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Original Articles

Identification of Smac mimetics as novel substrates for p-glycoprotein

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ABSTRACT

Multidrug resistance (MDR) in cancer patients undergoing chemotherapy is preventing effective treatment of multiple cancer types including pediatric tumors. Resistance to chemotherapeutic drugs in cancer cells is frequently associated with high expression of p-glycoprotein, a transporter in the plasma membrane that can mediate cellular drug export. Here, we generated pediatric cancer cells with acquired resistance to the chemotherapeutic drug vincristine (VCR). In these cells, acquired resistance is associated with increased expression of p-glycoprotein. VCR-resistant cells display an MDR phenotype and have acquired resistance to multiple other chemotherapeutic drugs including doxorubicin (DOXO) and etoposide (ETO). Notably, we discovered that these cells also display cross-resistance with several Smac mimetics, a novel class of experimental cancer therapeutics designed to induce apoptosis by inhibiting Inhibitor of Apoptosis (IAP) proteins. Resistance to Smac mimetics is reversible in the presence of p-glycoprotein inhibitors, highlighting Smac mimetics as novel substrates for p-glycoprotein. The identification of Smac mimetics as substrates for p-glycoproteins may influence the design of future clinical trials to prevent usage of Smac mimetics in the context of MDR or, alternatively, combine Smac mimetics with p-glycoprotein inhibitors to maximize their efficiency.

1. Introduction

The occurrence of acquired drug resistance severely impairs the successful treatment of cancer with chemotherapeutic drugs [1,2]. Cancer cells can develop resistance to anti-cancer drugs via two different mechanisms. Firstly, the intracellular drug concentration can be decreased, e.g. by enhancing cellular drug efflux. Secondly, intracellular signaling pathways can be altered which can cause drug resistance even though adequate intracellular drug levels have been achieved [3].

Cancer cells can extrude chemotherapeutic drugs by hijacking cellular systems used in non-malignant cells for extrusion of metabolic products and detoxification [4]. P-glycoprotein was the first member of the ATP-binding cassette sub-family B (ABC) transporter family to be discovered in cancer [5–8]. Many tumor cells express p-glycoprotein, and some carry genetic amplifications of the underlying gene MDR1 [9,10]. P-glycoprotein is localized in the plasma membrane and can pump substrates out of the cell in an ATP-dependent manner [11]. It has a broad affinity to various substrates including chemotherapeutic agents like DOXO, ETO, VCR and glucocorticoids [12]. Hence, chemotherapy-resistant tumors that display increased p-glycoprotein expression can acquire cross-resistance to multiple other, structurally unrelated drugs, which leads to MDR.

Inhibition of p-glycoprotein or similar ABC-transporters using small-molecule inhibitors that modulate drug efflux has long been discussed as a promising strategy to combat MDR in cancer [13]. Several pharmacological inhibitors have been developed, including first-generation compounds (e.g. Verapamil [14]), second-generation compounds (e.g. PSC833/Valspodar [15]) and third-generation compounds (e.g. Tariquidar [16]). However, the results of clinical trials aiming to use p-glycoprotein inhibitors to reverse drug resistance have overall been disappointing [17], which is mainly due to the highly toxic effects exerted by these inhibitors on normal cells, either directly or indirectly by decreasing the elimination of the anti-cancer drugs and hence increasing their toxicity on normal tissues when administered in combination [18]. Toxic effects on normal tissues could be overcome by cancer-specific drug delivery systems, which are currently under development.

Abbreviations: 5-FU, 5-Fluoruracil; ABC, ATP-binding cassette sub-family B; cIAP1, cellular IAP1; DOXO, doxorubicin; ETO, etoposide; FCS, fetal calf serum; IAP, Inhibitor of Apoptosis; MDR, multidrug resistance; MFI, mean fluorescence intensity; MRP1, MRD-associated protein 1; MTX, Methotraxate; PI, propidium iodide; RMS, rhabdomyosarcoma; UT, untreated; VCR, vincristine

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The occurrence of MDR in cancer patients undergoing chemotherapy can be mimicked in the laboratory by culturing cancer cells in the presence of chemotherapeutic drugs, thereby enforcing the evolution of acquired drug resistance. This has been described in multiple model systems including the rhabdomyosarcoma (RMS) cell line RD which develops resistance to VCR due to upregulation of p-glycoprotein [19]. In addition to p-glycoprotein, also several other ABC transporters have been found to be overexpressed in cancer cell lines cultured under selective pressure, including MRD-associated protein 1 (MRP1) [20].

Here, we generated pediatric cancer cell lines that display acquired resistance to VCR and concomitant upregulation of p-glycoprotein. Interestingly, we discovered that these cells display cross-resistance with several Smac mimetics, highlighting Smac mimetics as novel substrates for p-glycoprotein.

2. Material and methods

2.1. Chemicals

The Smac mimetics BV6, LCL161 and birinapant were purchased from Selleck Chemicals (Houston, TX, USA). Unless otherwise indicated, all other chemicals were purchased from Sigma (Deisenhofen, Germany).

2.2. Generation and culture of VCR-resistant cells

RD and SH-EP cells were obtained from the American Type Culture Collection (Manassas, VA, USA) and cultured in DMEM medium (Life Technologies, Inc., Darmstadt, Germany), supplemented with 10% fetal calf serum (FCS) (Biochrom, Berlin, Germany), 1% penicillin/streptomycin and 1 mM sodium pyruvate (Invitrogen, Karlsruhe, Germany). To generate cells with resistance to VCR cells were cultured in continuously increasing concentrations of VCR, starting with 1 nM. Once cells survived, doses were increased until resistance to 10 nM VCR was achieved (rVCR). rVCR RD cells were cultured continuously in 10 nM VCR and rVCR SH-EP cells in 15 nM of VCR.

