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Research paper

Subcellular redistribution of BAX during apoptosis induced by anticancer drugs

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BAX is the 192-amino acid, 21-kDa protein which is ubiquitously distributed in normal tissues and is regarded as a tumor suppressor sensitizing malignant cells to anticancer drugs. In spite of many studies, the molecular mechanism of BAX action is still obscure. In the present study subcellular BAX translocations in human colon adenocarcinoma COLO 205 cells exposed to various anticancer drugs [camptothecin (CPT), etoposide (ETO), staurosporine (STP), 2-chloro-2′-deoxyadenosine (2CdA) and nimesulide (NIM)] was examined. Cells were grown on coverslips under optimal conditions (10% FCS/DMEM) or were stimulated to apoptosis with the drugs examined. Laser scanning cytometry was applied for the quantitative analysis of BAX expression, and distribution in the cytoplasmic (BAX Cf) and nuclear (BAX Nf) area. BAX maximal pixel (BAX MP), the parameter corresponding to aggregation of BAX in the cell, was also measured. All examined drugs increased the number of cells with high BAX MP, reaching the peak at 60 min after drug administration. The most pronounced effect was in the case of 2CdA, CPT and STP. The increase in BAX MP was observed only when antibody recognizing the 43–61 amino acid sequence was used. When antibody binding the N-terminal epitope (11–30 amino acid sequence) was applied, the number of cells expressing high BAX MP significantly decreased. These results indicate that apoptotic stimuli delivered by anticancer drugs led to aggregation of BAX in cancer cells, which is dependent on BAX activation by its cleavage at the N-terminal epitope and exposure of the BH3 domain. It was shown that BAX Nf increased in cells treated with CPT, STP, ETO, 2CdA and NIM, whereas BAX Cf rose after STP and NIM. The increase in BAX Nf and, occurring in most treatments, the increase in the BAX Nf:Cf ratio indicates a BAX shift from the cytoplasm to the nucleus. Furthermore, staining with different antibodies showed that only the activated form of BAX was translocated to the nucleus. Immunoelectron microscopy revealed that CPT-induced apoptosis was associated with translocation of BAX from the cytosol to organellar membranes (mitochondrial, Golgi apparatus and endoplasmic reticulum) and via nuclear envelope pores to the nucleus, occurring within 60–180 min of cell exposure to the drug. The subcellular translocations of BAX preceded in time the appearance of morphological symptoms of apoptosis. In conclusion, (i) in spite of different molecular mechanisms of apoptosis induction by the anticancer drugs examined, BAX remains a common link in the chain of reactions leading to cell death, and (ii) BAX activation and subcellular translocations from the cytosol to organellar membranes and nucleus are key cellular responses to drugs bearing proapoptotic properties. [© 2001 Lippincott Williams & Wilkins.]

Key words: Anticancer drugs, apoptosis, BAX, laser scanning cytometry, nimesulide.

Introduction

BCL-2-related proteins are important regulators of apoptosis acting at the effector phase of cell death.[1–3] Overexpression of BCL-2, the most potent physiological inhibitor of apoptosis, occurs in many types of hematological proliferative diseases and malignant solid tumors, and has been associated with their resistance to chemo- and radiotherapy.[4–7] It has been observed that tumor cells with elevated BCL-2 expression survive oxidative stress,[8] starvation[9] and cytostatic drugs treatment.[10]

In contrast to BCL-2, BAX (the first described death promoter among BCL-2 family proteins) is regarded as a tumor suppressor, sensitizing malignant cells to

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anticancer drugs.[11,12] BAX is the 192-amino acid, 21-kDa protein, containing BH1, BH2, BH3 and C-terminal hydrophobic domains. In spite of many studies, the molecular mechanism of BAX action has not been fully elucidated. The following three main mechanisms have been proposed: (i) dimerization and interference with anti-apoptotic members of the BCL-2 family, (ii) the formation of ion channels at the mitochondrial level, and (iii) interference with mitochondrial permeability transition pore complexes through adenine nucleotide translocator protein or voltage-dependent anion channels.[3,13,14]

At least two conditions must be fulfilled for the proapoptotic function of BAX and other BCL-2-related death agonists: (i) activation, due to the exposure of the BH3 motif, and (ii) translocation from the cytosol to organelles.[15] Under physiological conditions BAX is present predominantly in the cytosol as a monomer.[16,17] The induction of apoptosis involves BAX activation throughout proteolytic cleavage of the N-terminus and exposure of the BH3 domain,[18] oligomerization of activated BAX particles,[19,20] and translocation to the mitochondrial membrane to form ion channels or polyprotein permeability transition pores.[14,21] The movement of BAX from the cytosol to the mitochondria during apoptosis is completed within 30 min, and precedes cell shrinkage and chromatin condensation.[22] Our previous experiments[23] and those performed by Mandal *et al.*[24] proved that, apart from mitochondria, BAX is translocated to the Golgi apparatus, endoplasmic reticulum and via nuclear envelope pores to the nucleus in cells stimulated to apoptosis.

