

Cathepsin B Mediates Caspase-Independent Cell Death Induced by Microtubule Stabilizing Agents in Non-Small Cell Lung Cancer Cells

Linda E. Bröker, Cynthia Huisman, Simone W. Span, José A. Rodriguez, Frank A. E. Kruyt, and Giuseppe Giaccone

Department of Medical Oncology, VU University Medical Center, Amsterdam, the Netherlands

Abstract

We have previously reported that the microtubule stabilizing agents (MSAs) paclitaxel, epothilone B and discodermolide induce caspase-independent cell death in non-small cell lung cancer (NSCLC) cells. Here we present two lines of evidence indicating a central role for the lysosomal protease cathepsin B in mediating cell death. First, inhibition of cathepsin B, and not of caspases or other proteases, such as cathepsin D or calpains, results in a strong protection against drug-induced cell death in several NSCLC cells. Second, MSAs trigger disruption of lysosomes and release and activation of cathepsin B. Interestingly, inhibition of cathepsin B prevents the appearance of multinucleated cells, an early characteristic of MSA-induced cell death, pointing to a central, proximal role for cathepsin B in this novel cell death pathway.

Introduction

Successful treatment of cancer with chemotherapy is largely dependent on its ability to trigger cell death in tumor cells. Activation of apoptosis has long been held responsible for the cell killing potential of anticancer drugs, but recent evidence suggests that there are forms of chemotherapy-induced cell death that cannot readily be classified as apoptosis or necrosis (1). Therefore, the contribution of the apoptotic cascade to the cytotoxic effects of chemotherapeutic agents and cytokines is currently being debated, and alternative forms of programmed cell death (PCD), such as “apoptosis-like PCD” and “necrosis-like PCD” have been described (2–4). Although caspases are well established as the main players in apoptosis, other proteases such as calpains, cathepsins, and serine proteases may account for alternative types of PCD (3). Cathepsins, in particular cathepsin B and D, act as the main executors of caspase-independent cell death induced by tumor necrosis factor α (TNF- α) in several systems (5, 6).

We have recently found that paclitaxel and two promising novel microtubule stabilizing drugs, epothilone B and discodermolide, exert their cytotoxic activity mainly via caspase-independent routes in non-small cell lung cancer (NSCLC) cell lines. This phenomenon was strongest for discodermolide and epothilone B, whereas paclitaxel-induced cell death was partially dependent on caspases and involved early triggering of the death receptor pathway, followed by activation of the mitochondria (7, 8). In the present study, we have investigated the potential role of other proteases in cell death induced by microtubule stabilizing agents (MSAs). Using a panel of specific protease inhibitors, we found that inhibition of the lysosomal protease cathepsin B, but not of caspases or other proteases such as calpains or cathepsin D, strongly protected against cell death, and prevented the development of multinucleated cells, an early feature of MSA-induced

cell death. We further show that paclitaxel, epothilone B, and discodermolide trigger disruption of the lysosomes, followed by release and activation of cathepsin B, providing evidence for a novel, cathepsin B-dependent cell death pathway induced by MSAs in NSCLC cells.

Materials and Methods

Cell Culture and Reagents. The human NSCLC cell lines NCI-H460 (H460), SW1573, and A549 were cultured in RPMI or DMEM (BioWhittaker, Verviers, Belgium), supplemented with 10% (v/v) FCS (Life Technologies, Inc., Breda, the Netherlands), 100 units/ml penicillin, and 100 μ g/ml streptomycin (Life Technologies, Inc., Grand Island, NY). Epothilone B and discodermolide were kindly provided by Dr. M. Wartmann (Novartis Pharma AG, Basel, Switzerland) and dissolved in DMSO. Paclitaxel (Bristol-Myers Squibb, Woerden, the Netherlands) was diluted in ethanol. For each experiment, we freshly diluted stock solutions of the drugs in culture medium to their final IC_{80} concentration (7, 8): for paclitaxel, 50 nM (H460), 15 nM (SW1573 and A549); for epothilone B, 2.0 nM (H460 and A549), 2.8 nM (SW1573); for discodermolide, 12 nM (H460), 19 nM (SW1573), and 26 nM (A549). zVAD-fmk (Enzyme Systems Products, Livermore, CA), calpeptin (Calbiochem, Darmstadt, Germany), CA-074 Me (Peptides International, Osaka, Japan), zFA-fmk (Enzyme Systems Products), and pepstatin A (Sigma Chemicals, St. Louis, MO) were dissolved in DMSO and added to the cells 1 h before the addition of the drugs at final concentrations of 50 μ M (zVAD-fmk), 100 μ M (calpeptin, CA-074 Me, pepstatin A), and 200 μ M (zFA-fmk).