2.3. Flow cytometry, microscopy and cell death assays

Surface staining for p-glycoprotein was performed on viable cells stained with 2 µl of MRK16 antibody (Kamiya) in PBS plus 2% FCS followed by staining with anti-mouse-IgG-PE (F0102B, R&D Systems) and flow cytometry. IgG2a (555571, BD Pharmingen) was used as isotype control. For determination of cell death, cells were seeded at 0.2×10^5 cells/cm^2 in a 96-well plate, allowed to settle overnight and treated with indicated drug concentrations. Cell death was determined by fluorescence microscopy of propidium iodide (PI)-stained nuclei using ImageXpress Micro XLS system (Molecular Devices, Biberach an der Riss, Germany). After indicated treatment times, cells were incubated with 10 µg/ml Hoechst 33342 and 1 µg/ml PI for 10–15 min at 37 °C with 5% CO_2 and cell death was visualized by fluorescence microscopy. For analysis of DNA fragmentation, cells were seeded in a 24-well plate, allowed to settle overnight and treated with indicated drug concentrations. After indicated treatment times, cells were washed with ice-cold PBS, resuspended in a buffer containing 0.05% trisodium citrate dihydrate pH 7.4, 0.05% Triton X-100, 50 µg/ml PI, incubated at 4 °C for 1 h and apoptosis was determined by flow cytometry (FACSCanto II, BD Biosciences, Heidelberg, Germany). Cell viability was determined by MTT assay according to the manufacturer's instructions (Roche Diagnostics, Mannheim, Germany). Caspase activity was assessed using Cell Event Caspase-3/7 Green Detection Reagent (Thermo Fisher).

2.4. Western blotting

Western blot analysis was performed using the following antibodies: p-glycoprotein (Cell Signaling, Beverly, MA, USA), cIAP1 (R&D Systems, Minneapolis, MS, USA), β-actin (Sigma) and GAPDH (HyTest, Turku, Finland). Infrared dye-labeled secondary antibodies and infrared imaging (Odyssey Imaging System, LICOR Bioscience, Bad Homburg, Germany) or enhanced chemiluminescence (Amersham Bioscience, Freiburg, Germany) were used for detection.

2.5. Statistical analysis

Statistical significance was assessed by Student's *t*-Test (two-tailed distribution, two-sample, unequal variance).

3. Results

3.1. Generation and characterization of pediatric cancer cell lines with acquired chemoresistance

To investigate drug resistance in pediatric tumors we generated SH-EP neuroblastoma and RD RMS cells that display resistance to the chemotherapeutic drug VCR. Continuous exposure of cells to increasing concentrations of VCR resulted in acquired resistance to VCR (Fig. 1A and B). VCR is a known substrate for the ABC-transporter p-glycoprotein/MDR1 [21]. In the case of RD cells, this selection resulted in reduced proliferation (population doubling time of 28 h for parental and 55 h for rVCR cells), but in the case of SH-EP cells, proliferation was only weakly affected (population doubling time of 23 h for parental and 28 h for rVCR cells). Since acquired resistance to VCR is often associated with upregulation of p-glycoprotein [22], we investigated expression levels of p-glycoprotein in parental and drug-resistant cells. Indeed, both SH-EP and RD cells rapidly upregulated p-glycoprotein upon continuous exposure to VCR as assessed by Western blotting or surface expression (Fig. 1C and D). This indicates that the observed resistance to VCR may be caused by an upregulation of p-glycoprotein and subsequently increased drug efflux. To assess intracellular drug accumulation, we used Doxorubicin (DOXO), a well-known p-glycoprotein substrate that exhibits red auto-fluorescence. Interestingly, VCR-resistant SH-EP and RD cells displayed reduced fluorescence upon DOXO treatment as compared to parental cells (Fig. 1E), indicating increased drug efflux or decreased drug uptake in the VCR-resistant cells.

3.2. Chemoresistant cells display cross-resistance with Smac mimetics

Next, we investigated the viability of VCR-resistant cells upon treatment with several chemotherapeutic drugs that are known p-glycoprotein substrates as well as experimental drugs. Both SH-EP and RD cells that are resistant to VCR also displayed reduced loss of viability to DOXO and ETO, indicating cross-resistance with other chemotherapeutic drugs. Interestingly, VCR-resistant cells also showed significant resistance towards the Smac mimetic BV6 with no loss of viability at concentrations that induced a profound loss of viability in the parental cells (Fig. 2A). To investigate whether intrinsic apoptosis is reduced in VCR-resistant subclones due to a general defect in the apoptotic signaling cascade, we included drugs that induce apoptosis but are not a substrate for p-glycoprotein. By comparison, sensitivity to 5-Fluoruracil (5-FU) or Methotraxate (MTX) was not affected by resistance to VCR, in line with the notion that these drugs are no substrates for p-glycoprotein and that p-Glycoprotein mediates apoptosis resistance in the VCR-resistant cells.

To confirm cross-resistance of VCR-resistant cells with the Smac mimetic BV6 we used additional assays to assess cell death. To this end, we investigated the effect of VCR and BV6 on PI uptake as a marker of cell death and on DNA fragmentation, a specific phenotype of apoptotic