In the present study we used laser scanning cytometry (LSC) for quantitative analysis of the subcellular redistribution of BAX in human colon adenocarcinoma COLO 205 cells exposed to the following cytostatic drugs: camptothecin (CPT; an inhibitor of DNA topoisomerase I), etoposide (ETO; an inhibitor of DNA topoisomerase II), staurosporine (STP; an inhibitor of serine-treonine kinase) and 2-chloro-2′-deoxyadenosine (2CdA; an antimetabolite), adenosine analog. We also examined the effect of nimesulide (NIM), a cyclooxygenase 2 (COX-2) inhibitor that belongs to the non-steroid anti-inflammatory drugs family (NSAIDs), but possesses anti-neoplastic properties.[25–27]

Materials and methods

Media and reagents

DMEM powdered medium (without L-glutamine), L-glutamine, phosphate buffer saline, pH 7.4 (PBS), FCS, fungizone and gentamycin sulfate were obtained from Gibco/BRL (Paisley, UK). Two polyclonal rabbit antibodies against human BAX recognizing amino acid sequences 11–30 and 43–61 were supplied by Santa Cruz Biotechnology (Santa Cruz, CA) and Dako (Glostrup, Denmark), respectively. FITC-conjugated $F(ab')_2$ fragment of swine anti-rabbit immunoglobulin was from Dako. Colloidal gold (18 nm)-conjugated donkey anti-rabbit IgG was from Jackson Immuno-Research (West Grove, PA). Horseradish peroxidase-conjugated goat anti-rabbit IgG (H+L) was supplied by BioRad (Hercules, CA). ECL Western blotting detection reagents and Hyperfilm ECL were purchased from Amersham Pharmacia Biotech (Little Chalfont, UK). Drugs and other chemicals were from Sigma (St Louis, MO). Sterile conical flasks, eight-chamber culture slides and sterile disposable pipettes were purchased from Nunc (Naperville, IL).

Cell culture

Human tumor cell line, colon adenocarcinoma COLO 205, was obtained from ATCC (Rockville, MD). Cell cultures were maintained in DMEM supplemented with 10% (v/v) FCS, 0.2% (w/v) L-glutamine, 50 μg/ml gentamycin and 2.5 μg/ml fungizone (10% FCS/DMEM) in an atmosphere of 5% CO_2/95% humidified air at 37°C and routinely subcultured every 2 or 3 days.

Drugs

Drugs were diluted in DMSO to create stock solutions and stored according to the manufacturers' suggestions. Concentrations used in the experiment were established after a series of screening tests in which COLO 205 cells were exposed to the increasing concentrations of drugs in 10% FCS/DMEM and assayed for apoptosis. Estimated final solutions were: 0.15 μM CPT, 0.5 μM STP, 25 μg/ml ETO, 5 μM 2-CdA and 1 μM NIM.

LSC

Exponentially growing cells were transformed to the eight-chamber culture slides, cultured for 24 h, and then incubated with the drugs in 10% FCS/DMEM for 1 and 3 h. The control cultures were treated with equivalent concentrations of DMSO suspended in 10% FCS/DMEM. Then cells were fixed in 0.25% formaldehyde for 15 min, washed twice with PBS, suspended in ice-cold 70% methanol and stored at 2°C for 30 min. Afterwards the cells were washed twice with PBS–1% BSA and incubated for 1 h with either primary rabbit anti-human BAX antibody (43–61 amino

acid sequence) diluted 1:20 with PBS–1% BSA or primary rabbit anti-human BAX antibody (11–30 amino acid sequence) diluted 1:100 with PBS–1% BSA. After primary incubation the cells were washed twice with PBS–1% BSA and incubated for 1 h with 1:20 secondary FITC-labeled antibody. The cells were washed twice in PBS–1% BSA and finally incubated with 5 μg/ml solution of 7-aminoactinomycin D (7-AAD) for 30 min to counterstain the DNA. As a negative control we used cells labeled with the rabbit immunoglobulin fraction (Dako) and the same FITC-conjugated secondary antibody. Then the chamber walls were removed and coverslips were mounted on microscope slides using anti-fade mounting medium (ICN, Aurora, OH). Probes were analyzed by LSC (CompuCyte, Boston, MA). At least 3×10^3 cells per chamber area were analyzed. The fluorescence excitation was provided by a 488 nm, 10 mW argon laser beam. The green fluorescence of FITC-labeled antibody was measured using a combination of dichroic mirrors and filters transmitting at 520 ± 20 nm wavelength (detector offset and gain set to 2000 and 32, respectively) and far red fluorescence of 7-AAD transmitting at >650 nm (offset 2000 and gain 30). BAX-associated green fluorescence was measured separately over the nucleus (Nf) and the cytoplasm (Cf). Nf was measured within the area outlined by the 'integration contour', located 1 pixel outside the 'threshold contour' triggered by the far red fluorescence of 7-AAD. Cf was measured within the rim of cytoplasm 9 pixels wide, located outside the 'integration contour'. The background green fluorescence was automatically measured within 2 pixels range outside the 'peripheral contour' and subtracted from both, nuclear and cytoplasmic green fluorescence, to obtain the final values of Nf and Cf, respectively. Another parameter measured was BAX maximal pixel (BAX MP) corresponding to the highest value of BAX-related fluorescence in the cell, regardless of cellular compartment. The obtained results were analyzed by Excel 2000 software (Microsoft, Redmond, WA).

Immunoelectron microscopy

For ultrastructural and immunocytochemical studies, tumor cells were fixed in 0.1% glutaraldehyde and 4% paraformaldehyde in 0.1 M PBS for 1 h at 4°C. The cells were washed in PBS for 30 min, treated with 1% OsO_4 for 1 h, dehydrated in ethanol gradient and embedded in Epon. For electron microscopy, ultrathin sections were processed according to the post-embedding immunogold procedure. Briefly, the sections were mounted on the formvar-coated nickel grids, incubated in 10% hydrogen peroxide for 10 min, rinsed in PBS for 15 min and blocked for 15 min in 5% bovine serum albumin (BSA) in PBS (PBS–5% BSA). The rabbit anti-human BAX antibody (Dako) was diluted 1:20 in PBS and applied on tissue slices for 1 h at 37°C. Then the grids were washed for 30 min in PBS and exposed to donkey anti-rabbit IgG conjugated with 18 nm colloidal gold particles diluted 1:50 in PBS. After 1 h incubation in the dark at 37°C, the grids were washed with PBS for 15 min, followed by a wash in distilled water for 15 min. The material was air-dried, and stained for 10 min with 4.7% uranyl acetate and for 2 min with lead citrate. The control experiments were carried out by incubating grids with the rabbit immunoglobulin fraction (Dako) instead of the anti-BAX antibody. The sections were examined and photographed by a JEOL 1200EX electron microscope.