Flow Cytometric Analysis. The extent of apoptosis was determined by flow cytometry, using propidium iodide (Sigma) staining of hypodiploid DNA-content, as previously described (9). We performed lysosomal integrity assays with the lysosomotropic probe Lysotracker Red (Molecular Probes, Eugene, OR), according to the literature (10).

Plasmid Construction and Transfection. We created an expression plasmid encoding yellow fluorescent protein-tagged cathepsin B by cloning full-length human cathepsin B cDNA (generated by reverse transcription-PCR using primers 5'-ATC TGT TCT CGA GCT ATG TGG CAG CTC TGG GCC TCC-3' and 5'-ATG GTA GGA TCC TTA GAT CTT TTC CCA GTA CTG-3') into the pEYFPC1 vector (Clontech Laboratories, Inc., Palo Alto, CA). Cells were seeded onto glass coverslips in a 6-well tray and were transfected with 1.5 μ g of plasmid DNA using the FuGene6 transfection reagent (Roche Molecular Biochemicals, Mannheim, Germany), according to the manufacturer's protocol.

Fluorescence Microscopy. We performed fluorescence stainings as described previously (7), using Hoechst 33342 (Sigma) or Lysotracker Red (Molecular Probes), according to the manufacturer's protocol. Slides were mounted in Vectashield (Vector Laboratories, Burlingame, CA) and were analyzed with a Leica DM IRBE fluorescence microscope.

Protein extraction and Western Blotting. Preparation of total cell extracts as well as Western blot analysis was performed as described previously (9). For immunodetection, anti-cathepsin B monoclonal antibody (Oncogene Research Products, Boston, MA; 1:400 dilution) and horseradish peroxidase-conjugated secondary antibody (Amersham, Braunschweig, Germany; 1:2000 dilution) were used. Enhanced chemiluminescence (Amersham) was used for detection.

Statistics. We analyzed quantitative experiments by the Student's *t* test. All *P*s resulted from the use of two-sided tests and were considered significant when <0.05 .

Received 9/29/03; revised 10/30/03; accepted 11/6/03.

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.

Notes: F. A. E. Kruyt and G. Giaccone share senior authorship.

Requests for reprints: Frank A. E. Kruyt, VU University Medical Center, Department of Medical Oncology, De Boelelaan 1117, 1081 HV Amsterdam, the Netherlands. Phone: 31-20-444-3374; Fax: 31-20-444-4355; E-mail: kruyt@vumc.nl.

Results

Identification of Cathepsin B As a Mediator of Caspase-Independent Cell Death Triggered by MSAs. To elucidate the caspase-independent pathways mediating cell death induced by MSAs, we evaluated the contribution of different cellular proteases, calpains, and cathepsin B and D, to the cytotoxic potential of paclitaxel, epothilone B and discodermolide. For this purpose, we screened several protease inhibitors for their ability to interfere with MSA-induced cell death in H460 cells. As shown in Table 1, the selective calpain inhibitor calpeptin did not reduce the cytotoxic effects of *IC*₈₀ concentrations of either drug, as determined by propidium iodide-staining based fluorescence-activated cell sorter-analysis of hypodiploid cells, a method that we previously reported to correlate well with induction of cell death (7, 8). The lack of effect of calpain-inhibition was independent of the duration of drug exposure (24–72 h) or concentration of the inhibitor (20–200 μM; data not shown). Interestingly, inhibition of cathepsin B by the selective chemical inhibitors CA-074 Me and zFA-fmk strongly protected H460 cells from induction of a hypodiploid cell population by paclitaxel, epothilone B, and discodermolide, thereby reducing their cell killing potential by 42 up to 70% (*P*s < 0.05). In a dose-response experiment, low concentrations of the inhibitors that also protect against TNF-α-induced cell death (25 μM of CA-074 Me and 100 μM of zFA-fmk (5)) already reduced the cytotoxic effects of MSAs significantly (data not shown), whereas optimal results were obtained when higher concentrations were used (100 and 200 μM respectively; Table 1). Additional studies indicated that cathepsin B inhibition provided protection from 16 up to 96 h posttreatment (data not shown). Protection was not observed when cells were pretreated with pepstatin A, a selective inhibitor of cathepsin D. As a control in these experiments, we used the pancaspase inhibitor zVAD-fmk. In line with our previous reports (7, 8), zVAD-fmk did not protect significantly against the cytotoxic effects of epothilone B or discodermolide, and it only reduced paclitaxel-induced cell death from 32.6 to 26.5%. We next analyzed the effect of cathepsin B-inhibition in other NSCLC cell lines, A549 and SW1573. As shown in Fig. 1, the cathepsin B inhibitors protected against paclitaxel-induced cell death in all of the investigated cell lines (*P*s < 0.05). This effect was strongest in SW1573 cells, in which CA-074 Me reduced paclitaxel-induced cell death from 33 to 2.5%. For epothilone B and discodermolide, the protection rates ranged from 45% in A549 cells up to 83% in SW1573 cells and from 42% in H460 cells up to 89% in SW1573 cells (*P*s < 0.05; data not shown). Taken together, these results provide evidence for a central role of cathepsin B as a mediator of caspase-independent cell death induced by MSAs in NSCLC cell lines.

