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Increased expression of VDAC1 sensitizes carcinoma cells to apoptosis induced by DNA cross-linking agents

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ABSTRACT

A major clinical problem regarding antitumoral treatment with DNA cross-linking agents such as cisplatin (Cisp), mechlorethamine (HN2) or its derivative melphalan (MLP) is intrinsic or acquired resistance to therapy, which frequently results from a resistance to apoptosis induction. In this study, aimed to identify novel sensitizing targets to DNA cross-linker-induced cell death, we demonstrated that MLP, Cisp and HN2 induce mitochondrial permeability transition pore (PTP)-mediated apoptosis in cervical and colon carcinoma cells. This apoptotic pathway is characterized by dissipation of the mitochondrial membrane potential, production of ROS, mitochondrial translocation of Bax, release of apoptogenic factors, caspase activation and nuclear alterations. The opening of PTP and subsequent apoptosis was reduced in Bax deficient cells and in cells with elevated Bcl-2 level, but not in cells invalidated for Bak. We further showed that, among the pro-apoptotic PTP regulators tested (VDAC1, creatine kinase, ANT1 and ANT3), exogenous overexpression of VDAC1 was the most effective in enhancing Cisp- and MLP-induced apoptosis. In addition, pharmacologically induced up-regulation of VDAC1 by the chemotherapeutic agent arsenic trioxide (As_2O_3) greatly sensitized HeLa cells to Cisp and MLP treatment. These data indicate that increased expression of VDAC1 appears as a promising strategy to improve DNA cross-linker-induced chemotherapy.

1. Introduction

DNA cross-linking agents such as cisplatin (Cisp), mechlorethamine (HN2) or its derivative melphalan (MLP) are among the oldest and most effective anticancer chemotherapeutics [1]. Although they are broadly used for their anticancer activity in many types of malignancies, including myeloma, cervix, breast, colon and lung cancers, there are still many uncertainties about their molecular pharmacology and mechanisms of action. A major limitation of their clinical use is the invariable development of resistance following prolonged administration [2,3]. Moreover, acquired resistance does not seem restricted to only one agent as MLP-resistant myeloma cells also display cross-resistance to Cisp, HN2 and radiation [4]. Therefore, elucidation of the mechanisms and factors that control the sensitivity to DNA cross-linking agents is crucial to improve the therapeutic outcome.

The antitumor activity of DNA cross-linkers has generally been attributed to their ability to prevent replication by forming intra- and interstrand cross-links on nuclear DNA. More recently, apoptosis was also proposed to account for their anticancer activity [5,6]. Most chemotherapeutic agents trigger the mitochondrial pathway of apoptosis, which results in mitochondrial membrane permeabilization (MMP) [7]. Diverse factors have been demonstrated to control MMP, including Bcl-2 family members, cardiolipin and/or the mitochondrial permeability transition pore (PTP). In many physio-pathological models, the prolonged opening of this polyprotein complex results in the mitochondrial permeability transition (MPT), mitochondrial matrix swelling and local ruptures of the outer membrane (OM) and leads to cell death. These events are accompanied by dissipation of mitochondrial transmembrane potential ($\Delta\Psi_m$) and release of pro-apoptotic proteins into the cytosol [7]. In cancer cells, the PTP would be composed or regulated by, at least, the voltage dependent anion channel (VDAC), the adenine nucleotide translocase (ANT) and the cyclophilin D (CypD) [8]. In human, four isoforms of ANT (ANT1–4) and three isoforms of VDAC (VDAC1–3) have been identified and

Abbreviations: AIF, apoptosis-inducing factor; ANT, adenine nucleotide translocase; Ars, arsenic trioxide (As_2O_3); Cisp, cisplatin; $\Delta\Psi_m$, mitochondrial transmembrane potential; HN2, mechlorethamine; IM, inner membrane; MLP, melphalan; MMP, mitochondrial membrane permeabilization; MPT, mitochondrial permeability transition; OM, outer membrane; PI, propidium iodide; PTP, permeability transition pore; ROS, reactive oxygen species; VDAC, voltage-dependent anion channel.

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demonstrated to exert opposite effects on apoptosis. Indeed, some isoforms (VDAC1, ANT1, ANT3) act predominantly as pro-apoptotic proteins [9–11], whereas others (VDAC2, ANT2, ANT4) protect from cell death [12–14]. Although VDAC proteins are highly conserved across species, the specific function of individual VDAC isoforms is poorly understood and remains to be elucidated. However, numerous studies, using silencing or overexpression approaches, suggest that the level of expression of VDAC1, which was shown to be the most abundant of the three isoforms in HeLa cells [15], plays a critical role in mitochondria-mediated apoptosis [16]. Thus, modulators of PTP have been proposed to be potent targets to improve mitochondria-dependent chemotherapeutic treatments.

Recently, we and others have demonstrated a central executioner role of mitochondria in the apoptotic process induced by DNA cross-linkers [6,17]. In this article, we showed that Cisp, MLP and HN2 triggered a PTP-mediated apoptotic process favored by Bax in cervix (HeLa) and colon (HCT116) carcinoma cells. In addition, our results indicate that the increase in expression levels of PTP regulators, and in particular of VDAC1, enhances DNA cross-linker-induced apoptosis.

2. Materials and methods

2.1. Cells, cell culture and reagents

HeLa Bcl-2 and HeLa vMIA were generously given by Dr V. Goldmacher (ImmunoGen, Cambridge, MA, USA). Parental HCT116 (HCT116 Bax+/Bak+) and HCT116-Bax-KO (HCT116 Bax−) were generously provided by Dr B. Vogelstein (Johns Hopkins University School of Medicine, Baltimore, MD, USA). The stable knockdown of Bak (HCT116 Bak− and HCT116 Bax−/Bak−) was constructed by Pr G. Chinnadurai (University School of Medicine, Saint Louis, MO, USA) and kindly given by Dr P. Daniel (Humboldt University, Berlin-Buch, Germany). The Rat-1 cell lines were kindly provided by Dr G. Kroemer (IGR, Villejuif, France). A549 cell line (#86012804) was purchased from ECACC (Salisbury, UK). Cells were cultured in DMEM:F12 medium supplemented with 100 U/ml penicillin, 100 μg/ml streptomycin, 1% glutamax (Invitrogen, Villebon sur Yvette, France) and 10% fetal bovine serum (Lonza, Levallois-Perret, France), at 37 °C under 5% CO2/95% air. All chemicals were purchased from Sigma–Aldrich (Lyon, France). The caspase inhibitor z-VAD-fmk was obtained from Bachem (GmbH, Germany). All fluorescent probes were from Invitrogen.

2.2. Flow cytometry analysis

For flow cytometry analysis of mitochondrial transmembrane potential ($\Delta\Psi_m$), cells were stained either with 100 nM $DiOC_6(3)$ or with 100 nM MitoTracker Red CMXRos for 20 min at 37 °C. Necrosis was estimated by adding 10 μg/ml of propidium iodide (PI) just before analysis. PTP opening was assessed as described previously [18]. Briefly, cells were preincubated for 15 min at 37 °C with 1 mM calcein-AM/1 mM $CoCl_2$ in HBSS (Invitrogen) supplemented with 1 mM HEPES, pH 7.3. HBSS was then replaced by complete culture medium during apoptosis induction. For the detection of DNA fragmentation (SubG1 population), cells were harvested, fixed and permeabilized with 70% cold ethanol for at least 24 h, washed three times with PBS and stained with 50 μg/ml PI in the presence of 12.5 Kunitz U/ml of RNase A. Intracellular ROS production was quantified by staining cells with 5 μM dihydroethidine (DHE) for 15 min at 37 °C and measuring the oxidation of DHE to red fluorescent ethidium (DHE → Eth). Fluorescence of cells was analyzed on Epics XL or Cell Lab Quanta MPL cytometers (Beckman Coulter, Villepinte, France).

2.3. Western blot analysis

After treatment, cells were lysed in lysis buffer (50 mM Hepes-KOH pH 7.4, 250 mM NaCl, 1% Triton X-100, 5 mM EDTA, 0.5 mM DTT, 1 mM PMSF and a cocktail of protease inhibitors (Roche, Meylan, France)) for 30 min at 4 °C. Proteins (30 μg) were separated by SDS-polyacrylamide gel electrophoresis and transferred to PVDF membranes (Millipore, Molsheim, France). Membranes were incubated overnight at 4 °C with the following antibodies: cyclophilin D (Mitosciences, OR, USA), ANTs and VDACs (directed against all isoforms of ANT and VDAC, generated by Genosphere Biotechnologies, Paris, France), α-tubulin, caspase-9, Bax, Bak, Bcl-2, mtCK, β-actin (all from Santa Cruz, CA, USA), VDAC1 (Calbiochem, MERCK, Fontenay-sous-bois, France), GAPDH (Abcam, Paris, France). Proteins were detected on a Chemidoc XRS (Bio-Rad, Marnes-la-Coquette, France) by using the ECL method according to the manufacturer's instructions (Millipore). To calculate the relative density (RD), ImageJ software was used and the intensity of each protein was normalized to α-tubulin, GAPDH or β-actin. The data obtained were then expressed as the ratio of the intensity of the protein in treated cells to that of the corresponding protein in untreated cells (Co.).

