inhibitor concentrations (0-1,000 nmol/L) and cell viability was related to the degree of proteasome inhibition. This method permits correlation of proteasome inhibitors with different IC_{50} values (BSc2118, 45 nmol/L; PS-341, 0.6 nmol/L; MG-132, 20 nmol/L) and to compare their antiproliferative efficiency on malignant cells (21). Correlating the reduction in cell viability with the ability to inhibit proteasome activity revealed that BSc2118 is considerably more efficient in affecting cell viability than MG-132. Furthermore, BSc2118 exhibited a similar antiproliferative effect as PS-341 (Fig. 1C). Together, these data show that inhibition of proteasome activity is able to effectively reduce the viability of MeWo and MeWo_{cis1} cells at low concentrations that are not toxic to primary human fibroblasts.
MeWo_{cis1} cells require higher inhibitor concentrations for cell cycle arrest. Because proteasome inhibitors are known to affect cell cycle progression, we next analyzed the effect of BSc2118 on the cell cycle in MeWo and MeWo_{cis1} cells. Flow cytometry-based cell cycle analysis revealed a BSc2118-triggered G2-M arrest in cisplatin-sensitive and cisplatin-resistant melanoma cells (Fig. 2). The presence of a sub-G1 peak that indirectly indicates apoptotic cell death (22) is detectable mainly after 48 hours of inhibitor treatment (Fig. 2). A similar effect was also observed when inhibitor-treated cells were analyzed by Annexin V staining (data not shown). Bromodeoxyuridine (BrdUrd) incorporation experiments also revealed an effect of proteasome inhibition on cell cycle progression in the G1-S phase in that both cell lines stopped to incorporate BrdUrd after 48 hours (Supplementary Fig. S1). As observed in the experiments above (Fig. 1), MeWo_{cis1} cells were again considerably more resistant against proteasome inhibition than MeWo cells.

Proteasomes of both MeWo and MeWo_{cis1} cells are equally sensitive to BSc2118. One important observation of the experiments done thus far is that both MeWo and MeWo_{cis1} cells react similarly to proteasome inhibition. However, significantly higher inhibitor concentration had to be applied to MeWo_{cis1} cells to obtain the same effects as in MeWo cells. Because it had been reported that proteasomes can become resistant to inhibitors (23), our data raised the possibility that proteasomes of MeWo_{cis1} cells may exhibit a reduced susceptibility toward proteasome inhibitors.

Therefore, we directly assessed the inhibitory effect of BSc2118 on cellular proteasome activity by exposing both MeWo and MeWo_{cis1} cells to different concentrations of BSc2118 and by measuring the chymotrypsin-like proteasome activity in cell lysates using the fluorogenic peptide substrate suc-LLVY-AMC. As shown in Fig. 3, reduction of the chymotrypsin-like proteasome activity by BSc2118 at two time points (1 and 4 hours), and at all inhibitor concentrations used, was almost identical in both cisplatin-sensitive and cisplatin-insensitive melanoma cells (Fig. 3A and B). These experiments confirm that BSc2118 penetrates both cell lines with the same efficiency, and, more importantly, that proteasomes of MeWo and MeWo_{cis1} cells are equally sensitive to BSc2118.

We also tested MeWo_{cis1} cells with respect to multidrug resistance by investigating activity of the multidrug resistance-associated protein (MRP1). However, no enhanced MRP-1 activity could be determined as defined by calcine-AM assay (data not shown). From these experiments, we therefore conclude that the relative resistance of MeWo_{cis1} cells toward proteasome inhibition is not caused by the different effect of proteasome inhibitors on MeWo and MeWo_{cis1} cells, but that the observed resistance is more likely the consequence of altered postproteasomal or proteasome-independent mechanisms.

