G. Kara / Hacettepe J. Biol. & Chem., 2022, 50 (4), 349-358

Research Article

Hacettepe Journal of Biology and Chemistry

journal homepage: www.hjbc.hacettepe.edu.tr

# siRNA targeting ABCB1 potentiates the efficacy of chemotherapy in human triple-negative breast cancer cells

# ABCB1'i hedefleyen siRNA insan üçlü-negatif meme kanseri hücrelerinde kemoterapinin etkinliğini güçlendirir

**Göknur Kara**[1,2]

[1]Department of Chemistry, Biochemistry Division, Ordu University, Ordu, Turkey.

[2]Department of Biomedical Engineering, Başkent University, Ankara, Turkey.

## ABSTRACT

Diminishing the efficacy of chemotherapy because of multidrug resistance (MDR) is a major clinical problem for triple-negative breast cancer (TNBC). MDR often occurs by overexpression of ATP-binding cassette B1 (ABCB1) protein that effuses various anticancer drugs from cancer cells. One of the newly developed techniques to address MDR is to knockdown ABCB1 by RNA interference (RNAi). RNAi is a gene-silencing process in that small interfering RNA (siRNA) blocks the expression of desired genes with high efficiency/specificity. The aim of this work is to examine the impact of ABCB1 inhibition via specific siRNAs on the efficacy of paclitaxel or etoposide in TNBC cells. The toxicity of increasing paclitaxel and etoposide concentrations on MDA-MB-231 cells was assessed using the MTT test. Cells were then co-treated with paclitaxel or etoposide in combination with ABCB1-siRNA, followed by cytotoxicity, colony formation, and migration assays. The administration of ABCB1-siRNA with paclitaxel or etoposide exhibited a synergistic effect and siRNA-drug treatments markedly reduced viability, clonogenicity, and migration of TNBC cells compared to siRNA or drug alone. Overall, these results indicate that TNBC cells become vulnerable even to sub-toxic doses of paclitaxel and etoposide after ABCB1-siRNA transfection, representing a promising approach to enhance the influence of chemotherapy in TNBC.

**Key Words**

Triple-negative breast cancer, multidrug resistance, chemotherapy, siRNA.

## ÖZ

Çoklu ilaç direnci (MDR) nedeniyle kemoterapinin etkinliğinin azalması, üçlü-negatif meme kanseri (TNBC) için önemli bir klinik sorundur. MDR sıklıkla, kanser hücrelerinden çeşitli antikanser ilaçları dışarı sızdıran ATP bağlayıcı kaset B1 (ABCB1) proteininin aşırı ekspresyonu ile oluşur. MDR'yi ele almak için yeni geliştirilen tekniklerden biri, ABCB1'i RNA interferans (RNAi) ile devre dışı bırakmaktır. RNAi, küçük interfere edici RNA'nın (siRNA) istenen genlerin ekspresyonunu yüksek verimlilik/spesifite ile bloke ettiği bir gen susturma mekanizmasıdır. Bu çalışmanın amacı, spesifik siRNA'lar aracılığıyla ABCB1 susturmasının TNBC hücrelerinde paklitaksel veya etoposidin etkinliği üzerindeki etkisini incelemektir. MDA-MB-231 hücrelerinde artan paklitaksel ve etoposid konsantrasyonlarının toksisitesi MTT testi ile değerlendirilmiştir. Hücreler daha sonra ABCB1-siRNA ile kombinasyon halinde paklitaksel veya etoposid ile birlikte muamele edilmiş, ardından sitotoksisite, koloni oluşumu ve göç deneyleri yapılmıştır. ABCB1-siRNA'nın paklitaksel veya etoposid ile uygulanması sinerjistik bir etki sergilemiş ve siRNA-ilaç tedavileri, siRNA veya tek başına ilaca kıyasla TNBC hücrelerinin canlılığını, klonojenisitesini ve göçünü belirgin şekilde azaltmıştır. Genel olarak, bu sonuçlar, TNBC hücrelerinin, ABCB1-siRNA transfeksiyonundan sonra alttoksik paklitaksel ve etoposid dozlarına karşı bile savunmasız hale geldiğini ve TNBC'de kemoterapinin etkisini iyileştirmek için umut verici bir stratejiyi temsil ettiğini göstermektedir.

**Anahtar Kelimeler**

Üçlü-negatif meme kanseri, çoklu ilaç direnci, kemoterapi, siRNA.

**Article History:** Received: Jul 28, 2021; Revised: Jan 18, 2022; Accepted: Apr 17, 2022; Available Online: Oct 5, 2022.

**DOI:** https://doi.org/10.15671/hjbc.975466

**Correspondence to:** G. Kara, Department of Chemistry, Biochemistry Division, Ordu University, Ordu, Turkey.

**E-Mail:** goknurkara@odu.edu.tr

## INTRODUCTION

Breast cancer continues to be the most lethal gynecological malignancy in many countries that threatens women's health. It is the most common type of female cancer and the main reason for deaths from cancer among women around the world [1,2]. According to Global Cancer Statistics, in 2020, breast cancer left behind lung cancer as the most frequently diagnosed cancer with approximately 2.3 million new patients, constituting 11.7% of all cancers. Moreover, female breast cancer is the fifth main reason for cancer mortems worldwide, with 685,000 total loss of life (6.9% of the total cancer deaths) in 2020 [2]. Oncologists clinically classify breast cancer into its subtypes in regard to the expression levels of estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor-2 (HER2) in tumors [3]. Subtypes of breast cancer exhibit considerable heterogeneity with different phenotypic and morphological profiles despite a common tissue of origin, and therefore have very distinct clinical behaviors and require specific treatment modalities [4,5].

Triple-negative breast cancer (TNBC) is a sub-class of breast cancer which represents roughly 20% of breast cancers and is described by the absence of ER and PR expressions, and HER2 overexpression/amplification [6,7]. TNBC is the most deadly and aggressive one among other breast cancer subtypes since it is associated with high molecular heterogeneity, metastatic capacity, and a worse prognosis. Because TNBC lacks ER, PR, and HER2, methods used to treat other breast cancers are not effective in patients with TNBC [8]. Chemotherapy is currently the mainstay as the only systemic treatment regimen for TNBC patients in the early and advanced stages of the disease, however, resistance to cytotoxic drugs has developed in the majority of TNBC tumors, leading to poor treatment outcomes. Unendurably high therapeutic doses causing grievous side effects to patients are mostly required to destroy these chemoresistant tumors. Therefore, standard-of-care and optimal chemotherapy protocols have not yet been established for TNBC [9-11].