Western blot analysis

The cultured cells were pelleted by centrifugation at 400 *g*, 2 min, 4°C and then frozen at −20°C. At the time of analysis the cells were suspended in ice-cold PBS. The fractionation of cells into nuclear and cytosolic fractions was performed according to Del Bufalo[28] with modifications as described.

After centrifugation at 400 *g*, 2 min, 4°C the supernatant was removed and the cell pellet was suspended in 0.1 ml Lysis Buffer I (pH 7.4) (10 mM Tris–HCl, 10 mM NaCl, 10 mM EDTA, 3 mM $MgCl_2$, 1% Triton X-100, 4 mM PMSF, and 20 μg/ml leupeptin as the protease inhibitor and 2 mM sodium orthovanadate as the alcaline/tyrosine phosphatases inhibitor) and incubated at 4°C for 30 min. The cell suspension was centrifuged at 500 *g*, 30 min, 4°C and the supernatant (containing cytoplasm with mitochondria and microsomes) was carefully removed and passed 6 times through a 20-gauge syringe needle. To obtain the nuclear fraction, the pellet inside a centrifuged Eppendorf tube was suspended in 0.5 ml Lysis Buffer II (pH 8.0) (20 mM Tris, 2% SDS, 4 mM PMSF, 20 μg/ml leupeptin and 2 mM sodium orthovanadate) and incubated at 4°C for 1 h. After incubation, the nuclear lysate was passed 10 times through a 20-gauge syringe needle. Protein concentration in both cellular fractions (nuclear or cytosolic) was determined by Lowry's method.[21] The lysates were mixed 1:2 (v/v) with Laemmli sample buffer (BioRad) containing 2.5% 2-mercaptoethanol and boiled for 5 min.

The samples containing identical quantities of proteins were subjected to SDS–PAGE (12.5% gel) together with Kaleidoscope Marker (BioRad). The electrophoresis was performed for 6 h at 50 V/120 mA using a Mini Protean II™ apparatus (BioRad). After electrophoresis the separated proteins were

electroblotted on a Sequi-Blot PVDF Membrane for Protein Sequencing (BioRad) for 1.5 h at 40 V/350 mA using a Mini Protean II™ apparatus. The membranes were blocked with 5% solution of non-fat dry milk in TBST (pH 7.5) for 1.5 h, washed in TBST at room temperature for 10 min and incubated with diluted 1:200 rabbit anti-human BAX monoclonal antibodies at 4°C for 16 h. The membrane was washed 3 times for 10 min in TBST containing 1% non-fat dry milk, incubated with diluted 1:1000 goat anti-rabbit IgG (H+L) antibody conjugated with horseradish peroxidase and washed again (twice for 10 min in TBST followed by once in TBS for 10 min). Labeled proteins were visualized using the ECL Western blotting detection reagents and Hyperfilm ECL high performance chemiluminescence film (Amersham Pharmacia Biotech).

Statistical evaluation

The results were statistically evaluated by ANOVA and Tukey's multiple range tests using Prism version 2.00 software (GraphPad Software, San Diego, CA). $p \leqslant 0.05$ was regarded as significant and $p \leqslant 0.01$ as highly significant.

Results

All examined drugs induced apoptosis of COLO 205 cells, manifested by a hypodiploidal area in the DNA frequency distribution histogram (Figure 1). The number of apoptotic cells in control culture (10% FCS/DMEM) was 2.5%, and was significantly increased after 3 h of incubation with CPT (14.1%), ETO (18.1%), STP (31.3%), 2CdA (23.2%) and NIM (11.9%). Apoptosis was confirmed by morphological evaluation of cells from the hypodiploid area. The gallery of cells relocated by CompuSort consists of cells with typical morphological features of apoptosis, e.g. cell shrinkage, condensation of chromatin (cells with high DNA maximal pixel) and fragmentation of the nucleus (Figure 1).

BAX expression in COLO 205 cells was evaluated using two parameters: (i) BAX MP, which reflects the highest BAX concentration (aggregation) in the cell, and (ii) BAX Cf and BAX Nf (the number of pixels in the area × each pixel fluorescence), which correspond to BAX content in the measured area. All drugs increased BAX MP with the peak at 60 min after administration to cell culture (Figure 2) (with the exception of CPT, where a further increase was observed). It indicates subcellular aggregation of BAX in the course of apoptotic death. The increase in cell number with high BAX MP was best seen in cultures exposed to 2CdA, CPT and STP. It should be pointed out that drug-induced BAX aggregation was detectable only when anti-BAX antibody recognizing 43–61 amino acid sequence was used. The number of COLO 205 cells with high BAX MP decreased, when the antibody binding 11–30 amino acid sequence was applied. It was shown in the case of CPT-treated cells, where a transient drop (within 60 min) of BAX MP was observed (Figure 2f). This effect was probably due to the cleavage and removal of the antibody-recognized epitope at the N-terminus in the course of BAX activation. The subsequent increase of BAX MP after 3 h of cell exposure to CPT could be a result of resynthesis of non-active BAX (Figure 2f).

To identify the sites of BAX aggregation in tumor cells exposed to apoptogenic drugs, post-embedment immunogold electron microscopy was applied. In control COLO 205 cells BAX-related gold particles were located mainly in the cytosol, on the nuclear envelope, plasma membrane and over the fibrous nucleoplasm. After 180 min of cell exposure to CPT numerous gold particles were located on cytoskeletal elements and organellar membranes: mitochondrial, endoplasmic reticulum and Golgi apparatus (Figure 3). Clusters of gold particles on mitochondrial cristae were present. The increase in BAX immunoreactivity on the nuclear envelope, within nuclear envelope pores and over fibrous nucleoplasm in tumor cells treated with CPT was also observed (Figure 3c).

