Biomedicine & Pharmacotherapy 192 (2025) 118698

Contents lists available at ScienceDirect

Biomedicine & Pharmacotherapy

journal homepage: www.elsevier.com/locate/biopha

SMARCA1 regulates PARP1-dependent ABC transporters expression and mediates doxorubicin resistance in triple-negative breast cancer

Karolina Gronkowska [a,c], Kinga Kołacz-Milewska [a,c], Sebastian Skoczylas [d], Éva Nagy [e], Damian Jacenik [b], Lóránt Székvölgyi [e], Agnieszka Robaszkiewicz [a], Magdalena Strachowska [a,b,c,*,1]

[a] Department of General Biophysics, Faculty of Biology and Environmental Protection, University of Lodz, Pomorska 141/143, Lodz 90-236, Poland
[b] Department of Cytobiochemistry, Faculty of Biology and Environmental Protection, University of Lodz, Pomorska 141/143, Lodz 90-236, Poland
[c] Bio-Med-Chem Doctoral School of the University of Lodz and Lodz Institutes of the Polish Academy of Sciences, University of Lodz, Banacha 12 /16, Lodz 90-237, Poland
[d] Department of Clinical Genetics, Medical University of Lodz, Pomorska 251, Lodz 92-213, Poland
[e] MTA-DE Momentum, Genome Architecture and Recombination Research Group, Department of Molecular and Nanopharmaceutics, Faculty of Pharmacy, University of Debrecen, H-4032, Hungary

ARTICLE INFO

Keywords:
Multidrug resistance
ABC transporters
PARP1/HPF1 complex
Triple negative breast cancer
ISWI family

ABSTRACT

Despite advances in cancer therapy, drug resistance remains a major obstacle in a range of cancer types, often driven by overexpression of ATP-binding cassette (ABC) transporters that restrict intracellular drug accumulation. Our previous studies identified the BRG1-p300 transcriptional complex at the promoters of specific ABC transporter genes. We used basal and induced doxorubicin-resistant triple-negative breast cancer (TNBC) cell lines to analyze changes in ABC transporter expression and drug accumulation following PARP1 inhibition. Moreover, the effect of commonly used antioxidants on the repressive effect of Veliparib against ABC genes was verified. ChIP-Seq was performed to identify transcription factor mediating PARP1-dependent ABC gene regulation. PARP1 inhibition or silencing of PARP1/HPF1 complex components downregulated ABCC and ABCG2 transporters, leading to increased intracellular accumulation of chemotherapeutic drugs in doxorubicin-resistant cells. Notably, suppression of genotoxic stress via antioxidant treatment reverses the inhibitory effect of Veliparib on ABC transporter expression. We identified SMARCA1 as a key regulator of PARP1-dependent expression of ABCC genes. SMARCA1 is a key effector of PARP1/p300-mediated regulation of ABC transporters and represents a potential therapeutic target in doxorubicin-resistant TNBC. These findings support the development of combinatorial strategies involving PARP1 inhibitors and chromatin remodeling modulators in refractory breast cancers.

1. Introduction

Despite decades of research aimed at developing novel cancer therapies, the disease remains one of the leading causes of death worldwide. According to WHO data, up to one in five people will develop cancer, with one in twelve women and one in nine men succumbing to the disease [1]. While significant progress has been made in the development of personalized therapies and immunotherapies, chemotherapy remains the most widely used treatment option [2]. Although chemotherapy initially improves survival rates, disease relapse is common due to the emergence of adaptive resistance mechanisms in cancer cells [3]. One such mechanism, multidrug resistance (MDR), reduces cellular sensitivity to therapeutic stressors and is a major contributor to chemotherapy failure. MDR can be induced by various factors, including cytostatic agents, hypoxia, and autophagy [4–7]. A key driver of MDR is the overexpression of ATP-binding cassette (ABC) transporters, which actively expel chemotherapeutic drugs from cells or sequester them in intracellular compartments [8]. These molecular pumps harness energy from adenosine triphosphate (ATP) hydrolysis to transport cytostatic agents against a chemical gradient, effectively diminishing drug efficacy

* Correspondence to: Department of Cytobiochemistry, Pomorska St. 141/143, Lodz 90-236, Poland.
E-mail address: magdalena.strachowska@biol.uni.lodz.pl (M. Strachowska).
[1] ORCID: https://orcid.org/0000-0002-3100-1183

https://doi.org/10.1016/j.biopha.2025.118698
Received 14 August 2025; Received in revised form 19 October 2025; Accepted 21 October 2025

[8]. Given their critical role in cancer progression, ABC transporters have become a central focus of the research presented in this study.

Our previous studies demonstrated that the transcription activation complex formed by Brahma-related gene 1 protein (BRG1) and E1A binding protein p300 (encoded by the EP300 gene) is present at the promoters of certain ABC transporter genes [9]. Given that ADP-ribosylation of p300 plays a pivotal role in the complex-mediated regulation of gene transcription, we investigated whether inhibition of poly(ADP-ribose) polymerase 1 (PARP1) activity influences the repression of ABC transporters. Additionally, we aimed to identify the transcription factor responsible for PARP1-dependent regulation of ABC genes critical for doxorubicin resistance in the MDA-MB-231 triple--negative breast cancer (TNBC) cell line.

PARP proteins constitute a crucial family of enzymes responsible for protein modification through PARylation. This family consists of 17 members, among which PARP1, PARP2, PARP5a/b are key mediators of ADP-ribose polymer synthesis, while the remaining members primarily catalyze the mono-ADP-ribosylation reactions [10]. The most well-documented role of PARP1 in cancer involves its participation in DNA repair, transcriptional regulation, and metabolic processes. Notably, PARP1 accounts for approximately 90 % of all PARylation events by attaching poly(ADP-ribose) (PAR) chains to transcription factors, chromatin remodeling proteins, and histones. Additionally, PARP1 undergoes auto-PARylation, a process essential for recruiting DNA repair proteins to sites of damage and modulating transcription. Our interest in PARP1 stemmed from its ability to ADP-ribosylate the BRG1-p300 transcription activation complex, which is present at the promoters of specific ABC transporter genes. PARP1 primarily catalyzes the attachment of poly (ADP-ribose) chains to aspartate or glutamate residues. However, when histone PARylation factor 1 (HPF1, also known as C4orf27) is recruited to DNA damage sites, ADP-ribosylation shifts to serine residues. Recent studies suggest that HPF1 plays a crucial role in regulating the auto-PARylation of PARP1 and PARP2, further influencing their activity in DNA damage response and transcriptional control [11].

In this study, we set out to investigate the impact of PARP1 loss of function on the repression of genes regulated by the p300 protein. To achieve this, we employed well-characterized PARP1 inhibitors with distinct mechanisms of action. Veliparib, a PARP inhibitor that does not induce the formation of trapped PARP-DNA complexes, offers a key advantage over other PARP inhibitors by avoiding myelosuppression, a condition marked by reduced bone marrow cell levels [12,13]. The second inhibitor, Olaparib, was approved by the U.S. Food and Drug Administration (FDA) in 2018 for the treatment of advanced ovarian, fallopian tube, and primary peritoneal cancers with germline or somatic BRCA1/BRCA2 mutations, which impair homologous recombination repair (HRR) [14]. Olaparib exerts its therapeutic effect by both inhibiting PARP enzymatic activity and inducing the formation of PARP-DNA "trap" complexes at sites of DNA damage, ultimately leading to cancer cell death [15]. When combined with other cytotoxic agents, PARP inhibitors further compromise the DNA repair capacity of cancer cells, resulting in the accumulation of genetic aberrations and subsequent cell death [16,17].

Here, we have tested the hypothesis that the PARP1/HPF1 complex is involved in the transcriptional regulation of ABC transporter genes that drive doxorubicin resistance in MDA-MB-231 triple-negative breast cancer cells. Additionally, we identified transcription factors that may contribute to the observed PARP1-mediated overexpression of ABC transporters in doxorubicin-resistant MDA-MB-231 cells. Our findings reveal new molecular targets and offer fresh insights into overcoming multidrug resistance in breast cancer refractory to standard chemotherapy. By identifying SWI/SNF-related matrix-associated actin-dependent regulator of chromatin subfamily A member 1 (SMARCA1) as a regulator of ABC transporters in a PARP1-dependent context, this study contributes to the growing understanding of how epigenetic and oxidative stress–related pathways could be leveraged to enhance therapeutic response in TNBC.

2. Materials and methods

2.1. Materials

Triple negative breast cancer wild-type MDA-MB-231 was obtained from Sigma-Aldrich.

Nunc™ Lab-Tek™ Chamber Slide and oligonucleotides for Real-Time PCR (ABC genes) were from Biokom (Janki/Warsaw, Poland).

L-Ascorbic acid from Chempur (Piekary Slaskie, Poland).

BIOFLOAT™ 96-well U-bottom Plate from faCellitate (Mannheim, Germany).

MGIEasy Cell-free DNA Library Prep Kit (Cat# MGI940–000191–00) and DNBSEQ-G400RS High-throughput Sequencing Kit (FCL SE100) from MGI (Shenzhen, China).

Olaparib and Veliparib (PARP1 inhibitors) from Cayman Chemical (Biokom, Janki/Warsaw, Poland).

siRNA Control (sc-37007), siRNA for PARP1 (Cat# sc-29437), anti-pADPr monoclonal antibody (Santa Cruz Biotechnology Cat# sc-56198, RRID:AB_785249) were purchased from Santa Cruz Biotechnology (AMX, Lodz, Poland).

NextSeq 500/550 High-Output v2 kit (75 cycles) (Cat# 20024906) from Illumina (California, United States).

DMEM High Glucose w/ L-Glutamine w/ Sodium Pyruvate, fetal bovine serum (FBS) and antibiotics (penicillin and streptomycin) were from Biowest (CytoGen, Zgierz, Poland).

NEBNext® Ultra™ II DNA Library Kit with Purification Beads, NEBNext® Multiplex Oligos for Illumina (Index Primers Set 1), NEBNext Library Quant Kit for Illumina were from New England Biolabs (LabJOT, Warsaw, Poland).

Leibovitz's L-15 Medium, albumin from human serum, Nunc® MicroWell™ 384 well optical bottom plates, Triton X-100, DAPI, resazurin sodium salt, probenecid, doxorubicin hydrochloride, daunorubicin hydrochloride, methotrexate, paclitaxel, etoposide, glutathione, anti-HPF1 antibody (Cat# HPA043467, RRID:AB_10793949) were from Sigma Aldrich (Poznan, Poland).

Anti-MRP5 (ABCC5) Polyclonal Antibody (Thermo Fisher Scientific Cat# PA5–102678, RRID:AB_2852074), High-Capacity cDNA Reverse Transcription Kit, SuperSignal™ West Pico Chemiluminescent Substrate, TRI Reagent™, Lipofectamine RNAiMAX, OptiMem, PageRuler™ Prestained Protein Ladder (10–180 kDa), Pierce™ Protease Inhibitor Tablets (EDTA-free; PIC), PMSF Protease Inhibitor, RNAse, Dithiothreitol (DTT), PowerUp™ SYBR™ Green Master Mix for qPCR, ProLong™ Diamond Antifade Mountant, SlowFade™ Glass Soft-set Antifade Mountant (with DAPI), Paclitaxel Oregon Green™ 488 Conjugate, Texas Red™-X Phalloidin, Dynabeads™ Protein G, UltraPure™ Phenol:Chloroform:Isoamyl Alcohol (25:24:1, v/v) (#15593031), oligonucleotides for Real-Time PCR (PARP1, HPF1, SMARCA1, selected regions for ChIP-qPCR), Mono-Methyl Lysine Polyclonal Antibody (Thermo Fisher Scientific Cat# PA5–116817, RRID:AB_2901448), Phosphoserine/threonine/tyrosine Polyclonal Antibody (Thermo Fisher Scientific Cat# 61–8300, RRID:AB_2533941), SMARCA1 Monoclonal Antibody (Cat# MA5–31912, ID: 2H7B9, RRID:AB_2787535), siRNA for SMARCA1 (Cat# AM16708, ID: 142645), siRNA for HPF1/C4orf27 (Cat# AM16709, ID: 215741) were from Thermo Fisher Scientific (Thermo Fisher Scientific, Warsaw).

Anti-ABCC1 (D7O8N) Rabbit mAb (CST Cat# 14685, RRID: AB_2798572), anti-ABCC3 (D8V8J) Rabbit mAb (CST Cat# 39909, RRID:AB_2799164), anti-ABCC4 (D2Q2O) Rabbit mAb (CST Cat# 12705, RRID:AB_2797999), anti-Histone H3 (1B1B2; for Western Blot) Mouse mAb (CST Cat# 14269, RRID:AB_2756816), anti-p300 (CST D2X6N - Rabbit mAb #54062, RRID:AB_2799450), anti-H3K4me3 (CST C42D8 - Rabbit mAb #9751, RRID:AB_2616028), anti-rabbit IgG HRP-linked Antibody (CST Cat# 7074, RRID:AB_2099233), anti-Acetylated Lysine (CST Cat# 9441S), anti-PARP1 Monoclonal Antibody (Rabbit

mAb, Cat# 9532, ID: 46D11, RRID:AB_659884), anti-p300 Monoclonal Antibody (Rabbit mAb, Cat# 54062, ID: D2X6N, RRID:AB_2799450) were from Cell Signaling Technologies (LabJOT, Warsaw, Poland).