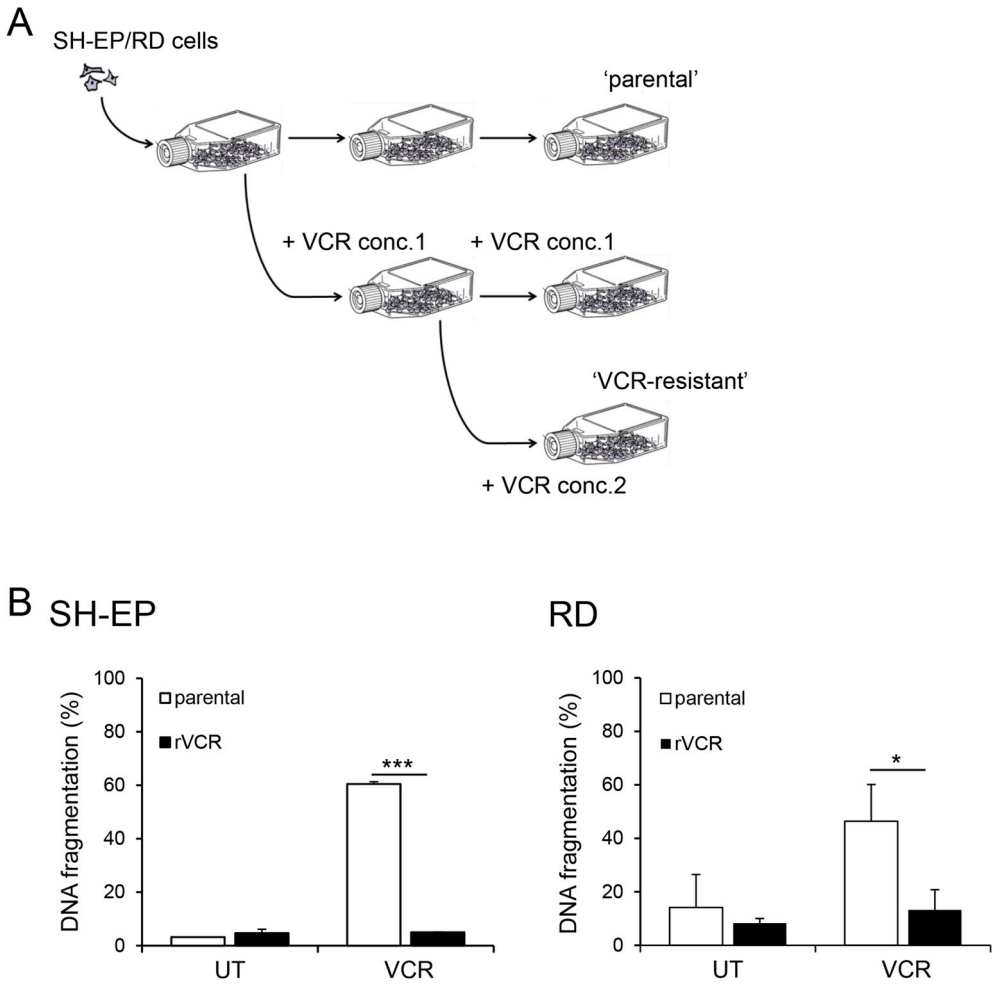

Fig. 1. Generation of VCR-resistant pediatric tumor cells. **A.** Generation of SH-EP neuroblastoma and RD RMS cells with acquired resistance to VCR by continuous culture of cells with increasing concentrations of VCR. **B.** SH-EP (left) or RD (right) cells were either left untreated (UT) or incubated with 10 nM of VCR for 72 h before analysis of apoptosis using PI staining and flow cytometric analysis of DNA fragmentation (data shown are mean + SD, n = 4). **C.** Expression of p-glycoprotein was examined by Western blotting in parental and rVCR SH-EP and RD cells. **D.** Surface expression of p-glycoprotein (solid lines) was assessed by staining of viable cells with anti-p-glycoprotein (MRK16) antibody and flow cytometry. As controls, no primary antibody (dotted lines) or IgG2a isotype antibody (dashed lines) were included. **E.** Parental and rVCR SH-EP (left) and RD (right) cells were incubated with DOXO (0.05 μg/ml) for 24 h. Red fluorescence (PE channel) was measured using flow cytometry. The upper graph displays the quantification of PE mean fluorescence intensity (MFI), n = 3. The lower graph displays an example of the histograms. *, $p < 0.05$, **, $p < 0.01$, ***, $p < 0.001$. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

cells. PI uptake (Fig. 2B) as well as DNA fragmentation (Fig. 2C) were significantly reduced in VCR-resistant cells as compared to parental SH-EP or RD cells.

Smac mimetics lead to cell death by inducing proteasomal degradation and protein loss of the IAP protein cellular IAP1 (cIAP1) [23]. Therefore, we monitored cIAP1 expression as a pharmacodynamic marker of BV6's intracellular activity. Interestingly, loss of cIAP1 protein expression occurred rapidly upon BV6 treatment in parental cells, but was significantly delayed in VCR-resistant cells (Fig. 3A and B). This indicates that cIAP1 protein is less inhibited in VCR-resistant cells than in parental cells, which may be explained by a reduced activity of BV6 due to increased drug efflux via p-glycoprotein. To assess whether IAP degradation results in apoptosis or another form of cell death, we asked whether caspases are activated following treatment with BV6. In parental SH-EP or RD cells, exposure to BV6 resulted in increased caspase activation, whereas in rVCR cells caspases were not activated upon exposure to BV6 (Fig. 3C). In conclusion, these experiments demonstrate that the on-target activity of the Smac mimetic BV6 is reduced in rVCR cells, thus resulting in apoptosis resistance.

3.3. P-glycoprotein inhibitors sensitize for Smac mimetic-induced apoptosis

To further investigate the role of p-glycoprotein in MRD observed in VCR-resistant cells we utilized the specific p-glycoprotein inhibitor PSC-833. First, we examined the uptake of DOXO using its own fluorescence and flow cytometry. Notably, addition of PSC-833 reversed the loss of DOXO uptake in VCR-resistant cells back to the levels observed in the parental cells (Fig. 4A), showing that the reduced DOXO uptake in VCR-resistant cells was dependent on p-glycoprotein.

Next, we asked whether apoptosis can be restored upon inhibition of p-glycoprotein. Importantly, PSC-833 significantly restored VCR- and BV6-induced cell death in VCR-resistant cells, while its addition had little effect on apoptosis induced by VCR or BV6 in the parental cells (Fig. 4B). In parallel, PSC-833 restored loss of cIAP1 upon BV6 treatment in VCR-resistant cells (Fig. 4C), suggesting that, upon inhibition of p-glycoprotein, cellular levels of BV6 were restored and cIAP1 was subsequently targeted for proteasomal degradation.

Further confirmation of p-glycoprotein-dependent loss of BV6 activity in VCR-resistant cells was provided by detailed dose-response analyses demonstrating that PSC-833 restored the effect of BV6 in a concentration-dependent manner (Fig. 4D). These data indicate that the