LSC provided the possibility for quantitative evaluation of BAX redistribution between cytoplasmic and nuclear compartments in tumor cells exposed to anticancer drugs. All examined drugs significantly increased BAX Nf within 60 min, with the highest response in the case of CPT, NIM and 2CdA (Figure 4). The simultaneous increase in BAX Cf was observed in STP- and NIM-treated cells. For this reason BAX Nf:Cf ratio increased only in COLO 205 cells exposed to CPT, 2CdA and, to a lesser extent, in ETO-treated cells (Figure 4). Staining with different anti-BAX antibodies revealed that only the activated (cleaved) form of BAX was translocated to the nucleus. This was shown on BAX Nf histograms of COLO 205 cells exposed to CPT and STP (Figure 5). Both drugs increased BAX-related fluorescence in the nuclear area (BAX Nf) within 60 min, but only when the antibody recognizing the active form of BAX (43–61 amino acid sequence) was used (Figure 5a and c). The opposite effect, i.e. the decrease in BAX Nf, was observed when antibody recognizing a non-active form of BAX (11–30 amino acid sequence) was applied (Figure 5b and d). Activation of BAX by proteolytic cleavage was confirmed by Western blotting by showing a decrease in the non-active and a simultaneous increase in the

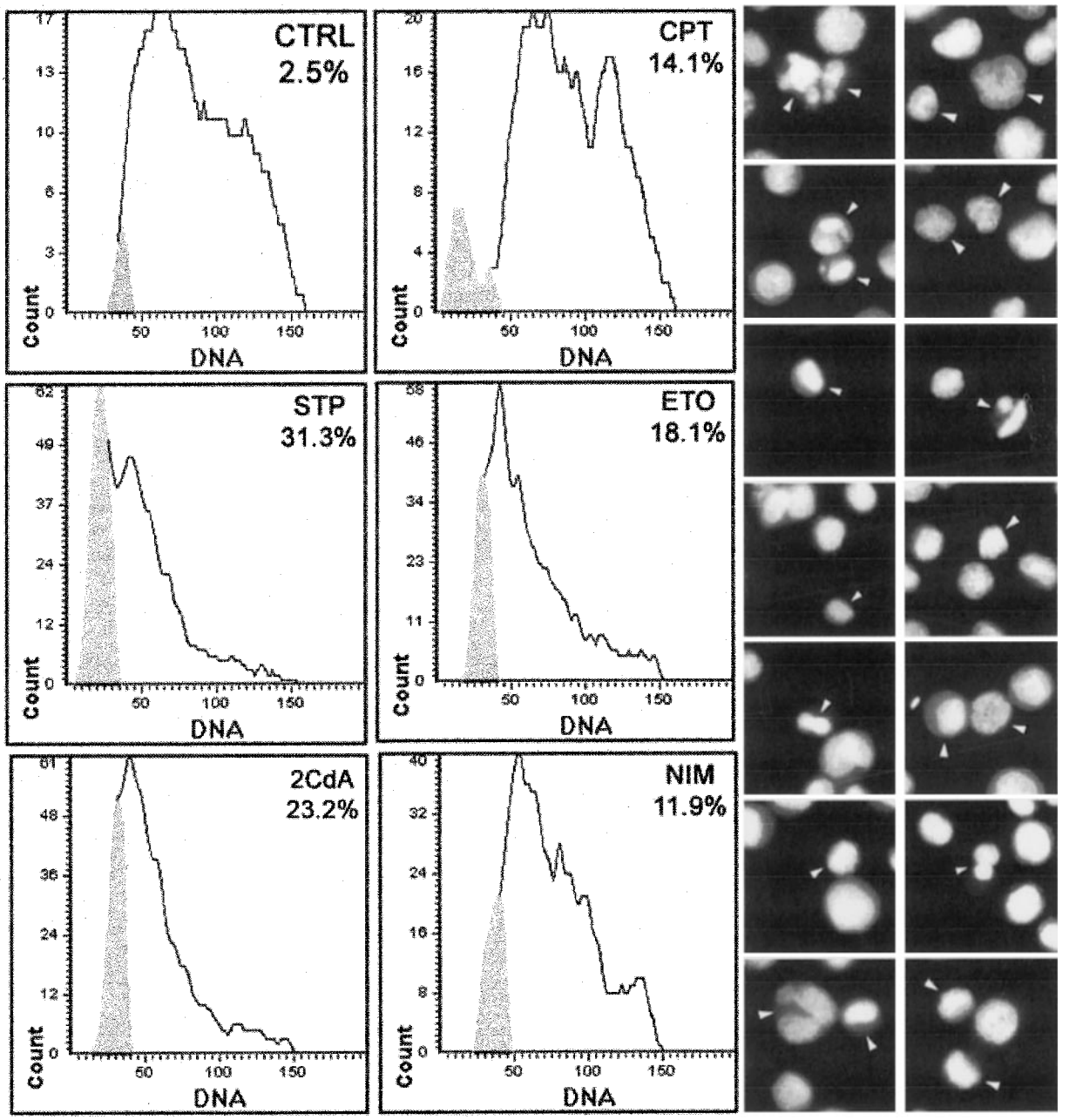

Figure 1. Percent of apoptotic cells in control culture (CTRL) and in cultures treated with 0.15 μM CPT, 0.5 μM STP, 25 μg/ml ETO, 5 μM 2-CdA and 1 μM NIM. The right panel shows photographs of cells with typical features of apoptosis, i.e. nucleus fragmentation, DNA condensation and margination, and formation of apoptotic bodies (cells marked with arrows). Figures are representative from three experiments. Apoptotic cells were relocated using CompuSort.

active form of BAX within 60 min after proapoptotic stimulation by CPT (Figure 6).