Today, chemotherapy resistance is responsible for up to 90% of treatment failure, especially in metastatic cancers, and constitutes an important obstacle to successful cancer treatment [12]. Multidrug resistance (MDR) is identified as the resistance of cancerous cells to a wide diversity of chemotherapy drugs. The reason why neoplastic cells develop a multidrug-resistant phenotype is multifactorial and one of the most important is attributed to transporter-mediated drug efflux machinery [10,13]. The ATP-binding cassette (ABC) superfamily are transmembrane proteins that are known to possess a major role in the MDR of various solid tumors, such as breast cancer [14,15]. With ATP hydrolysis, these proteins act as drug effluxers and pump a broad range of antineoplastic agents from the cytosol to the outside of the cell, resulting in MDR. In this way, the intracellular accumulation of anticancer drugs is reduced below the required level for cell killing, thereby leading to low therapeutic efficacy [16,17]. ABCB1, also known as permeability glycoprotein (P-gp) or MDR1, is the first and most well-identified member of the ABC transporters [13, 18]. Emerging evidence has shown that ABCB1 is overexpressed in a variety of types of malignancies and its high expression is significantly related to poor prognosis, higher relapse rates, low survival rates, and MDR [19-22]. In a study, it was reported that TNBC patients with chemotherapy resistance had upregulated ABCB1 expression compared to chemotherapy-responsive patients, revealing the correlation between ABCB1 status and MDR in TNBC [23].

Strategies to reverse and overcome MDR and increase response to the chemotherapeutics by inhibiting the drug efflux activity of ABCB1 in cancer cells have attracted a great deal of interest from researchers in recent years [24,25]. RNA interference (RNAi) is a proper approach with its superior capability for silencing the target gene specifically without causing significant side effects than other techniques using low-molecular-weight therapeutics such as chemosensitizers or MDR modulators [17,26,27]. RNAi is a natural cellular process found in many eukaryotes, including animals that modulates gene expression. This mechanism is triggered by the existence of chemically synthesized, double-stranded, 21-23 nucleotide-long RNA molecules called small interfering RNA (siRNA) [28]. Exogenous siRNA comes together with the RNA-induced silencing complex (RISC) where its double-strand is separated and the passenger (sense) strand is discarded. The guide (antisense) strand RNA remaining in the RISC induces target mRNA recognition through excellent base pair complementarity, thereby causing cleavage and degradation of the mRNA, resulting in silencing of the target gene [29,30]. Over the last decades, several siRNA cancer therapeutics have been studied in clinical trials and have demons-

trated highly effective and persistent gene silencing, emerging as a promising approach for the therapy of various solid cancers [31]. However, siRNA faces several challenges of great concern in its clinical use, including its short half-life in blood (easily degraded by nucleases) and insufficient cellular uptake (it has a negative charge and large molecular weight). This can be overcome by encapsulating naked siRNAs into nanomaterial-based delivery systems so that these nanocarriers can protect siRNA from enzymatic degradation, renal clearance and enhance its half-life in the circulation [32,33].

Combination therapy of anticancer agents with nanocarrier-mediated specific siRNAs that inhibit ABCB1 may sensitize cancer cells to the drugs to even lower doses, thereby improving the therapeutic effects and reducing the side effects of conventional chemotherapy [34]. Based on this, in the current work, the anticancer potential of the combination treatment of ABCB1 downregulating siRNAs and two different conventional chemotherapeutics (paclitaxel and etoposide) was sought to evaluate in TNBC cells in vitro. A commercial transfection reagent, HiPerFect, consisting of a mixture of neutral and cationic lipids was used as an ABCB1-siRNA nanocarrier. The obtained results revealed that silencing ABCB1 increased the sensitivity of paclitaxel- and etoposide-induced suppression of cell viability, colony formation, and migration of MDA-MB-231 cells.

## MATERIALS and METHODS

### Cell lines, culture conditions, and reagents

TNBC cell line; MDA-MB-231 cells were supplied from the American Type Culture Collection (ATCC). Cells were cultured and maintained in an RPMI-1640 medium containing 10% fetal bovine serum (FBS) and a 1% penicillin-streptomycin solution. Cultured cells were incubated in a humidified atmosphere (5% $CO_2$) at 37°C. Experiments were performed with cells having 60–80% confluence in culture dishes. All cell culture reagents were purchased from Pan-Biotech. Paclitaxel and etoposide were obtained from Carbosynth Ltd. and Kocak Farma, respectively.

### siRNA transfections and drug treatments

ABCB1-siRNA (sense strain: 5′-CGAGUCACUGCCUAAUAAATT-3′ and antisense strain: 5′-UUUAUUAGGCAGUGACUCGAT-3′) and non-silencing control-siRNA were purchased from Qiagen. MDA-MB-231 cells were grown in well-plates 24 h before transfection. Cells were transfected with either ABCB1-siRNA or control-siRNA (final concentration: 50 nM) through HiPerFect transfection reagent (Qiagen) based on the manufacturer's directions. Control-siRNA transfected cells were used as negative controls. 48 h after siRNA transfections, cells were interacted with paclitaxel or etoposide for an additional 48 h.

Paclitaxel was dissolved in DMSO having a final stock concentration of 10 mM and the stock concentration of etoposide was 34 mM. The anticancer agents were diluted to the highest concentrations required in the RPMI medium and serial dilutions were also made in the culture medium for subsequent determined lower dosages. In this way, MDA-MB-231 cells were interacted with the stated concentrations. At least two independent experiments were carried out for each treatment; in each experiment, at least three technical replicates were conducted.

### In vitro cytotoxicity test

Cytotoxicity was investigated using 3-(4,5-dimethylthiazol-2-yl)-2,5- diphenyltetrazolium bromide (MTT) (Glentham Life Sciences) test based on measurement of mitochondrial activity. Briefly, $5 \times 10^3$ cells were grown in 96-well plates for overnight incubation and exposed to the test samples for the indicated times. Later, 100 µl of MTT reagent was pipetted to the wells, and the cells were cultured at 37°C, 5% $CO_2$, for 4 h. After MTT reagent removal, 100 µl of solubilization solution (a mixture of isopropyl alcohol and HCl) was added and all plates were incubated for 30 min at dark. A microplate reader was utilized to carry out the absorbance measurements at 570 nm. Results referred to as the percentages of reduced MTT and the absorbance of the control cells was presumed as 100%. Cell viabilities (%) were determined based on the following equation:

$$\text{Cell viability}(\%) = \frac{\text{A570}(\text{sample})}{\text{A570}(\text{control})} \text{ x100}$$

### Clonogenic survival assay

The clonogenic test is a cell survival and proliferation assay based upon the capability of a single cell to form a colony and grow into a colon [35]. The reduction of the colony formation of the cells after treatments was determined. Briefly, MDA-MB-231 cells were grown in 6-well plates ($1 \times 10^3$ cells/per well). After incubation for 24 h, the cells were interacted with the experimental groups and cultured for 10-14 days for colony formation. The obtained colonies were stained using crystal violet and counted.