2.4. Immunofluorescence

To determine intracellular localization of cytochrome *c* (Cyt *c*), AIF and Bax, cells were seeded on slides in six-well multidishes, treated with DNA cross-linkers, washed in PBS 1X, fixed in 3.7% paraformaldehyde for 10 min at RT and permeabilized for 3 min in acetone at −20 °C. Cells were saturated in PBS/3% bovine serum albumin (BSA) for 30 min and incubated for 1 h with anti-Cyt *c* (mAb 6H2.B4, BD Biosciences, Le Pont de Claix, France), anti-AIF (Millipore) or anti-Bax (N-20, Santa Cruz) in PBS/1% BSA at RT. After two washes, the secondary antibody (Jackson ImmunoResearch, Suffolk, England) was added in PBS/1% BSA. To detect apoptotic cells, nuclei were stained with 2.5 μg/ml Hoechst 33348 for 5 min. Micrographs were taken on a Leica fluorescence microscope (Leica Microsystèmes, Nanterre, France).

2.5. Transfection experiments

To determine the effect of PTP member overexpression on DNA cross-linker-induced mitochondrial alterations, cells were co-transfected using ExGen 500 (Fermentas, St. Leon-Rot, Germany) with an expression vector encoding a member of PTP (pcDNA3.1 constructs) and a plasmid carrying the *gfp* gene (pEGFP-N2, Takara Bio Europe/Clontech, Saint-Germain-en-Laye, France) with a 9:1 ratio for 48 h, then incubated in the absence or the presence of MLP or Cisp for 24 h and stained with the $\Delta\Psi_m$ specific probe Mitotracker Red CMXRos. The fluorescence was determined by cytometric analysis of samples gated on GFP-positive cells. To evaluate the influence of the overexpression of PTP members on cross-linker-induced mitochondrial apoptosis, results are expressed as the percentage of ΔCMXRos low cells, which corresponds to the difference between the % of CMXRos low cells obtained after transfection with the vector encoding the protein of interest and the % of CMXRos low cells obtained after transfection with the empty vector. Accordingly, positive values represent sensitizing effects. The effect of overexpression of VDAC1 on DNA-crosslinker-induced cell death was investigated by co-transfecting cells with an expression vector encoding VDAC1 or an empty vector and a plasmid encoding the TurboRFP protein (Evrogen, Moscow, Russia) with a 9:1 ratio for 48 h. Then, cells were treated with MLP and Cisp for 24 h and stained with 0.1 μg/ml Fluorescein diacetate (FDA) for 5 min at RT. The percentage of FDA negative cells (dead cells) was recorded in TurboRFP positive cells

(transfected cells) by flow cytometry. Results were expressed as the percentage of ΔFDA negative cells, which corresponds to the difference between the % of FDA negative cells obtained after transfection with the vector encoding VDAC1 and the % of FDA negative cells obtained after transfection with the empty vector. Accordingly, positive values represent sensitizing effects.

2.6. VDAC1 knockdown by RNA interference

pSM2 vectors encoding scrambled and VDAC1 microRNA-adapted short hairpin RNAs (shRNAmir) were purchased from Open Biosystems (Lafayette, CO, USA). The sense sequence of VDAC1 shRNAmir was 5′-GAATTTCAAGCATAAATGAATA-3′ (#RHS1764-9395854). HeLa cells were co-transfected for 72 h with pSM2 vectors and a plasmid encoding GFP protein (ratio 9:1) using TurboFect (Fermentas, St. Leon-Rot, Germany), according to the manufacturer's instructions. After 72 h, cells were incubated with MLP and Cisp for 24 h and stained with Mitotracker Red CMXRos for 30 min at 37 °C. The fluorescence of CMXRos was determined by cytometric analysis of samples gated on GFP-positive cells.

2.7. Statistical analysis

Data were analyzed using Prism 4 (GraphPad version 4.03, La Jolla, CA, USA). One-way ANOVA was used to assess differences among groups followed by Dunnett's post hoc test. When two groups were compared, differences were assessed by Student's *t*-test. Differences between groups were considered significant if the *P* value was less than 0.05.

3. Results

3.1. Apoptosis triggered by DNA cross-linking agents involves PTP opening

To characterize cell death induced in HeLa and HCT116 carcinoma cell lines by DNA cross-linking agents, cells were incubated with MLP, Cisp and HN2 for 24 h and then analyzed by flow cytometry after staining with propidium iodide (PI) to measure necrosis, $DiOC_6(3)$ to record mitochondrial transmembrane potential ($\Delta\Psi_m$) and calcein/cobalt to determine PTP opening [18]. In addition, after 48 h of treatment, the percentage of DNA fragmentation (SubG1) was determined. In both cell lines, the percentage of PI-positive cells (PI high) remained very low, while a strong increase in SubG1 population was observed (Fig. 1A and B), demonstrating that cell death induced by DNA cross-linking agents is apoptosis rather than necrosis in our conditions. As observed with lonidamine (LND), that provokes apoptosis via MMP [19], the three DNA damaging agents induced a significant increase of PTP opening (calcein low cells) and a dissipation of the $\Delta\Psi_m$ ($DiOC_6(3)$ low cells). As a control, the protonophore CCCP uncoupled mitochondria without promoting PTP opening and subsequent nuclear apoptosis in both cell lines. Western blot analysis indicates that two major components of PTP, VDAC and CypD, were up-regulated in response to MLP, Cisp and HN2, further suggesting the involvement of this lethal pore in the apoptotic process (Fig. 1C). Reactive oxygen species (ROS) have been demonstrated to be a cause or a consequence of PTP opening and mitochondrial alterations in apoptosis [20]. Production of ROS induced by MLP, Cisp and HN2 was thus investigated by staining cells with the ROS-sensitive probe dihydroethidium. In comparison with control cells, the three DNA cross-linkers increased the percentage of oxidation of DHE to ethidium (DHE → Eth), showing that the apoptotic process induced by these DNA damaging agents is associated with generation of ROS in cervix and colon carcinoma cell lines (Fig. 1D). The immunofluorescence analyses (Fig. 1E) revealed that after the treatment with DNA cross-linkers the punctuated staining pattern of the intermembrane space proteins cytochrome *c* (Cyt *c*) and apoptosis-inducing factor (AIF) became diffuse in apoptotic cells presenting a condensed or fragmented nucleus (arrows). The release of cytochrome *c* subsequently triggered the activation of the initiator caspase-9 as shown by the cleavage of the 47 kDa procaspase-9 to its 35/37 kDa active forms (Fig. 2A). In addition, pretreatment of HeLa cells with the pan-caspase inhibitor zVAD-fmk (50 μM) efficiently blocked DNA cross-linker-induced PTP opening (Fig. 2B), dissipation of $\Delta\Psi_m$ and DNA fragmentation (not shown), confirming the involvement of caspases in this apoptotic process. Altogether, these data indicate that, in HeLa and HCT116 cancer cells, apoptosis induced by MLP, Cisp and HN2 is associated with PTP opening and MMP with subsequent release of apoptogenic factors, caspase activation and nuclear alterations.

3.2. The level of Bcl-2 family proteins is modulated by MLP, Cisp and HN2 treatments

We next examined the level of expression of Bcl-2 family proteins following incubation with DNA cross-linking agents. As observed in Fig. 3A, the level of the pro-apoptotic proteins Bax and Bak is significantly increased, while in contrast, the expression of the anti-apoptotic protein Bcl-2 decreased drastically, this protein being almost undetectable after 24 h of treatment with Cisp and HN2. In addition, after 24 h, Bax showed a typical diffuse cytoplasmic distribution in the absence of drug, while in the presence of MLP, Cisp and HN2, a punctuated staining pattern was observed in cells with fragmented nucleus (Fig. 3B, arrows), demonstrating a relocalization of Bax from the cytoplasm to the OM of mitochondria. Taken together, these results suggest an involvement of the Bcl-2 family proteins in the regulation of the apoptotic process either via their expression level and/or their intracellular localization.