Discussion

Results of the present study clearly indicate that in spite of different molecular mechanisms of apoptosis induction for the anticancer drugs examined (CPT, ETO, STP, 2CdA and NIM), all apoptotic pathways converge on BAX as a common death promoter. Although the role of Bcl-2 family proteins as a universal mechanism in apoptosis regulation was postulated earlier,[2,3] the influence of different anticancer drugs on post-translational modifications and subcellular redistribution of BAX has not been compared.

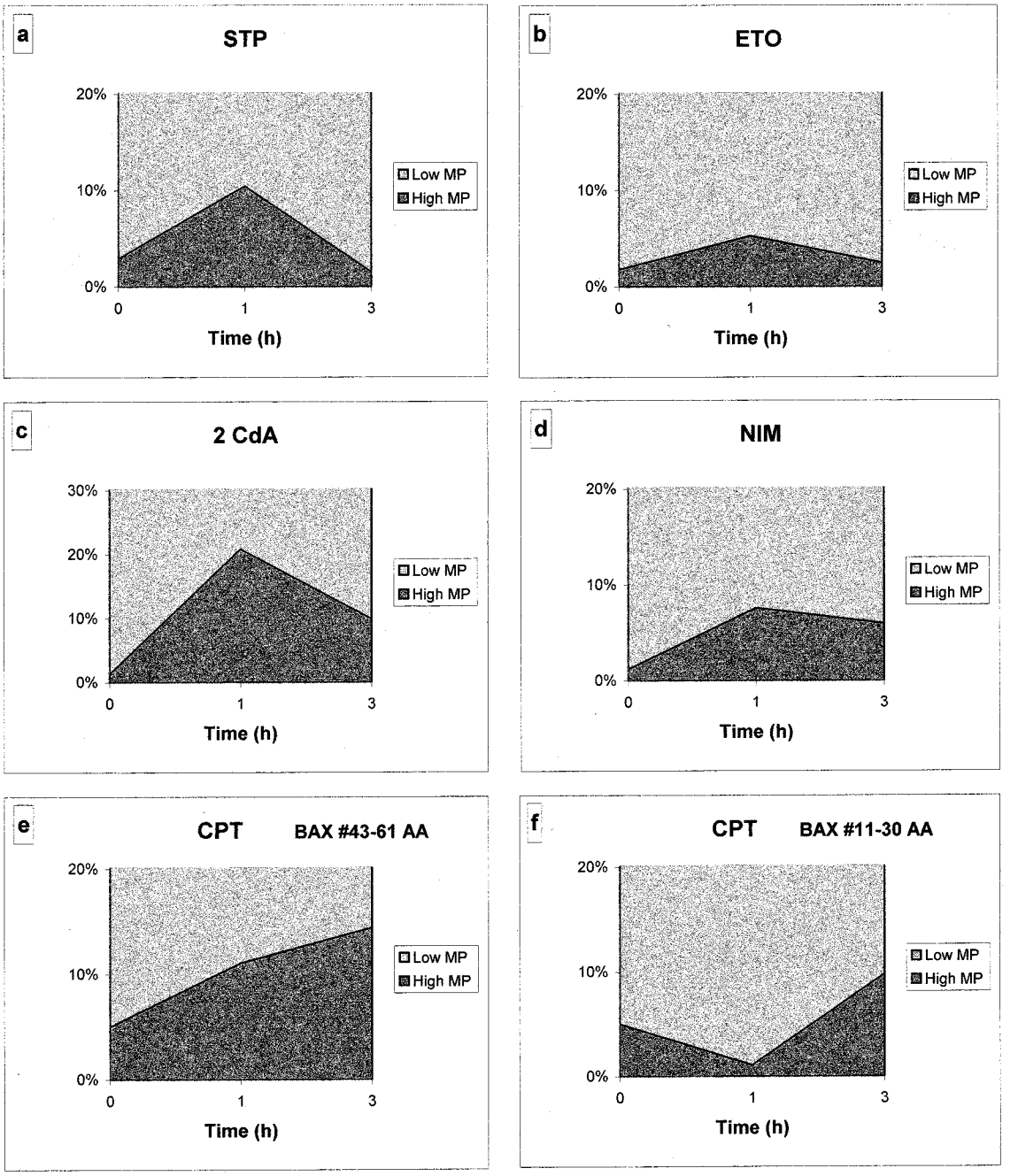

Figure 2. Change in percentage of COLO 205 cells expressing high BAX MP, a parameter referring to the concentration of BAX particles. Cells were treated with 0.5 μM STP, 25 μg/ml ETO, 5 μM 2-CdA, 1 μM NIM and 0.15 μM CPT for 1 and 3 h. The increase in BAX MP with a peak after 1 h incubation occurs only with the antibody recognizing the 43–61 amino acid sequence, referring to the active form of BAX (a–e). An opposite effect was observed when antibodies recognizing the N-terminal sequence 11–30 amino acids, referring to the inactive form of BAX, were used (significant decrease in percentage of cells expressing high BAX MP after 1 h incubation with camptothecin) (f). The increase in cells expressing high BAX MP in (f) after 3 h incubation was probably a result of the resynthesis of inactive BAX.