Cathepsin B Is Released from the Lysosomes and Activated on Treatment with Paclitaxel, Epothilone B, and Discodermolide. The lysosomal protease cathepsin B can be released from its cellular compartment on triggering with apoptotic stimuli such as TNF-α, result-

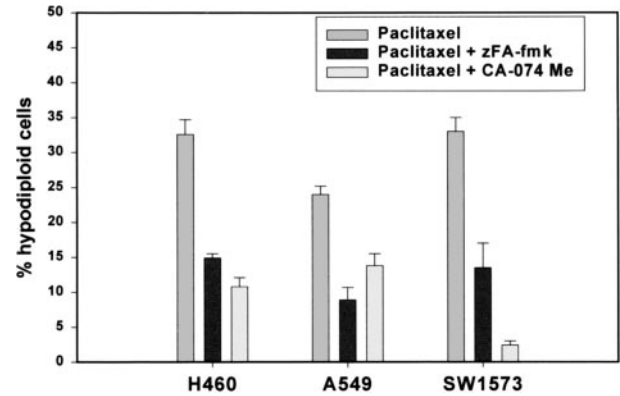


Fig. 1. Inhibition of cathepsin B protects against cell death in a panel of non-small cell lung cancer (NSCLC) cells. H460, A549, and SW1573 were treated for 48 h with paclitaxel, with or without pretreatment with the cathepsin B inhibitors CA-074 Me or zFA-fmk and were stained with propidium iodide and analyzed by flow cytometry. Mean and SD of at least three independent experiments are shown.

ing in tumor cell apoptosis (5). To assess whether treatment with MSAs results in a similar lysosomal membrane permeabilization, we performed lysosomal integrity assays using the lysosomotropic fluorescence probe LysoTracker Red. In H460 cells treated with paclitaxel, increased numbers of cells with a weak fluorescence (so-called “pale cells”) were detected (Fig. 2A), a phenomenon reported to be due to leakage of the probe into the cytoplasm, indicating lysosomal rupture (10). This effect was also observed in SW1573 cells (data not shown), or when cells were treated with epothilone B or discodermolide, and was already present from 8 h after treatment (Fig. 2B). Next, we studied whether the disruption of the lysosomal integrity would facilitate the release of cathepsin B. We, therefore, engineered a yellow fluorescent protein-tagged cathepsin B fusion protein into the expression vector pEYFP1, to allow easy detection by fluorescence microscopy. Colocalization studies with LysoTracker Red confirmed that cathepsin B-yellow fluorescent protein was present mainly in the lysosomes (data not shown). As shown in Fig. 2C, cathepsin B was released from the lysosomes of SW1573 cells on incubation with either drug, as indicated by the diffuse staining pattern of the fusion protein throughout the cells after treatment. As expected based on the integrity studies, this release was evident after 16 h of incubation, and was also observed in H460 cells (data not shown). Inhibition of cathepsin B only slightly prevented disruption of the lysosomes (data not shown), suggesting that cathepsin B can stimulate its own release, as reported previously (11), and confirming that cathepsin B release occurs downstream of the disturbance of the lysosomal membrane integrity. To examine the activation of cathepsin B after its release into the cytoplasm, we performed Western Blot analysis on cellular extracts derived from SW1573 cells, treated for 16 up to 72 h with paclitaxel or epothilone B. Cathepsin B is synthesized as an inactive pro-enzyme (*M*_r 43,000) which is processed into active 25,000 or 31,000 forms (12). As shown in Fig. 2D, a clear increase in the cellular amount of the cleaved, active *M*_r 25,000 product of cathepsin B was observed from 48 h after treatment with either agent, whereas levels of the inactive *M*_r 43,000 pro-enzyme and the active *M*_r 31,000 product remained unchanged. Incubation of H460 cells with paclitaxel or epothilone B produced similar results (data not shown). Taken together, these results indicate that lysosomal disruption and release and subsequent activation of cathepsin B accompany the induction of cell death in NSCLC cells by MSAs.