IκBα degradation and NF-κB activation in MeWo_{cis1} cells are proteasome independent. To study a potential proteasome-independent mechanism of resistance in melanoma cells at the molecular level, we focused on the IκBα expression as an indicator for NF-κB activation. In this context, it has been shown that IκBα is mainly degraded in a proteasome-independent manner, but that in some cells and under certain physiologic conditions IκBα degradation can also occur independently of the proteasome (24). Because it is established that NF-κB can interfere with the induction of cell death, and based on the results obtained above, we hypothesized that the increased resistance of MeWo_{cis1} cells to proteasome inhibition may be due to an enhanced constitutive NF-κB activation as it has been described for other tumor cells (25, 26). In the canonical NF-κB activation pathway, TNF stimulates the phosphorylation and proteasome-dependent degradation of IκBα. This process results in the release and transfer of NF-κB into the nucleus. Because the activation of NF-κB in the cytosol requires proteasome activity, the inhibition of proteasome-dependent IκBα degradation by proteasome inhibitors should also impair NF-κB activation (27).

At first, we analyzed IκBα expression in both cell lines after stimulation of the cells with TNF, and in the absence or presence of BSc2118. In comparison to MeWo cells, longer TNF stimulation of MeWo_{cis1} cells was required to induce degradation of IκBα (data not shown). As expected, TNF-induced IκBα degradation was stabilized by proteasome inhibition in parental MeWo cells (Fig. 4A, top). However, in complete contrast, proteasome inhibition by BSc2118 did not result in the expected stabilization of IκBα in MeWo_{cis1} cells (Fig. 4A, bottom), suggesting that the NF-κB pathway is defective or might have been altered in MeWo_{cis1} cells.

To assess nuclear NF-κB translocation, EMSAs were done. As expected from the data shown above, activation of NF-κB was impaired by proteasome inhibition in MeWo cells (Fig. 4B). In contrast, and in complete accordance with the results shown in Fig. 4A, inhibition of proteasomes in MeWo_{cis1} cells did not prevent nuclear translocation of NF-κB. The low NF-κB baseline binding observed in both cell lines (also see Fig. 6A) excludes the above
hypothesis that high constitutive NF-κB activity is responsible for the observed effects in MeWo<sub>cis1</sub> cells. Together, however, these data also indicate the existence of an imbalance in the NF-κB signaling pathway in MeWo<sub>cis1</sub> cells and suggest that IκBα degradation, and hence NF-κB activation, might be controlled by a proteasome-independent mechanism.

**Role for calpain in IκBα degradation in MeWo<sub>cis1</sub> cells.** Although IκBα degradation is attributed primarily to the ubiquitin-proteasome pathway (28), it has also been shown that alternative proteases, i.e., calpain, are involved in the regulation of IκBα stability (8). To investigate whether calpain might be responsible for the observed TNF-induced IκBα degradation in MeWo<sub>cis1</sub> cells, the calpain-specific inhibitor PD150606 was used (29). PD150606 binds to the noncatalytic site of activated calpain when the substrate is bound to the protease (29) and importantly does not inhibit proteasome activity. To this end, melanoma cells were preincubated with BSc2118, MG-132, or PD150606 for 1 hour, followed by TNF stimulation for 20 minutes for MeWo cells and 40 minutes for MeWo<sub>cis1</sub> cells. As a control and to confirm IκBα degradation, these melanoma cells were exposed to TNF alone. As shown in Fig. 5A (top), TNF-induced IκBα degradation was prevented in the parental MeWo cell line by proteasome inhibitors BSc2118 and MG-132. Interestingly, inhibition of calpain also was able to confer IκBα stability, indicating that in MeWo cells two parallel pathways for NF-κB activation coexist. In contrast, although the proteasome inhibitors failed to confer IκBα stability in MeWo<sub>cis1</sub> cells, only the exposure of MeWo<sub>cis1</sub> cells to calpain inhibitor abolished TNF-induced degradation of IκBα (Fig. 5A, bottom) and this effect was dose dependent (Fig. 5B). Similar results were obtained using different calpain inhibitors, such as E64 (data not shown).

In addition, comparison of cellular calpain- and proteasome-specific proteolytic activities in both cell lines revealed different biological effects; whereas both melanoma cell lines exhibited similar proteasome activity, calpain activity was significantly up-regulated in MeWo<sub>cis1</sub> cells (Fig. 5C), suggesting a shift toward an increased use of the calpain-dependent proteolytic pathway in MeWo<sub>cis1</sub> Cells.