Apoptosis is believed to be a relatively quick process occurring within 60 min,[29] especially in tumor cells 'primed' to apoptosis. It has been suggested that the subcellular redistribution rather than the synthesis of BAX and other death promoters belonging to the BH3 and BAX subfamilies plays a key role in apoptosis

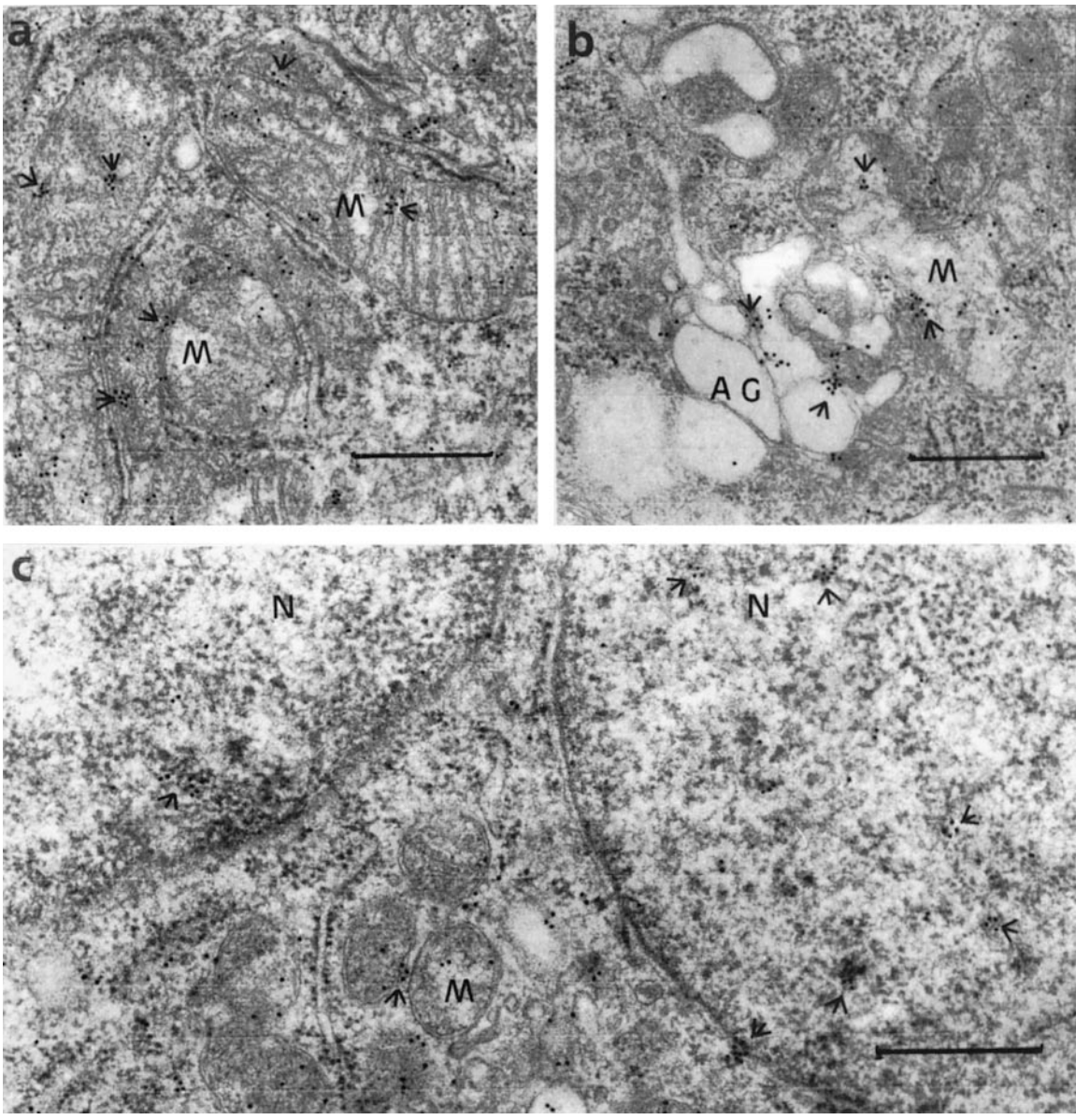

Figure 3. Immunogold labeling of COLO 205 cells treated with CPT (0.15 μM, 3 h). Arrows point to the aggregations of BAX particles on: (a) mitochondria membranes (M), (b) Golgi apparatus (AG) and (c) in the nucleus (N).

induction.[15] Apoptosis induced by all examined drugs (Figure 1) was associated with aggregation of BAX molecules (increase in BAX MP) in COLO 205 cells within 60 min after drug administration (Figure 2). Immunoelectron microscopy showed that the increase of BAX MP was a result of aggregation of BAX molecules on organellar membranes (mitochondrial, Golgi apparatus and endoplasmic reticulum) and in the nucleus where it moved through the nuclear envelope pores (Figure 3). A similar redistribution of BAX was observed in HC11 mouse mammary epithelial cells exposed to transforming growth factor-β1.[23] Translocation of BAX from cytosol to mitochondria was also described in mouse lymphoma HL60 cell line after STP and ETO treatment.[30]

In spite of intensive study, the molecular mechanism of BAX proapoptotic action remains unclear. It is suggested that BAX may form ion channels. Another possibility is that together with other proteins (porin, adenine nucleotide translocator and cyclophilin D) BAX forms permeability transition pore complexes in the external mitochondrial membrane, which in turn facilitates the release of cytochrome *c*, caspase-9 and AIF from intermembrane spaces.[3,20,31,32] Cytochrome