3.3. The pro-apoptotic protein Bax, but not Bak, promotes PTP opening and apoptosis induced by DNA cross-linking agents

To directly assess the involvement of Bax and Bak in the mitochondriotoxic activity of DNA cross-linkers, we took advantage of human colon carcinoma HCT116 cells lacking either Bax (HCT Bax−) or Bak (HCT Bak−) or both pro-apoptotic proteins (HCT Bax− Bak−). After 24 h of treatment with DNA damaging drugs, inactivation of Bak failed to protect cells against MLP-, Cisp- or HN2-induced PTP opening, while cells lacking Bax were partially preserved from the decrease of calcein fluorescence observed in parental HCT116 cells (Fig. 4A). Thus the pro-apoptotic protein Bax, but not Bak, sensitizes HCT116 cells to the opening of PTP in response to these DNA damaging agents. As expected, comparable results were observed when assessing the subsequent apoptotic events such as the $\Delta\Psi_m$ and the nuclear alterations (Fig. 4B and D). In contrast with the other mitochondrial events, the cytometric analysis of the ethidium fluorescence (DHE → Eth) demonstrated no noteworthy difference between the parental HCT116 cells and the Bax or Bak deficient mutants (Fig. 4C), suggesting that the generation of ROS is either a Bax and Bak independent event or an early event in the signaling of MLP-, Cisp- and HN2-induced apoptosis. Interestingly, after 42 h of treatment, only a very weak protection against PTP opening and dissipation of $\Delta\Psi_m$ was detected in Bax-deficient cells (Fig. 4E, about 6–10% less calcein low cells and $DiOC_6(3)$ low cells in the presence of drugs compared to controls), suggesting that Bax could favor DNA cross-linker-induced apoptosis, but would not be indispensable for this process to occur. Altogether, our results demonstrate that the pro-apoptotic

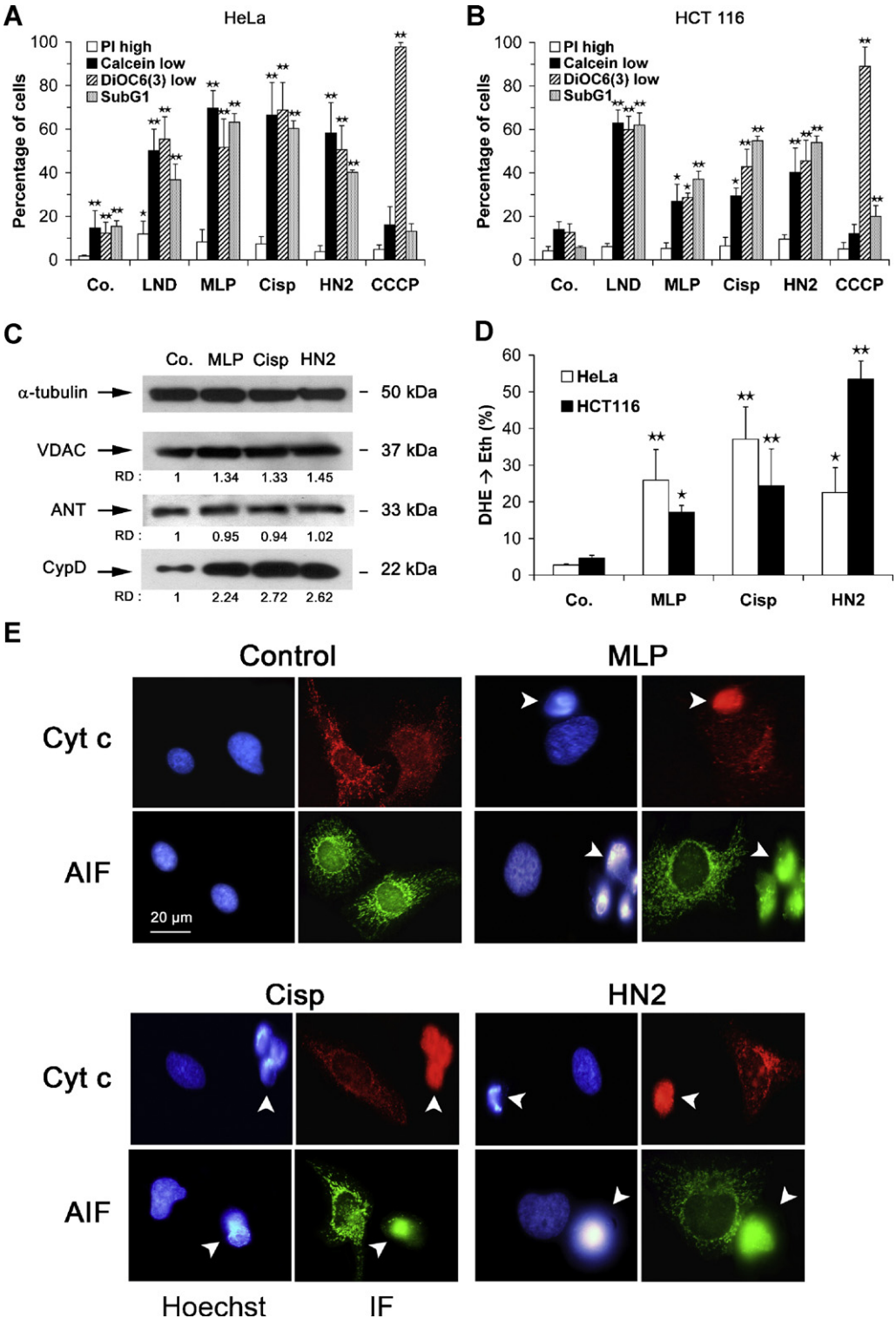

Fig. 1. DNA cross-linkers induce PTP opening, $\Delta\Psi_m$ loss, ROS generation and release of apoptotic factors. (A) HeLa cells were treated with 25 μM MLP, 20 μM Cisp or 10 μM HN2, (B) HCT116 cells were treated with cross-linkers at 100 μM. As controls, cells were left untreated (Co.) or incubated with 250 μM LND or 20 μM CCCP. After 24 h of treatment, necrosis (PI high), PTP opening (calcein low) and $\Delta\Psi_m$ loss ($DiOC_6(3)$ low) were analyzed by flow cytometry. DNA fragmentation (SubG1) was recorded after 48 h. (C) Total protein extracts from HeLa cells treated or not during 24 h, were immunoblotted with anti-α-tubulin, anti-Cyp D, anti-ANTs or anti-VDACs antibodies. RD: relative density as described in materials and methods. (D) HeLa and HCT116 cells were left untreated (Co.) or treated as in (A) for 24 h, and ROS production was quantified by flow cytometry after DHE staining. (E) HeLa cells treated or not for 24 h, were immunostained for cytochrome *c* (Cyt *c*) or apoptosis inducing factor (AIF). Apoptotic cells (white arrows) were identified by Hoechst staining as cells presenting bright condensed or fragmented nucleus. The data in the bar graph represent mean ± s.d ($n = 4$, $^{*}P < 0.05$, $^{**}P < 0.01$ by one-way ANOVA/Dunnett's post-test *versus* control).

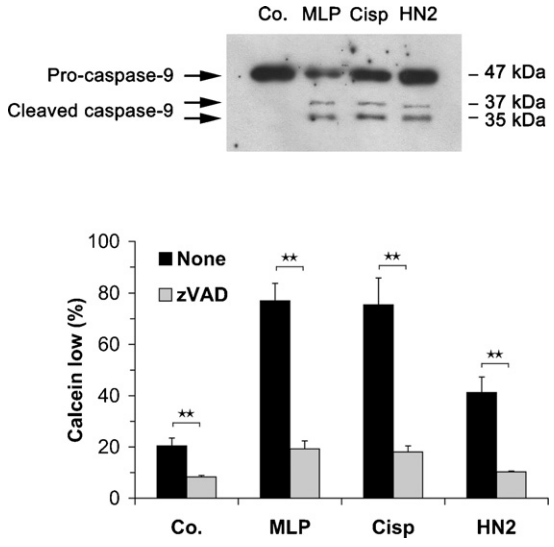

Fig. 2. Activation of caspases in response to DNA cross-linkers. (A) Total protein extracts from HeLa cells incubated or not with cross-linkers were immunoblotted with anti-caspase-9 antibody. (B) HeLa cells were pre-treated for 1 h with 50 μM zVAD-fmk, then incubated or not with DNA damaging agents for 24 h. PTP opening was analyzed by flow cytometry. The data in the bar graphs represent mean ± s.d. ($n = 4$, $^{**}P < 0.01$ compared with the absence of zVAD (none) by Student's t-test).

protein Bax sensitizes cancer cells, but is not absolutely required, to the opening of PTP and subsequent apoptosis in response to MLP, Cisp and HN2.

3.4. DNA cross-linker-induced PTP opening and subsequent apoptosis are inhibited by Bcl-2 and vMIA

In order to determine the effect of Bcl-2 and vMIA, two well-known MMP inhibitors [21,22], on apoptosis induced by DNA damaging agents, cytometric analysis were performed on HeLa cells overexpressing either Bcl-2 (Hela Bcl-2) or vMIA (HeLa vMIA) and compared with parental HeLa cells. Both Bcl-2 and vMIA almost totally inhibited the opening of PTP (calcein low, Fig. 5A) induced by MLP, Cisp and HN2. This inhibition of PTP opening was associated with inhibition of other apoptosis-related alterations, including the loss of the $\Delta\Psi_m$ ($DiOC_6(3)$, Fig. 5B), the production of ROS (DHE → Eth, Fig. 5C), and the DNA fragmentation (SubG1, Fig. 5D). Thus, efficient inhibition of PTP opening by Bcl-2 and vMIA and subsequent inhibition of other mitochondrial and nuclear apoptotic events indicate that PTP opening is truly a critical step in response to MLP, Cisp and HN2. Based on the fact that Bcl-2 acts either at ER or mitochondrial level to inhibit apoptosis [22,23], we wondered whether its protective effects were dependent from its subcellular localization. To this end, we took advantage of Rat-1 cell lines overexpressing Bcl-2 in whole cell (Bcl-2) or targeted to the mitochondrion (Bcl-2 mt) or to the ER (Bcl-2 ER). Cells were incubated for 24 h in the absence or the presence of MLP, Cisp, HN2 and thapsigargin (TG), an inhibitor of SERCA pumps that induces