2.2. Methods

2.2.1. Cell culture and treatment with inhibitors

For the first five passages, the MDA-MB-231 triple-negative breast cancer line was cultured in L15 medium with supplementation of 15 % FBS, penicillin-streptomycin antibiotics (sequentially 50 U/mL and 50 μg/mL) without CO2 (as recommended by the manufacturer Sigma-Aldrich). Then, over the next two weeks, the cells were adapted to DMEM medium with 10 % bovine serum FBS, including the antibiotics penicillin-streptomycin (successively 50 U/mL and 50 μg/mL) and cultured in an atmosphere of 5 % CO2.

Cells were seeded on a plate at least 24 h before the scheduled experiment, and incubation with 1 μM Olaparib (iPARP1) and 1 μM Veliparib (iPARP1) inhibitors lasted 72 h [18–21].

2.2.2. Induction of doxorubicin resistance

Doxorubicin at a concentration of 1 μM was added to proliferating MDA-MB-231 cells. Cells were treated with selected anthracycline for 48 h every 4 weeks for a total number of 6 cycles in single doses, bearing in mind recovery of the cells to a normal proliferative process. Then, after 48 h, the drug was removed from the culture, and the cells were cultured in DMEM medium without the addition of doxorubicin until the next treatment cycle [22]. The full characteristics of the doxorubicin-resistant MDA-MB-231 line can be found in our previous publication by Strachowska et al. [22].

2.2.3. Real-Time PCR

Depending on the experiment performed, after the designated incubation time, total RNA was isolated using TRI Reagent™. RNA concentration thereafter was measured using a Quantus™ Fluorometer. In the next step, the reverse transcription reaction was performed using the High-Capacity cDNA Reverse Transcription Kit according to the protocol provided by the manufacturer Thermo Fisher Scientific (25 °C -> 10 min, 36 °C -> 120 min, 95 °C -> 5 min, 4 °C -> ∞). Expression of selected genes was measured using PowerUp™ SYBR™ Green Master Mix on a Bio-Rad CFX96 C1000 Touch Real-Time system (polymerase activation: 95 °C, 10 min; PCR cycles: denaturation at 95 °C, 15 s; annealing and extension at 60 °C, 1 min). All primers listed for the PARP1, HPF1, SMARCA1 and ABCC genes were designed by our team using Primer-BLAST (RRID:SCR_003095) and verified experimentally in this study.

✓ primers

	Forward	Reverse
PARP1	5′-AAGCCCTAAAGGCTCAGAACG−3′	5′-ACCATGCCATCAGCTACTCGGT−3′
HPF1	5’-TCGCTTGAACAGAGAACCGTGA−3’	5’-TGGAACAACCAAGCCTGCAC−3’
SMARCA1	5’-GCTTGGCATGGTGGAATGGA−3’	5’-TTTGGCTGTTTTGGAGGCCG−3’

2.2.4. Western Blot

Cells after appropriate treatments were lysed with RIPA buffer containing 1 mM phenylmethylsulfonyl fluoride (PMSF) and 1x protease inhibitor cocktail (PIC) and then sonicated on a Bandelin Sonopuls HD2070 instrument. In the next step, the concentration of samples was equilibrated based on dsDNA content determined with a Quantus™ fluorometer. Proteins were separated by SDS-PAGE electrophoresis and transferred to a nitrocellulose membrane. The blocking step was carried out in 5 % albumin solution in PBS with 0.1 % Tween 20. After an overnight incubation at 4ºC with the primary antibody in 5 % albumin solution in PBS-Tween, the membranes were washed three times with PBS-Tween. In the next step, horseradish peroxidase (HRP)-conjugated secondary antibody was added to the membranes for 2 h of incubation at room temperature in 1 % albumin solution in PBS-Tween. SuperSignal™ West Pico PLUS chemiluminescent substrate was used to visualize the proteins of interest (Supplementary material - Western Blot).

For each experiment, histone H3 served as a control for equal concentrations of protein in each sample.

2.2.5. Confocal microscopy

2.2.5.1. ABC transporters expression. Cells were seeded onto a Nunc™ Lab-Tek™ chamber at a density of 10,000 cells per well. After 24 h cells were treated with Olaparib and Veliparib and incubated for 72 h. The cells were washed three times with PBS solution and fixed in 1 % formaldehyde solution in PBS. Following a 15-minute incubation at room temperature, the cells were permeabilized and blocked with 1 % FBS solution in PBS with 0.1 % Triton X-100 at room temperature for 1 h. After the designated time, primary antibody in a solution of 1 % BSA in PBS with 0.1 % Triton X-100 was added to the cells overnight at 4°C. In the next step, the cells were washed three times with PBS solution, and then secondary antibody conjugated to a fluorescent probe was added to the cells. Incubation with the secondary antibody lasted 2 h at room temperature. In the final preparation step, the cells were washed and the slide was sealed using SlowFade™ Glass Soft-set Antifade Mountant, which contained DAPI.

2.2.5.2. Drug accumulation. To measure drug accumulation, cells in adherent form were seeded onto a Nunc™ Lab-Tek™ chamber at a density of 10,000 cells per well. Cells in 2D culture were then treated with PARP protein inhibitors or transfected with siRNA for PARP1 and HPF1. Silencing of selected PARP1 and HPF1 genes was also performed for 3D cultures - spheroids. After 72 h, cells were treated with anthracyclines which have autofluorescence features, such as doxorubicin, daunorubicin and paclitaxel Oregon Green 488 and then incubated for 24 h. After the designated time, the cells were washed three times with PBS solution and then fixed with 1 % formaldehyde solution. The slide was then protected with SlowFade™ Glass Soft-set Antifade Mountant (with DAPI).

2.2.6. Transient silencing of PARP1 and HPF1 genes

Cells of the wild-type and doxorubicin-resistant MDA-MB-231 were seeded into a 24-well plate at a density of 100,000 cells per well, and into a Nunc™ Lab-Tek chamber at a density of 10,000 cells per well. After 24 h, cells in 2D cultures or three-week-old spheroids were transfected with RNAiMAX lipofectamine:siRNA complex in OptiMEM medium as recommended by the manufacturer Thermo Fisher Scientific. After a 4-hour incubation, DMEM medium with 10 % FBS and antibiotics was supplemented to a predetermined volume. After 72 h, TRIzol was added to the transfected cells, and RNA isolation was performed, followed by reverse transcription under conditions corresponding to the manufacturer’s Thermo Fisher Scientific recommendations. In the final step, the Real-Time PCR method was chosen to determine changes in ABC gene expression after silencing the components of the complex.

2.2.7. Resazurin toxicity assay

MDA-MB-231 cells were seeded into a 96-well plate at a density of 10,000 cells per well. After 24 h, the cells were transfected with siSMARCA1, siABCC3, siABCC4 and siABCC10 according to a previously described protocol. In order to compare viability after SMARCA1 silencing, probenecid (an inhibitor of ABCC group proteins) was also introduced into the experiment. After 72 h of incubation, the cells were treated with paclitaxel and doxorubicin for another 48 h. In the next

step, the cells were washed with PBS, followed by resazurin medium with a final concentration of 5 μM, and a 4-hour incubation at 37 °C. The fluorescence intensity (excitation = 530 nm, emission = 590 nm), which corresponds to the metabolic activity of the cells, was measured using a BioTek Synergy HTX fluorescence microplate reader.

2.2.8. Co-immunoprecipitation and immunoprecipitation

2.2.8.1. General description of the method. MDA-MB-231 cell lines were seeded at a density of 5 million cells per 90 mm diameter dish. The following day, after rinsing three times with PBS, the cells were resuspended in IP buffer (20 mM HEPES - KOH; 50 mM KCl; 5 mM MgCl2; 0.2 mM EDTA; 20 % glycerol; 0.1 % NP-40) with PIC, PMSF and DTT inhibitors and collected in an Eppendorf tube. The cell suspension was sonicated on a Diagenode Bioruptor 300 apparatus for 35 cycles/high. After centrifugation of the cells, 10 % of the supernatant was collected as a control for the total amount of proteins analyzed in the sample (input) and the rest of the sample was separated into an antibody sample (IP) and an IgG control. After a 2-hour incubation of the lysate with the antibody, magnetic beads were added to the samples. After an additional 2 h of incubation, the beads were washed with IP buffer, resuspended in electrophoresis lysis buffer with β-mercaptoethanol, and loading buffer. Subsequently, the mixture prepared in this way was incubated at 70°C for 10 min. After detaching the beads from the lysate, the proteins were separated and visualized by Western Blot.

An experiment involving co-immunoprecipitation to verify the appearance of the PARP1/HPF1 complex in response to damaging agents in the MDA-MB-231 baseline began by seeding the cells. Then, after 24 h, the cells were treated with etoposide (25 μM) and H2O2 (25 μM) and incubated for two and four hours, respectively. In the next step, co-immunoprecipitation combined with protein detection by Western Blot technique was performed during which anti-PARP1 and anti-HPF1 antibodies were used. The co-immunoprecipitation also included an assay to determine the appearance of physical interaction of SMRACA1 protein with PARP1, p300 and HPF1 in the doxorubicin-resistant MDA-MB-231 line compared to the baseline. The analysis involving immunoprecipitation and identification of post-translational modifications of PARP1 protein in the baseline, doxorubicin-resistant and paclitaxel-resistant MDA-MB-231 lines was based on antibodies specific for methylation, acetylation and phosphorylation. For experiments involving PARP1 and HPF1 complex formation and verification of post-translational modifications, an anti-PARP1 antibody was selected for immunoprecipitation.

2.2.9. ROS detection - DCFDA / H2DCFDA

Cells were seeded into a black 96-well plate at a density of 10,000 cells per well. After 24 h, H2DCFDA reagent at a concentration of 2 μM was added to the cells. After 3 h, fluorescence was measured on a Biotek HTX reader at excitation/emission wavelengths λex= 485 nm and λem= 530 nm. The fluorescence intensity of the probe is directly proportional to the level of ROS reacting with.

2.2.10. ChIP-Seq and ChIP-qPCR

The chromatin immunoprecipitation (ChIP) method was performed according to the published protocol included in the publication by Wisnik et al. [23]. MDA-MB-231 wild-type and doxorubicin-resistant cell lines were seeded at a density of 10 million cells per 150 mm diameter dish. After 24 h, the cells were fixed with 1 % formaldehyde, and after a 10-minute incubation, the process was arrested with 125 mM glycine and cell were rinsed 3-times with cold PBS. After incubation with appropriate buffers containing PIC and PMSF inhibitors, cells were resuspended in lysis buffers. Isolated chromatin was sonicated using a Diagenode Bioruptor sonicator, followed by centrifugation for 10 min at 10,000 rpm at 4°C. Conjugated antibodies with magnetic beads were added to the prepared samples. After a 24-hour incubation at 4°C, the immunoprecipitated chromatin was rinsed in purification buffers, which was followed by an overnight desquamation process at 65°C. DNA was isolated by phenol:chloroform:isoamyl (PCI) alcohol extraction.

For ChIP-Seq involving PARP1 the library for sequencing was constructed using the NEBNext® Ultra™ DNA Library Prep Kit with Sample Purification Beads for Illumina® according to the protocol provided by the manufacturer, and sequenced using the NextSeq 500/550 High Output Kit v2.5 (75 Cycles) at the Department of Clinical Genetics, University of Medical Sciences, Lodz, Poland.

For ChIP-Seq targeting histone modifications such as histone H3 lysine 4 trimethylation (H3K4me3) and histone H3 lysine 27 acetylation (H3K27ac) the library for sequencing was prepared with MGIEasy Cell-free DNA Library Prep Set and sequenced on DNBSEQ-G400 using DNBSEQ-G400RS High-throughput Sequencing Kit from MGI at the Department of Clinical Genetics, University of Medical Sciences, Lodz, Poland.

For ChIP-qPCR after PCI isolation qPCR method was performed for selected regions of *ABCC3*, *ABCC4* and *ABCC5*, where SMARCA1 was present. The control for immunoprecipitation was IgG, while the exon PARP1 was used for the confidence of the regions.

	Forward	Reverse
ABCC3	5′- TCTTGAATGTTTAGTT TAATGAATTTTTAC −3′	5′- GTGCTAGGATTACAG GCATGAGCCATGAGG −3′
ABCC4	5’- ACAATGAGATACCAT CTCACACCAGTTAGA −3’	5’- AGGAATCGCCACAC TGACTTCCACAATGGT −3’
ABCC5	5’- CAGAAAGCATCCTTCCT GCCAGCACAGCCA −3’	5’- ACCTCAGCCTCTCAAAGT GCTGGGATTGTAGG −3’

PARP1 was immunoprecipitated with anti-PARP1 antibody in both the wild-type and doxorubicin-resistant line MDA-MB-231. Moreover, two modifications H3K4me3 and H3K27ac were selected for the doxorubicin resistant cell line. Data covering the selected modifications in the MDA-MB-231 wild-type were obtained from the Short Reads Achieve database from NCBI with numbers SRR22007612 (H3K4me3) and SRR15130683 (H3K27ac). Additionally, for ChIP-qPCR immunoprecipitation was performed using anti-SMARCA1 antibody.

2.2.11. Bioinformatics analysis

In silico stage of the study, which aimed to identify the transcription factor playing a crucial role in PARP1-dependent expression of ABC transporters, was initiated by analyzing the results obtained by the ChIP-Seq method using the UseGalaxy platform (RRID:SCR_006281) [24]. First, after removing the adapters, the quality of the obtained reads was checked. Then the reads were mapped to the human hg19 genome using the Bowtie 2 tool. In the next step, PARP1-rich regions were selected via the MACS2 peak calling tool at p-value = 0.001. Next, the bedtools intersect intervals tool found common regions for PARP1-enriched narrow peak sequences and regions occurring within ± 170,000 from the transcription initiation site (TSS) of PARP1-dependent genes encoding ABCC3, ABCC4 and ABCC5 transporters. The XSTREME tool from the MEME Suite - Motif-based sequence analysis tools (RRID: SCR_001783) allowed the generation of a list of transcription factors for the identified motifs [25]. The list of factors included p53, p73 and SMARCA1. Among the three identified transcription factors, SMARCA1, a ISWI family chromatin remodeling protein, emerged as the most relevant candidate and was selected for further analysis.