Inhibition of Cathepsin B Potently Protects Against Drug-Induced Nuclear Changes. Next, we studied the effect of cathepsin B inhibition on nuclear changes elicited by MSAs. Paclitaxel, epothilone B, and discodermolide trigger mitotic anomalies, resulting in abnormal chromosome segregation and the appearance of so-called “multinucleated cells” with multiple nuclei of various sizes. This early event is then

Table 1 Protective effect of various protease inhibitors on cell death induced by paclitaxel, epothilone B, and discodermolide in H460 cells

Percentages of cells with a hypodiploid DNA-content are shown, as determined by propidium iodide-staining and flow cytometric analysis after 48-h exposure to the drugs. Results represent the mean ± SD of three independent experiments.

Inhibitor (target protease)	Percentage of cells with a hypodiploid DNA-content		
	Paclitaxel	Discodermolide	Epothilone B
None (control)	32.6 ± 2.1%	32.5 ± 1.7%	33.2 ± 2.7%
zVAD-fmk (caspases)	26.5 ± 1.7% ^a	30.8 ± 1.2%	31.2 ± 1.7%
Calpeptin (calpains)	35.8 ± 3.6%	32.0 ± 3.7%	31.9 ± 3.8%
CA-074 Me (cathepsin B)	10.8 ± 1.3% ^a	14.2 ± 2.0% ^a	14.9 ± 2.3% ^a
zFA-fmk (cathepsin B)	14.9 ± 0.6% ^a	18.9 ± 1.9% ^a	14.5 ± 2.6% ^a
Pepstatin A (cathepsin D)	32.0 ± 3.9%	30.5 ± 1.0%	34.1 ± 3.6%

^a *P* < 0.05.