### Cell motility and migration assay

The motility and migration capacity of the cells was investigated through an in vitro wound-healing analysis. For this purpose, MDA-MB-231 cells were grown in 6-well plates ($8 \times 10^4$ cells/per well) and then maintained for 24 h. After transfection with the test groups for the indicated time, the wells were scratched using a 200 µl sterile micropipette tip (time 0) and the culture medium was changed with a new one. The photographs of the cells were taken at time point 0 h using an inverted phase-contrast microscope (Olympus CK40) to establish the wound width. Images of cell motility and migration were taken again at time point 36 h. Wound healing was stated as a percentage of migration, assuming the gap width at 0 h is 100%, and normalizing those at the 36 h to the 0 h.

### Statistical analyses

The results shown represent as mean ± standard deviation (SD). The data of any two groups were compared and the Student's t-test was used to define the statistical significance between the groups. p-values ≤ 0.05 were considered statistically significant and were indicated by an asterisk. Statistical analyses were carried out through a GraphPad Prism Software Version 7.0.

## RESULTS and DISCUSSION

The fact that TNBC cells acquire MDR against many conventional anticancer agents, including taxanes (such as paclitaxel) and epipodophyllotoxins (such as etoposide), becomes a critical complication in the clinic [36,37]. Overexpression of ABCB1 drives MDR in chemoresistant tumors where ABCB1 effluxes chemotherapeutic drugs out of cells leading to a decrease in intracellular drug concentration. siRNA shows tremendous potential to interfere with ABCB1 and be used in combination with traditional chemotherapeutics in the treatment of TNBC [38,39]. This strategy exhibits a noteworthy clinical impact in reversing chemoresistance by increasing the intracellular concentration of the drug (Figure 1).

### MTT cytotoxicity test

To investigate whether ABCB1 knockdown influences the short-term cytotoxic effects of chemotherapeutic agents; paclitaxel or etoposide on MDA-MB-231 cells, an MTT test was carried out. For this purpose, first, the effect of paclitaxel and etoposide on cells was examined. MDA-MB-231 cells were interacted with a range of concentrations of drugs for 48 h to determine low