2.2.12. Statistical analysis

The results were presented as means ± SEM. Normality of distribution was checked using the Shapiro-Wilk test. The difference between two means was checked using the non-parametric Mann-Whitney test in a data set with a non-normal distribution. The Student’s *t*-test was chosen for comparisons of two means in samples meeting the Gaussian distribution requirement. For multiple comparisons, a one-way ANOVA1 analysis of variance was conducted, and then the Tukey test

or Bonferroni Correction was chosen to determine potential differences between samples. Statistically significant data were marked with "*" when $p < 0.05$, otherwise marked with "ns" (Supplementary excel spreadsheet). Statistical analyses were performed using GraphPad Prism software, Boston, Massachusetts USA, www.graphpad.com (RRID: SCR_002798).

3. Results

In our previous work, we showed that inhibition of the bromodomain acetyltransferase CREB-binding protein (CBP) and p300 leads to decreased expression of genes encoding ABC transporters. We therefore set out to verify whether we would achieve a similar effect using inhibitors and silencing the PARP1 protein, which is a cofactor of p300.

To assess the impact of PARP inhibition on the expression of ABC transporter genes, we first analyzed changes in mRNA and protein levels following treatment with selected PARP inhibitors. In wild-type MDA-MB-231 cells, both Olaparib and Veliparib significantly reduced the expression of *ABCC1*, *ABCC2*, *ABCC3*, and *ABCC4*, while Olaparib also downregulated *ABCC5* and *ABCC10* at the mRNA level (Fig. 1A). Western blot analysis further confirmed the inhibitory effects at the protein level for ABCC1 and *ABCC4* (Fig. 1B). Additionally, confocal microscopy revealed reduced *ABCC1* expression in response to Veliparib and decreased *ABCC5* levels following Olaparib treatment (Fig. 1C).

Functionally, inhibition of PARP1 activity led to increased intracellular accumulation of daunorubicin and methotrexate, as demonstrated by fluorescence-based drug retention assays (Fig. 1C and D, Supplementary Excel spreadsheet). We then extended our analysis to doxorubicin-resistant MDA-MB-231 cells. In these resistant cells, treatment with both inhibitors led to a marked decrease in the expression of all tested *ABCC* transporters as well as *ABCG2* (Fig. 1E). Moreover, drug accumulation assays revealed that Olaparib and Veliparib significantly enhanced intracellular retention of methotrexate, paclitaxel, and daunorubicin in doxorubicin-resistant cells (Fig. 1F and G, Supplementary Excel spreadsheet), further supporting the role of PARP1 in the regulation of ABC transporter-mediated drug efflux. To determine whether changes in ABC transporter expression and drug accumulation are specific to PARP1 inhibition and not the result of non-specific cytotoxic stress caused by prolonged drug exposure, we performed cytotoxicity tests on selected PARP1 inhibitors. These tests showed no toxicity in the cell lines tested (Supplementary Excel spreadsheet). These findings suggest that PARP1 inhibition effectively suppresses the expression of key ABC transporters involved in multidrug resistance, thereby enhancing intracellular drug retention in both wild-type and drug-resistant MDA-MB-231 cells.

Given the increasing evidence supporting the role of HPF1 in PARP1-dependent ADP-ribosylation during DNA damage, along with the observed downregulation of ABC transporters upon PARP inhibition, we further investigated the impact of silencing key components of the PARP1/HPF1 complex on ABC transporter expression.

Using real-time PCR, we demonstrated that silencing PARP1 and HPF1 significantly influences the regulation of ABC transporters in doxorubicin-resistant MDA-MB-231 cells. Knockdown of these factors resulted in a marked reduction in the expression levels of all tested ABC transporters, reinforcing their critical role in maintaining drug resistance.

In wild-type MDA-MB-231 cells, PARP1 silencing led to a significant reduction in the transcription of *ABCC1*, *ABCC3*, *ABCC4*, and *ABCC5*, while HPF1 silencing specifically inhibited *ABCC1* expression. In contrast, in doxorubicin-resistant MDA-MB-231 cells, where DNA damage was present even in the absence of drug treatment, silencing either PARP1 or HPF1 resulted in the downregulation of all tested ABC transporter genes (Fig. 2A). This suggests that the regulatory effect of the PARP1/HPF1 complex on ABC transporters is stress-dependent and plays a crucial role in multidrug resistance.

To further investigate the functional impact of PARP1/HPF1 silencing, we measured cytostatic drug accumulation in 2D and 3D spheroid cultures following knockdown of PARP1 and HPF1 (Fig. 2B and C, Supplementary Excel spreadsheet). In doxorubicin-resistant cells, silencing PARP1 and HPF1 significantly increased intracellular accumulation of anticancer drugs, whereas this effect was not observed in wild-type cells.

To determine whether PARP1/HPF1 complex formation is induced by increased DNA damage in resistant cells, we exposed wild-type MDA-MB-231 cells to DNA-damaging agents H_2O_2 and etoposide. Co-immunoprecipitation followed by Western blot analysis confirmed a direct interaction between PARP1 and HPF1 in response to genotoxic stress (Fig. 2D). Confocal microscopy further validated the colocalization of PARP1 and HPF1 within the cell nucleus (Fig. 2E).

Consistent with these findings, ADP-ribosylation levels were significantly higher in doxorubicin-resistant cells compared to wild-type MDA-MB-231, indicating elevated PARP1 activity and a higher degree of DNA damage in the resistant phenotype (Fig. 2F). Since the PARP1/HPF1 complex regulates ABC transporter expression in response to genotoxic stress, we tested whether its effect could be counteracted by antioxidants, which mitigate reactive oxygen species (ROS).

Interestingly, glutathione (GSH) significantly inhibited Veliparib-induced repression of *ABCC3* (at 2.5 μM), *ABCC4* (at 1 μM and 2.5 μM), and *ABCC5* (at 2.5 μM), genes critical for doxorubicin resistance. Similarly, Vitamin C counteracted Veliparib-mediated repression of *ABCC3*, *ABCC4*, and *ABCC5* at 1 μM, whereas N-acetylcysteine (NAC) only inhibited *ABCC4* when combined with Veliparib at 2.5 μM. Notably, an opposite and statistically significant effect was observed for *ABCC3* in cells treated with the Vitamin C (2.5 μM) + Veliparib combination (Fig. 3A, Supplementary Material). In wild-type MDA-MB-231 cells: 1) GSH significantly decreased *ABCC1* and *ABCC5* expression, while increasing *ABCC3*. 2) NAC increased the expression of *ABCB1*, *ABCC1*, and *ABCC10*. 3) Vitamin C induced *ABCC1* repression. In doxorubicin-resistant MDA-MB-231 cells: 1) GSH significantly reduced *ABCC3*, *ABCC4*, and *ABCC5* expression. 2) NAC repressed *ABCC3*, *ABCC4*, *ABCC5*, and *ABCG2*. 3) Vitamin C increased *ABCB1*, *ABCC10*, and *ABCC1* while decreasing *ABCC3*, *ABCC4*, *ABCC5*, and *ABCG2* (Fig. 3B). Given the profound impact of antioxidants on ABC transporter expression, we further compared ROS levels between wild-type and doxorubicin-resistant MDA-MB-231 cells. Notably, ROS levels were significantly elevated in resistant cells compared to the wild-type (Fig. 3C).

The above findings indicate that PARP1/HPF1 complex formation is driven by genotoxic stress and plays a critical role in ABC transporter overexpression in doxorubicin-resistant breast cancer cells. The ability of GSH and NAC to repress ABC transporter expression suggests their potential therapeutic application in counteracting multidrug resistance by modulating oxidative stress and PARP1 activity. These results provide preliminary information on the role of PARP1/HPF1 in chemoresistance and suggest that antioxidants may have the potential to modulate the response to chemotherapy in TNBC, although further studies are required to confirm their functional relevance.

Given the observed differences in PARP1 ADP-ribosylation levels between the studied cell lines, we sought to investigate whether intracellular mechanisms beyond DNA damage could influence PARP1 activity. Previous studies have suggested that post-translational modifications (PTMs) play a crucial role in modulating PARP1 enzymatic activity [26,27]. Therefore, we explored whether specific PTMs contribute to the differential regulation of PARP1 function in wild-type and doxorubicin-resistant MDA-MB-231 cells. (Fig. 4).

The doxorubicin-resistant MDA-MB-231 cells exhibited elevated levels of all tested post-translational modifications (PTMs) compared to the wild-type cells (Fig. 4). According to previous reports, methylation and acetylation of specific amino acid residues are known to enhance PARP1 activity. Additionally, phosphorylation plays a dual role in regulating PARP1 function: phosphorylation at the N-terminal BRCA1 C-terminal (BRCT) domain activates PARP1, whereas phosphorylation at

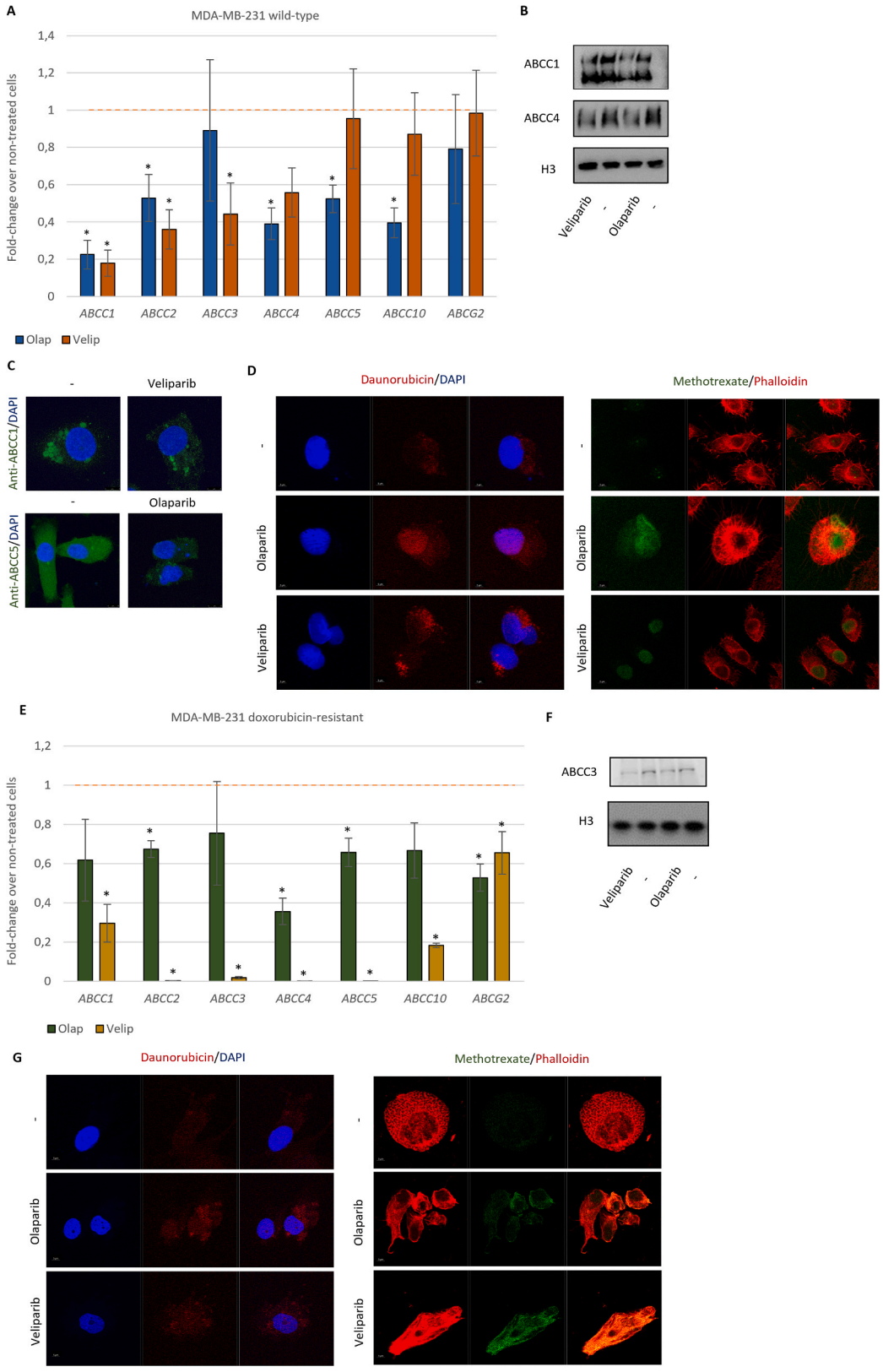
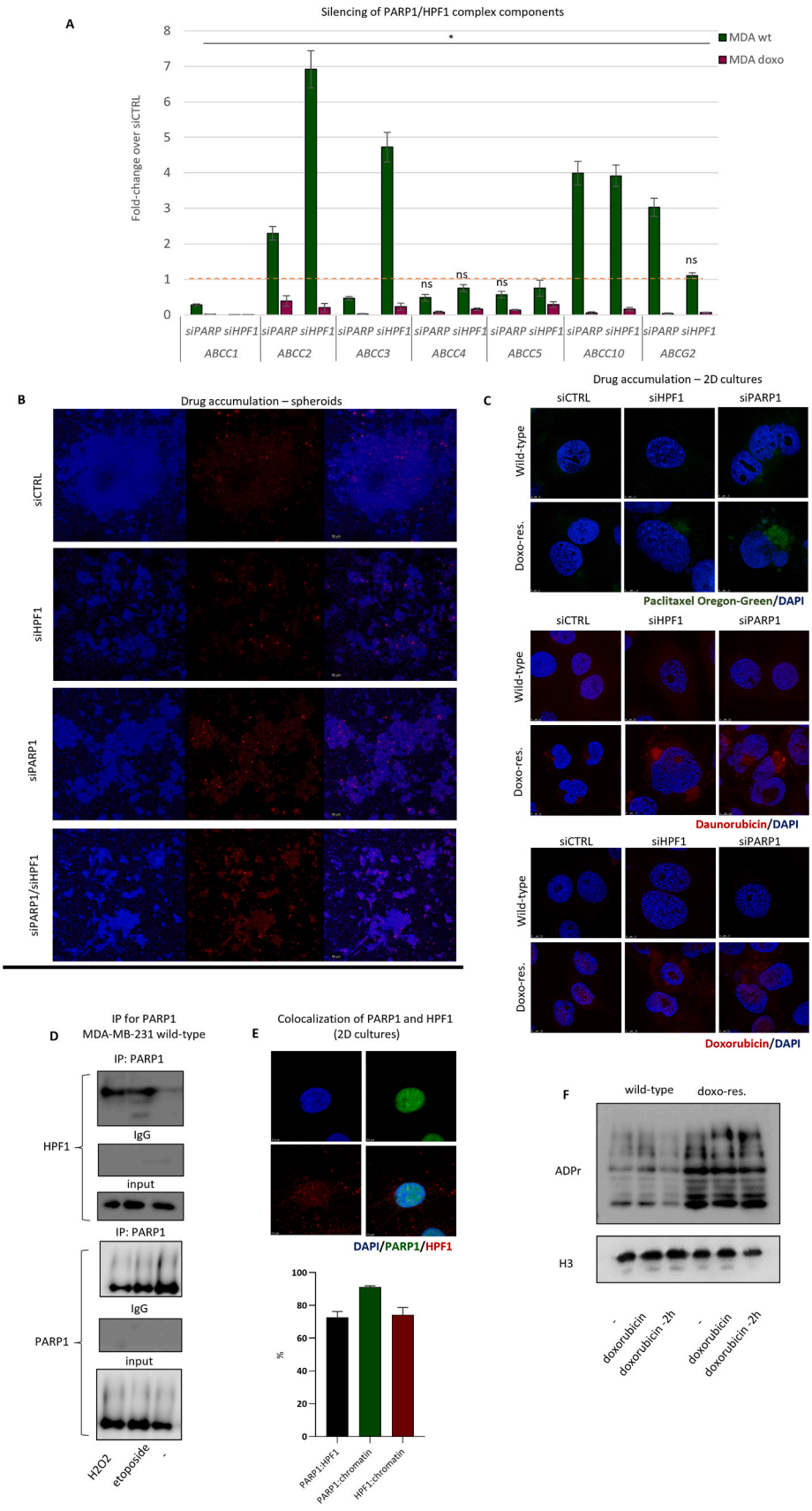