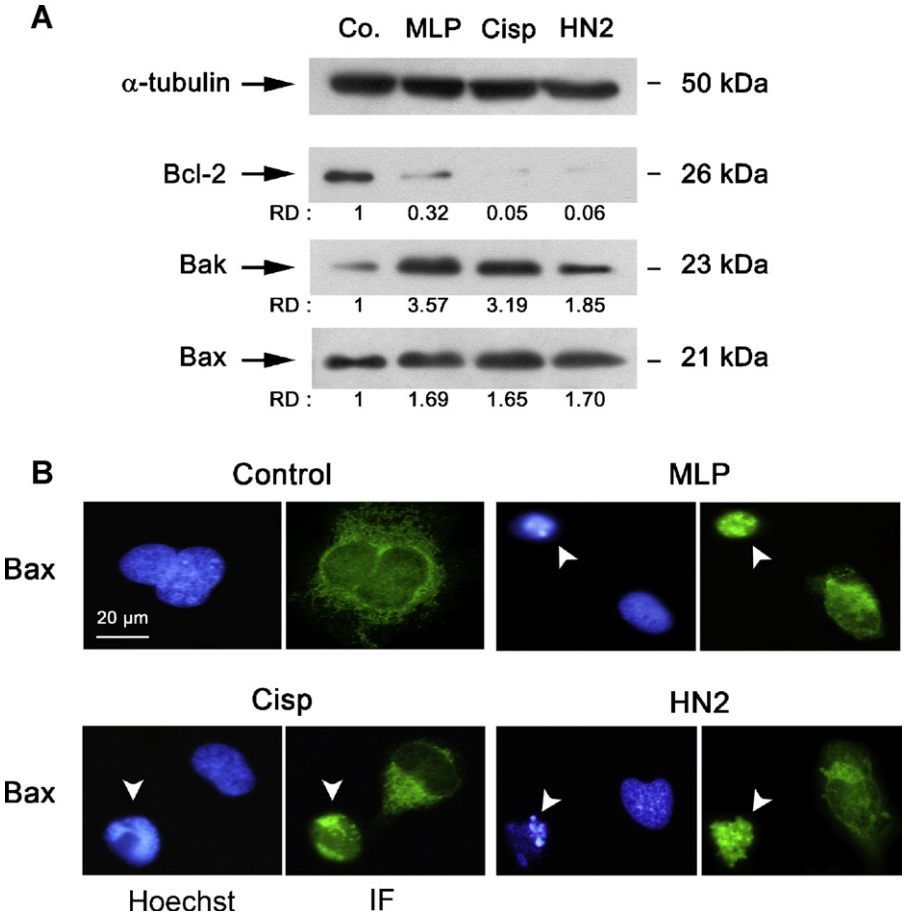

Fig. 3. DNA damaging agents modulate the level of Bcl-2 family proteins and induce Bax relocalization to mitochondria. (A) Total protein extracts from HeLa cells treated or not with MLP, Cisp or HN2 for 24 h, were immunoblotted with anti-Bax, anti-Bak, anti-Bcl-2 or anti-α-tubulin antibodies. RD: relative density as described in materials and methods. (B) After 24 h of treatment with DNA cross-linkers, Bax was detected in HeLa cells with an anti-Bax antibody. Apoptotic cells (white arrows) were identified by Hoechst staining as cells with bright condensed or fragmented nucleus.

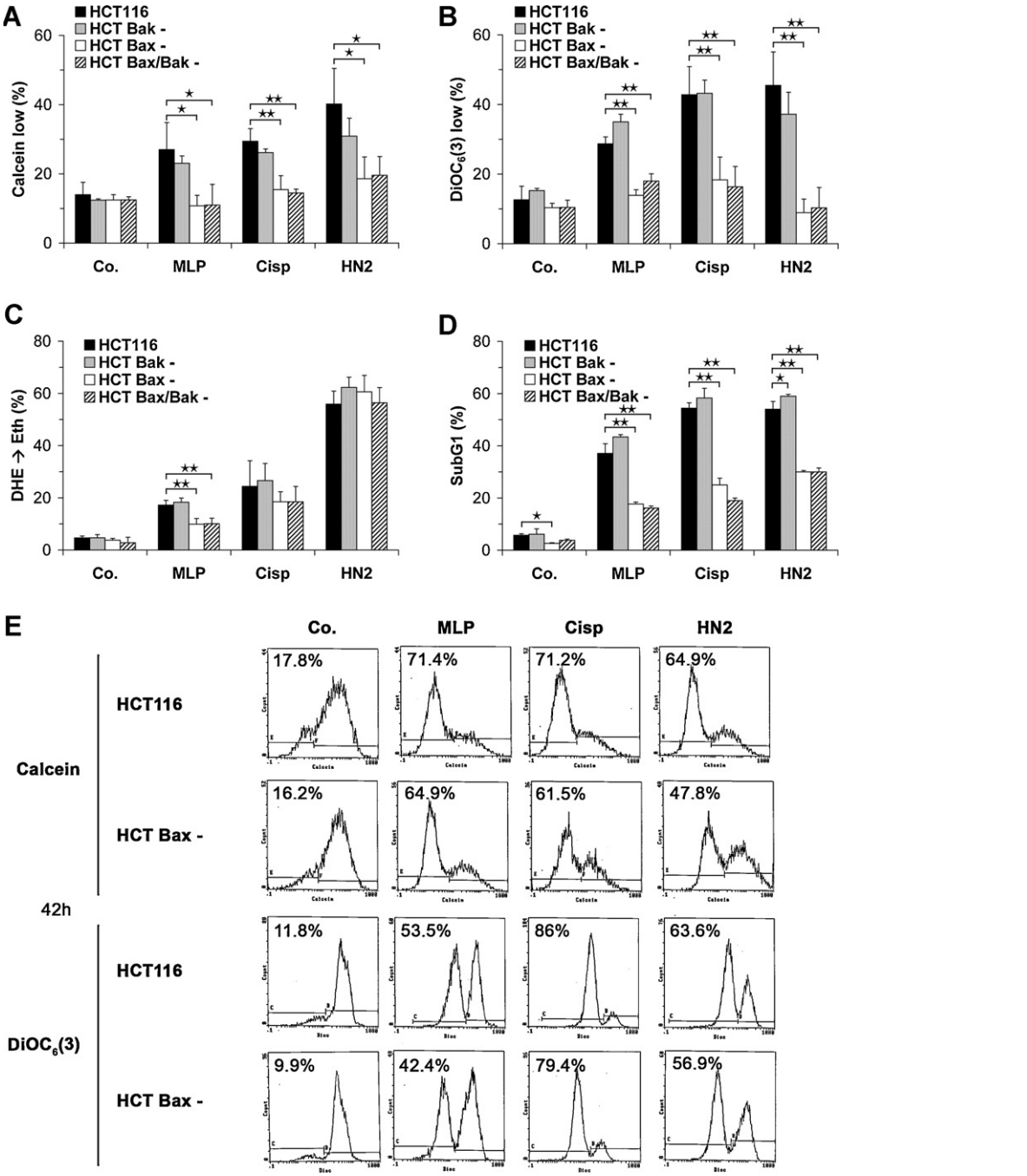

Fig. 4. PTP-dependent apoptosis induced by DNA cross-linkers is favored by Bax but not by Bak. HCT116 parental cells *versus* cells deficient either for the pro-apoptotic proteins Bax (Bax−) or Bak (Bak−), or both (Bax/Bak−) were incubated with DNA cross-linking agents. After 24 h, cells were analyzed by flow cytometry for (A) PTP opening, (B) $\Delta\Psi_m$ loss, (C) ROS production and (D) DNA fragmentation. The data represent mean ± s.d. ($n = 3$, $^*P < 0.05$, $^{**}P < 0.01$ by one-way ANOVA/Dunnett's post-test *versus* parental cells). (E) HCT116 or HCT Bax- cells were incubated during 42 h with DNA damaging drugs, and calcein (*top*) or $DiOC_6(3)$ (*bottom*) fluorescence was analyzed by flow cytometry. The percentage of calcein low and $DiOC_6(3)$ low cells is specified on each histogram. The data are representative of three independent experiments.

ER stress-dependent opening of PTP and subsequent apoptosis [18], and the fluorescence of calcein and $DiOC_6(3)$ was recorded by flow cytometry. As expected, in response to TG, Bcl-2 strongly inhibited the drop in calcein fluorescence induced by ER stress, whatever its subcellular localization (Fig. 5E). In contrast, in presence of either of the DNA damaging agents, a prevention of PTP opening and $\Delta\Psi_m$ dissipation was observed in Rat-1 Bcl-2 and Bcl-2 mt but not in Rat-1 Bcl-2 ER (Fig. 5E and F). Taken together, these data indicate that in response to DNA cross-linking agents, Bcl-2 has to be localized at the mitochondrion to exert a protective effect against MMP.