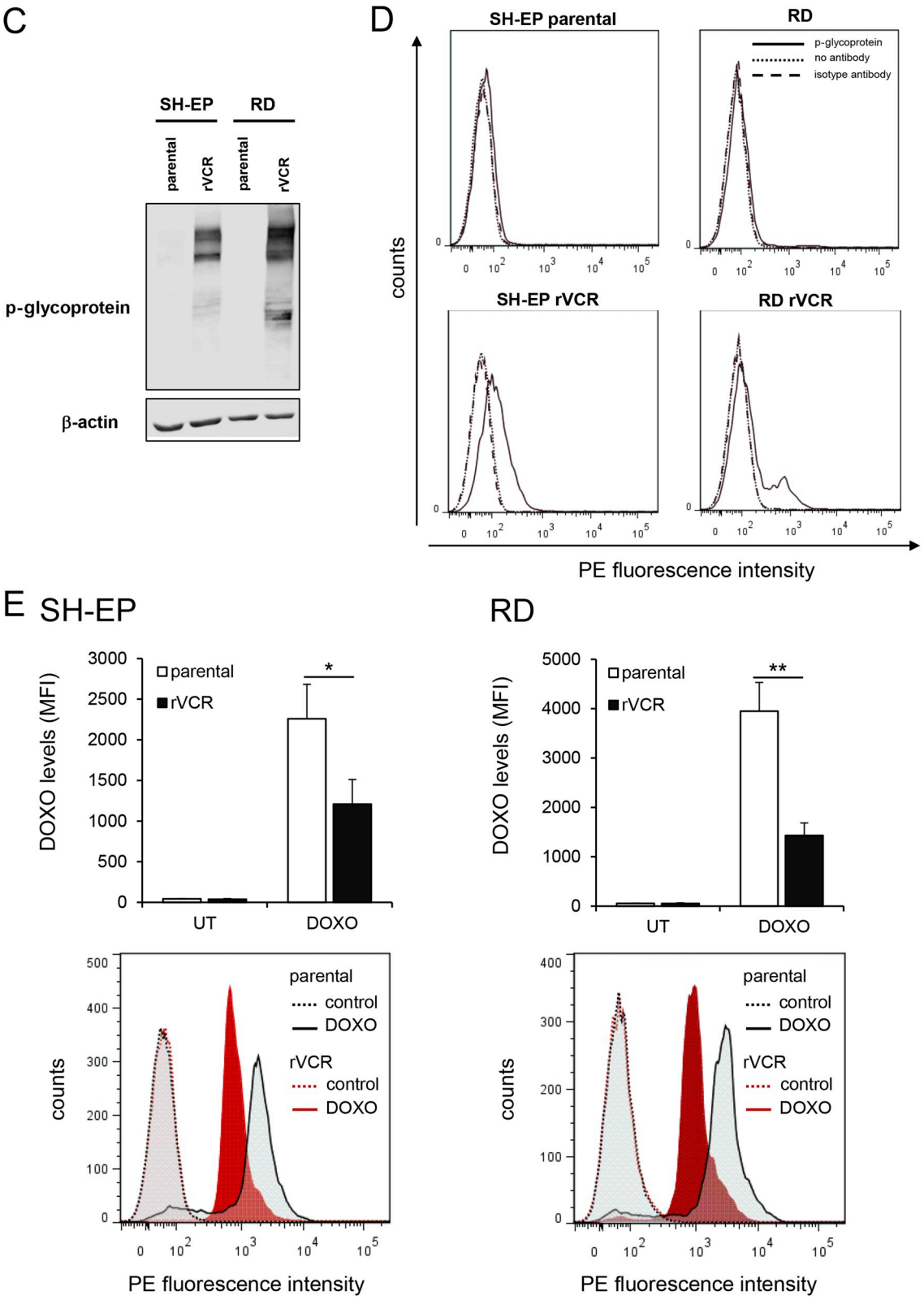

Fig. 1. (*continued*)

cross-resistance with BV6 is mediated by p-glycoprotein, implying that BV6 is a novel substrate for p-glycoprotein.

To investigate whether this effect of p-glycoprotein on BV6 was compound-specific or a more generally observed effect for Smac mimetics, we extended our studies to two other Smac mimetics, i.e. LCL161 and birinapant. Importantly, VCR-resistant RD cells also responded less to LCL161 and birinapant than parental cells, and addition of PSC-833 significantly increased the activity of both Smac mimetics (Fig. 5). These findings indicate that besides BV6 also other Smac mimetics could be potential substrates for p-glycoprotein.