Fig. 1. PARP inhibitors silence the transcription of p300-related ABC genes and contribute to increased drug accumulation in the MDA-MB-231 basal and doxorubicin-resistant lines. (A)(E) The bar graphs represent the decrease in ABC gene transcription after incubation with PARP1 protein inhibitors, which was determined by Real-Time PCR. Results are presented as fold-change expression to untreated cells. Data are expressed as mean ± SEM, n = 18 – 36 for wild-type MDA-MB-231 and n = 3 – 26 for doxorubicin-resistant MDA-MB-231, *$p < 0.05$. (B)(F) Results at the mRNA level, so repression of selected genes was also confirmed at the protein level using Western Blot. The internal control for all samples was histone H3. (C) Confocal microscopy allowed confirmation of changes in the expression of ABC transporters at the protein level, visualizing their localization (D)(G) and the effect of inhibitors on the accumulation of selected cytokines.



(caption on next page)

Fig. 2. Silencing the components of the PARP1/HPF1 complex reduces the expression of ABC transporters, increases drug accumulation in 2D and 3D cultures, and moreover, the formation of the complex is dependent on DNA-damaging agents. (A) Alterations in ABC transporter expression after silencing of components of the PARP1/HPF1 complex were quantified by real-time PCR and presented as fold-change relative to untreated cells. Data are expressed as mean ± SEM, n = 4 – 12, *p < 0.05. (B)(C)(E) Confocal microscopy was used to determine the effect of silencing on drug accumulation in 2D and 3D cultures. In addition, the results allowed us to confirm the association between a decrease in transporter expression due to silencing of PARP1/HPF1 complex components and an increase in cytostatic drug accumulation in the doxorubicin-resistant line compared to the MDA-MB-231 baseline. (D) Co-immunoprecipitation of PARP1 protein with HPF1 detection was performed to verify the dependence of complex formation on the appearance of DNA-damaging conditions that are present in the doxorubicin-resistant line. Input indicates the total amount of proteins analyzed in the sample, and IgG was used as a control. (E) Colocalization of PARP1 and HPF1 in the doxorubicin-resistant MDA-MB-231 line was visualized by confocal microscopy. (F) The level of ADP-ribosylation was determined using Western Blot to assess PARP1 protein activity and the level of DNA damage in MDA-MB-231 baseline and doxorubicin-resistant line cells treated and untreated with doxorubicin.

the Ser177 residue inhibits its enzymatic activity [28]. Since the pan-phosphorylation antibody used in our analysis detects multiple phosphorylation sites, we cannot definitively conclude whether increased phosphorylation in the doxorubicin-resistant cells results in PARP1 activation or inhibition. Further site-specific phosphorylation studies will be required to determine its precise regulatory impact on PARP1 function in drug-resistant breast cancer cells.

Next, we sought to identify PARP1 and HPF1 binding sites across the genome in both wild-type and doxorubicin-resistant MDA-MB-231 cells using ChIP-Seq analysis. However, HPF1 was not detectable on chromatin, preventing its inclusion in further analysis. Therefore, we focused exclusively on PARP1-enriched regions for downstream bioinformatics assessment. Our analysis revealed that PARP1 binding sites were located within ±170,000 bp of the transcriptional start sites (TSS) of several ABC transporter genes (Fig. 5). These findings suggest a direct regulatory role for PARP1 in the transcriptional control of ABC transporters, further supporting its involvement in multidrug resistance mechanisms in triple-negative breast cancer cells.

Due to the modest increase in *ABCC2* and *ABCG2* expression in doxorubicin-resistant cells compared to wild-type, we focused our motif analysis on the PARP1-enriched regions of genes that exhibited both significant PARP1 binding and expression changes upon PARP1 silencing, namely: *ABCC3*, *ABCC4*, *ABCC5*, and *ABCC10*. These genes play a crucial role in doxorubicin resistance, as their expression is strongly linked to drug efflux mechanisms. Using the XSTREME tool from MEME-Suite, we identified a set of transcription factors (TFs) common to the PARP1-enriched regions of these genes in resistant cells. However, we were unable to detect motifs for the PARP1-rich region within ±10,000 bp of *ABCC10*, prompting us to assess whether ABCC10 plays a functional role in acquired resistance. To test this, we silenced *ABCC10* using siRNA and evaluated cellular sensitivity to selected anticancer drugs. Interestingly, *ABCC10* knockdown did not significantly affect drug sensitivity, suggesting that ABCC10 is not a critical determinant of acquired resistance in this model (Fig. 6). Consequently, the PARP1-rich region associated with *ABCC10* was excluded from further analyses and interpretation.

Since *ABCC10* silencing did not significantly impact cell viability in response to the tested drugs, we focused our analysis on identifying key transcription factors involved in PARP1-dependent gene expression for the PARP1-enriched regions common to *ABCC3*, *ABCC4*, and *ABCC5* (Fig. 7).

SMARCA1 (SNF2L1) belongs to the ISWI (Imitation Switch) family of proteins and is part of the NURF (Nucleosome Remodeling Factor) complex. ISWI proteins are responsible for ATP-dependent changes in chromatin structure, nucleosome positioning, DNA replication, and maintenance of repression [29]. Considering the role of this factor in chromatin remodeling, we selected this factor for further analysis. Notably, SMARCA1 binding motifs were exclusively identified in the PARP1-enriched regions of *ABCC3*, *ABCC4*, and *ABCC5* in doxorubicin-resistant cells, whereas these motifs were absent in the wild-type (baseline) cell line (Fig. 7 A-C). This suggests that SMARCA1 may be a key regulator of ABCC transporter gene expression in drug-resistant cells, linking chromatin remodeling to PARP1-mediated transcriptional regulation in the context of doxorubicin resistance.

In the next step, we examined the effect of *SMARCA1* silencing on ABC transporter expression to confirm its role as a transcriptional coregulator of selected PARP1-dependent genes. Knockdown of SMARCA1 significantly reduced the expression of *ABCC3* and *ABCC4*, but had no effect on these transporters in wild-type MDA-MB-231 cells, suggesting that its regulatory role is specific to the doxorubicin-resistant phenotype. Interestingly, SMARCA1 silencing also led to a marked reduction in *ABCC2* expression, despite the inability of MEME-Suite motif analysis to identify transcription factors within the PARP1-rich region (±10,000 bp from TSS) of this gene. This suggests that SMARCA1 may regulate *ABCC2* independently of identifiable sequence motifs. Conversely, *ABCC5* transcription was significantly upregulated following *SMARCA1* silencing, indicating that SMARCA1 may act as a repressor of *ABCC5* expression in doxorubicin-resistant cells (Fig. 8A). To assess the functional impact of SMARCA1 depletion, we performed a viability assay based on resazurin metabolism in doxorubicin-resistant MDA-MB-231 cells with normal and reduced SMARCA1 levels. *SMARCA1* silencing, in combination with a pan-ABCC inhibitor, led to a similar reduction in cell viability upon treatment with doxorubicin (1 μM) and paclitaxel (0.01 μM), reinforcing the role of SMARCA1 in multidrug resistance (Fig. 8B). These findings highlight SMARCA1 as a key regulatory factor in PARP1-dependent ABC transporter expression, with gene-specific activator and repressor functions, making it a potential target for overcoming drug resistance in triple-negative breast cancer.

Finally, we assessed the effect of SMARCA1 silencing on the intracellular accumulation of doxorubicin and paclitaxel to establish a functional correlation between ABC transporter repression, increased drug retention, and enhanced chemosensitivity. Since SMARCA1 silencing may influence broader chromatin remodeling mechanisms beyond ABC transporter transcription, this experiment aimed to confirm its direct role in drug efflux regulation. Two independent methods, fluorescence-based quantification and confocal microscopy, consistently demonstrated significantly higher intracellular drug accumulation following SMARCA1 silencing, supporting its role in mediating drug efflux via ABC transporters (Figs. 8C and 8D; Supplementary Excel spreadsheet). These findings strongly suggest that SMARCA1 is functionally linked to the transcriptional regulation of *ABCC3* and *ABCC4* at PARP1-enriched sites, reinforcing its role in PARP1-dependent multidrug resistance mechanisms. Given that HPF1 silencing also reduced the expression of p300/PARP1/SMARCA1-dependent genes, we hypothesized that HPF1 might be a component of this regulatory complex. To test this, we performed co-immunoprecipitation (co-IP), which confirmed a physical interaction between PARP1, HPF1, p300, and SMARCA1, establishing a direct molecular link between these factors in doxorubicin-resistant MDA-MB-231 cell line (Fig. 8E). To further validate the association of SMARCA1 with PARP1-enriched chromatin regions, we conducted chromatin immunoprecipitation (ChIP-qPCR). This analysis confirmed the presence of SMARCA1 at the PARP1-rich regulatory regions of *ABCC3*, *ABCC4*, and *ABCC5* in doxorubicin-resistant MDA-MB-231 cells, providing direct evidence of its transcriptional role in drug resistance (Fig. 8F). These results demonstrate that SMARCA1 directly regulates PARP1-dependent ABC transporter expression, enhances drug efflux, and contributes to chemoresistance in triple-negative breast cancer. Furthermore, the identification of a functional PARP1/HPF1/p300/SMARCA1 complex highlights a novel regulatory mechanism that could be targeted therapeutically to