3.5. The level of VDAC1 expression modulates DNA cross-linker-induced apoptosis

The demonstration that the PTP plays a role in response to DNA damaging agents led us to determine whether modulation of the level of several PTP regulators (VDAC, ANT and mitochondrial creatine kinase (mtCK)) might potentiate the apoptotic activity of DNA cross-linkers. Therefore, we examined the ability of the pro-apoptotic isoforms of these proteins (*i.e.* VDAC1, mtCK, ANT1 and ANT3) to sensitize HeLa cells to Cisp and MLP, this latter molecule being currently more widely used than HN2 in anticancer

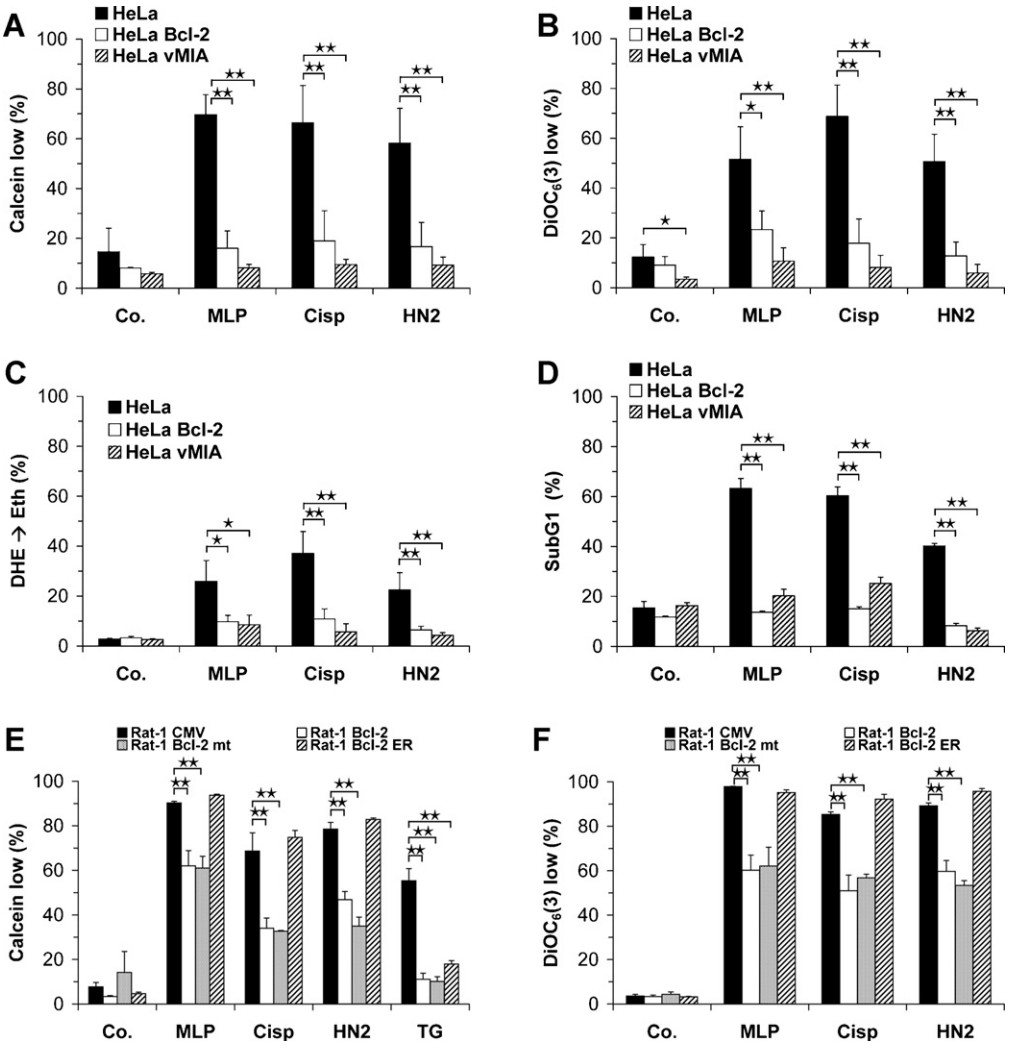

Fig. 5. Bcl-2 and vMIA inhibit PTP opening and apoptosis induced by DNA cross-linkers. Parental HeLa cells or overexpressing either Bcl-2 or vMIA were left untreated (Co.) or treated with DNA damaging drugs. After 24 h of treatment, cells were submitted to flow cytometric analysis of (A) PTP opening (calcein low), (B) $\Delta\Psi_m$ loss ($DiOC_6(3)$ low), (C) ROS production (DHE → Eth) and (D) DNA fragmentation (SubG1). (E and F) Parental Rat-1 cell line (CMV) was compared to Rat-1 cells overexpressing Bcl-2 in whole cell (Bcl-2) or targeted either to the mitochondria (Bcl-2 mt) or to the ER (Bcl-2 ER). After 24 h, cells were analyzed for (E) calcein or (F) $DiOC_6(3)$ fluorescence by flow cytometry. The data represent mean ± s.d ($n = 3$, $^*P < 0.05$, $^{**}P < 0.01$ by one-way ANOVA/Dunnett's post-test *versus* parental cells).

chemotherapy. To this aim, the dissipation of $\Delta\Psi_m$ was evaluated by flow cytometry in cells co-transfected with plasmids encoding the GFP and a member of PTP. In the absence of DNA cross-linkers (white bars), transfection with either VDAC1, mtCK or ANT1 resulted in a slight increase (about 10%) of cells with low $\Delta\Psi_m$ and enforced expression of ANT3 strongly induced MMP *per se* (Fig. 6A). These data corroborate the previously described pro-apoptotic activity of these proteins. Furthermore, we observed that VDAC1 overexpression sensitized HeLa cells to Cisp (hatched bars), while MLP-induced MMP was significantly improved by expression of each PTP regulators (black bars). These results show that, among the different pro-apoptotic PTP modulators tested, increased expression of VDAC1 was able to sensitize HeLa cells to the mitochondrial alterations triggered both by melphalan and cisplatin. In addition, VDAC1 not only promoted MLP- and Cisp-induced mitochondrial alterations in HeLa cells but also in A549 lung carcinoma cells (Fig. 6B), indicating that the sensitization by VDAC1 to DNA cross-linkers is not cell-specific. As shown in Fig. 6C, enforced expression of VDAC1 increased the percentage of dead cells (FDA negative) induced by DNA cross-linkers, demonstrating that the sensitizing effect of VDAC1 overexpression was not restricted to mitochondrial events. The importance of VDAC1 in the apoptotic process triggered by DNA cross-linkers was reinforced by RNA interference experiments. Indeed, knockdown of VDAC1 with shRNAmir (inset in Fig. 6D, shVDAC1) reduced mitochondrial alterations by more than 50% as compared to scrambled shRNAmir (shSCR, Fig. 6D). Taken together, these results identify VDAC1 as a prominent target to enhance the efficiency of Cisp and MLP treatments of cancer cells.

3.6. Pharmacological increase of VDAC1 expression level promotes the apoptotic activity of DNA cross-linkers

Since exogenous overexpression of VDAC1 increased the sensitivity of carcinoma cells to MLP and Cisp treatments, we next investigated whether a pharmacologically induced augmentation of the expression level of VDAC1 would also promote the apoptotic response to DNA cross-linking agents. Interestingly, the antineoplastic chemotherapeutic agent arsenic trioxide (As_2O_3), which has received U.S. Food and Drug Administration approval for the treatment of acute promyelocytic leukemia [24] and is under investigation in clinical trials for the treatment of multiple myeloma and solid tumors including bladder cancer, glioma, breast cancer, cervical cancer, colorectal cancer, germ cell tumors,