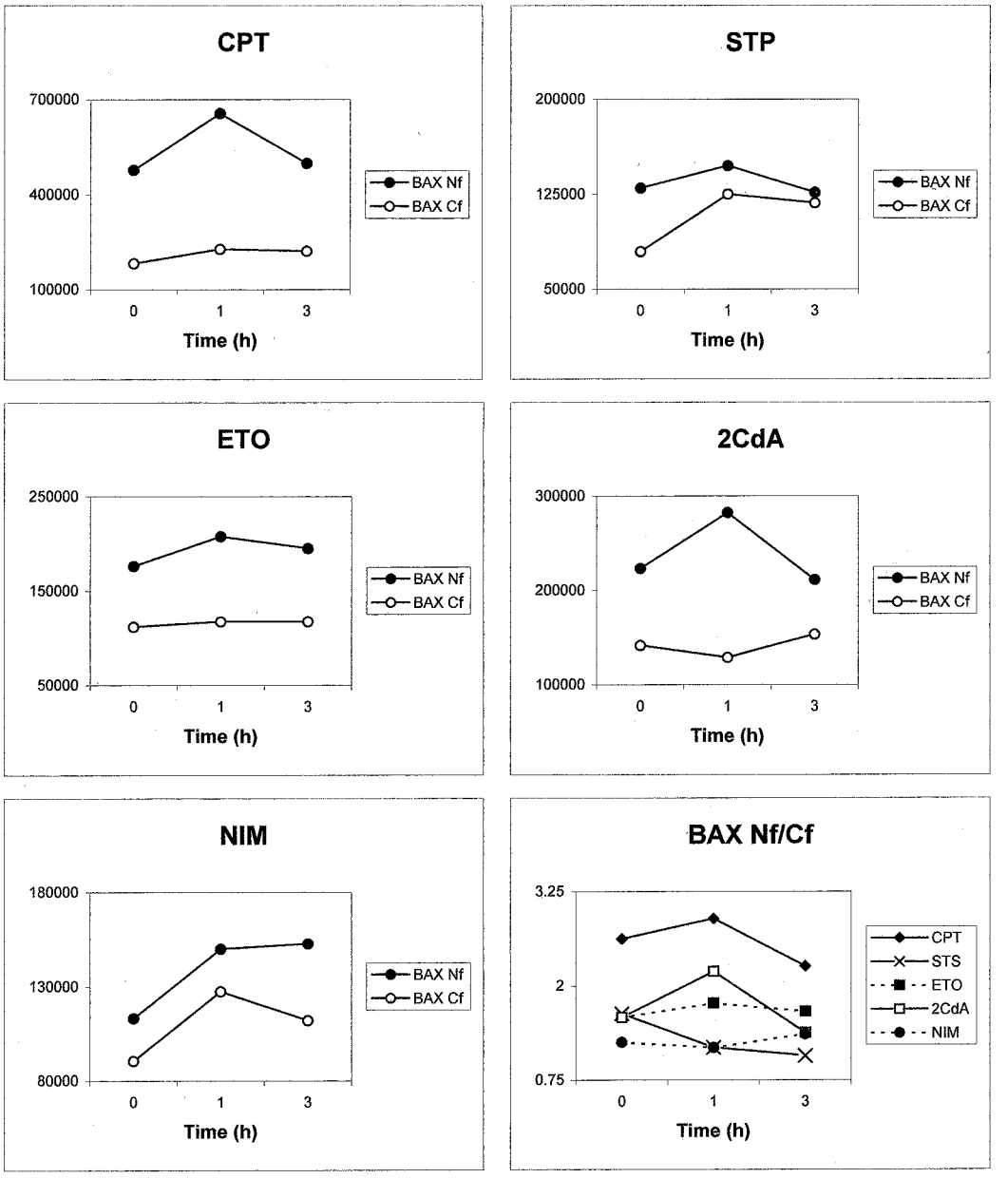

Figure 4. Changes in BAX Nf, BAX Cf and BAX Nf : Cf ratio in COLO 205 cells treated with 0.15 μM CPT, 0.5 μM STP, 25 μg/ml ETO, 5 μM 2-CdA and 1 μM NIM. Results are the means from three experiments.

c and caspase-9 form a complex with cytosolic Apaf-1 that eventually leads to the activation of caspase cascade and caspase-dependent DNase. AIF degrades nuclear proteins and partly DNA (to about 50-kb fragments) independently from caspases and ATP.[31] It is believed that BAX translocation from cytosol to organellar membranes is dependent on its activation occurring by proteolytic cleavage of the N-terminus and exposure of the BH3 domain, which permits its oligomerization up to heptamers.[18–20] Oligomerization of BAX is probably based on conformational changes leading to hydrophobic domain exposure, which produces an anchoring point for membrane lipids.[20] It is interesting that BID (being the BH3 only protein) facilitates BAX translocation from the cytosol to organellar membranes under the influence of STP.[33]

The results of our study indicate that activation of BAX occurring through proteolytic cleavage of the N-terminal epitope and BH3 motif exposure is the condition for BAX redistribution in COLO 205 cells