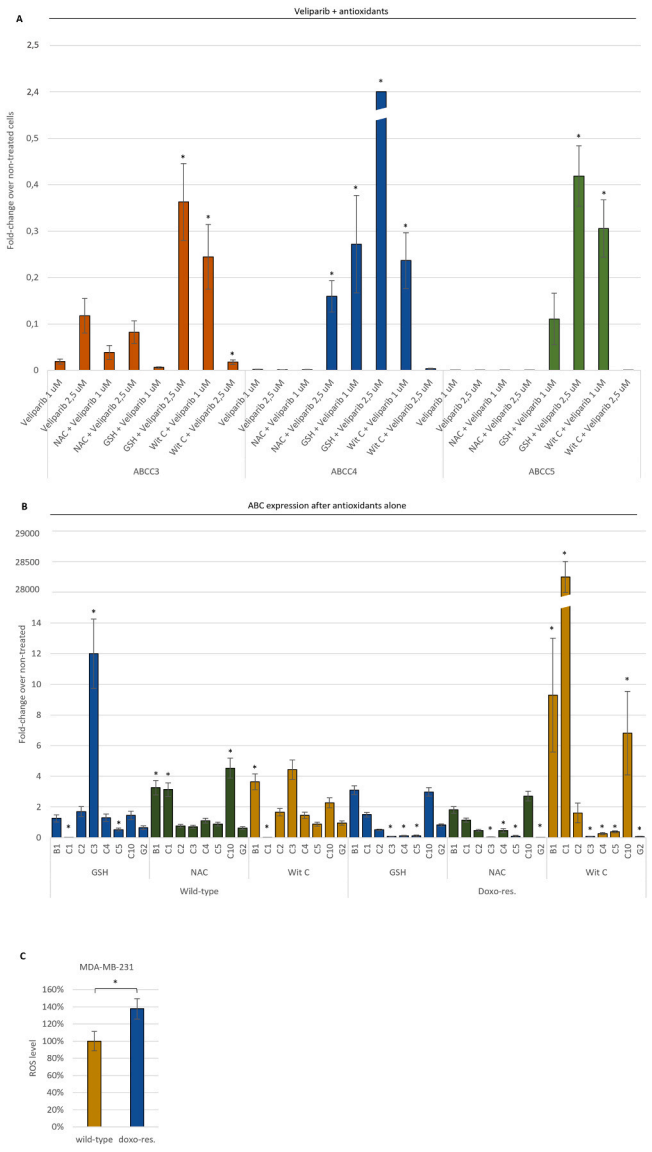


Fig. 3. Effects of antioxidants on Veliparib repression and direct expression of ABC transporters. Real-Time PCR technique was used to determine the effect of antioxidants relative to the previously described repressive effect of Veliparib on ABC transporters important for resistance (A) and to identify changes in ABC transporter expression after treatment with the commonly used antioxidants GSH, NAC and vitamin C in basal and resistant MDA-MB-231 lines (B). For (A) data are expressed as mean ± SEM, n = 16–20, *p < 0.05. For (B) data are expressed as mean ± SEM, n = 4 for wild-type MDA-MB-231, n = 4 – 20 for doxorubicin-resistant MDA-MB-231, *p < 0.05. The results are shown in a bar graph as the multiplicity of change relative to untreated cells. (C) In order to compare the differential effects of antioxidants on cells of the baseline and doxorubicin-resistant lines, the level of reactive oxygen species was determined using a fluorescence reader. Oxidation of DCFH by ROS transformed the molecule into DCF, which has the ability to emit green fluorescence. Measurements were made at wavelengths for λex= 485 nm and λem= 530 nm.

overcome multidrug resistance.

4. Discussion

Resistance to anticancer drugs remains a critical obstacle in chemotherapy, often leading to treatment failure and disease progression. Overcoming MDR requires a comprehensive understanding of the molecular mechanisms that drive its development. Various cellular processes contribute to doxorubicin resistance, including protective autophagy, which allows cancer cells to withstand cytotoxic stress, and the inhibition of tumor suppressor pathways such as phosphatase and tensin homolog (PTEN), which disrupts key regulatory mechanisms that normally limit tumor growth. At the same time, the activation of tumor-promoting factors like protein kinase B (Akt) enhances cell survival and proliferation, further diminishing the effectiveness of chemotherapy. The ability of cancer cells to evade apoptosis also plays a crucial role in MDR, allowing them to persist despite exposure to chemotherapeutic agents. However, one of the most significant contributors to resistance is the upregulation of ABC transporters, which actively expel drugs from the cell, reducing intracellular drug accumulation and limiting therapeutic efficacy. [30].

One of the most critical mechanisms underlying doxorubicin resistance is the overexpression of ABC transporters, which actively expel chemotherapeutic agents such as doxorubicin from cancer cells or sequester them within intracellular organelles, reducing drug efficacy. Previous studies have identified various transcription factors and chromatin remodeling enzymes involved in ABC transporter gene regulation in drug-resistant cancers. For instance, FOXO3a, HDAC2, EZH2, and NRF2 have been implicated in the transcriptional control of *ABCB1*, *ABCC2*, and *ABCG2* in different cancer types. [31]; HDAC2 was involved in the regulation of *ABCB1* expression in doxorubicin resistant hepatocarcinoma cells [32]; EZH2 regulates expression of *ABCB1* in doxorubicin-resistant breast cancer cells [33]; NRF2 was associated with the activation of *ABCB1* [34] as well as *ABCC2* and *ABCG2* in hepatocarcinoma cells [35]. There is still little known about the transcriptional regulation of ABCC group transporters although they constitute a large group of transporters that remove drugs from the cell. In our previous work, we described a mechanism in which the acquisition of cisplatin resistance by A549 non-small cell lung cancer cells and MDA-MB-231 triple-negative breast cancer cells led to the appearance of histone acetyltransferase p300 on the promoter of the *ABCC10* gene. In addition, the use of the bromodomain inhibitor CBP/p300 led to decreased expression of few ABC transporters in cisplatin, paclitaxel and doxorubicin resistant cancer cell lines [22,36]. In the present study, we verified that a similar effect would be obtained by using inhibitors and silencing PARP1 protein, whose role includes being a cofactor of histone acetyltransferase p300 that regulates gene expression. Previous reports showed that PARP1 directs the transcription of some proliferation and DNA repair genes in breast cancer cells by the ADP-ribosylation of p300, thereby facilitates the expression of genes that confer a cancer cell phenotype [37]. In this paper, the important role of the PARP1/HPF1 complex in regulating the expression of genes encoding ABC transporter proteins is presented. PARP1 protein inhibitors are used in monotherapy or in combination to treat many types of cancer. We were able to show that inhibition and silencing of PARP1 protein significantly decreases (Wilcoxon matched-pairs signed rank test, $p < 0,05$) the expression of ABC genes, indicating that PARP1 actively contributes to the regulation of transcription of these genes. The observed decrease in transporter expression correlated with increased accumulation of doxorubicin in cells, suggesting a functional relationship between PARP1 activity and drug efflux, which may determine the observed resistance to chemotherapy. By simultaneously downregulating the expression of transporters and inhibiting the DNA repair process, cancer cell death can be induced. Inhibition of PARP1 led to a significant increase in intracellular drug accumulation. This effect is consistent with the reduced expression of ABC transporters (ABCC2, ABCC3, ABCC4) observed during PARP1 inhibition. The most likely explanation for this phenomenon is that PARP1, through interaction with HPF1 and recruitment of SMARCA1, maintains a chromatin environment promoting ABC gene transcription. When PARP1 activity is blocked, these loci become less transcriptionally active, leading to reduced efflux capacity and higher intracellular drug levels. These results suggest that PARP1 contributes to chemotherapy resistance in TNBC cells not only through DNA repair, but also by maintaining the expression of transporters responsible for removing drugs from the tumor cell. In addition, the demonstrated relationship

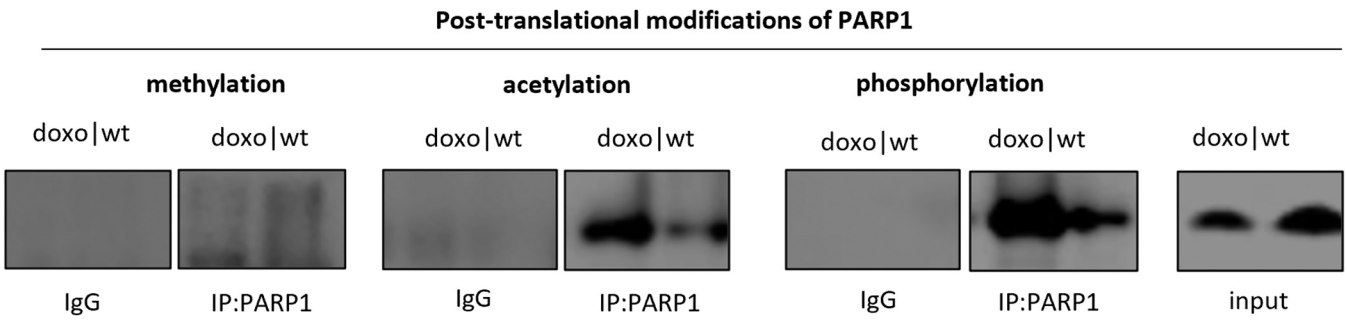


Fig. 4. Post-translational modifications of the PARP1 protein. The Western Blot technique allowed visualization and comparison of changes in PARP1 protein post-translational modifications such as acetylation, methylation and phosphorylation between the MDA-MB-231 basal and doxorubicin-resistant lines.

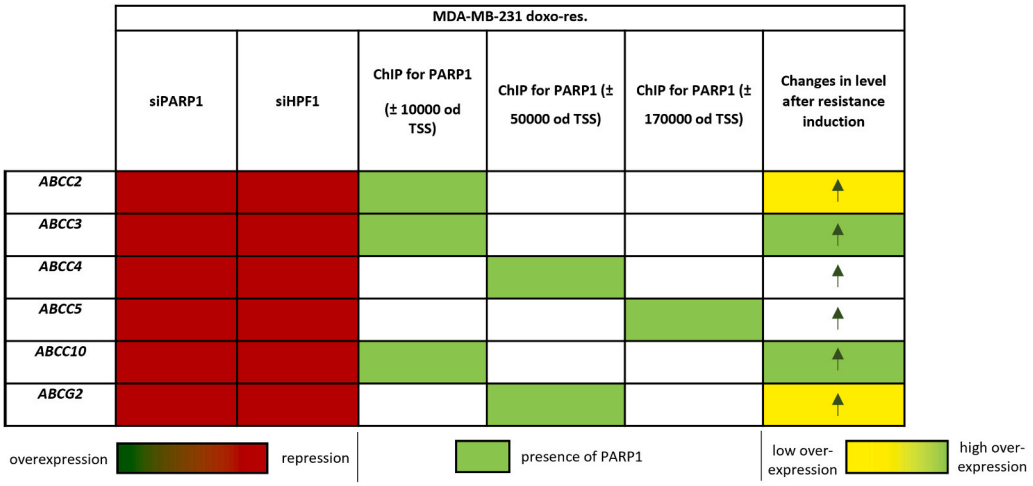


Fig. 5. Summary of the dependence of the transcription of *ABCC3*, *ABCC4*, and *ABCC5* genes on PARP1 and HPF1 proteins, the presence of PARP1 on chromatin at specific distances from the transcription initiation site of ABC genes, and changes in their levels after induction of resistance.

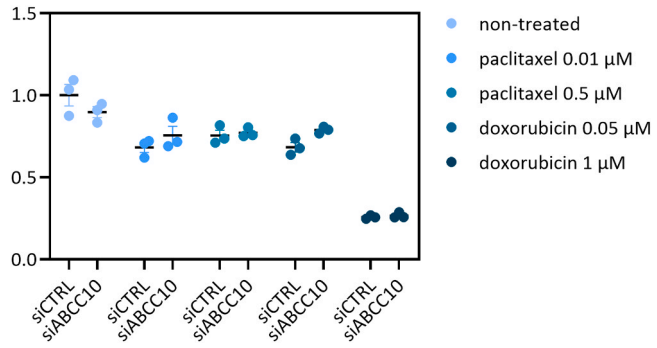


Fig. 6. Effect of ABCC10 silencing on cell viability. To confirm the minor role of ABCC10 in the induction of doxorubicin resistance, the level of viable cells after treatment with selected anticancer drugs in combination with silencing of the selected gene was determined. The results obtained are presented in the form of a heat-map. Scatter dot plot show individual replicate values (n = 3; independently treated wells within a single experiment); horizontal bars represent mean ± SEM.

may be important for the design of future therapies, which may aim at dual inhibition of PARP1 and HPF1 proteins.

We also demonstrated the important role of damage factors on the appearance of PARP1/HPF1 complex in the MDA-MB-231 baseline. Interestingly, we showed that GSH and vitamin C significantly down-regulate the expression of *ABCC3*, *ABCC4* and *ABCC5* genes with Veliparib at least one concentration (Mann Whitney test, $p < 0,05$), while NAC further significantly downregulates ABCC4 ($p < 0,0001$). Moreover, among all antioxidants, most notably GSH and NAC, downregulate the expression of PARP1-dependent ABC transporters. A mounting literature demonstrates an important role for GSH in tumor initiation and progression, as well as resistance, and GSH depletion itself is extremely important in ROS-based therapy [38]. In addition, one paper highlighted that GSH was involved in generating cisplatin resistance in several types of cancer [39]. In hepatocellular carcinoma, GSH and NAC induced proliferation and tumor growth [40]. Antioxidants indirectly modulate the expression of ABC transporters by altering intracellular ROS levels and the activity of redox-dependent and redox-sensitive transcription factors. GSH neutralizes ROS and modifies redox-dependent proteins, including Nrf2, thereby facilitating its translocation to the cell nucleus and increasing the expression of Nrf2-dependent genes [41]. NAC is a precursor of GSH, therefore it has a similar effect on gene expression and also influences ROS-dependent kinase pathways. Vitamin C acts as a reducing agent, alters the NADP/NADPH balance and may indirectly modulate the activity of transcription factors [42,43].