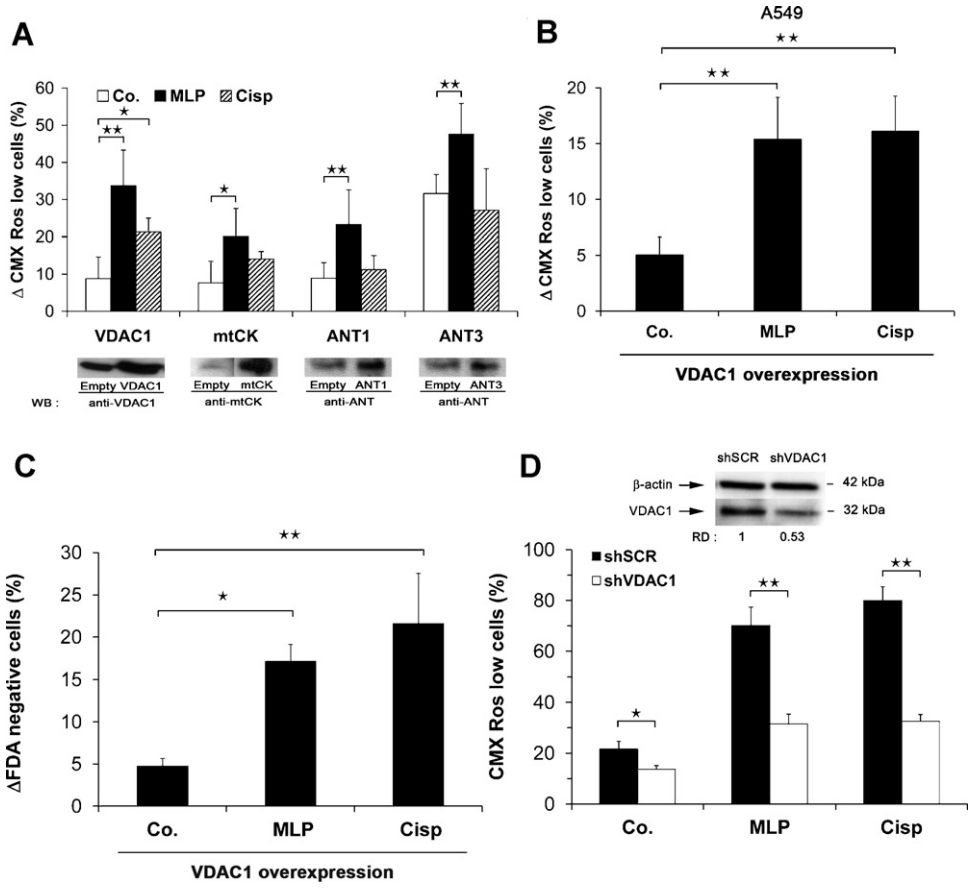

Fig. 6. The expression level of VDAC1 modulates the apoptotic response of cells to DNA cross-linkers. (A) HeLa cells were transiently co-transfected with plasmids encoding a member of PTP and the GFP protein. After 48 h, cells were incubated for 24 h without (Co.) or with MLP or Cisp, stained with CMXRos for flow cytometry analysis or lysed for western blot investigations. For the determination of $\Delta\Psi_m$ loss, analysis was gated on GFP-positive cells. Results are expressed as the percentage of ΔCMXRos low cells as described in materials and methods. The data represent mean ± s.d. ($n = 4$; $^*P < 0.05$, $^{**}P < 0.01$ compared to PTP member alone by one-way ANOVA/Dunnett's post-test). (B) A549 cells were transiently co-transfected with plasmids encoding VDAC1 and the GFP protein. After 48 h, cells were incubated for 24 h without (Co.) or with MLP or Cisp and stained with CMXRos for flow cytometry analysis. Results are expressed as the percentage of ΔCMXRos low cells ($n = 3$; $^*P < 0.05$, $^{**}P < 0.01$ compared to VDAC1 alone (Co.) by one-way ANOVA/Dunnett's post-test). (C) HeLa cells were transiently co-transfected with plasmids encoding VDAC1 and the turboRFP protein for 48 h and then incubated 24 h in the absence (Co.) of the presence of MLP and Cisp, stained with the viability marker FDA and analyzed by flow cytometry. Analysis was gated on turboRFP-positive cells. Results are expressed as the percentage of ΔFDA negative cells (dead cells) as described in materials and methods. The data represent mean ± s.d. ($n = 3$; $^*P < 0.05$, $^{**}P < 0.01$ compared to VDAC1 alone (Co.) by one-way ANOVA/Dunnett's post-test). (D) HeLa cells were co-transfected with plasmids encoding scrambled (shSCR) or VDAC1 (shVDAC1) shRNAmir and the GFP protein for 72 h, lysed for western blot analysis (inset, RD: relative density) or incubated 24 h more without (Co.) or with MLP or Cisp and then stained with CMXRos for flow cytometry analysis The data represent mean ± s.d. ($n = 3$; $^*P < 0.05$, $^{**}P < 0.01$ compared to scrambled shRNAmir (shSCR) by Student's t-test).

liver cancer, lung cancer and melanoma [25], has been demonstrated to increase VDAC1 expression and oligomerization by two independent groups [26,27]. As shown in Fig. 7A, we also observed by western blot that arsenic trioxide used at 20 μM (Ars) clearly increased the expression level of VDAC1 protein in HeLa cells without inducing notable mitochondrial alterations, ROS production or DNA fragmentation. Consequently, we then examined whether Ars has the ability to modify the apoptotic response to MLP and Cisp treatments. To mimic a resistance of HeLa cells to DNA cross-linkers, suboptimal concentrations of MLP (10 μM) and Cisp (7.5 μM) were used. As expected, these low concentrations of DNA cross-linkers only slightly induced loss of $\Delta\Psi_m$ (Fig. 7B and C), ROS generation (Fig. 7D and E) and DNA fragmentation (Fig. 7F and G), leading to less than 8% of augmentation when compared to control cells. By contrast, the cotreatment of cells with Ars in combination with either MLP or Cisp stimulated more than a 6-fold increase in mitochondrial alterations (dissipation of $\Delta\Psi_m$ and ROS production) and more than a 1.5-fold augmentation of the percentage of cells in SubG1. These findings demonstrate a synergistic amplification of the apoptotic process when Ars is used in combination with the DNA cross-linkers and strongly suggest that As_2O_3-mediated increase of VDAC1 expression greatly sensitizes carcinoma cells to MLP and Cisp.

4. Discussion

Our results reveal a role of PTP in the apoptotic pathway triggered by different genotoxic agents widely used in chemotherapy. We demonstrated that DNA cross-linkers induce a mitochondrion-controlled apoptotic process orchestrated by PTP. Thus, in HeLa and HCT116 cell lines, Cisp, HN2 and MLP cause PTP opening with dissipation of $\Delta\Psi_m$, production of ROS, release of apoptogenic factors, caspase activation and DNA fragmentation (Figs. 1 and 2). Interestingly, the role of PTP in response to DNA damage is further illustrated by the demonstration that although the chemotherapeutic agents used herein generate DNA adducts by different mechanisms (platination for Cisp and alkylation for HN2 and MLP), they ultimately lead to activation of PTP opening and apoptosis. Nevertheless, molecular mechanisms other than generation of DNA adducts might also trigger PTP activation. Indeed, cisplatin and the other platinum compound oxaliplatin were demonstrated to induce MMP and apoptosis in enucleated HCT116 cells by two independent groups, indicating that these agents can initiate apoptosis independently of their DNA damaging effects by targeting a cellular compartment other than the nucleus [28,29], such as the endoplasmic reticulum or the mitochondrion [17,29]. Therefore, PTP could be directly activated by cisplatin, a hypothesis