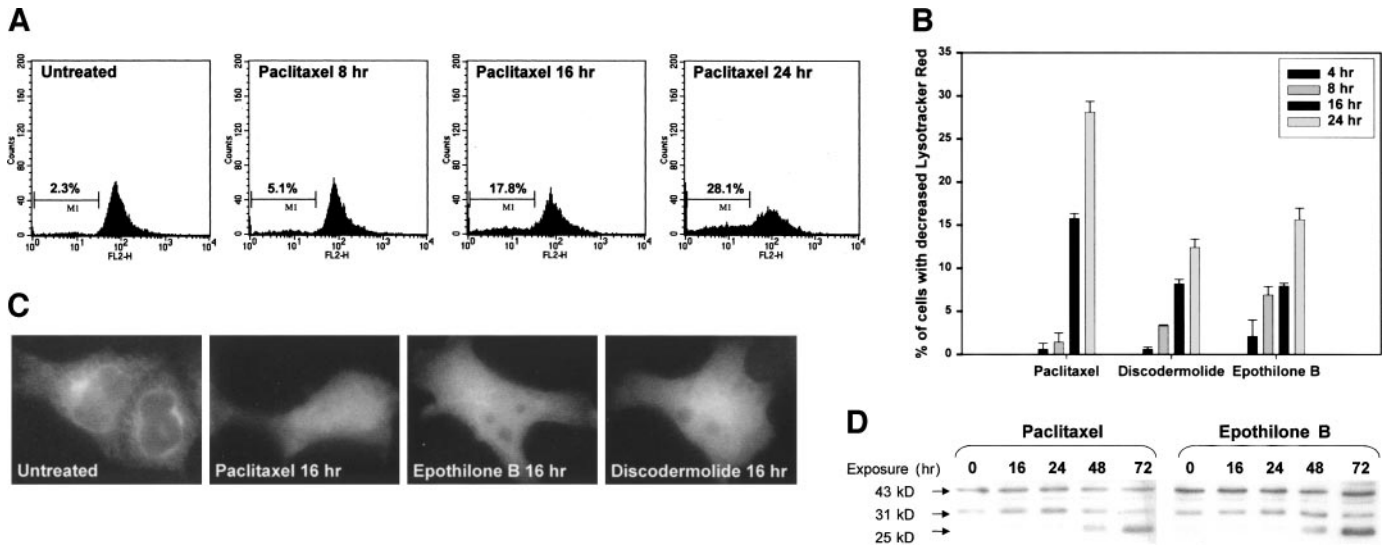


Fig. 2. Cathepsin B is released from the lysosomes and is activated on treatment with microtubule stabilizing agents (MSAs). **A**, H460 cells were treated for 8–24 h with paclitaxel, stained with LysoTracker Red, and analyzed by flow cytometry. The marker indicates pale cells with disrupted lysosomes. Representative figures of three independent experiments are shown. **B**, time course experiment of H460 cells treated for 4–24 h with paclitaxel, etoposide B, or discodermolide, as described under **A**. The results represent the mean and SD of at least three independent experiments. **C**, Cathepsin B-yellow fluorescent protein diffuses throughout H460 cells on 16 h treatment with paclitaxel, etoposide B, or discodermolide. **D**, Western blot analysis of SW1573 cells, showing activation of cathepsin B on treatment with paclitaxel or etoposide B, as indicated by the cleaved, active M_r 25,000 band. *kD*, M_r in thousands.

followed by the onset of apoptotic features and cell death (7, 13). As illustrated in Fig. 3A, the cathepsin B inhibitor zFA-fmk prevented the development of multinucleated cells on 48-h treatment with paclitaxel in the H460 cell line. Similar results were obtained with etoposide B and discodermolide (data not shown) and in SW1573 cells (Fig. 3B). In this cell line, the vast majority of untreated cells displayed normal nuclear morphology with only 1% of cells showing characteristics of apoptotic cells with chromatin condensation, nuclear fragmentation, and apoptotic bodies. On treatment with paclitaxel for 48 h, this pattern changed dramatically: 76% of cells became multinucleated, and 9.5% of cells showed apoptotic morphology. The cathepsin B inhibitors CA-074 Me and zFA-fmk almost completely prevented the induction of multinucleated cells and reduced their incidence to 3 and 7%, respectively. As reported before (8), the pancaspase-inhibitor zVAD-fmk did not affect the induction of multinucleated cells by paclitaxel but only reduced the percentage of cells with apoptotic morphology from 9.5 to 3%. There were no significant differences between the three drugs in either H460 or SW1573 cells (data not shown). This protective effect of cathepsin B-inhibition on drug-induced nuclear changes was reflected in the cell cycle profile. As shown in Fig. 3C, CA-074 Me largely restored the cell cycle profile of SW1573 cells treated with etoposide B and reduced the amount of cells with a hypodiploid DNA-content from 30.3 to 7.7%. Similar results were found in H460 and A549 cells, and for paclitaxel and discodermolide (data not shown). These findings confirm the pivotal role of cathepsin B in cell death induced by MSAs and suggest that activation of cathepsin B represents an early step in this process.

Discussion

We have previously reported that paclitaxel, etoposide B, and discodermolide trigger cell death primarily via caspase-independent routes in NSCLC cells (7, 8). In this study, we demonstrate that MSAs can trigger disruption of lysosomes leading to release and subsequent activation of the lysosomal protease cathepsin B, representing an early stage of the cell death process in this type of cell. Inhibition of cathepsin B, and not of caspases or other proteases such as calpains or cathepsin D, strongly protected against cell death, indicating that cathepsin B is essential for the caspase-independent cell death triggered by paclitaxel, etoposide B, and

discodermolide. Although our observation of cathepsin-B dependency in MSA-induced cell death is novel, involvement of lysosomal proteases in cell death is not unprecedented. Cathepsin D mediates PCD induced by IFN- γ , Fas, and TNF- α in HeLa cells (14), and this protease can activate cathepsin B in hepatocytes on treatment with camptothecin and thereby lead to cell death (6). The pivotal role of cathepsin B in hepatocyte apoptosis has further been demonstrated in cathepsin B knockout mice, which were resistant to TNF- α -mediated apoptosis and liver injury (15).