The quick and dysregulated expansion of tumors frequently surpasses the formation of new blood vessels, leading to a deficient and disorganized system that fails to deliver enough oxygen to all parts of the tumor. This dysfunctional angiogenesis contributes to uneven oxygen distribution and areas of chronic hypoxia within the tumor. During hypoxia, reactive oxygen species ROS are produced through several mechanisms, mainly mitochondrial dysfunctions and the activation of various pro-oxidant enzymes [44]. Previous reports link hypoxia with expression of *ABCB1* and *ABCG2* drug transporters [45]. In our study we provide the experimental evidence that oxidative stress increase

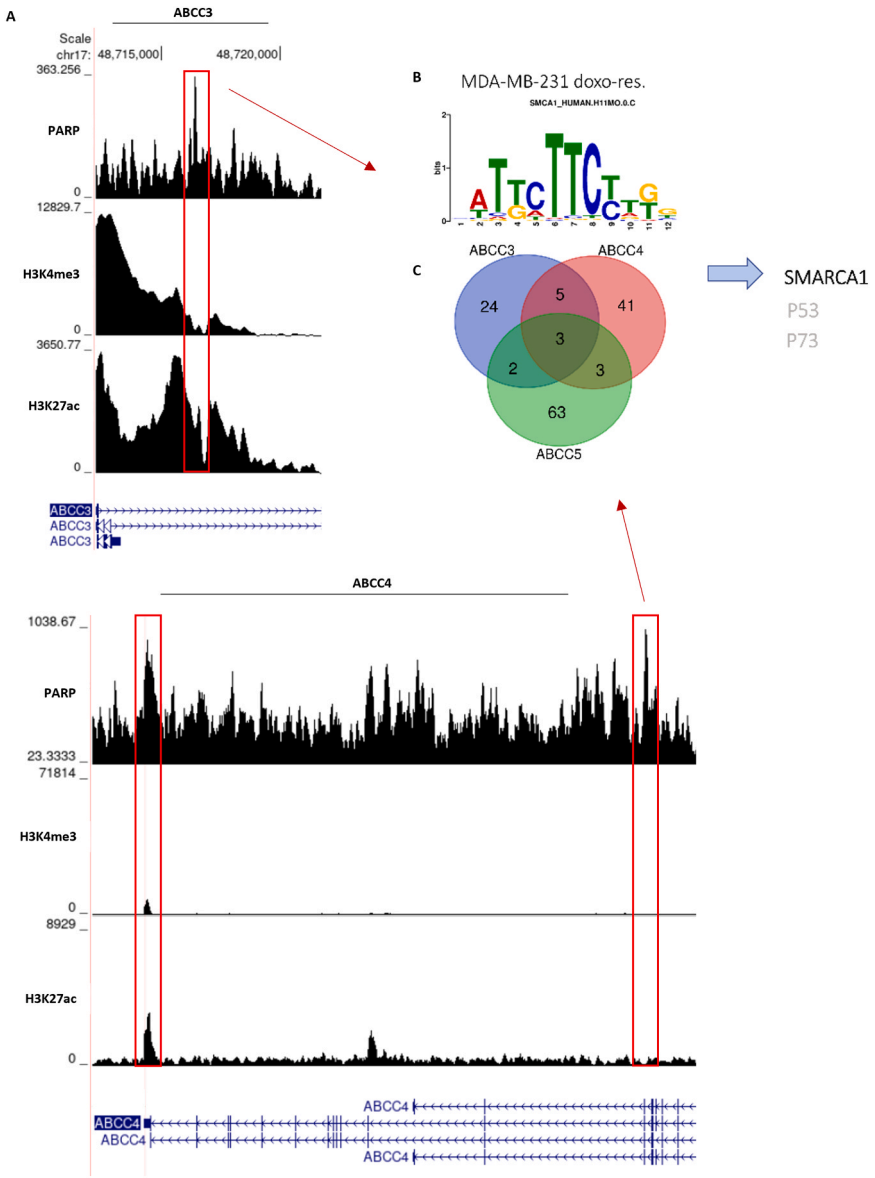
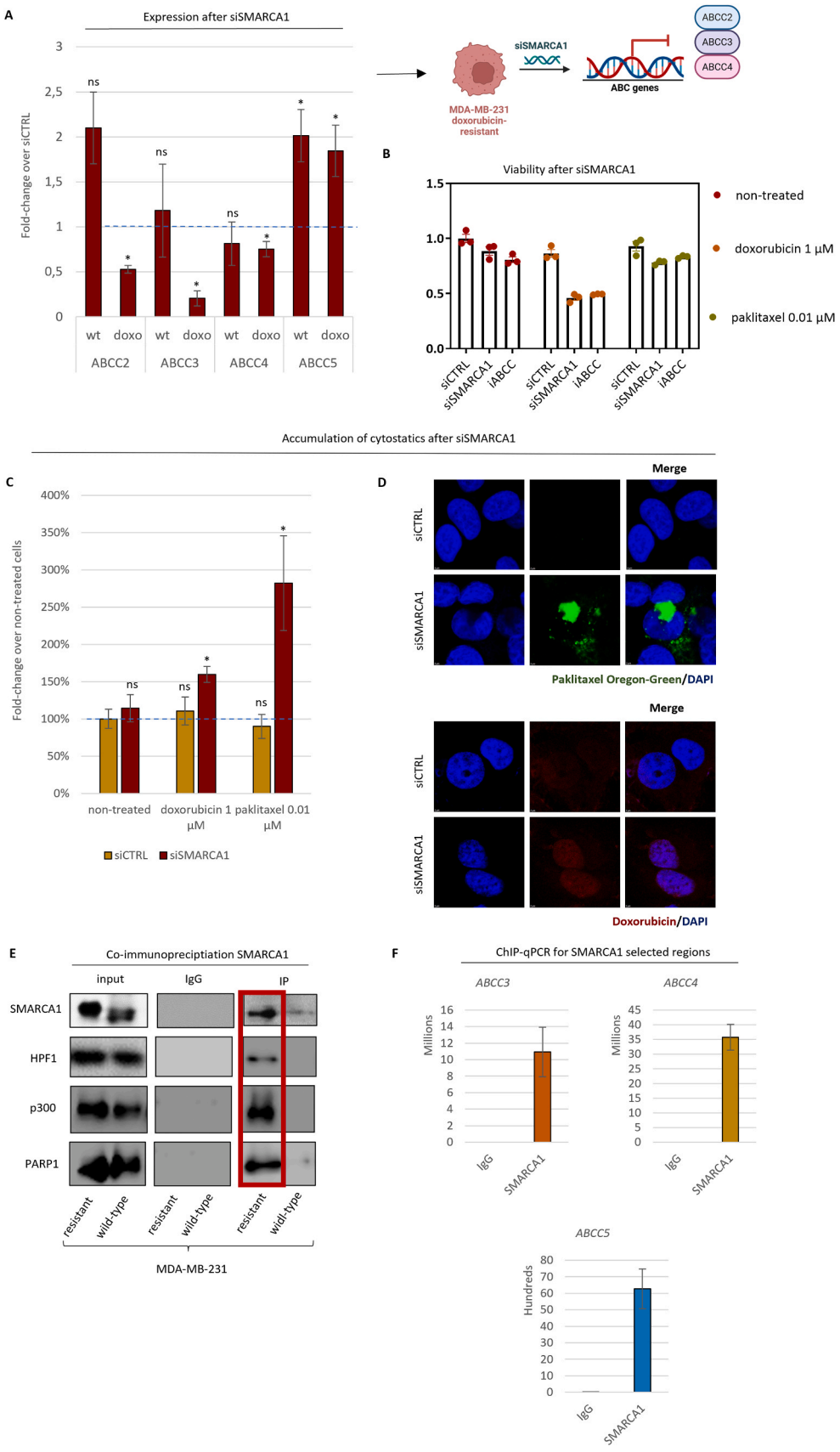


Fig. 7. Analysis of regions selected to generate a list of factors responsible for PARP1-dependent expression of *ABCC3*, *ABCC4* and *ABCC5* transporters. (A) Analysis of regions selected to generate a list of factors responsible for PARP1-dependent expression of *ABCC3*, *ABCC4* and *ABCC5* transporters. Regions of selected PARP1-rich ABC genes taken for further analysis were visualized using UCSC Genome Browser. Peaks for selected regions were highlighted in red. (B) A motif for SMARCA1 generated by the XSTREME tool in the MEME-Suite is also shown. (C) The number of common and individual factors for each gene is summarized in Venn Diagram form. Common factors for *ABCC3*, *ABCC4* and *ABCC5* alongside SMARCA1 include p53 and p73.

expression of ABCC group ABC transporters. In wild-type MDA-MB-231, GSH significantly ($p < 0,05$) raised *ABCC3* while significantly ($p < 0, 05$) lowering *ABCC1* and *ABCC5* (one-way ANOVA followed by Dunnet's multiple comparison test, $p < 0,05$). In resistant cell line GSH significantly decreased *ABCC3*, *ABCC4* and *ABCC5* expression (Kruskal-Wallis test followed by Dunnet's multiple comparison test, $p < 0, 05$). In the wild-type, NAC increased the levels of *ABCB1*, *ABCC1* and *ABCC10*, while in the resistant cell line it decreased the escalation of *ABCC3* and *ABCC5*. This result suggests that the effects of GSH and NAC may depend on the nature of resistance. It is important to conduct further studies to compare the effects of GSH and NAC in different types of cancer.

Furthermore, we identified a transcription factor that may be responsible for PARP1-dependent expression of *ABCC2*, *ABCC3*, *ABCC4* and *ABCC5*. SMARCA1 belongs to a family of chromatin remodeling complexes, and its appearance in regions for *ABCC3* and *ABCC4* suggested that it may play a role in regulating their expression. SMARCA1 is a mammalian ISWI gene that encodes the SNF2L protein. SNF2L plays a key role in regulating the transition of cells from a progenitor state to a differentiated state. Silencing of this factor significantly decreased viability (ANOVA followed by Dunnett's multiple comparisons test, $p < 0,05$) and increased accumulation of selected cytostatic agents. indicating that its presence plays an important role in maintaining resistance. Statistical significance confirms that these effects are unlikely to be due to chance and suggests a functional relationship between this factor and drug efflux mechanisms. Previous studies indicate that members of the SWI/SNF family may contribute to the genesis of resistance. For example, loss of the SMARCB1 subunit belonging to the SWI/SNF complex led to the induction of resistance to doxorubicin [46]. In turn, increased BRG1 and BRM activity regulates the response to commonly used chemotherapeutics. BRM activity has been linked to resistance to cisplatin [47] and gemcitabine [48]. BRG1, on the other hand, has been linked to resistance to doxorubicin [46] and paclitaxel [49]. Little is known about the relationship between the ISWI complex



(caption on next page)

Fig. 8. Effect of SMARCA1 silencing on *ABCC3*, *ABCC4* and *ABCC5* gene expression and cell sensitivity to selected drugs. (A) Alterations in the expression of selected ABCC genes in the doxorubicin-resistant MDA-MB-231 line after SMARCA1 factor silencing using siRNA-selected were measured by Real-Time PCR. The expression level of ABC transporters was visualized as a bar graph, and the results were presented as fold change against siCTRL. Data are expressed as mean ± SEM, n = 7–10, *p < 0.05. The siSMARCA1 functional scheme was created with BioRender.com (RRID:SCR_018361). (B) The resazurin assay was performed to compare cell viability after SMARCA1 silencing and application of the well-known pan-ABCC inhibitor probenecid in a system with doxorubicin (1 μM) and paclitaxel (0.01 μM). Data represent mean ± SEM of three independently treated wells (n = 3) from one representative experiment. Accumulation of cytostatic drugs after SMARCA1 silencing was measured using a fluorescence reader (C) and confocal microscopy (D). (E) Co-immunoprecipitation of SMARCA1 protein allowed verification of the physical interaction of the immunoprecipitated protein with PARP1, HPF1 and p300. (F) The ChIP-qPCR method allowed us to confirm the presence of SMARCA1 in PARP1-rich ABC gene regions in the doxorubicin-resistant MDA-MB-231 line. Data are expressed as mean ± SEM, n = 4, *p < 0.05.

and chemotherapy resistance. Previous studies indicate that SMARCA5 plays a role in the development of paclitaxel resistance in ovarian cancer [29]. In this work SMARCA1, as a ISWI chromatin remodeler, seems to selectively regulate the expression of ABC transporters in a phenotype-dependent manner contributing to cancer cell doxorubicin resistance. In resistant cells, which were characterized by the activation of doxorubicin resistance mechanisms, we observed increased recruitment of SMARCA1 to the ABCC2, ABCC3 and ABCC4 promoters, which correlated with higher expression of these genes and increased efflux activity. In contrast, in the case of ABCC5, SMARCA1 acted as a repressor, and its silencing resulted in increased gene expression. In basal cells, SMARCA1 was not selectively recruited, and ABC transporter expression remained at a lower level. These findings suggest that the regulatory function of SMARCA1 depends on the cell phenotype and may be altered in response to chemotherapeutic stress signals. Previous reports indicated that, high SMARCA1 expression was correlated with the metastatic potential of colorectal cancer and poor prognosis in colorectal cancer patients [50], as well as lung adenocarcinoma patients [51]. It should be noted that SMARCA1 silencing, besides significant downregulating the expression of *ABCC2*, *ABCC3* and *ABCC4*, also significantly upregulated the expression of *ABCC5* (*t*-test for independent samples or Mann-Whitney test, depending on whether the assumptions of normality, $p < 0{,}05$), which could suggest its role as a transcriptional repressor of this gene. Our results indicate that SMARCA1 regulates ABC transporter expression in a context-dependent manner, functioning as an activator for ABCC2 and ABCC3, and as a repressor for ABCC5. Comparable bidirectional roles have been reported for other chromatin remodelers, including BRG1/SMARCA4 [49], CHD4 [52–54], and ISWI [55,56]. The regulatory outcome of SMARCA1 appears to be determined by the local chromatin context, interacting protein partners, and epigenetic modifications. While our model is still hypothetical, it warrants further experimental validation. Our data suggest that the regulation of ABC transporter expression by SMARCA1 may be modulated by the PARP1/HPF1/p300/SMARCA1 complex. The formation of this complex likely occurs in regions of active chromatin in response to cellular stress or chemotherapy treatment, enabling nucleosome remodeling and histone acetylation. Depending on the local genomic context, this mechanism can lead to both activation (ABCC2, ABCC3, ABCC4) and repression (ABCC5) of ABC transporter genes, which may explain the context-dependent function of SMARCA1. In our previous study, we demonstrated a similar relationship in which the absence of a repressive CoREST complex in the promoter of the *ABCC10* gene led to enhanced transcription in non-small cell lung cancer cells and triple-negative breast cancer cells (treated with cisplatin) through binding of the p300 protein [36]. Taken together, these results indicate that, depending on the type of cancer, SMARCA1 can be anti- or pro-tumor. In the context of TNBC, our findings suggest that SMARCA1 may represent a novel druggable epigenetic regulator of chemoresistance. This opens up the possibility of designing combinatory therapies involving PARP1 inhibitors and SMARCA1 modulators to enhance drug efficacy in patients with drug-resistant tumors. In the case of doxorubicin-resistant MDA-MB-231, we demonstrated possible tumor-suppressive function involving ABC transporters that are important in resistance.