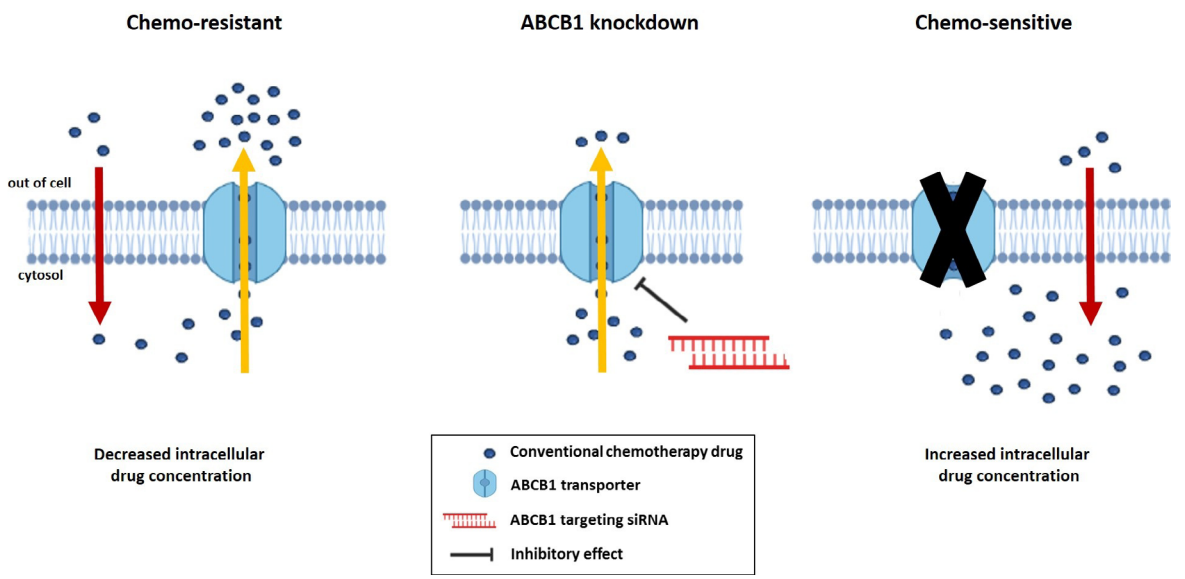


**Figure 1.** Schematic description of the reversal of ABCB1-mediated MDR in chemo-resistant cancer cells.

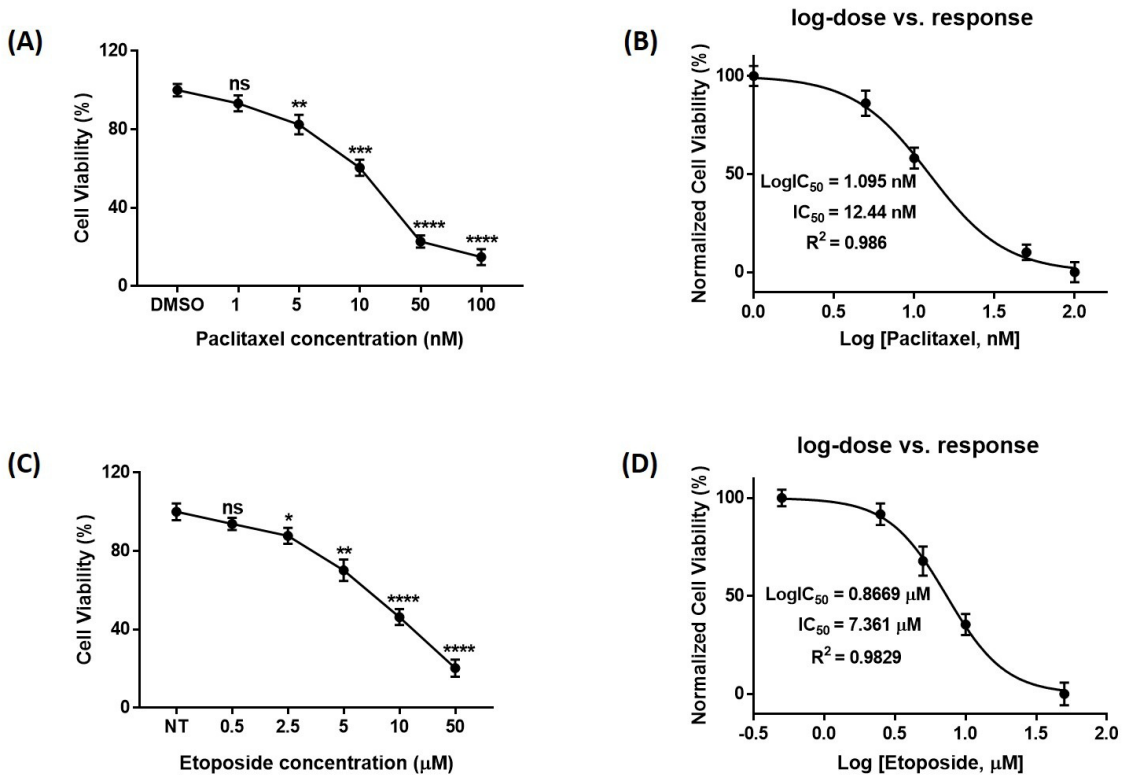


**Figure 2.** Cell viability of MDA-MB-231 cells carried out by MTT after the treatments with varying concentrations of paclitaxel (A) and etoposide (C) for 48 h and $IC_{50}$ values of paclitaxel (B) and etoposide (D) against MDA-MB-231cells (n = 3).

doses to be combined with ABCB1 silencing siRNA. As shown in Figure 2A and C, both compounds caused a reduction in cell viability proportionally with increasing concentration. No significant toxicity was observed after treating the cells either with 1 nM paclitaxel (93.28% cell viability) or 0.5 µM etoposide (93.83% cell viability). $IC_{50}$ doses for paclitaxel and etoposide were established using GraphPad Prism Software Version 7.0. To create log dose-therapeutic response curves, obtained data points were merged by carrying out non-linear regression analysis. In each case, the cell viability data were normalized to the largest value in the data set that was defined as 100%. The $IC_{50}$ values of paclitaxel and etoposide when incubated with the cells for 48 h were found to be as 12.44 nM and 7.361 µM, respectively, (Figure 2B and D).

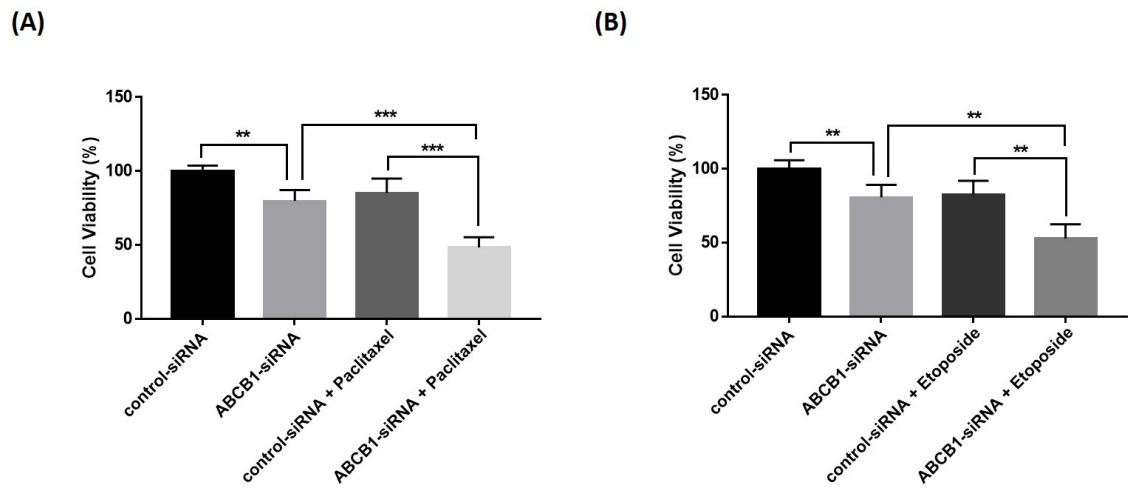


**Figure 3.** The short-term effect of ABCB1-siRNA on the chemosensitivity of the MDA-MB-231 cells to paclitaxel (A) and etoposide (B) examined by the MTT cytotoxicity test (n = 4).

For combination treatments, 1 nM and 0.5 µM concentrations were selected as low doses of paclitaxel and etoposide, respectively. MDA-MB-231 cells were transfected with ABCB1-siRNA and then interacted with the drugs. The obtained results are given in Figure 3A and B. The combined treatment of ABCB1-siRNA and paclitaxel or etoposide, separately, significantly reduced cell growth as compared with those from each treatment. Control-siRNA+paclitaxel and Control-siRNA+etoposide treated cells had 85.33% and 82.54% viability, and combination with ABCB1-siRNA drastically decreased cell viability values were determined as 48.42% (***p=0.0008) and 52.87% (**p=0.0044), respectively. These results elicit that silencing ABCB1 in TNBC cells increases the cytotoxic effects of paclitaxel or etoposide even when low drug concentrations are used.

**Colony formation assay**

The effect of combined therapy of ABCB1-siRNA and paclitaxel or etoposide on MDA-MB-231 clonogenicity was examined using a colony formation assay 14 days after treatments. As illustrated in Figure 4A and B, MDA-