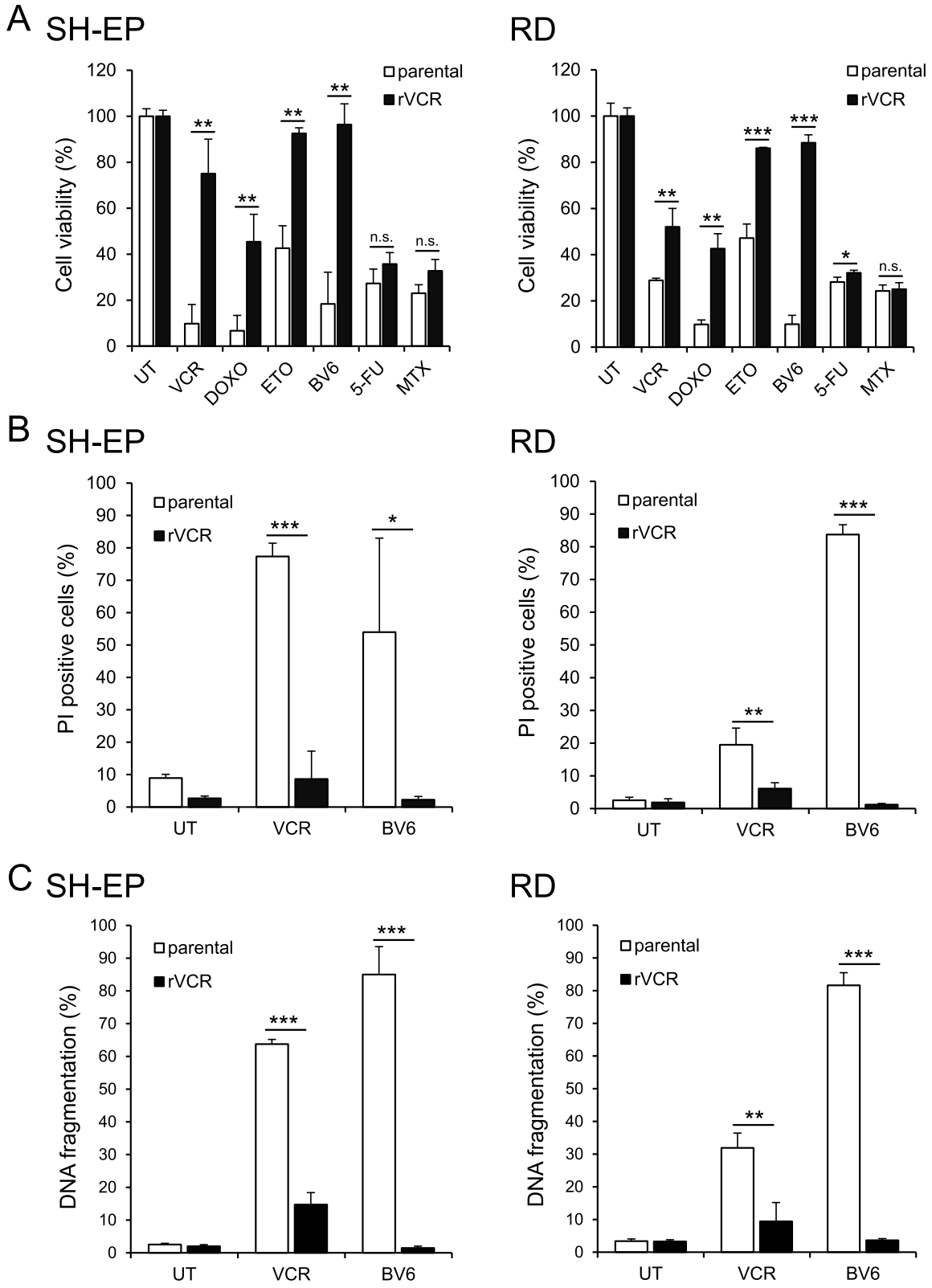

Fig. 2. VCR-resistant tumor cells display a multidrug-resistant phenotype. **A.** Parental or rVCR SH-EP (left) or RD (right) cells were either left untreated (UT) or exposed to VCR (10 nM), DOXO (0.05 μg/ml), ETO, BV6 (10 μM), 5-FU or MTX for 72 h prior to analysis of cell viability using MTT assay (data shown are mean + SD, n = 3). B-C. Parental or rVCV SH-EP (left) or RD (right) cells were either left untreated (UT) or exposed to VCR (10 nM) or BV6 (10 μM) before analysis of cell death using **(B)** PI staining and microscopy at 72 h (n = 4) or **(C)** PI staining and flow cytometric analysis of DNA fragmentation at 48 h (data shown are mean + SD, n = 4). *, $p < 0.05$, **, $p < 0.01$, ***, $p < 0.001$. n.s., not significant.

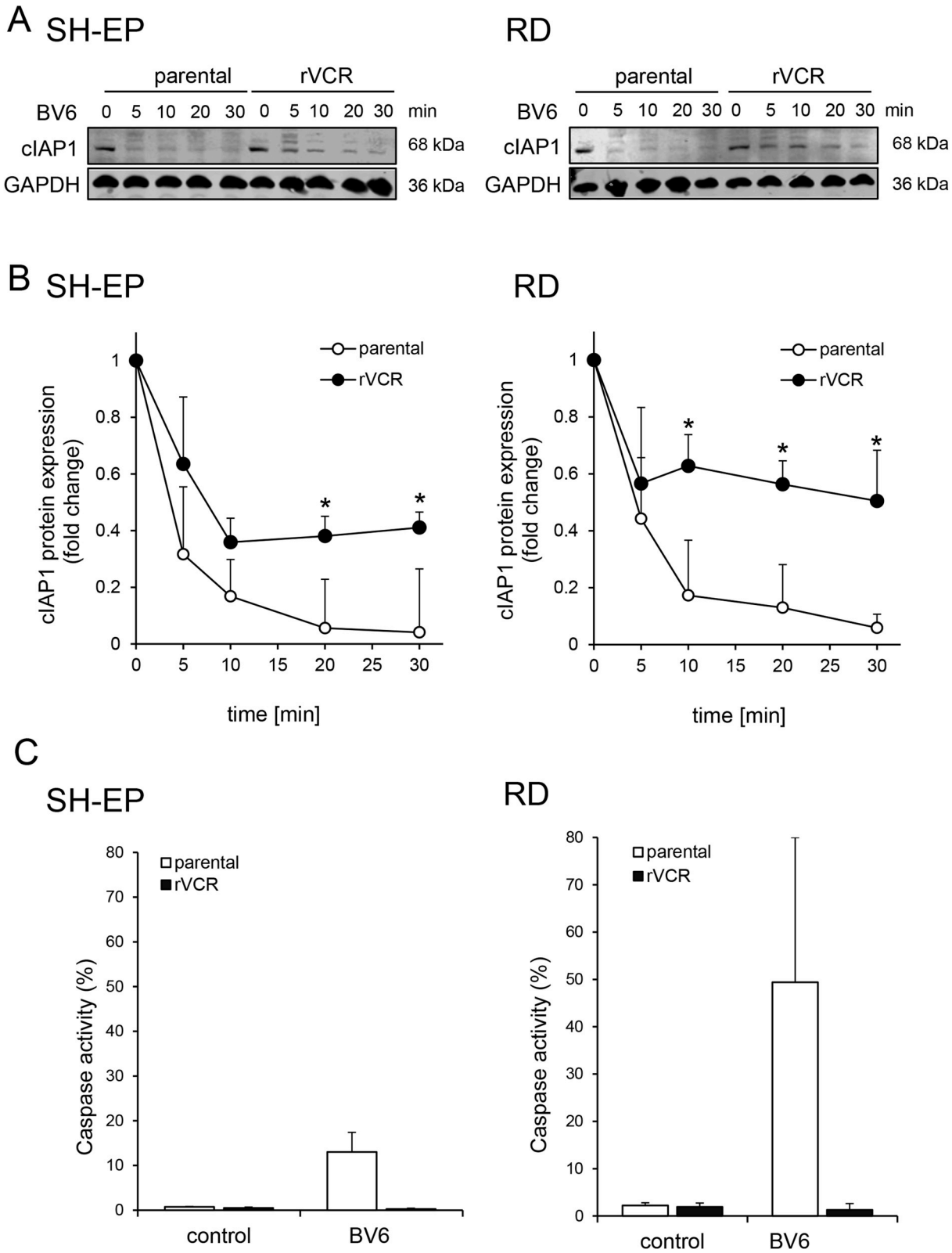

Fig. 3. Multidrug-resistant cells are less sensitive to BV6. **A-B.** SH-EP (left) or RD (right) cells were incubated with BV6 (10 μM) for up to 30 min. **A.** Loss of cIAP1 on BV6 treatment was analyzed using Western blotting. **B.** Levels of cIAP1 in parental or rVCR cells were quantified using Licor Image Studio Lite (n = 3). *, $p < 0.05$. **C.** Caspase activity was assessed in parental and rVCR SH-EP (left) or RD (right) cells at 24 h after exposure to BV6 (10 μM) (data shown are mean + SD, n = 2).