The main advantage of our study is the identification of SMARCA1 as a chromatin remodeler and regulator of ABC transporters in TNBC cells with established resistance to doxorubicin, which is supported by both gene expression analyses and functional drug accumulation assays. Another advantage is the inclusion of PARP1/HPF1, p300 and SMARCA1 in a common regulatory complex controlling chemotherapy resistance, which reveals a new mechanistic perspective. In the future, it would be worthwhile to perform studies on a cell model other than TNBC, as well as to verify the effect of the discussed combination therapy on healthy cells. Future studies should include additional experiments on antioxidants that would focus on the functional validation of drug accumulation and cytotoxic response. It would also be important to perform genomic mapping of SMARCA1 binding sites after PARP1 inhibition (ChIP-seq) and global transcriptomic profiling after SMARCA1 silencing (RNA-seq). Such analyses would help confirm the direct regulation of ABC transporters and identify potential SMARCA1 co-factors. Our data suggest the existence of additional potential regulators, such as p53 and p73, which emerged in the analysis. Investigating their role in regulating the expression of *ABCC3*, *ABCC4* and *ABCC5* opens up additional avenues for future research. Finally, in vivo validation was not possible within the current project due to financial constraints, but will be crucial to confirm the translational relevance of our findings. Future studies should therefore extend these observations to additional resistant TNBC models, animal systems, and genome-wide chromatin and transcriptomic analyses. Performing RNA-Seq after silencing SMARCA1 could reveal transcriptomic changes that were induced by the loss of this remodeler, not just selected ABCCs. This would allow confirmation of which ABC transporters are directly regulated, as well as the identification of potential SMARCA1 co-factors.

The potential clinical application of our discoveries lies in combination therapies using PARP1 inhibitors together with SMARCA1 modulators to improve the effectiveness of treating tumors with increased overexpression of ABC transporters characterized by multidrug resistance. Such strategies may allow for the use of lower doses of cytotoxic agents while maintaining therapeutic efficacy, reducing systemic toxicity and minimize the risk of cancer cells resistance development. Given that epigenetic changes often alter the sensitivity of cancer cells to therapies, this highlights the importance of epigenetics-targeted therapy as an effective complementary treatment. Although monotherapy with epigenetic drugs has shown high efficacy in treating various types of cancer, it has also been associated with side effects. A growing body of research points to the potential benefits of combination therapies that would include epigenetic drugs with other therapeutic methods, including chemotherapy and targeted therapy [57]. It has been shown that combination therapy involving DNMT and HDAC inhibitors simultaneously induces the expression of major tumor suppressor genes and inhibits the expression of key oncogenes such as MYC and IRF4, demonstrating greater anticancer efficacy than monotherapy [58]. Low doses of DNMT and HDAC inhibitors reprogram cancer cells to respond to cytotoxic agents and overcome resistance to chemotherapy [57,59]. Our study suggests that SMARCA1 targeting could also sensitized cells to therapy. However, our research leaves several challenges unresolved. Although MDA-MB-231 is a widely used TNBC model and allows for the study of ABC transporter regulation mechanisms, it should be noted that this line does not reflect the full heterogeneity and microenvironment observed in patient tumors. Therefore, the results should be interpreted in the context of the limitations of the in vitro model. A more detailed understanding of the mechanisms of interaction

between PARP1/HPF1 and SMARCA1 in ABC regulation is needed. In addition, the safety of combined modulation of these proteins for healthy cells should be carefully evaluated, and potential limitations related to pharmacokinetics and drug interactions should be verified in preclinical studies.

5. Conclusions

SMARCA1 plays a role in the PARP1-dependent expression of *ABCC2*, *ABCC3*, *ABCC4* and *ABCC5* transporters, which contribute to the resistance observed after doxorubicin treatment in triple-negative breast cancer cell lines. Further studies are warranted to elucidate the precise mechanism of action of SMARCA1. Nevertheless, these findings support its potential as a molecular target for therapeutic strategies aimed at overcoming multidrug resistance in breast cancer.

Ethics approval and consent to participate

Not applicable.

Funding information

This research was funded by National Science Centre Poland, grant number 2022/45/N/NZ5/01371 awarded to M. Strachowska.

Lorant Szekvolgyi was supported by NKFIH-K-142137 and the Thematic Excellence Programme TKP2021-EGA-18 provided from the National Research, Development and Innovation Fund of Hungary.

Consent for publication

Not applicable.

CRediT authorship contribution statement

Karolina Gronkowska: Writing – review & editing, Writing – original draft, Investigation, Formal analysis. **Kinga Kołacz-Milewska:** Writing – review & editing, Investigation. **Magdalena Strachowska:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Lóránt Székvölgyi:** Writing – review & editing, Methodology, Funding acquisition. **Agnieszka Robaszkiewicz:** Writing – review & editing, Supervision, Methodology, Conceptualization. **Éva Nagy:** Investigation. **Damian Jacenik:** Writing – review & editing. **Sebastian Skoczylas:** Investigation.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

The authors have no acknowledgements to report.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.biopha.2025.118698.

Data availability

Sequencing data are available at NCBI SRA (accession SRR32846407, SRR32846406, SRR32845362, SRR32845363), all other datasets are included in the Supplementary Material.

References

[1] World Health Organization (WHO), (n.d.). ⟨https://www.who.int/⟩ (accessed September 22, 2025).
[2] A. Zafar, S. Khatoon, M.J. Khan, J. Abu, A. Naeem, Advancements and limitations in traditional anti-cancer therapies: a comprehensive review of surgery, chemotherapy, radiation therapy, and hormonal therapy, Discov. Oncol. 16 (2025) 607, https://doi.org/10.1007/s12672-025-02198-8.
[3] Y.-J. Chern, I. T. Tai, Adaptive response of resistant cancer cells to chemotherapy, Cancer Biol. Med. 17 (2020) 842–863, https://doi.org/10.20892/j.issn.2095-3941.2020.0005.
[4] T.B. Emran, A. Shahriar, A.R. Mahmud, T. Rahman, M.H. Abir, MohdF.-R. Siddiquee, H. Ahmed, N. Rahman, F. Nainu, E. Wahyudin, S. Mitra, K. Dhama, M.M. Habiballah, S. Haque, A. Islam, M.M. Hassan, Multidrug resistance in cancer: understanding molecular mechanisms, immunoprevention and therapeutic approaches, Front. Oncol. 12 (2022) 891652, https://doi.org/10.3389/fonc.2022.891652.
[5] X.-T. Fu, K. Song, J. Zhou, Y.-H. Shi, W.-R. Liu, G.-M. Shi, Q. Gao, X.-Y. Wang, Z.-B. Ding, J. Fan, Tumor-associated macrophages modulate resistance to oxaliplatin via inducing autophagy in hepatocellular carcinoma, Cancer Cell Int. 19 (2019) 71, https://doi.org/10.1186/s12935-019-0771-8.
[6] Y. Lv, S. Zhao, J. Han, L. Zheng, Z. Yang, L. Zhao, Hypoxia-inducible factor-1α induces multidrug resistance protein in colon cancer, Onco Targets Ther. 8 (2015) 1941–1948, https://doi.org/10.2147/OTT.S82835.
[7] L. Sivak, V. Subr, J. Tomala, B. Rihova, J. Strohalm, T. Etrych, M. Kovar, Overcoming multidrug resistance via simultaneous delivery of cytostatic drug and P-glycoprotein inhibitor to cancer cells by HPMA copolymer conjugate, Biomaterials 115 (2017) 65–80, https://doi.org/10.1016/j.biomaterials.2016.11.013.
[8] J. Goebel, J. Chmielewski, C.A. Hrycyna, The roles of the human ATP-binding cassette transporters P-glycoprotein and ABCG2 in multidrug resistance in cancer and at endogenous sites: future opportunities for structure-based drug design of inhibitors, Cancer Drug Resist. 4 (2021) 784–804, https://doi.org/10.20517/cdr.2021.19.
[9] M. Strachowska, K. Gronkowska, S. Michlewska, A. Robaszkiewicz, CBP/p300 bromodomain Inhibitor–I–CBP112 declines transcription of the key ABC transporters and sensitizes cancer cells to chemotherapy drugs, Cancers 13 (2021) 4614, https://doi.org/10.3390/cancers13184614.
[10] M.-F. Langelier, T. Eisemann, A.A. Riccio, J.M. Pascal, PARP family enzymes: regulation and catalysis of the poly(ADP-ribose) posttranslational modification, Curr. Opin. Struct. Biol. 53 (2018) 187–198, https://doi.org/10.1016/j.sbi.2018.11.002.
[11] T.A. Kurgina, N.A. Moor, M.M. Moor, K.N. Naumenko, A.A. Ukraintsev, O.I. Lavrik, HPF1 stimulates PARP1 and PARP2 to autoPARylation and histone modification, Sema 27 (2023), 163–163.
[12] M. Borgini, P. Wipf, Synthesis of veliparib prodrugs and determination of Drug-Release-Dependent PARP-1 inhibition, ACS Med. Chem. Lett. 14 (2023) 652–657, https://doi.org/10.1021/acsmedchemlett.3c00065.
[13] L.M. Wagner, Profile of veliparib and its potential in the treatment of solid tumors, Onco Targets Ther. 8 (2015) 1931–1939, https://doi.org/10.2147/OTT.S69935.
[14] FDA approved olaparib (LYNPARZA, AstraZeneca Pharmaceuticals LP) for the maintenance treatment of adult patients with deleterious or suspected deleterious germline or somatic BRCA-mutated (gBRCAm or sBRCAm) advanced epithelial ovarian, fallopian tube or primary peritoneal cancer who are in complete or partial response to first-line platinum-based | FDA, (n.d.). ⟨https://www.fda.gov/drugs/fda-approved-olaparib-lynparza-astrazeneca-pharmaceuticals-lp-maintenance-treatment-adult-patients⟩ (accessed September 22, 2025).
[15] X. Fu, P. Li, Q. Zhou, R. He, G. Wang, S. Zhu, A. Bagheri, G. Kupfer, H. Pei, J. Li, Mechanism of PARP inhibitor resistance and potential overcoming strategies, Genes Dis. 11 (2024) 306–320, https://doi.org/10.1016/j.gendis.2023.02.014.
[16] N.E. Muvarak, K. Chowdhury, L. Xia, C. Robert, E.Y. Choi, Y. Cai, M. Bellani, Y. Zou, Z.N. Singh, V.H. Duong, T. Rutherford, P. Nagaria, S.M. Bentzen, M. M. Seidman, M.R. Baer, R.G. Lapidus, S.B. Baylin, F.V. Rassool, Enhancing the cytotoxic effects of PARP inhibitors with DNA demethylating agents - a potential therapy for cancer, Cancer Cell 30 (2016) 637–650, https://doi.org/10.1016/j.ccell.2016.09.002.
[17] J.K. Horton, S.H. Wilson, Strategic combination of DNA-Damaging agent and PARP inhibitor results in enhanced cytotoxicity, Front Oncol. 3 (2013) 257, https://doi.org/10.3389/fonc.2013.00257.
[18] K. Balaji, S. Vijayaraghavan, L. Diao, P. Tong, Y. Fan, J.P. Carey, T.N. Bui, S. Warner, J.V. Heymach, K.K. Hunt, J. Wang, L.A. Byers, K. Keyomarsi, AXL inhibition suppresses the DNA damage response and sensitizes cells to PARP inhibition in multiple cancers, Mol. Cancer Res. 15 (2017) 45–58, https://doi.org/10.1158/1541-7786.MCR-16-0157.
[19] R.A. Sankaranarayanan, J. Peil, A.T.J. Vogg, C. Bolm, S. Terhorst, A. Classen, M. Bauwens, J. Maurer, F. Mottaghy, A. Morgenroth, Auger emitter conjugated PARP inhibitor for therapy in triple negative breast cancers: a comparative In-Vitro study, Cancers (Basel) 14 (2022) 230, https://doi.org/10.3390/cancers14010230.
[20] W.-K. Wong, F.D.C. Guerra Liberal, S.J. McMahon, DNA repair inhibitors potentiate fractionated radiotherapy more than Single-Dose radiotherapy in breast cancer cells, Cancers (Basel) 14 (2022) 3794, https://doi.org/10.3390/cancers14153794.
[21] E.A.M. Ruigrok, N.S. Verkaik, E. de Blois, C. de Ridder, D. Stuurman, S.J. Roobol, D.C. Van Gent, M. de Jong, W.M. Van Weerden, J. Nonnekens, Preclinical assessment of the combination of PSMA-Targeting radionuclide therapy with PARP