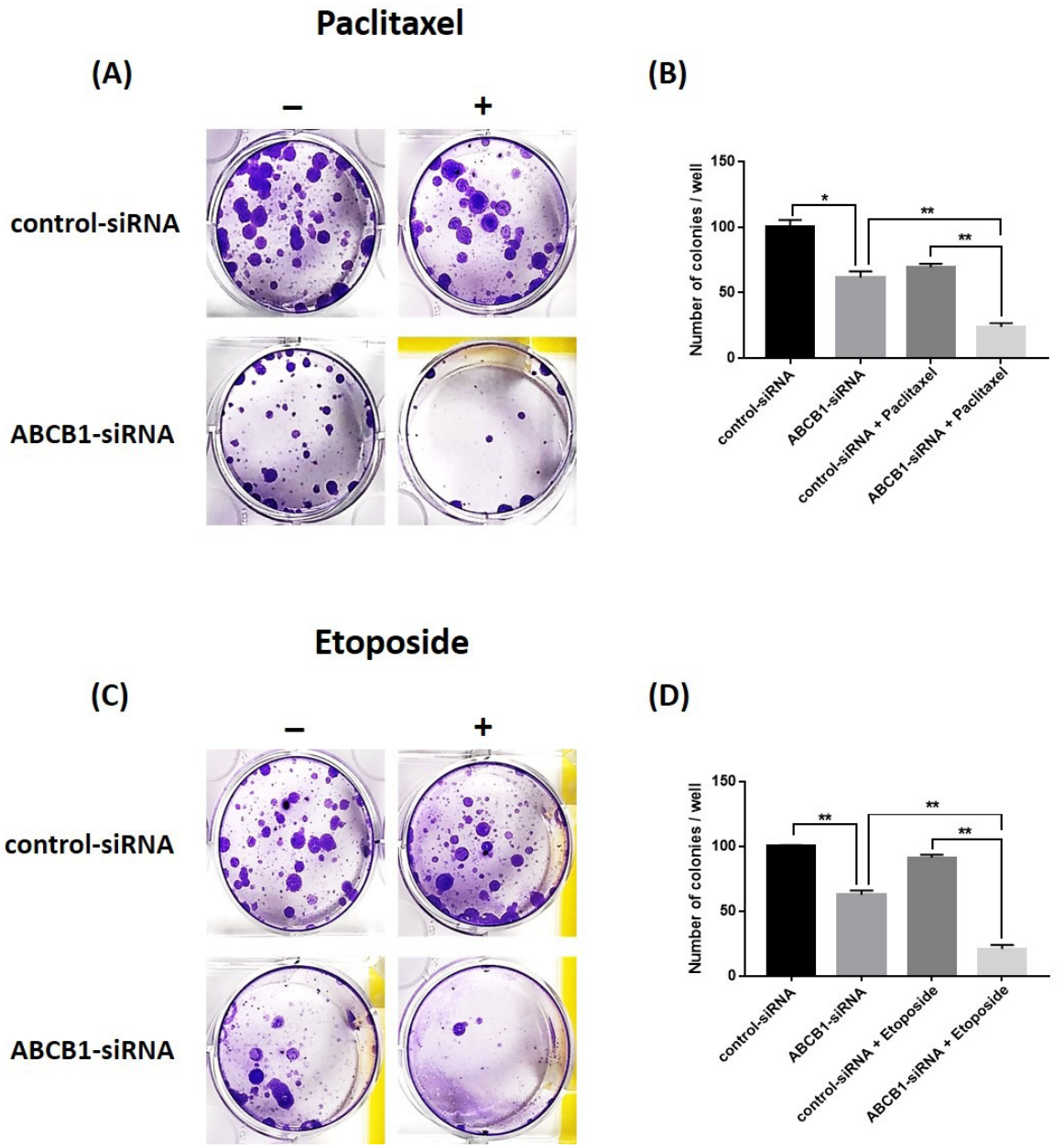


**Figure 4.** Effect of combination treatment of ABCB1-siRNA with paclitaxel (A, B) and etoposide (C, D) on the clonogenic ability of MDA-MB-231 cells (n = 3).

MB-231 cells transfected with ABCB1-siRNA+paclitaxel possessed markedly lower colony formation capacity (24.03 ± 2.76 colonies/well, **p=0.0037) than these cells treated with control-siRNA+paclitaxel (69.48 ± 2.32 colonies/well,). In a similar manner, ABCB1-siRNA+etoposide treatment (20.93 ± 3.29 colonies/well, **p=0.0016) remarkably reduced colony formation in MDA-MB-231 cells compared with control-siRNA+etoposide treated cells (91.47 ± 2.19 colonies/well) (Figure 4C and D). Obtained results show the long-term growth-inhibiting effects of ABCB1 silencing on paclitaxel- or etoposide-mediated chemotherapy in TNBC.

**Migration assay**

The main reason for cell death in advanced breast cancers is metastasis. The effect of ABCB1 knockdown together with paclitaxel or etoposide treatments on the motility and migration of highly aggressive MDA-MB-231 cells was evaluated. The results are demonstrated in Figure 5A and B. Combined treatment of ABCB1-siRNA and paclitaxel led to dramatically less migration (48.47%) by MDA-MB-231 cells than either ABCB1-siRNA (66.87%, ***p=0.0007) or control-siRNA+paclitaxel (78.96%, ***p=0.0001) alone. Similarly, the percentage of migrating cells treated with ABCB1-siRNA and etoposide together was determined as 54.54% when compared with that of cells interacted with solely ABCB1-siRNA (66.87%, **p=0.0016) or control-siRNA+etoposide (84.93%, ***p=0.0002). Furthermore, silencing ABCB1 also significantly suppressed migration compared to control-siRNA transfected cells (*p=0.0282). This confirms the previous study reporting that ABCB1 expression is related to TNBC metastatic spread and ABCB1 may be a good biomarker of metastatic spread [13].

Because of its "triple-negative" nature arising from the absence of hormone receptors or HER2 receptors, endocrine therapies or targeted therapies for HER2 are not clinically feasible for patients suffering from TNBC. Following resistance to chemotherapy as a result of the emerging MDR mechanism in TNBC cells, the US FDA has not currently approved a standard treatment or proprietary drug for TNBC [40,41]. Even so, paclitaxel, a mitotic-inhibitor-based anticancer drug, is still suggested as the first-line regimen for the treatment of TNBC. It binds to the b-subunit of tubulin and causes cytoskeleton framework distortion, a requirement for tumor cell replication along with metastatic spread, suppressing cell proliferation [42,43]. In conjunction with the possibility of developing resistance to paclitaxel in a large number of patients with TNBC, there is an incentive to introduce more chemotherapeutic alternatives or combinations with other molecules to prevent chemoresistance and high dose-related toxicity [44]. Etoposide, which is a semi-synthetic derivative of podophyllotoxin and has a topoisomerase II inhibitory effect, is an agent effective against various cancers including TNBC. It acts to generate a ternary complex with topoisome-