Strong evidence is now accumulating for the involvement of alternative proteases, such as cathepsin B, in PCD (16), but the molecular identity of the mediators and the necessity of activation of the apoptotic pathways remains to be elucidated in most cases and may vary on the type of cells and the applied death stimulus (17). For example, cathepsin B has been reported to contribute to apoptosis via induction of mitochondrial membrane permeabilization, possibly via cleavage of Bid, in some systems, thereby acting upstream of the caspase cascade (18–21). Others, however, have found that cathepsin B can act as an effector protease, downstream of caspases (5, 22). Additionally, cathepsin B is capable of executing cell death completely independent of the apoptotic machinery in WEHI-S fibrosarcoma cells (5). In our system, the inhibition of caspases did not affect the cytotoxicity of etoposide B or discodermolide, and it only slightly reduced cell death by paclitaxel, indicating that cathepsin B is the main mediator of MSA-induced cell death in NSCLC cells, without requirement of the apoptotic cascade. Loss of mitochondrial membrane potential may, however, contribute to this process as a secondary event, because we have previously reported that these drugs trigger release of cytochrome *c* in a late stage of the cell death process (7, 8). In this respect, our data indicate that the three drugs may not act fully identically; whereas paclitaxel may partially exploit caspases as effector proteases, etoposide B and discodermolide induce cell death completely independently from the apoptotic machinery in NSCLC cells. Caspase-independent cell death is, however, not an intrinsic characteristic of this type of cell, because the apoptotic cascade is functional and accounts for the cytotoxic activity of DNA-damaging agents (9). Our study, therefore, provides further evidence for the view that the cellular death response depends on the type and dose of chemotherapeutic stress within the cellular context.

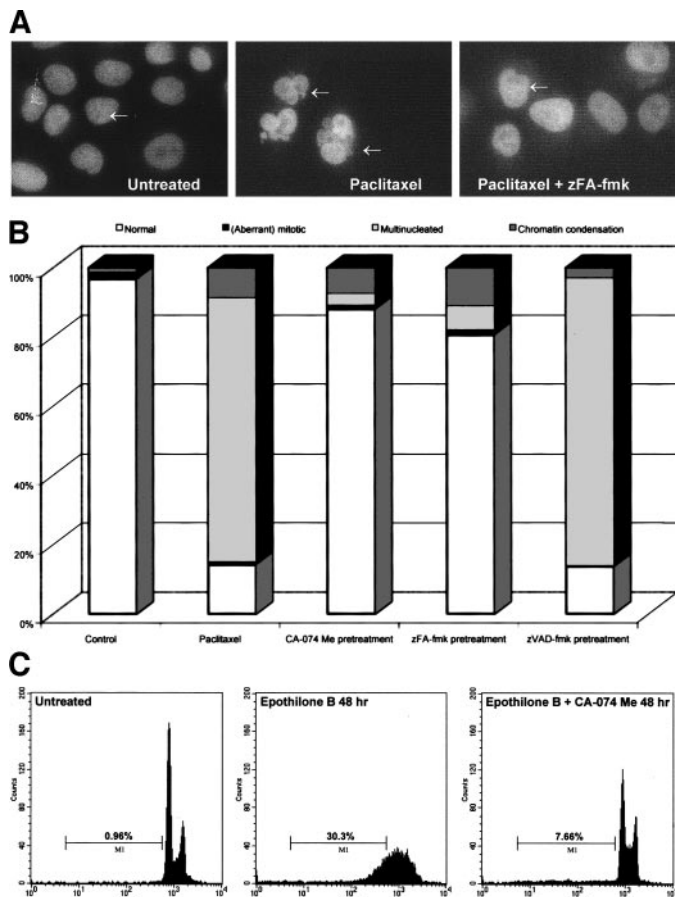


Fig. 3. Inhibition of cathepsin B prevents drug-induced nuclear changes. **A**, H460 cells were treated for 48 h with paclitaxel or epothilone B, with or without pretreatment with the cathepsin B inhibitor zFA-fmk and were stained with Hoechst 33342 and analyzed by fluorescence microscopy. *Arrowheads*, normal (left and right panel) and multinucleated (middle panel) cells. Representative figures of multiple experiments are shown. **B**, morphological changes [as depicted under **A**, and described previously (7)] in SW1573 cells treated for 48 h with paclitaxel, with or without the cathepsin B inhibitors CA-074 Me and zFA-fmk, or the pancaspase inhibitor zVAD-fmk. Results represent the mean of three independent experiments, in each of which 300 cells were counted. All SDs were <10%. **C**, SW1573 cells were treated for 48 h with epothilone B, with or without the cathepsin B inhibitor CA-074 Me and were stained with propidium iodide and analyzed by flow cytometry. Representative figures of three independent experiments are shown.