inhibitors for prostate cancer treatment, Int. J. Mol. Sci. 23 (2022) 8037, https://doi.org/10.3390/ijms23148037.
[22] M. Strachowska, K. Gronkowska, M. Sobczak, M. Grodzicka, S. Michlewska, K. Kołacz, T. Sarkar, J. Korszun, M. Ionov, A. Robaszkiewicz, I-CBP112 declines overexpression of ATP-binding cassette transporters and sensitized drug-resistant MDA-MB-231 and A549 cell lines to chemotherapy drugs, Biomed. Pharmacother. 168 (2023) 115798, https://doi.org/10.1016/j.biopha.2023.115798.
[23] E. Wiśnik, T. Płoszaj, A. Robaszkiewicz, Downregulation of PARP1 transcription by promoter-associated E2F4-RBL2-HDAC1-BRM complex contributes to repression of pluripotency stem cell factors in human monocytes, Sci. Rep. 7 (2017) 9483, https://doi.org/10.1038/s41598-017-10307-z.
[24] The Galaxy Community, The galaxy platform for accessible, reproducible, and collaborative data analyses: 2024 update, Nucleic Acids Res. 52 (2024) W83–W94, https://doi.org/10.1093/nar/gkae410.
[25] T.L. Bailey, J. Johnson, C.E. Grant, W.S. Noble, The MEME suite, Nucleic Acids Res. 43 (2015) W39–W49, https://doi.org/10.1093/nar/gkv416.
[26] R. Hamamoto, Regulation of poly ADP-Ribose polymerase 1 functions by post-translational modifications, Front. Biosci. 23 (2018) 13–26, https://doi.org/10.2741/4578.
[27] U.M. Muthurajan, M.R.D. Hepler, A.R. Hieb, N.J. Clark, M. Kramer, T. Yao, K. Luger, Automodification switches PARP-1 function from chromatin architectural protein to histone chaperone, Proc. Natl. Acad. Sci. U. S. A. 111 (2014) 12752–12757, https://doi.org/10.1073/pnas.1405005111.
[28] F. Shang, J. Zhang, Z. Li, J. Zhang, Y. Yin, Y. Wang, T.L. Marin, B. Gongol, H. Xiao, Y. Zhang, Z. Chen, J.Y.-J. Shyy, T. Lei, Cardiovascular protective effect of metformin and telmisartan: reduction of PARP1 activity via the AMPK-PARP1 cascade, PLoS ONE 11 (2016) e0151845, https://doi.org/10.1371/journal.pone.0151845.
[29] Y. Li, H. Gong, P. Wang, Y. Zhu, H. Peng, Y. Cui, H. Li, J. Liu, Z. Wang, The emerging role of ISWI chromatin remodeling complexes in cancer, J. Exp. Clin. Cancer Res. 40 (2021) 346, https://doi.org/10.1186/s13046-021-02151-x.
[30] M.D.A. Paskeh, H. Saebfar, M.K. Mahabady, S. Orouei, K. Hushmandi, M. Entezari, M. Hashemi, A.R. Aref, M.R. Hamblin, H.L. Ang, A.P. Kumar, A. Zarrabi, S. Samarghandian, Overcoming doxorubicin resistance in cancer: siRNA-loaded nanoarchitectures for cancer gene therapy, Life Sci. 298 (2022) 120463, https://doi.org/10.1016/j.lfs.2022.120463.
[31] R.C.-Y. Hui, R.E. Francis, S.K. Guest, J.R. Costa, A.R. Gomes, S.S. Myatt, J. J. Brosens, E.W.-F. Lam, Doxorubicin activates FOXO3a to induce the expression of multidrug resistance gene *abcb1* (*mdr1*) in K562 leukemic cells, Mol. Cancer Ther. 7 (2008) 670–678, https://doi.org/10.1158/1535-7163.MCT-07-0397.
[32] Y. Yang, J. Zhang, T. Wu, X. Xu, G. Cao, H. Li, X. Chen, Histone deacetylase 2 regulates the doxorubicin (Dox) resistance of hepatocarcinoma cells and transcription of ABCB1, Life Sci. 216 (2019) 200–206, https://doi.org/10.1016/j.lfs.2018.11.043.
[33] B. Jiang, Y. Li, X. Qu, H. Zhu, Y. Tan, Q. Fan, Y. Jiang, M. Liao, X. Wu, Long noncoding RNA cancer susceptibility candidate 9 promotes doxorubicin-resistant breast cancer by binding to enhancer of zeste homolog 2, Int. J. Mol. Med. (2018), https://doi.org/10.3892/ijmm.2018.3812.
[34] X. Xia, Q. Wang, T. Ye, Y. Liu, D. Liu, S. Song, C. Zheng, NRF 2/ ABCB 1-mediated efflux and PARP 1-mediated dampening of DNA damage contribute to doxorubicin resistance in chronic hypoxic HepG2 cells, Fundamental Clin. Pharmacy 34 (2020) 41–50, https://doi.org/10.1111/fcp.12505.
[35] T. Adachi, H. Nakagawa, I. Chung, Y. Hagiya, K. Hoshijima, N. Noguchi, M.T. Kuo, T. Ishikawa, Nrf2-dependent and -independent induction of ABC transporters ABCC1, ABCC2, and ABCG2 in HepG2 cells under oxidative stress, J. Exp. Ther. Oncol. 6 (2007) 335–348.
[36] M. Sobczak, M. Strachowska, K. Gronkowska, A. Robaszkiewicz, Activation of ABCC genes by cisplatin depends on the CoREST occurrence at their promoters in A549 and MDA-MB-231 cell lines, Cancers 14 (2022) 894, https://doi.org/10.3390/cancers14040894.
[37] M. Sobczak, A.R. Pitt, C.M. Spickett, A. Robaszkiewicz, PARP1 Co-Regulates EP300–BRG1-Dependent transcription of genes involved in breast cancer cell proliferation and DNA repair, Cancers 11 (2019) 1539, https://doi.org/10.3390/cancers11101539.
[38] L. Kennedy, J.K. Sandhu, M.-E. Harper, M. Cuperlovic-Culf, Role of glutathione in cancer: from mechanisms to therapies, Biomolecules 10 (2020) 1429, https://doi.org/10.3390/biom10101429.
[39] B. Niu, K. Liao, Y. Zhou, T. Wen, G. Quan, X. Pan, C. Wu, Application of glutathione depletion in cancer therapy: enhanced ROS-based therapy, ferroptosis, and chemotherapy, Biomaterials 277 (2021) 121110, https://doi.org/10.1016/j.biomaterials.2021.121110.
[40] V.X. Zhang, K.M.-F. Sze, L.-K. Chan, D.W.-H. Ho, Y.-M. Tsui, Y.-T. Chiu, E. Lee, A. Husain, H. Huang, L. Tian, C.C.-L. Wong, I.O.-L. Ng, Antioxidant supplements promote tumor formation and growth and confer drug resistance in hepatocellular carcinoma by reducing intracellular ROS and induction of TMBIM1, Cell Biosci. 11 (2021) 217, https://doi.org/10.1186/s13578-021-00731-0.
[41] X. Chen, Q. Zhou, H. Chen, J. Bai, R. An, K. Zhang, X. Zhang, H. An, J. Zhang, Y. Wang, M. Li, Glutathione induces Keap1 S-Glutathionylation and mitigates oscillating Glucose-Induced β-Cell dysfunction by activating Nrf2, Antioxidants (Basel) 13 (2024) 400, https://doi.org/10.3390/antiox13040400.
[42] Y. Xu, H. Zheng, I. Slabu, E.A. Liehn, M. Rusu, Vitamin c in cardiovascular disease: from molecular mechanisms to clinical evidence and therapeutic applications, Antioxidants 14 (2025) 506, https://doi.org/10.3390/antiox14050506.
[43] J. Kaźmierczak-Barańska, K. Boguszewska, A. Adamus-Grabicka, B.T. Karwowski, Two faces of vitamin C-Antioxidative and Pro-Oxidative agent, Nutrients 12 (2020) 1501, https://doi.org/10.3390/nu12051501.
[44] E.N. Mendoza, M.R. Ciriolo, F. Ciccarone, Hypoxia-Induced reactive oxygen species: their role in cancer resistance and emerging therapies to overcome it, Antioxidants (Basel) 14 (2025) 94, https://doi.org/10.3390/antiox14010094.
[45] M.L. Pinzón-Daza, Y. Cuellar-Saenz, F. Nualart, A. Ondo-Mendez, L. Del Riesgo, F. Castillo-Rivera, R. Garzón, Oxidative stress promotes Doxorubicin-Induced pgp and BCRP expression in colon cancer cells under hypoxic conditions, J. Cell. Biochem. 118 (2017) 1868–1878, https://doi.org/10.1002/jcb.25890.
[46] R. Dubey, A.M. Lebensohn, Z. Bahrami-Nejad, C. Marceau, M. Champion, O. Gevaert, B.I. Sikic, J.E. Carette, R. Rohatgi, Chromatin-Remodeling complex SWI/SNF controls multidrug resistance by transcriptionally regulating the drug efflux pump ABCB1, Cancer Res. 76 (2016) 5810–5821, https://doi.org/10.1158/0008-5472.CAN-16-0716.
[47] I. Jancewicz, J.A. Siedlecki, T.J. Sarnowski, E. Sarnowska, BRM: the core ATPase subunit of SWI/SNF chromatin-remodelling complex-a tumour suppressor or tumour-promoting factor? Epigenetics Chromatin 12 (2019) 68, https://doi.org/10.1186/s13072-019-0315-4.
[48] Z. Zhang, F. Wang, C. Du, H. Guo, L. Ma, X. Liu, M. Kornmann, X. Tian, Y. Yang, BRM/SMARCA2 promotes the proliferation and chemoresistance of pancreatic cancer cells by targeting JAK2/STAT3 signaling, Cancer Lett. 402 (2017) 213–224, https://doi.org/10.1016/j.canlet.2017.05.006.
[49] K. Gronkowska, S. Michlewska, T. Płoszaj, M. Strachowska, A. Stępień, M. Borowiec, A. Bednarek, A. Robaszkiewicz, BRG1 targeting overcomes ABCC-based multidrug resistance induced by paclitaxel, (2025) 2025.05.01.651609. https://doi.org/10.1101/2025.05.01.651609.
[50] C. Fu, S. Duan, C. Zhang, X. Cui, F. Wang, L. Wang, J. Hu, T. Li, L. Li, Identification of SMARCA1 as a key regulator for Colorectal Cancer, (2024). https://doi.org/10.1101/2024.12.24.630213.
[51] Y. Wu, H. Ni, D. Yang, Y. Niu, K. Chen, J. Xu, F. Wang, S. Tang, Y. Shi, H. Zhang, J. Hu, D. Xia, Y. Wu, Driver and novel genes correlated with metastasis of non-small cell lung cancer: a comprehensive analysis, Pathol. Res. Pract. 224 (2021) 153551, https://doi.org/10.1016/j.prp.2021.153551.
[52] Y. Zhong, B.P. Paudel, D.P. Ryan, J.K.K. Low, C. Franck, K. Patel, M.J. Bedward, M. Torrado, R.J. Payne, A.M. van Oijen, J.P. Mackay, CHD4 slides nucleosomes by decoupling entry- and exit-side DNA translocation, Nat. Commun. 11 (2020) 1519, https://doi.org/10.1038/s41467-020-15183-2.
[53] J.V. Goodman, T. Yamada, Y. Yang, L. Kong, D.Y. Wu, G. Zhao, H.W. Gabel, A. Bonni, The chromatin remodeling enzyme Chd4 regulates genome architecture in the mouse brain, Nat. Commun. 11 (2020) 3419, https://doi.org/10.1038/s41467-020-17065-z.
[54] Y. Munakata, M. Hu, Y. Kitamura, A.L. Bynder, A.S. Fritz, R.M. Schultz, S. H. Namekawa, Chromatin remodeler CHD4 establishes chromatin states required for ovarian reserve formation, maintenance, and germ cell survival, 2024.08.12.607691, bioRxiv (2024), https://doi.org/10.1101/2024.08.12.607691.
[55] M. Oppikofer, T. Bai, Y. Gan, B. Haley, P. Liu, W. Sandoval, C. Ciferri, A. G. Cochran, Expansion of the ISWI chromatin remodeler family with new active complexes, EMBO Rep. 18 (2017) 1697–1706, https://doi.org/10.15252/embr.201744011.
[56] B. Bartholomew, ISWI chromatin remodeling: one primary actor or a coordinated effort? Curr. Opin. Struct. Biol. 24 (2014) 150–155, https://doi.org/10.1016/j.sbi.2014.01.010.
[57] L. Tao, Y. Zhou, Y. Luo, J. Qiu, Y. Xiao, J. Zou, Y. Zhang, X. Liu, X. Yang, K. Gou, J. Xu, X. Guan, X. Cen, Y. Zhao, Epigenetic regulation in cancer therapy: from mechanisms to clinical advances, MedComm Oncol. 3 (2024) e59, https://doi.org/10.1002/mog2.59.
[58] X. Yu, H. Zhao, R. Wang, Y. Chen, X. Ouyang, W. Li, Y. Sun, A. Peng, Cancer epigenetics: from laboratory studies and clinical trials to precision Medicine, Cell Death Discov. 10 (2024) 28, https://doi.org/10.1038/s41420-024-01803-z.
[59] Q. Babar, A. Saeed, T.A. Tabish, S. Pricl, H. Townley, N. Thorat, Novel epigenetic therapeutic strategies and targets in cancer, Biochim. Et. Biophys. Acta (BBA) Mol. Basis Dis. 1868 (2022) 166552, https://doi.org/10.1016/j.bbadis.2022.166552.