**Inhibition of calpain prevents NF-κB activation in cisplatin-resistant human melanoma cells.** To address the question whether stabilization of IκBα by calpain inhibition also accompanies impeded NF-κB nuclear translocation in MeWo<sub>cis1</sub> cells, EMSA assays were done. In agreement with the data shown above, Figure 4.

![Figure 4](https://cancerres.aacrjournals.org)
A

MeWo

TNF

lxBα

β-Tubulin

1 2 3 4 5

MeWo cis1

lxBα

β-Tubulin

1 2 3 4 5

B

MeWo cis1

PD150606 (µM)

TNF

lxBα

β-Tubulin

1 2 3 4 5

C

Proteasome activity

Fluorescence (LLV:AMC)

MeWo

MeWo cis1

p > 0.05

Fluorescence (L:AMC)

MeWo

MeWo cis1

p < 0.005

Discussion

Cisplatin resistance, or more generally chemotherapy resistance, is a major obstacle in the treatment of metastatic melanoma and other solid tumors. However, in most cases, severe toxicity precludes the administration of higher doses of chemotherapeutic agents. One of the practical ways to solve this problem in the clinic is to combine traditional chemotherapeutics with their synergistic modulators, thus improving therapeutic efficacy without increasing toxicity. Moreover, analysis of mechanisms leading to chemotherapy resistance might result in the identification of targets that will safely and effectively enhance therapeutic success. In this context, inhibition of the proteasome represents a new target for cancer therapy (30).

In this study, we first analyzed the effects of the new proteasome inhibitor BSc2118 on both cisplatin-resistant and cisplatin-sensitive melanoma cells. We found that BSc2118 induced reduction in cell viability in both MeWo and MeWo cis1 cells. Calpain inhibitor treatment at 25 µmol/L by itself affected cell viability in both cell lines only marginally (Fig. 6C). As shown before, BSc2118 alone reduced cell viability by ~60%. However, although the combination of both inhibitors resulted only in a slight additional reduction of MeWo cell viability, combined inhibitor treatment of MeWo cis1 cells had a significantly stronger effect and reduced cell viability to ~10% (Fig. 6C). It is of note that addition of cisplatin to proteasome/calpain inhibitor–treated MeWo cis1 cells did not affect cell viability stronger than both inhibitors alone (Supplementary Fig. S2).

Thus, our data show that the combined treatment of MeWo cis1 cells with BSc2118 and PD150606 affects two different proteolytic pathways and can enhance the proteasome inhibitor–mediated death of the tumor cells, especially of the cisplatin-resistant cells.

Inhibition of calpain activity in MeWo cis1 cells completely abolished TNF-induced NF-κB activation (Fig. 6A, right). In contrast, no significant effect was observed on MeWo cells whose NF-κB activation system is BSc2118 sensitive (Fig. 6A, left).

To study a possible effect of calpain inhibition with PD150606 and the concomitant inhibition of NF-κB activation in MeWo cis1 cells, we examined the induction of apoptosis. Annexin V staining revealed a correlation with increasing calpain inhibitor concentrations and an increase in the relative amount of apoptotic MeWo cis1 cells. In contrast, the relative amount of Annexin V–positive MeWo cells remained almost unchanged (Fig. 6B, a-c, bottom right quadrant). In fact, considerably more MeWo cells underwent necrosis as evidenced by double-positive staining for propidium iodide and Annexin V. However, the relative number of MeWo cis1 necrotic cells remained constant when the cells were treated with the calpain inhibitor (Fig. 6B, a-c, top right quadrant). Interestingly, the inhibitor of TNF-induced proteasome-dependent lxBα degradation, BAY 11-7082, induced more cell death in MeWo cells than in the MeWo cis1 cells (Fig. 6B, d, top and bottom right quadrants).

These experiments show that in a concentration-dependent manner, calpain inhibition can affect cell viability of cisplatin-resistant cells. However, given the lack of a precise molecular mechanism, the observed effect on apoptosis of chemoresistant cells may only be a correlative one.