4. Discussion

By studying MDR in pediatric cancers, we identified Smac mimetics as novel substrates for p-glycoprotein. While this is the first report connecting the Smac mimetic BV6 with MDR, our conclusions are supported by two previous studies that have linked the activity of Smac mimetics to p-glycoprotein expression [24,25]. In this regard, the Smac mimetic T-3256336 developed by Takeda [26] has been described as a

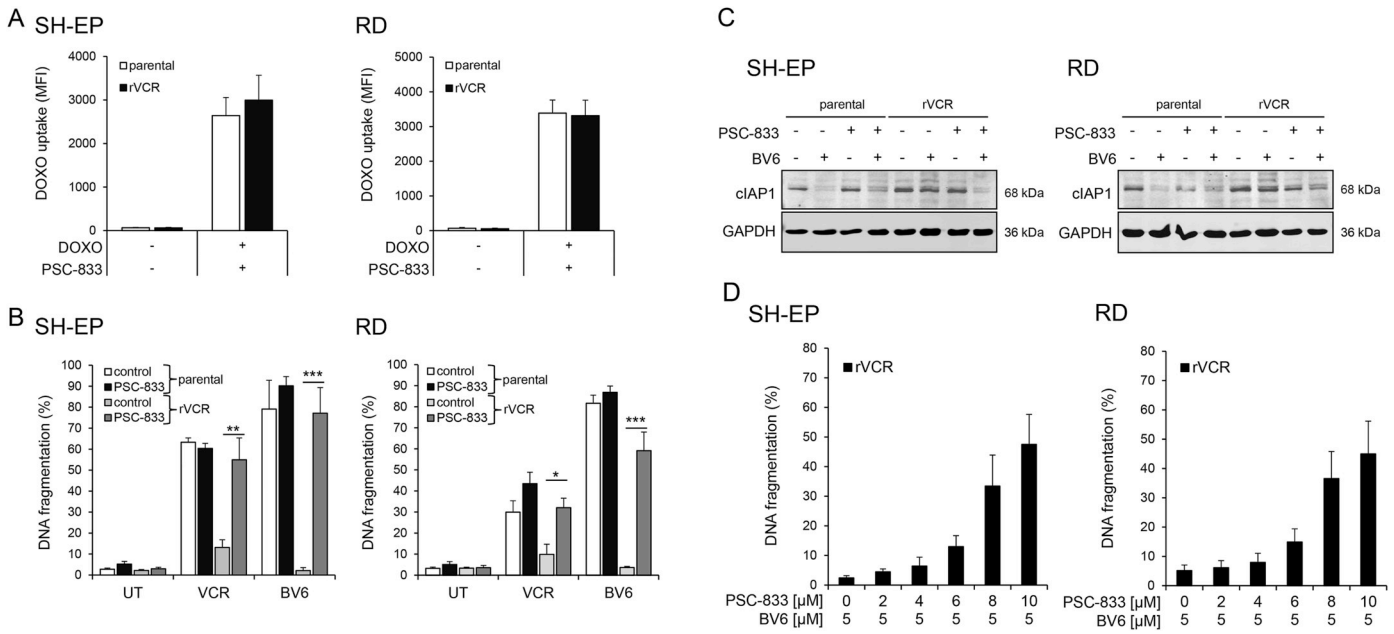

Fig. 4. BV6 is a substrate for p-glycoprotein. **A.** Parental and rVCR SH-EP (left) and RD (right) cells were either left untreated (UT) or exposed to DOXO (0.05 μg/ml) and PSC-833 (10 μM) for 24 h. Red fluorescence due to DOXO uptake was measured using flow cytometry and analysis of MFI (n = 3). **B.** Parental and rVCR SH-EP (left) and RD (right) cells were either left untreated (UT) or exposed to VCR (10 nM) or BV6 (10 μM) in the absence or presence of PSC-833 (10 μM) for 48 h prior to PI staining and flow cytometric analysis of DNA fragmentation (data shown are mean + SD, n = 3). **C.** Parental and rVCR SH-EP (left) and RD (right) cells were either left untreated (UT) or exposed to PSC-833 (10 μM) and/or BV6 (10 μM) for 10 min prior to analysis of cIAP1 expression using Western blotting. *, $p < 0.05$, **, $p < 0.01$, ***, $p < 0.001$. **D.** rVCR SH-EP (left) or RD (right) cells were incubated with the indicated concentrations of PSC-833 or BV6 for 72 h before analysis of apoptosis using staining with PI and flow cytometric analysis of DNA fragmentation (data shown are mean + SD, n = 4).

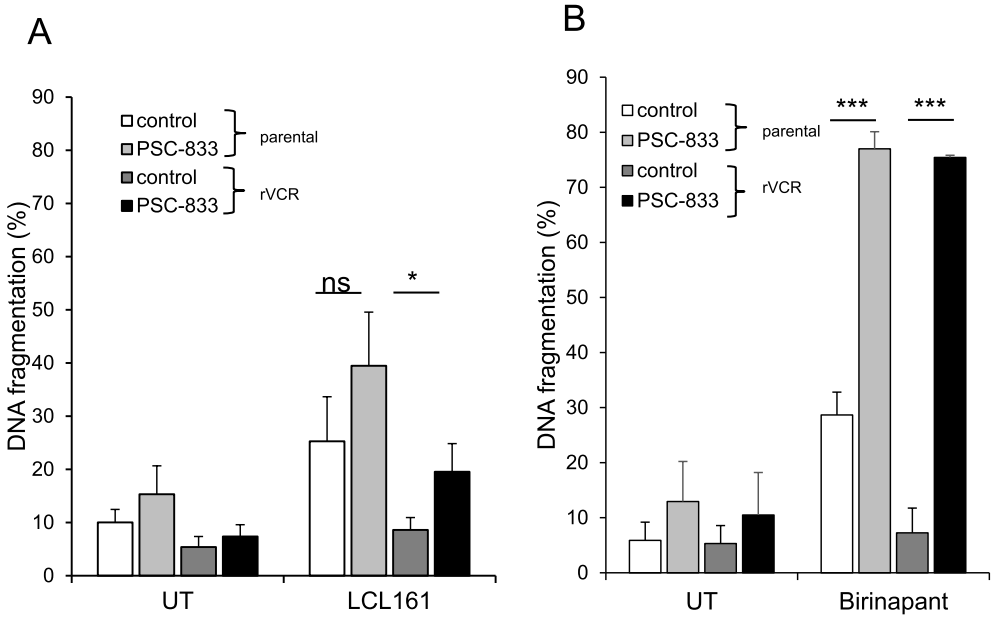

Fig. 5. Other Smac mimetics are substrates for p-glycoprotein. **A-B.** Parental or rVCR RD cells were either left untreated (UT) or exposed to 40 μM of LCL161 **(A)** or 60 μM of birinapant **(B)** in the absence or presence of PSC-833 (10 μM) for 48 h before analysis of DNA fragmentation using PI staining and flow cytometry (data shown are mean + SD, n = 3). *, $p < 0.05$, **, $p < 0.01$, ***, $p < 0.001$.

potential substrate for p-glycoprotein. In addition, birinapant and some other novel Smac mimetics have also been shown to display reduced activity in p-glycoprotein-expressing cells [24].