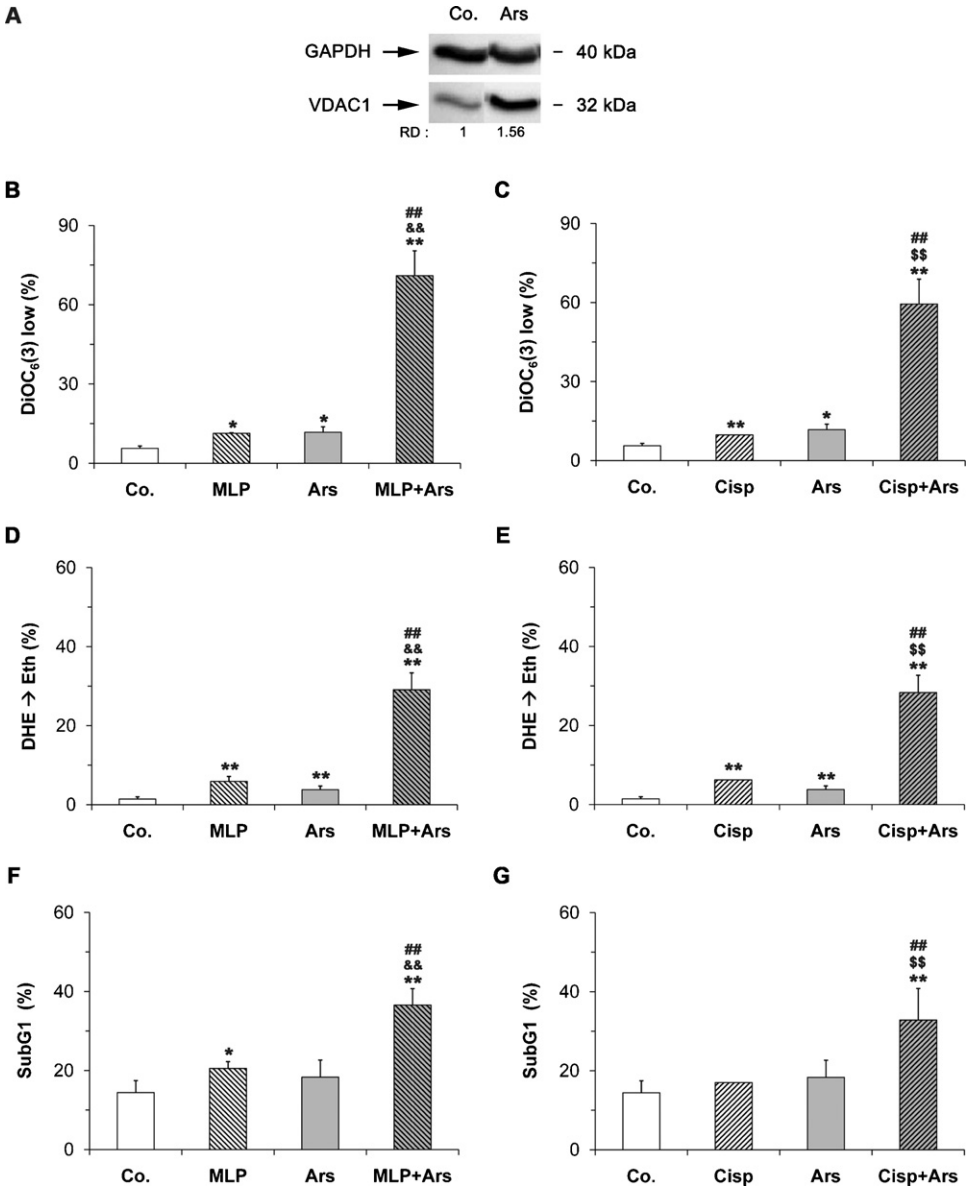

Fig. 7. Pharmacological increase of VDAC1 expression level sensitizes HeLa cells to mitochondrial alterations and nuclear apoptosis induced by MLP and Cisp. (A) Total protein extracts from HeLa cells treated or not for 24 h with 20 μM Ars were immunoblotted with anti-VDAC1 and anti-GAPDH antibodies. RD: relative density as described in materials and methods. HeLa cells treated with 10 μM MLP, 7.5 μM Cisp or 20 μM Ars alone and in combination (MLP + Ars or Cisp + Ars) were analyzed by flow cytometry for (B and C) $\Delta\Psi_m$ dissipation, (D and E) ROS production and (F and G) DNA fragmentation (SubG1). The data represent mean ± s.d ($n = 3$; $^{*}P < 0.05$, $^{**}P < 0.01$ compared to control; $^{\#\#}P < 0.01$ compared to Ars alone; $^{\&\&}P < 0.01$ compared to MLP alone; $^{\$\$}P < 0.01$ compared to Cisp alone by Student's *t*-test).

reinforced by the observation that, among the mitochondrial membrane proteins, this chemotherapeutic agent preferentially binds the PTP regulator VDAC [17], but this requires further investigations.

We also showed that knockout or neutralization by vMIA [30] of the pro-apoptotic protein Bax impedes the opening of PTP, the $\Delta\Psi_m$ loss and the DNA fragmentation induced by DNA cross-linkers (Figs. 4 and 5). Nevertheless, we observed that the protection conferred by the knockout of Bax was not long-lasting (Fig. 4E), suggesting that Bax deficiency delays but does not prevent the opening of PTP. Therefore, we can assume that Bax favors the opening of PTP, but is not essential to PTP-dependent MMP during DNA cross-linker-induced apoptosis. Such Bax-mediated activation of PTP has been proposed to result from the interaction of Bax with the PTP proteins VDAC [31] or ANT [32]. Conversely, no involvement of Bak was found in cross-linker-mediated mitochondrial apoptosis, the Bak-deficient cancer cells being as responsive as the parental cells to MLP, Cisp and HN2. This differential role of Bax and Bak was previously observed in response to other genotoxic agents by two independent groups who reported that HCT116 cells deficient for Bax, but not for Bak, are more resistant to apoptosis induced by the DNA alkylating agent irinotecan [33], and that knockdown of Bax by RNA interference increases the resistance of HCT116 cells to the cisplatin analogue oxaliplatin, whereas Bak knockdown has no effect [28]. The absence of Bak effect in HCT116 cells in response to DNA damaging agents could be due to its sequestration by the mitochondrial VDAC2, a VDAC isoform that specifically interacts with the inactive conformer of Bak and prevents its activation [12]. The overexpression of the anti-apoptotic protein Bcl-2, which inhibits the opening of PTP and the subsequent mitochondrial and nuclear alterations (Fig. 5), greatly improves the resistance of HeLa cells to DNA cross-linkers. Since Bcl-2 has also been shown to exert indirect effects on mitochondria via the inhibition of apoptotic

signals originating from ER [23,34], we tested the importance of the subcellular localization of Bcl-2 in its protective activity. A prevention of DNA cross-linker-induced PTP opening and $\Delta\Psi_m$ drop was observed in cells with mitochondria-restricted Bcl-2, but not in cells with ER-restricted Bcl-2, demonstrating that only a rigorous mitochondrial localization of Bcl-2 conferred a protection against PTP-dependent MMP in response to DNA damaging agents.

The demonstration of the central role of PTP and of the importance of its regulation by Bcl-2 family members in the response of different carcinoma cells to DNA cross-linker-induced MMP and apoptosis led us to evaluate the capacity of pro-apoptotic PTP regulators (VDAC1, mtCK, ANT1 and ANT3) to sensitize cancer cells to Cisp and MLP treatments. We demonstrated that mitochondrial alterations induced by Cisp were significantly increased by exogenous VDAC1 overexpression. In response to MLP treatment, although all the PTP members tested sensitize cancer cells to mitochondrial alterations, the overexpression of VDAC1 was the most effective in enhancing MLP-induced MMP (Fig. 6). In addition, we showed that knockdown of VDAC1 with shRNAmir greatly impaired the pro-apoptotic effects of MLP and Cisp (Fig. 6D). These results thus support our hypothesis that PTP regulators, and in particular VDAC1, are valuable targets to improve Cisp- and MLP-based chemotherapy. This is consistent with recent observations of Tajeddine et al., who demonstrated that knockdown of VDAC1 is particularly efficient in preventing cisplatin-induced mitochondrial apoptosis in A549 and HeLa cells and that pharmacological inhibition of Bcl-2/Bcl-X_L restores the sensitivity of VDAC1-depleted cells to cisplatin [35]. To explain the poorly understood mechanism by which VDAC1 contributes to cisplatin-induced apoptosis, they suggest that VDAC1 might be an activator of the pro-apoptotic protein Bax (but not of Bak). The biochemical mechanisms underlying the VDAC1-mediated activation of Bax are still unresolved and require further investigations. Nevertheless, Strasser et al. [36] have recently proposed a new model of Bax activation namely the priming-capture-displacement model. In this model, direct activation by BH3-only proteins (*e.g.* Bim, tBid, PUMA) generates primed Bax, which is immediately captured by anti-apoptotic members (such as Bcl-2), until BH3-only proteins displace it, allowing primed Bax to form oligomers and induce MMP. Therefore, it is tempting to assume that Bax/VDAC1 interaction could also displace anti-apoptotic proteins from primed Bax allowing Bax oligomerization and MMP induction, a hypothesis that remains to be experimentally validated. Besides, the findings that cisplatin preferentially binds to VDAC in the mitochondrial membranes have led to the proposition that Cisp binding to this PTP core protein could significantly alter its structure or function, facilitating MMP and subsequent apoptosis [17].

Considering the finding that VDAC1 appears rate-limiting in Cisp- and MLP-induced apoptosis, we postulated that a pharmacologically induced increase of VDAC1 expression level might be effective in sensitizing cancer cells to DNA cross-linkers. Therefore, we chose to focus our research on the effect of the combined treatment of As_2O_3 with DNA cross-linkers. Indeed, As_2O_3, which is approved for treatment of relapsed and refractory acute promyelocytic leukemia [24], has shown promising activity against solid tumors in many clinical trials [25]. In addition, As_2O_3 has been demonstrated to increase VDAC1 expression and oligomerization in multiple myeloma [27], HeLa and HEK cells [26]. Nevertheless, the apoptotic potential of As_2O_3 was also attributed to its ability to increase ROS production in cells [37] or to target PTP [38]. Therefore, in our experiments, this molecule was used at a concentration that induced an increase of VDAC1 protein level without triggering a notable generation of ROS, MMP or nuclear fragmentation (Fig. 7). The resistance of HeLa cells to DNA cross-linkers was mimicked by incubating cells with suboptimal concentrations of MLP and Cisp, which *per se* induced only few mitochondrial and nuclear alterations (Fig. 7). In these conditions, we demonstrated that the combined treatment of DNA damaging agents with As_2O_3 greatly sensitizes carcinoma cells to dissipation of $\Delta\Psi_m$, ROS production and DNA fragmentation. Although the mechanisms by which the As_2O_3-increased expression of VDAC1 synergistically enhances sensitivity of carcinoma cells to DNA cross-linkers need further studies, Keinan et al. [26] proposed that VDAC1 homo-oligomers induced by As_2O_3, or other molecules such as staurosporine or selenite, could form large pores in the outer mitochondrial membrane, thus mediating an alternative route for cytochrome *c* release. However, the question arises of whether VDAC1 upregulation mediated by arsenite might also cause DNA cross-linker-induced toxicity in normal tissues. Despite the necessity to investigate this issue *in vivo*, it has very recently been reported in an *in vivo* model of epithelial ovarian cancer, that arsenite sensitizes cancer cells to cisplatin by enhancing tumor platinum uptake [39], suggesting that tumors could be more sensitive to arsenite/cisplatin treatment than normal tissues.