Additive effects of proteasome and calpain inhibition on cell viability. If the increased resistance of MeWo cis1 cells to proteasome inhibitor is caused by a shift to calpain-mediated lxBα degradation, treatment of melanoma cells with calpain inhibitor should also affect the viability of the cells. To address this question, we analyzed the effects of BSc2118 and/or calpain inhibitor PD150606 on the viability of MeWo and MeWo cis1 cells. Calpain inhibitor treatment at 25 µmol/L by itself affected cell viability in both cell lines only marginally (Fig. 6C). As shown before, BSc2118 alone reduced cell viability by ~60%. However, although the combination of both inhibitors resulted only in a slight additional reduction of MeWo cell viability, combined inhibitor treatment of MeWo cis1 cells had a significantly stronger effect and reduced cell viability to ~10% (Fig. 6C). It is of note that addition of cisplatin to proteasome/calpain inhibitor–treated MeWo cis1 cells did not affect cell viability stronger than both inhibitors alone (Supplementary Fig. S2).

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In WEHI-231 B cells, it has been shown that prolonged NF-κB activation is associated with continued degradation of IκBα and that the NF-κB pathway may participate in proteasome inhibitor resistance (24). On the other hand, Hideshima et al. (31) showed that NF-κB blockade by proteasome inhibition cannot account for all of the antitumor activity observed in multiple myeloma. Interestingly, our analysis of the NF-κB pathway in MeWo cis1 melanoma cells revealed a proteasome-independent but calpain inhibitor–mediated IκBα stabilization and inhibition of NF-κB activation. In neuronal cells, it was shown previously that binding of glutamate to its receptor induces a shift from a proteasome-dependent to a calpain-dependent IκBα degradation followed by NF-κB activation and that calpain-dependent IκBα degradation plays a role in inflammation, as well as in neuronal cell survival and cell death (32). Interestingly, in MeWo cells, both the proteasome and the calpain-sensitive IκBα degradation seem to coexist, whereas in MeWo cis1 cells the calpain-sensitive IκBα degradation predominates. Furthermore, calpain activity in lysates of MeWo cis1 cells was strongly increased, suggesting for the first time that calpain may play a role in chemotherapy resistance in melanoma cells. Thus far, it has been acknowledged that calpain activity can lead tumor cells to apoptosis (33) and is involved in genistein-induced or cisplatin-mediated apoptosis (5, 34). In HCT 116 human colon carcinoma cells, it was shown that cisplatin induced...
increased cytosolic calcium level, calpain activation, as well as endoplasmic reticulum stress (5). Interestingly, the caspase-resistant MeWoΔcalpain cells studied here exhibit increased calpain activity even in the absence of caspilin and already the inhibition of calpain activity is able to induce apoptosis. This latter result may be explained in part by the recently described apoptotic defects in MeWoΔcalpain cells (11) and by a shift in the NF-κB signaling pathway toward an ubiquitin-proteasome system independent IκBα degradation. Calpain- and proteasome-dependent NF-κB activation following TNF treatment has also been observed in human HepG2 cells (8). When ubiquitinating enzymes were inactivated, IκBα proteolysis occurred only in a strictly calpain-dependent manner (8). However, it is not currently defined which steps in the signaling cascade are affected in the MeWoΔcalpain cells that result in an almost complete shift toward TNF-induced, calpain-sensitive IκBα degradation. In this context, it is important to note that it has recently been shown that bortezomib and caspilin induce apoptosis via endoplasmic reticulum stress in pancreatic cancer cells. Therefore, selecting caspilin-resistant cells could have resulted in selection of endoplasmic reticulum stress–resistant cells and thus may in part account for the observed differences in growth arrest and apoptosis induction between MeWo and MeWoΔcalpain cell lines (35).

Our experiments also show that the combined inhibition of both the proteosome and calpain affects the viability of MeWoΔcalpain cells considerably more than when each agent is applied alone, resulting in an almost complete cell death of MeWoΔcalpain cells. It is interesting to note that inhibition of calpain with nontoxic concentrations of PD150606 alone had no significant effect on cell viability of either MeWo or MeWoΔcalpain cells and that only the application of PD150606 together with Bc2118 significantly increased the antitumor activity of proteosome inhibition on caspilin-resistant cells. Thus, by combining proteosome and calpain inhibitors, our data may display new therapeutic strategies for the treatment of chemotherapy-resistant melanoma cells.

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References


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