Drug exclusion by p-glycoprotein or related plasma membrane transporters may lead to inefficient IAP inhibition in cancer cells, in particularly in those cancers that have previously been exposed to chemotherapy and may have acquired high p-glycoprotein expression as resistance mechanism. Although Smac mimetics have extensively been studied in clinical trials, no Smac mimetic has so far been approved for cancer treatment [27]. Up until now, the limited clinical efficacy of Smac mimetics has mainly been attributed to the lack of a biomarker that could be used to preselect susceptible tumors. However, our study may provide an alternative explanation, as most clinical studies have been performed in patients that have previously received several rounds of chemotherapy, and hence the cancers may already display high p-glycoprotein expression levels.

Interestingly, there have already been several approaches aiming at reducing the exclusion of Smac mimetics by p-glycoprotein. To this end, modification of T-325636 has resulted in less affinity for p-glycoprotein while retaining IAP-binding capacity [25]. In an alternative approach, the linear structures of Smac mimetics have been modified to macrocyclic structures, which has resulted in enhanced cellular activity in p-

glycoprotein-expressing cells [24]. Taken together, these studies demonstrate that modifications of Smac mimetics can reduce the affinity for p-glycoprotein, retaining higher intracellular drug levels and better target inhibition in the context of multidrug-resistant cancers. However, so far none of the modified Smac mimetics with reduced affinity for p-glycoproteins has been tested in clinical studies.

Inhibition of IAP proteins by Smac mimetics is often being discussed in the context of chemoresistant cancers and may represent a promising strategy to resensitize tumor cells to anticancer therapeutics. In this regard, it is worth noting that several reports have indicated high IAP expression levels in chemoresistant cells and have suggested a connection between high IAP levels and bad prognosis [28,29]. A study on multiple myeloma has shown particularly high IAP expression levels in cells that also express high p-glycoprotein upon chemotherapy and has identified high IAP expression levels as a marker for poor prognosis in chemotherapy-resistant cells [28]. Also, multidrug-resistant HL60 cells display higher cIAP2 levels than their parental counterparts [30]. While the link between IAPs and chemoresistance has been studied extensively [31,32], so far no mechanistic explanation has been provided for this association. Our findings showing that Smac mimetics are substrates for p-glycoprotein and hence limited in their ability to combat chemoresistance adds another layer of complexity to the field of IAP proteins.

Pediatric cancers are fundamentally different from adult tumors and usually display a lower mutational burden and a faster growth rate. About 30% of all childhood malignancies are solid tumors and, in a majority of cases, respond well to chemotherapy. However, in particular in neuroblastoma the success of chemotherapy is hampered by the occurrence of MDR which represents a major clinical problem leading to poor outcome associated with chemoresistant disease [33]. In contrast to adult tumors, MDR has not yet been studied intensively in pediatric malignancies [34]. Here, we provide evidence showing that p-glycoprotein is highly relevant in neuroblastoma and RMS, two of the most common childhood cancers. Targeted therapies are being discussed as treatment options for chemoresistant pediatric cancers [33,35], and hence our findings suggesting that Smac mimetics are substrates for p-glycoprotein are important to guide the design of clinical studies.

Our data imply that currently used Smac mimetics like BV6, LCL161 and birinapant are much more likely to efficiently induce cell death in cancer cells before they have been exposed to chemotherapy or in combination with chemotherapeutics. It also suggests that Smac mimetics are less likely to be beneficial in advanced tumors with multidrug-resistant phenotype acquired after chemotherapy treatment.

Conflicts of interest

The authors declare that they do not have any conflict of interest.