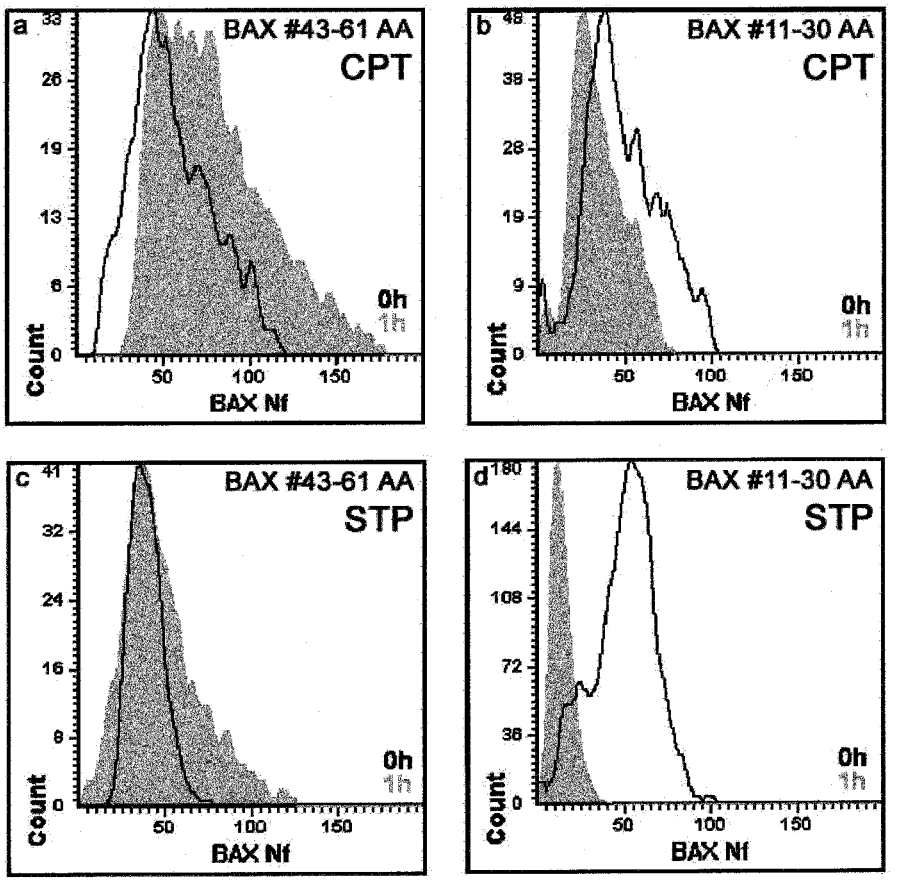

Figure 5. Increase in BAX Nf was observed only when antibodies recognizing the 43–61 amino acid epitope of BAX (referring to the activated form of BAX) were used (a and c). When cells were stained with antibodies recognizing the N-terminal, 11–30 amino acid sequence of BAX, a decrease in BAX Nf was observed (b and d). COLO 205 cells were treated with 0.15 μM CPT (a and b) and 0.5 μM STP (c and d) for 1 h.

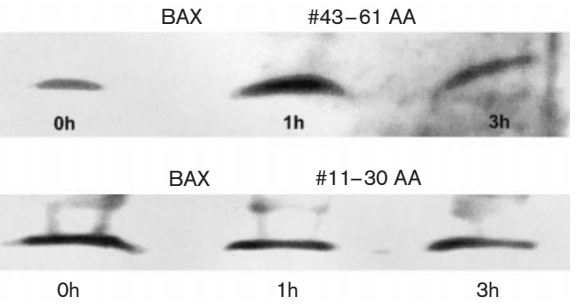

Figure 6. Western blot analysis of BAX in COLO 205 cells treated with 0.15 μM CPT for 1 and 3 h. The quantity of the activated form of BAX (antibodies recognizing 43–61 amino acid sequence) was increased, reaching its peak after 1 h (upper panel), whereas the quantity of the inactive form (antibodies recognizing 11–30 amino acid sequence) significantly decreased during the experiment (lower panel).

under the influence of supplemented drugs. This was demonstrated by using two anti-BAX antibodies recognizing different amino acid sequences: 11–30 amino acids, binding the N-terminal sequence encoded by exon 2, or 43–61 amino acids, binding the sequence encoded by exon 3 of the *bax* gene. The increase in BAX MP, indicating subcellular BAX aggregation (Figures 2 and 4), and the increase in BAX Nf, reflecting enhanced BAX expression in nuclear area (Figures 5 and 6), were observed only when 43–61 amino acid antibody was used. The opposite effect was observed when antibody against N-terminal epitope was applied, i.e. a decrease in BAX MP (Figure 4) and BAX Nf (Figure 6). The decrease in the non-active form with a simultaneous increase in the active form of BAX in COLO 205 cells exposed to CPT was confirmed by Western blotting (Figure 7). Particularly intriguing is the redistribution of BAX to the nucleus after drug stimulation, which manifests itself as an increase in BAX Nf (Figures 5 and 6), BAX Nf : Cf ratio (with the exception of STP and NIM effect; Figure 5), and the number of BAX-related gold

particles localized on the nuclear envelope and in the nucleus (Figure 3c). The lack of BAX Nf/Cf increase in the case of STP- and NIM-treated cells (in spite of an evident rise in BAX Nf) was probably due to increased synthesis of BAX in the cytoplasm (manifested by a BAX Cf increase). The translocation of BAX from the cytoplasm to the nucleus was described both in normal[23] and tumor cells [24] exposed to apoptogenic stimuli. Our study with the embedment-free immunogold technique showed a structural association of BAX with intermediate filaments of the karyo- and cytoskeleton in COLO 205 cells.[34] Moreover, we observed an increase in BAX immunoreactivity on fine filaments and lamina–pore complex of the nuclear matrix in colon adenocarcinoma cells treated with CPT.[35] The association of BAX with the nuclear matrix was also observed in glioblastoma cells.[36] The physiological role of BAX in the nucleus remains unexplained. We speculate that BAX could be involved in reorganization of the karyo- and cytoskeleton in the course of apoptotic death. The cooperation of other BCL-2-related death promoters with caspases in disassembly of the cytoskeleton has also been postulated.[37]

Conclusions

- Regardless of the different molecular mechanisms of apoptosis induction by CPT, ETO, STP, 2CdA and NIM, BAX remains a common link in the chain of reactions leading to cell death.
- Post-translational modification of BAX, i.e. activation via proteolytic cleavage of the N-terminal epitope, and subcellular redistribution to organellar membranes (mitochondrial, Golgi apparatus and endoplasmic reticulum) and via nuclear envelope pores to the nucleus, belong to early (occurring within 60 min) reactions of human colon adenocarcinoma COLO 205 cells to the apoptosis-inducing drugs.
- NIM, a specific COX-2 inhibitor belonging to NSAID family, shows proapoptotic properties similar to those of classic anticancer drugs in colon adenocarcinoma COLO 205 cells.

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