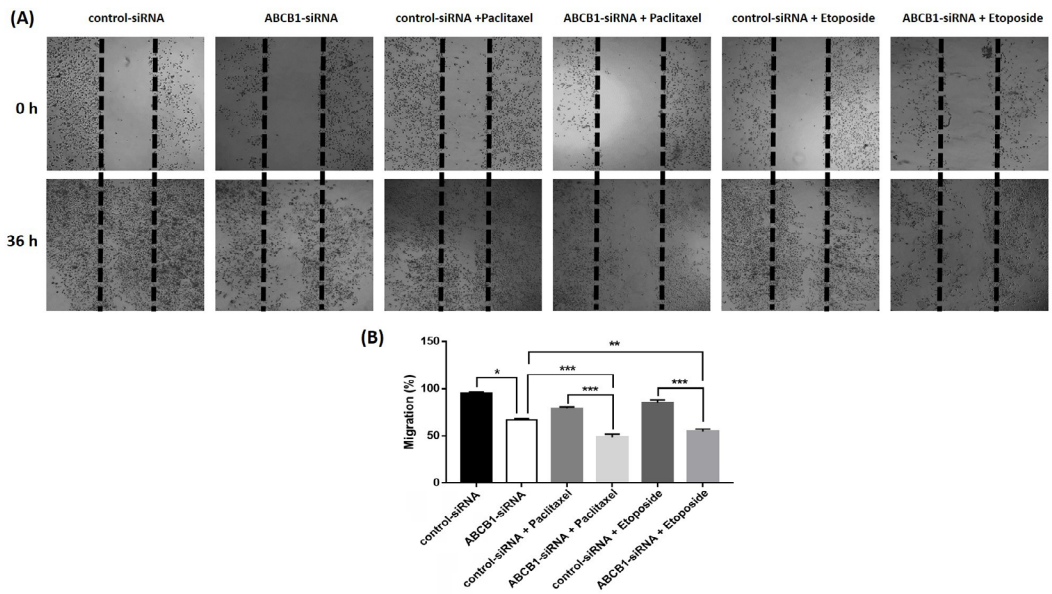


**Figure 5.** Effect of ABCB1 inhibition on motility and migration ability of MDA-MB-231 cells treated with paclitaxel or etoposide, as performed by wound healing assay (A, B) (n = 3).

rase II and DNA, resulting in the double-strand breaking of DNA, and apoptosis [36,44,45].

It is obvious from long experience in clinical cancer treatment that the preferred therapeutic outcome cannot be achieved with a single therapeutic-based monotherapy due to the development of MDR. With the combination of chemotherapeutic drugs and MDR-reversing therapeutics such as ABCB1 silencing siRNA, the potency of each therapeutic strategy can be selectively and synergistically enhanced [34,46]. Kachalaki et al. transfected etoposide-resistant HL-60 cells with ABCB1-siRNA via a commercial transfection agent for the therapy of acute myeloid leukemia (AML). The researchers revealed that inhibition of ABCB1 expression at protein and mRNA levels is accompanied by an increase in sensitivity of HL-60 cells to etoposide having a lower cytotoxic index and in apoptosis [47]. Since ABCB1 expression is also detected in non-malignant tissues to maintain homeostasis of several pathways, attempts to specifically target ABCB1 only in tumors that minimize damage to normal tissues are vital [11,48]. In this respect, nanoparticles can be utilized to increase the intracellular localization of cancer therapeutics in tumors. Nanoparticles have the ability on carrying, protecting, and delivering siRNAs to the cells. Besides that, these nanocarrier systems offer great advantages on tumor targeting either with passive targeting strategies by enhanced permeability and retention effect (EPR) or active targeting strategies by decorating the nanomaterials with specific molecules recognizing the malignant cells [41,49]. In recent work, a polyplex; composed of polyethylene glycol (PEG), L-arginine**,** and poly (ethyleneimine, PEI) was formulated to deliver ABCB1-siRNA to MCF-7/ADR cells (doxorubicin-resistant breast cancer cells) [50]. Transfecting the cells with the polyplex**-**loaded siRNA significantly down-regulated ABCB1 leading to more cellular uptake and cytotoxic impact of doxorubicin. In 2015, Yang et al. demonstrated an active-targeting nanoparticle system that is a cluster of differentiation 44 (CD44) targeting hyaluronic acid (HA)-based (HA-PEI/HA-PEG) nanocarrier loaded with MDR1-siRNA (ABCB1-siRNA) can efficaciously transport MDR1-siRNA to drug-resistant ovarian cancer cells. Administration of MDR1-siRNA mediated by CD44 targeted HA-PEI/HA-PEG nanocarriers with paclitaxel treatment remarkably suppressed P-gp expression and tumor growth, and enhanced apoptosis level in SKOV-3TR ovarian cancer models in mice [51].

In the current work, HiPerFect, which is a mixture of neutral and cationic lipids was used as an ABCB1-siRNA nanocarrier. The obtained results provide evidence that combined ABCB1-siRNA and paclitaxel or etoposide treatments display synergistic antitumor effects in TNBC cells in vitro. This work also indicates that the ABCB1 gene carries an important role in resistance to paclitaxel and etoposide in TNBC.

## CONCLUSION

In this study, the therapeutic potential of targeting ABCB1 in chemoresistance to paclitaxel and etoposide in TNBC was tested. ABCB1 silencing siRNA and antineoplastic drugs were administered together to the MDA-MB-231 TNBC cell line and the efficacy of combination therapy was investigated, separately. The results here showed that siRNA targeting ABCB1 dramatically increased paclitaxel- and etoposide-induced cell death and reduced colony formation and migration in MDA-MB-231 cells. Silencing ABCB1 increased the sensitivity of TNBC cells to sub-toxic doses of paclitaxel and etoposide. This chemo-siRNA combination therapy may allow the use of low doses of anticancer agents and thereby decrease chemotherapy-induced toxicity in non-cancerous cells. Additionally, it should be considered that it is crucial to target ABCB1 specifically in tumor cells as its expression is also found in healthy cells. Taken together, these findings reveal that a combination of ABCB1 knockdown used with traditional chemotherapeutic agent approach may offer a dual effect on inhibition of TNBC tumor growth and progression, suggesting an innovative therapeutic option for patients with an aggressive, unresponsive, or relapsed form of TNBC.