The nearly complete prevention of drug-induced nuclear changes suggests that activation of cathepsin B represents an early step in the cell death process. The mechanism underlying MSA-induced disruption of the lysosomal membrane, which preceded release and activation of cathepsin B in our studies, remains, however, speculative; and other, yet-unidentified factors are likely to contribute to this process. For example, p53 has been reported to trigger lysosomal destabilization via yet unknown pathways (10), but also the generation of reactive oxygen species may account for this phenomenon (19). Additionally, TNF- α has been postulated to trigger lysosomal permeabilization via formation of the lysosomotropic detergent sphingosine from ceramide (11). As paclitaxel induces ceramide formation (23), similar processes may be involved in the release and activation of cathepsin B triggered by this class of agents. Alternatively, interaction between the drugs and microtubules may trigger a yet-uncharacterized signal transduction cascade leading to lysosomal permeabilization. In this respect, it would be interesting to see whether similar characteristics of cell death occur after treatment with cytotoxic drugs that act via destabilizing microtubules, such as the *Vinca* alkaloids. Currently, additional studies are in progress to unravel the molecular pathways underlying the exciting process of caspase-independent cell death

induced by microtubule-interacting agents and to evaluate a potential role of cathepsin B as a determinant of sensitivity to MSA-based chemotherapy.

Acknowledgments

We are grateful to Dr. M. Wartmann (Novartis Pharma AG, Basel, Switzerland), who kindly provided discodermolide and epothilone B.