In conclusion, our results demonstrate that PTP regulators represent interesting targets to sensitize cancer cells to DNA cross-linkers. Although the subtle differences of sensitizing capacities of PTP members remain to investigate, molecules that increase expression of VDAC1 hold promise as a way to overcome inherited or acquired resistance to DNA damaging agents for cancer treatment. Consequently, the development of small organic compounds or the design of therapeutic peptides truly selective for VDAC1 is of great interest in order to enhance the chemotherapeutic activity of DNA cross-linking agents.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgments

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References

[1] Kohn KW. Beyond DNA cross-linking: history and prospects of DNA-targeted cancer treatment—fifteenth Bruce F. Cain Memorial Award Lecture. Cancer Res 1996;56:5533–46.
[2] Sarkaria JN, Kitange GJ, James CD, Plummer R, Calvert H, Weller M, et al. Mechanisms of chemoresistance to alkylating agents in malignant glioma. Clin Cancer Res 2008;14:2900–8.
[3] Siddik ZH. Cisplatin: mode of cytotoxic action and molecular basis of resistance. Oncogene 2003;22:7265–79.
[4] Bellamy WT, Dalton WS, Gleason MC, Grogan TM, Trent JM. Development and characterization of a melphalan-resistant human multiple myeloma cell line. Cancer Res 1991;51:995–1002.
[5] Henkels KM, Turchi JJ. Cisplatin-induced apoptosis proceeds by caspase-3-dependent and -independent pathways in cisplatin-resistant and -sensitive human ovarian cancer cell lines. Cancer Res 1999;59:3077–83.
[6] Sourdeval M, Lemaire C, Deniaud A, Taysse L, Daulon S, Breton P, et al. Inhibition of caspase-dependent mitochondrial permeability transition protects airway epithelial cells against mustard-induced apoptosis. Apoptosis 2006;11:1545–59.
[7] Kroemer G, Galluzzi L, Brenner C. Mitochondrial membrane permeabilization in cell death. Physiol Rev 2007;87:99–163.
[8] Brenner C, Grimm S. The permeability transition pore complex in cancer cell death. Oncogene 2006;25:4744–56.
[9] Bauer MK, Schubert A, Rocks O, Grimm S. Adenine nucleotide translocase-1, a component of the permeability transition pore, can dominantly induce apoptosis. J Cell Biol 1999;147:1493–502.

[10] Shimizu S, Matsuoka Y, Shinohara Y, Yoneda Y, Tsujimoto Y. Essential role of voltage-dependent anion channel in various forms of apoptosis in mammalian cells. J Cell Biol 2001;152:237–50.
[11] Zamora M, Granell M, Mampel T, Vinas O. Adenine nucleotide translocase 3 (ANT3) overexpression induces apoptosis in cultured cells. FEBS Lett 2004;563: 155–60.
[12] Cheng EH, Sheiko TV, Fisher JK, Craigen WJ, Korsmeyer SJ. VDAC2 inhibits BAK activation and mitochondrial apoptosis. Science 2003;301:513–7.
[13] Gallerne C, Touat Z, Chen ZX, Martel C, Mayola E, Sharaf el dein O, et al. The fourth isoform of the adenine nucleotide translocator inhibits mitochondrial apoptosis in cancer cells. Int J Biochem Cell Biol 2010;42:623–9.
[14] Le Bras M, Borgne-Sanchez A, Touat Z, El Dein OS, Deniaud A, Maillier E, et al. Chemosensitization by knockdown of adenine nucleotide translocase-2. Cancer Res 2006;66:9143–52.
[15] De Pinto V, Guarino F, Guarnera A, Messina A, Reina S, Tomasello FM, et al. Characterization of human VDAC isoforms: a peculiar function for VDAC3. Biochim Biophys Acta 2010;1797:1268–75.
[16] Shoshan-Barmatz V, De Pinto V, Zweckstetter M, Raviv Z, Keinan N, Arbel N. VDAC, a multi-functional mitochondrial protein regulating cell life and death. Mol Aspects Med 2010;31:227–85.
[17] Yang Z, Schumaker LM, Egorin MJ, Zuhowski EG, Guo Z, Cullen KJ. Cisplatin preferentially binds mitochondrial DNA and voltage-dependent anion channel protein in the mitochondrial membrane of head and neck squamous cell carcinoma: possible role in apoptosis. Clin Cancer Res 2006;12:5817–25.
[18] Deniaud A, Sharaf el dein O, Maillier E, Poncet D, Kroemer G, Lemaire C, et al. Endoplasmic reticulum stress induces calcium-dependent permeability transition, mitochondrial outer membrane permeabilization and apoptosis. Oncogene 2008;27:285–99.
[19] Belzacq AS, El Hamel C, Vieira HL, Cohen I, Haouzi D, Metivier D, et al. Adenine nucleotide translocator mediates the mitochondrial membrane permeabilization induced by lonidamine, arsenite and CD437. Oncogene 2001;20:7579–87.
[20] Le Bras M, Clement MV, Pervaiz S, Brenner C. Reactive oxygen species and the mitochondrial signaling pathway of cell death. Histol Histopathol 2005;20: 205–19.
[21] Goldmacher VS, Bartle LM, Skaletskaya A, Dionne CA, Kedersha NL, Vater CA, et al. A cytomegalovirus-encoded mitochondria-localized inhibitor of apoptosis structurally unrelated to Bcl-2. Proc Natl Acad Sci USA 1999;96:12536–41.
[22] Zamzami N, Brenner C, Marzo I, Susin SA, Kroemer G. Subcellular and submitochondrial mode of action of Bcl-2-like oncoproteins. Oncogene 1998;16: 2265–82.
[23] Thomenius MJ, Wang NS, Reineks EZ, Wang Z, Distelhorst CW. Bcl-2 on the endoplasmic reticulum regulates Bax activity by binding to BH3-only proteins. J Biol Chem 2003;278:6243–50.
[24] Cohen MH, Hirschfeld S, Flamm Honig S, Ibrahim A, Johnson JR, O'Leary JJ, et al. Drug approval summaries: arsenic trioxide, tamoxifen citrate, anastrazole, paclitaxel, bexarotene. Oncologist 2001;6:4–11.
[25] Emadi A, Gore SD. Arsenic trioxide—an old drug rediscovered. Blood Rev 2010;24:191–9.
[26] Keinan N, Tyomkin D, Shoshan-Barmatz V. Oligomerization of the mitochondrial protein voltage-dependent anion channel is coupled to the induction of apoptosis. Mol Cell Biol 2010;30:5698–709.
[27] Zheng Y, Shi Y, Tian C, Jiang C, Jin H, Chen J, et al. Essential role of the voltage-dependent anion channel (VDAC) in mitochondrial permeability transition pore opening and cytochrome c release induced by arsenic trioxide. Oncogene 2004;23:1239–47.
[28] Gourdier I, Crabbe L, Andreau K, Pau B, Kroemer G. Oxaliplatin-induced mitochondrial apoptotic response of colon carcinoma cells does not require nuclear DNA. Oncogene 2004;23:7449–57.
[29] Mandic A, Hansson J, Linder S, Shoshan MC. Cisplatin induces endoplasmic reticulum stress and nucleus-independent apoptotic signaling. J Biol Chem 2003;278:9100–6.
[30] Poncet D, Larochette N, Pauleau AL, Boya P, Jalil AA, Cartron PF, et al. An anti-apoptotic viral protein that recruits Bax to mitochondria. J Biol Chem 2004;279:22605–14.
[31] Shimizu S, Ide T, Yanagida T, Tsujimoto Y. Electrophysiological study of a novel large pore formed by Bax and the voltage-dependent anion channel that is permeable to cytochrome c. J Biol Chem 2000;275:12321–5.
[32] Marzo I, Brenner C, Zamzami N, Jurgensmeier JM, Susin SA, Vieira HL, et al. Bax and adenine nucleotide translocator cooperate in the mitochondrial control of apoptosis. Science 1998;281:2027–31.
[33] Wang S, El-Deiry WS. Requirement of p53 targets in chemosensitization of colonic carcinoma to death ligand therapy. Proc Natl Acad Sci USA 2003;100: 15095–100.
[34] Lam M, Dubyak G, Chen L, Nunez G, Miesfeld RL, Distelhorst CW. Evidence that BCL-2 represses apoptosis by regulating endoplasmic reticulum-associated Ca2+ fluxes. Proc Natl Acad Sci USA 1994;91:6569–73.
[35] Tajeddine N, Galluzzi L, Kepp O, Hangen E, Morselli E, Senovilla L, et al. Hierarchical involvement of Bak, VDAC1 and Bax in cisplatin-induced cell death. Oncogene 2008;27:4221–32.
[36] Strasser A, Cory S, Adams JM. Deciphering the rules of programmed cell death to improve therapy of cancer and other diseases. EMBO J 2011;30:3667–83.
[37] Chen YC, Lin-Shiau SY, Lin JK. Involvement of reactive oxygen species and caspase 3 activation in arsenite-induced apoptosis. J Cell Physiol 1998;177: 324–33.
[38] Larochette N, Decaudin D, Jacotot E, Brenner C, Marzo I, Susin SA, et al. Arsenite induces apoptosis via a direct effect on the mitochondrial permeability transition pore. Exp Cell Res 1999;249:413–21.
[39] Muenyi CS, States VA, Masters JH, Fan TW, Helm CW, States JC. Sodium arsenite and hyperthermia modulate cisplatin-DNA damage responses and enhance platinum accumulation in murine metastatic ovarian cancer xenograft after hyperthermic intraperitoneal chemotherapy (HIPEC). J Ovarian Res 2011;4:9.