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References

[1] K. Cheung-Ong, G. Giaever, C. Nislow, DNA-damaging agents in cancer chemotherapy: serendipity and chemical biology, Chem. Biol. 20 (2013) 648–659.
[2] M.M. Gottesman, Mechanisms of cancer drug resistance, Annu. Rev. Med. 53 (2002) 615–627.
[3] R.K. Vadlapatla, A.D. Vadlapudi, D. Pal, A.K. Mitra, Mechanisms of drug resistance in cancer chemotherapy: coordinated role and regulation of efflux transporters and metabolizing enzymes, Curr. Pharmaceut. Des. 19 (2013) 7126–7140.
[4] T. Efferth, M. Volm, Multiple resistance to carcinogens and xenobiotics: P-glycoproteins as universal detoxifiers, Arch. Toxicol. 91 (2017) 2515–2538.
[5] C.J. Chen, J.E. Chin, K. Ueda, D.P. Clark, I. Pastan, M.M. Gottesman, et al., Internal duplication and homology with bacterial transport proteins in the mdr1 (P-glycoprotein) gene from multidrug-resistant human cells, Cell 47 (1986) 381–389.
[6] D.R. Bell, J.H. Gerlach, N. Kartner, R.N. Buick, V. Ling, Detection of P-glycoprotein in ovarian cancer: a molecular marker associated with multidrug resistance, J. Clin. Oncol. 3 (1985) 311–315.
[7] N. Kartner, J.R. Riordan, V. Ling, Cell surface P-glycoprotein associated with multidrug resistance in mammalian cell lines, Science 221 (1983) 1285–1288.
[8] R.L. Juliano, V. Ling, A surface glycoprotein modulating drug permeability in Chinese hamster ovary cell mutants, Biochim. Biophys. Acta 455 (1976) 152–162.
[9] D.W. Shen, A. Fojo, J.E. Chin, I.B. Roninson, N. Richert, I. Pastan, et al., Human multidrug-resistant cell lines: increased mdr1 expression can precede gene amplification, Science 232 (1986) 643–645.
[10] I.B. Roninson, J.E. Chin, K.G. Choi, P. Gros, D.E. Housman, A. Fojo, et al., Isolation of human mdr DNA sequences amplified in multidrug-resistant KB carcinoma cells, Proc. Natl. Acad. Sci. U. S. A. 83 (1986) 4538–4542.
[11] S.V. Ambudkar, I.W. Kim, Z.E. Sauna, The power of the pump: mechanisms of action of P-glycoprotein (ABCB1), Eur. J. Pharmaceut. Sci. 27 (2006) 392–400.
[12] S.V. Ambudkar, S. Dey, C.A. Hrycyna, M. Ramachandra, I. Pastan, M.M. Gottesman, Biochemical, cellular, and pharmacological aspects of the multidrug transporter, Annu. Rev. Pharmacol. Toxicol. 39 (1999) 361–398.
[13] G. Szakacs, J.K. Paterson, J.A. Ludwig, C. Booth-Genthe, M.M. Gottesman, Targeting multidrug resistance in cancer, Nat. Rev. Drug Discov. 5 (2006) 219–234.
[14] K. Yusa, T. Tsuruo, Reversal mechanism of multidrug resistance by verapamil: direct binding of verapamil to P-glycoprotein on specific sites and transport of verapamil outward across the plasma membrane of K562/ADM cells, Cancer Res. 49 (1989) 5002–5006.
[15] P.R. Twentyman, N.M. Bleehen, Resistance modification by PSC-833, a novel non-immunosuppressive cyclosporin, Eur. J. Canc. 27 (1991) 1639–1642 [corrected].
[16] C. Martin, G. Berridge, P. Mistry, C. Higgins, P. Charlton, R. Callaghan, The molecular interaction of the high affinity reversal agent XR9576 with P-glycoprotein, Br. J. Pharmacol. 128 (1999) 403–411.
[17] S.B. Kaye, Reversal of drug resistance in ovarian cancer: where do we go from here? J. Clin. Oncol. 26 (2008) 2616–2618.
[18] Z. Binkhathlan, A. Lavasanifar, P-glycoprotein inhibition as a therapeutic approach for overcoming multidrug resistance in cancer: current status and future perspectives, Curr. Cancer Drug Targets 13 (2013) 326–346.
[19] H.A. Cocker, N. Tiffin, K. Pritchard-Jones, C.R. Pinkerton, L.R. Kelland, In vitro prevention of the emergence of multidrug resistance in a pediatric rhabdomyosarcoma cell line, Clin. Canc. Res. 7 (2001) 3193–3198.
[20] P. Borst, R. Evers, M. Kool, J. Wijnholds, A family of drug transporters: the multidrug resistance-associated proteins, J. Natl. Cancer Inst. 92 (2000) 1295–1302.
[21] W.S. Dalton, B.G. Durie, D.S. Alberts, J.H. Gerlach, A.E. Cress, Characterization of a new drug-resistant human myeloma cell line that expresses P-glycoprotein, Cancer Res. 46 (1986) 5125–5130.
[22] D. Imrichova, M. Coculova, L. Messingerova, Z. Sulova, A. Breier, Vincristine-induced expression of P-glycoprotein in MOLM-13 and SKM-1 acute myeloid leukemia cell lines is associated with coexpression of nestin transcript, Gen. Physiol. Biophys. 33 (2014) 425–431.
[23] E. Varfolomeev, J.W. Blankenship, S.M. Wayson, A.V. Fedorova, N. Kayagaki, P. Garg, et al., IAP antagonists induce autoubiquitination of c-IAPs, NF-kappaB activation, and TNFalpha-dependent apoptosis, Cell 131 (2007) 669–681.
[24] R.L. Talbott, R.M. Borzilleri, C. Chaudhry, J. Fargnoli, H. Shen, C. Fairchild, et al., Pharmacology of smac mimetics; chemotype differentiation based on physical association with caspase regulators and cellular transport, Exp. Cell Res. 338 (2015) 251–260.
[25] Z. Shiokawa, K. Hashimoto, B. Saito, Y. Oguro, H. Sumi, M. Yabuki, et al., Design, synthesis, and biological activities of novel hexahydropyrazino[1,2-a]indole derivatives as potent inhibitors of apoptosis (IAP) proteins antagonists with improved membrane permeability across MDR1 expressing cells, Bioorg. Med. Chem. 21 (2013) 7938–7954.
[26] H. Sumi, M. Yabuki, K. Iwai, M. Morimoto, R. Hibino, M. Inazuka, et al., Antitumor activity and pharmacodynamic biomarkers of a novel and orally available small-molecule antagonist of inhibitor of apoptosis proteins, Mol. Canc. Therapeut. 12 (2013) 230–240.
[27] S. Fulda, Smac mimetics to therapeutically target IAP proteins in cancer, Int. Rev. Cell. Mol. Biol. 330 (2017) 157–169.
[28] Y. Nakagawa, S. Abe, M. Kurata, M. Hasegawa, K. Yamamoto, M. Inoue, et al., IAP family protein expression correlates with poor outcome of multiple myeloma patients in association with chemotherapy-induced overexpression of multidrug resistance genes, Am. J. Hematol. 81 (2006) 824–831.
[29] T. Nomura, M. Yamasaki, Y. Nomura, H. Mimata, Expression of the inhibitors of apoptosis proteins in cisplatin-resistant prostate cancer cells, Oncol. Rep. 14 (2005) 993–997.
[30] M. Notarbartolo, S. Lo Cicero, M. Meli, P. Poma, M. Labbozzetta, M. Cervello, et al., Induction of apoptosis by the adenosine derivative IB-MECA in parental or multidrug-resistant HL-60 leukemia cells: possible relationship to the effects on inhibitor of apoptosis protein levels, Chemotherapy 51 (2005) 272–279.
[31] R.B. Lopes, R. Gangeswaran, I.A. McNeish, Y. Wang, N.R. Lemoine, Expression of the IAP protein family is dysregulated in pancreatic cancer cells and is important for resistance to chemotherapy, Int. J. Canc. 120 (2007) 2344–2352.
[32] C.G. Ferreira, P. van der Valk, S.W. Span, J.M. Jonker, P.E. Postmus, F.A. Kruyt, et al., Assessment of IAP (inhibitor of apoptosis) proteins as predictors of response

to chemotherapy in advanced non-small-cell lung cancer patients, Ann. Oncol. 12 (2001) 799–805.
[33] K.A. Cole, J.M. Maris, New strategies in refractory and recurrent neuroblastoma: translational opportunities to impact patient outcome, Clin. Canc. Res. 18 (2012) 2423–2428.
[34] D. Fruci, W.C. Cho, V. Nobili, F. Locatelli, A. Alisi, Drug transporters and multiple drug resistance in pediatric solid tumors, Curr. Drug Metabol. 17 (2016) 308–316.
[35] C.U. Louis, J.M. Shohet, Neuroblastoma: molecular pathogenesis and therapy, Annu. Rev. Med. 66 (2015) 49–63.