**Acknowledgments**
I would like to thank Prof. Dr. Emir Baki Denkbas for his technical support.

## References


1. A. Lee, M.B.A. Djamgoz, Triple negative breast cancer: emerging therapeutic modalities and novel combination therapies, Cancer Treat. Rev., 62 (2018) 110-122.
2. H. Sung, J. Ferlay, R.L. Siegel, M. Laversanne, I. Soerjomataram, A. Jemal, F. Bray, Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J. Clin., 71 (2021) 209-249.
3. K.M. Hirshfield, S. Ganesan, Triple-negative breast cancer: molecular subtypes and targeted therapy, Curr. Opin. Obstet. Gynecol., 26 (2014) 34-40.
4. P. Kumar, R. Aggarwal, An overview of triple-negative breast cancer, Arch. Gynecol. Obstet., 293 (2016) 247-269.
5. M. De Laurentiis, D. Cianniello, R. Caputo, B. Stanzione, G. Arpino, S. Cinieri, V. Lorusso, S. De Placido, Treatment of triple negative breast cancer (TNBC): current options and future perspectives, Cancer Treat. Rev., 36 (2010) S80-S86.
6. N.K.R. Chalakur-Ramireddy, S.B. Pakala, Combined drug therapeutic strategies for the effective treatment of Triple Negative Breast Cancer, Biosci. Rep., 38 (2018) BSR20171357.
7. Y. Tang, Y. Wang, M.F. Kiani, B. Wang, Classification, treatment strategy, and associated drug resistance in breast cancer, Clin. Breast Cancer, 16 (2016) 335-343.
8. Y. Chang-Qing, L. Jie, Z. Shi-Qi, Z. Kun, G. Zi-Qian, X. Ran, L. Hui-Meng, Z. Ren-Bin, Z. Gang, Y. Da-Chuan, Z. Chen-Yan, Recent treatment progress of triple negative breast cancer, Prog. Biophys. Mol. Biol., 151 (2020) 40-53.
9. L. Wein, S. Loi, Mechanisms of resistance of chemotherapy in early-stage triple negative breast cancer (TNBC), The Breast, 34 (2017) S27-S30.
10. M. Nedeljković, A. Damjanović, Mechanisms of chemotherapy resistance in triple-negative breast cancer—how we can rise to the challenge, Cells, 8 (2019) 957.
11. N. Fultang, A. Illendula, J. Lin, M.K. Pandey, Z. Klase, B. Peethambaran, ROR1 regulates chemoresistance in Breast Cancer via modulation of drug efflux pump ABCB1, Sci. Rep.,10 (2020) 1-12.
12. M.A. Medina, G. Oza, A. Sharma, L.G. Arriaga, J.M. Hernández Hernández, V.M. Rotello, J.Y. Ramirez, Triple-negative breast cancer: a review of conventional and advanced therapeutic strategies, Int. J. Environ. Res. Public Health, 17 (2020) 2078.
13. M. Nedeljković, N. Tanić, M. Prvanović, Z. Milovanović, N. Tanić, Friend or foe: ABCG2, ABCC1 and ABCB1 expression in triple-negative breast cancer, Breast Cancer, 28 (2021) 727-736.
14. K.L. Lee, Y.C. Kuo, Y.S. Ho, Y.H. Huang, Triple-negative breast cancer: current understanding and future therapeutic breakthrough targeting cancer stemness, Cancers, 11 (2019) 1334.
15. F.J. Sharom, ABC multidrug transporters: structure, function and role in chemoresistance, Pharmacogenomics, 9 (2008) 105-127.
16. Y.S. Abd El-Aziz, A.J. Spillane, P.J. Jansson, S. Sahni, Role of ABCB1 in mediating chemoresistance of triple-negative breast cancers, Biosci. Rep., 41 (2021) BSR20204092.
17. Y.T. Li, M.J. Chua, A.P. Kunnath, E.H. Chowdhury, Reversing multidrug resistance in breast cancer cells by silencing ABC transporter genes with nanoparticle-facilitated delivery of target siRNAs, Int. J. Nanomed., *7* (2012) 2473.
18. C.P. Wu, A.M. Calcagno, S.V. Ambudkar, Reversal of ABC drug transporter-mediated multidrug resistance in cancer cells: evaluation of current strategies, Curr. Mol. Pharmacol., 1 (2008) 93-105.
19. D.H. Sabnis, L.C. Storer, J.F. Liu, H.K. Jackson, J.P. Kilday, R.G. Grundy, I.D. Kerr, B. Coyle, A role for ABCB1 in prognosis, invasion and drug resistance in ependymoma, Sci. Rep., 9 (2019) 1-9.
20. S. Sun, J. Cai, Q. Yang, Y. Zhu, S. Zhao, Z. Wang, Prognostic value and implication for chemotherapy treatment of ABCB1 in epithelial ovarian cancer: a meta-analysis, PloS one, 11 (2016) e0166058.
21. B.J. Trock, F. Leonessa, R. Clarke, Multidrug resistance in breast cancer: a meta-analysis of MDR1/gp170 expression and its possible functional significance, J. Natl. Cancer Inst., 89 (1997) 917-931.
22. H.S. Chan, T.M. Grogan, G. Haddad, G. DeBoer, V. Ling, P-glycoprotein expression: critical determinant in the response to osteosarcoma chemotherapy, J. Natl. Cancer Inst., 89 (1997) 1706-1715.
23. A.A.A. Aziz, M.S.M. Salleh, I. Mohamad, V.M.K., Bhavaraju, M.M. Yahya, A.D. Zakaria, S.H. Gan, R. Ankathil, Genotypes and haplotypes of ABCB1 contribute to TAC chemotherapy response in Malaysian triple negative breast cancer patients, Meta Gene, 16 (2018) 21-27.
24. M. Saraswathy, S. Gong, Different strategies to overcome multidrug resistance in cancer, Biotechnol. Adv., 31 (2013) 1397-1407.
25. M. Majidinia, M. Mirza-Aghazadeh-Attari, M. Rahimi, A. Mihanfar, A. Karimian, A. Safa, B. Yousefi, Overcoming multidrug resistance in cancer: Recent progress in nanotechnology and new horizons, IUBMB life, 72 (2020) 855-871.
26. J. Zhang, Z. Du, S. Pan, M. Shi, J. Li, C. Yang, H. Hu, M. Qiao, D. Chen, X. Zhao, Overcoming multidrug resistance by Codelivery of MDR1-targeting siRNA and doxorubicin using EphA10-mediated pH-sensitive Lipoplexes: In Vitro and In Vivo evaluation, ACS Appl. Mater. Interfaces, 10 (2018) 21590-21600.
27. T. Fatemian, I. Othman, E.H. Chowdhury, Strategies and validation for siRNA-based therapeutics for the reversal of multi-drug resistance in cancer, Drug Discov. Today, 19 (2014) 71-78.
28. A. Singh, P. Trivedi, N.K. Jain, Advances in siRNA delivery in cancer therapy, Artif. Cells Nanomed. Biotechnol., 46 (2018) 274-283.
29. P. Zhang, K. An, X. Duan, H. Xu, F. Li, F. Xu, Recent advances in siRNA delivery for cancer therapy using smart nanocarriers, Drug Discov. Today, 23 (2018) 900-911.
30. Y. Xin, M. Huang, W.W. Guo, Q. Huang, L.Z. Zhang, G. Jiang, Nano-based delivery of RNAi in cancer therapy, Mol. Cancer, 16 (2017) 1-9.
31. J.E. Zuckerman, M.E. Davis, Clinical experiences with systemically administered siRNA-based therapeutics in cancer, Nat. Rev. Drug Discov., 14 (2015) 843-856.
32. C.G. Zhang, W.J. Zhu, Y. Liu, Z.Q. Yuan, S.D. Yang, W.L. Chen, J.Z. Li, X.F. Zhou, C. Liu, X.N. Zhang, Novel polymer micelle mediated co-delivery of doxorubicin and P-glycoprotein siRNA for reversal of multidrug resistance and synergistic tumor therapy, Sci. Rep., 6 (2016) 1-12.
33. R. Misra, M. Das, B.S. Sahoo, S.K. Sahoo, Reversal of multidrug resistance in vitro by co-delivery of MDR1 targeting siRNA and doxorubicin using a novel cationic poly (lactide-co-glycolide) nanoformulation, Int. J. Pharm., 475 (2014) 372-384.
34. C. Sarisozen, J. Pan, I. Dutta, V.P. Torchilin, Polymers in the co-delivery of siRNA and anticancer drugs to treat multidrug-resistant tumors, Int. J. Pharm. Investig., 47 (2017) 37-49.