References

- Blagosklonny, M. V. Cell death beyond apoptosis. *Leukemia* (Baltimore), *14*: 1502–1508, 2000.
- Brown, J. M., and Wouters, B. G. Apoptosis: mediator or mode of cell killing by anticancer agents? *Drug Resist. Upd.*, *4*: 135–136, 2001.
- Leist, M., and Jäättelä, M. Four deaths and a funeral: from caspases to alternative mechanisms. *Nat. Rev. Mol. Cell. Biol.*, *2*: 589–598, 2001.
- Schmitt, C. A., and Lowe, S. W. Apoptosis is critical for drug response in vivo. *Drug Resist. Upd.*, *4*: 132–134, 2001.
- Foghsgaard, L., Wissing, D., Mauch, D., Lademann, U., Bastholm, L., Boes, M., Elling, F., Leist, M., and Jäättelä, M. Cathepsin B acts as a dominant execution protease in tumor cell apoptosis induced by tumor necrosis factor. *J. Cell Biol.*, *153*: 999–1010, 2001.
- Roberts, L. R., Adjei, P. N., and Gores, G. J. Cathepsins as effector proteases in hepatocyte apoptosis. *Cell Biochem. Biophys.*, *30*: 71–88, 1999.
- Bröker, L. E., Huisman, C., Ferreira, C. G., Rodriguez, J. A., Kruyt, F. A. E., and Giaccone, G. Late activation of apoptotic pathways plays a negligible role in mediating the cytotoxic effects of discodermolide and epothilone B in non-small cell lung cancer cells. *Cancer Res.*, *62*: 4081–4088, 2002.
- Huisman, C., Ferreira, C. G., Bröker, L. E., Rodriguez, J. A., Smit, E. F., Postmus, P. E., Kruyt, F. A. E., and Giaccone, G. Paclitaxel triggers cell death primarily via caspase-independent routes in the non-small cell lung cancer cell line NCI-H460. *Clin. Cancer Res.*, *8*: 596–606, 2002.
- Ferreira, C. G., Span, S. W., Peters, G. J., Kruyt, F. A. E., and Giaccone, G. Chemotherapy triggers apoptosis in a caspase-8-dependent and mitochondria-controlled manner in non-small cell lung cancer cells. *Cancer Res.*, *60*: 7133–7141, 2000.
- Yuan, X. M., Li, W., Dalen, H., Lotem, J., Kama, R., Sachs, L., and Brunk, U. T. Lysosomal destabilization in p53-induced apoptosis. *Proc. Natl. Acad. Sci. USA*, *99*: 6286–6291, 2002.
- Werneburg, N. W., Guicciardi, M. E., Bronk, S. F., and Gores, G. J. Tumor necrosis factor- α -associated lysosomal permeabilization is cathepsin B dependent. *Am. J. Physiol. Gastrointest. Liver Physiol.*, *283*: G947–G956, 2002.
- Chan, S. J., San Segundo, B., McCormick, M. B., and Steiner, D. F. Nucleotide and predicted amino acid sequences of cloned human and mouse preprocathepsin B cDNAs. *Proc. Natl. Acad. Sci. USA*, *83*: 7721–7725, 1986.
- Chen, J. G., and Horwitz, S. B. Differential mitotic responses to microtubule-stabilizing and -destabilizing drugs. *Cancer Res.*, *62*: 1935–1938, 2002.
- Deiss, L. P., Galinka, H., Berissi, H., Cohen, O., and Kimchi, A. Cathepsin D protease mediates programmed cell death induced by interferon- γ , Fas/APO-1 and TNF- α . *EMBO J.*, *15*: 3861–3870, 1996.
- Guicciardi, M. E., Miyoshi, H., Bronk, S. F., and Gores, G. J. Cathepsin B knockout mice are resistant to tumor necrosis factor- α -mediated hepatocyte apoptosis and liver injury: implications for therapeutic applications. *Am. J. Pathol.*, *159*: 2045–2054, 2001.
- Wyllie, A. H., and Golstein, P. More than one way to go. *Proc. Natl. Acad. Sci. USA*, *98*: 11–13, 2001.
- Herr, I., and Debatin, K. M. Cellular stress response and apoptosis in cancer therapy. *Blood*, *98*: 2603–2614, 2001.
- Boya, P., Gonzalez-Polo, R. A., Poncet, D., Andreau, K., Vieira, H. L., Roumier, T., Perfettini, J. L., and Kroemer, G. Mitochondrial membrane permeabilization is a critical step of lysosome-initiated apoptosis induced by hydroxychloroquine. *Oncogene*, *22*: 3927–3936, 2003.
- Boya, P., Andreau, K., Poncet, D., Zamzami, N., Perfettini, J. L., Metivier, D., Ojcius, D. M., Jäättelä, M., and Kroemer, G. Lysosomal membrane permeabilization induces cell death in a mitochondrion-dependent fashion. *J. Exp. Med.*, *197*: 1323–1334, 2003.
- Guicciardi, M. E., Deussing, J., Miyoshi, H., Bronk, S. F., Svigen, P. A., Peters, C., Kaufmann, S. H., and Gores, G. J. Cathepsin B contributes to TNF- α -mediated hepatocyte apoptosis by promoting mitochondrial release of cytochrome c. *J. Clin. Invest.*, *106*: 1127–1137, 2000.
- Stoka, V., Turk, B., Schendel, S. L., Kim, T. H., Cirman, T., Snipas, S. J., Ellerby, L. M., Bredesen, D., Freeze, H., Abrahamson, M., Bromme, D., Krajewski, S., Reed, J. C., Yin, X. M., Turk, V., and Salvesen, G. S. Lysosomal protease pathways to apoptosis. Cleavage of bid, not pro-caspases, is the most likely route. *J. Biol. Chem.*, *276*: 3149–3157, 2001.
- Jones, B., Roberts, P. J., Faubion, W. A., Kominami, E., and Gores, G. J. Cystatin A expression reduces bile salt-induced apoptosis in a rat hepatoma cell line. *Am. J. Physiol.*, *275*: G723–G730, 1998.
- Asakuma, J., Sumitomo, M., Asano, T., and Hayakawa, M. Selective Akt inactivation and tumor necrosis factor-related apoptosis-inducing ligand sensitization of renal cancer cells by low concentrations of paclitaxel. *Cancer Res.*, *63*: 1365–1370, 2003.

Cancer Research

The Journal of Cancer Research (1916–1930) | The American Journal of Cancer (1931–1940)

Cathepsin B Mediates Caspase-Independent Cell Death Induced by Microtubule Stabilizing Agents in Non-Small Cell Lung Cancer Cells

Linda E. Bröker, Cynthia Huisman, Simone W. Span, et al.

Cancer Res 2004;64:27-30.

Updated version Access the most recent version of this article at:
<http://cancerres.aacrjournals.org/content/64/1/27>

Cited articles This article cites 20 articles, 13 of which you can access for free at:
<http://cancerres.aacrjournals.org/content/64/1/27.full.html#ref-list-1>

Citing articles This article has been cited by 23 HighWire-hosted articles. Access the articles at:
</content/64/1/27.full.html#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.