35. M.A. Erdogan, A. Ashour, E. Yuca, K. Gorgulu, B. Ozpolat, Targeting eukaryotic elongation factor-2 kinase suppresses the growth and peritoneal metastasis of ovarian cancer, Cell. Signal., 81 (2021) 109938.
36. L. Chen, H. Cui, J. Fang, H. Deng, P. Kuang, H. Guo, X. Wang, L. Zhao, Glutamine deprivation plus BPTES alters etoposide- and cisplatin-induced apoptosis in triple negative breast cancer cells, Oncotarget, 7 (2016) 54691.
37. X.Y. Liu, W. Jiang, D. Ma, L.P. Ge, Y.S. Yang, Z.C. Gou, X.E. Xu, Z.M. Shao, Y.Z. Jiang, SYTL4 downregulates microtubule stability and confers paclitaxel resistance in triple-negative breast cancer, Theranostics, 10 (2020) 10940.
38. M. Creixell, N.A. Peppas, Co-delivery of siRNA and therapeutic agents using nanocarriers to overcome cancer resistance, Nano today, 7 (2012) 367-379.
39. I.U. Khan, R.U. Khan, H. Asif, Alamgeer, S.H. Khalid, S. Asghar, M. Saleem, K.U. Shah, S.U. Shah, S.A.A. Rizvi, Y. Shahzad, Co-delivery strategies to overcome multidrug resistance in ovarian cancer, Int. J. Pharm., 533 (2017) 111-124.
40. Y. Qi, X. Fu, Z. Xiong, H. Zhang, S.M. Hill, B.G. Rowan, Y. Dong, Methylseleninic acid enhances paclitaxel efficacy for the treatment of triple-negative breast cancer, PloS one, 7 (2012) e31539.
41. A.K. Bakrania, B.C. Variya, S.S. Patel, Novel targets for paclitaxel nano formulations: hopes and hypes in triple negative breast cancer, Pharmacol. Res., 111 (2016) 577-591.
42. L.Y. Sha, Y. Zhang, W. Wang, X. Sui, S.K. Liu, T. Wang, H. Zhang, MiR-18a upregulation decreases Dicer expression and confers paclitaxel resistance in triple negative breast cancer, Eur. Rev. Med. Pharmacol. Sci., 20 (2016) 2201-2208.
43. B.T. McGrogan, B. Gilmartin, D.N. Carney, A. McCann, Taxanes, microtubules and chemoresistant breast cancer, Biochim. Biophys. Acta Rev. Cancer, 1785 (2008) 96-132.
44. M. Gaber, K.A. Elhasany, S. Sabra, M.W. Helmy, J.Y. Fang, S.N. Khattab, A.A. Bekhit, M. Teleb, K.A. Elkodairy, A.O. Elzoghby, Co-Administration of Tretinoin Enhances the Anti-Cancer Efficacy of Etoposide via Tumor-Targeted Green Nano-Micelles, Colloids Surf. B Biointerfaces, 192 (2020) 110997.
45. S. Das, N. Tripathi, S. Siddharth, A. Nayak, D. Nayak, C. Sethy, P.V. Bharatam, C.N. Kundu, Etoposide and doxorubicin enhance the sensitivity of triple negative breast cancers through modulation of TRAIL-DR5 axis, Apoptosis, 22 (2017) 1205-1224.
46. G. Kara, S. Tuncer, M. Türk, E.B. Denkbaş, Downregulation of ABCE1 via siRNA affects the sensitivity of A549 cells against chemotherapeutic agents, Med. Oncol., 32 (2015) 103.
47. S. Kachalaki, B. Baradaran, J. Majidi, M. Yousefi, D. Shanehbandi, S. Mohammadinejad, B. Mansoori, Reversal of chemoresistance with small interference RNA (siRNA) in etoposide resistant acute myeloid leukemia cells (HL-60), Biomed. Pharmacother., 75 (2015) 100-104.
48. J.M. de Azevedo Delou, G.M. Vignal, V. Índio-do-Brasil, M.T. de Souza Accioly, T.S.L. da Silva, D.N. Piranda, M. Sobral-Leite, M.A. de Carvalho, M.A.M. Capella, R. Vianna-Jorge, Loss of constitutive ABCB1 expression in breast cancer associated with worse prognosis, Breast Cancer, 9 (2017) 415-428.
49. N.R. Patel, B.S. Pattni, A.H. Abouzeid, V.P. Torchilin, Nanopreparations to overcome multidrug resistance in cancer, Adv. Drug Deliv. Rev., 65 (2013) 1748-1762.
50. S. Lu, V.B. Morris, V. Labhasetwar, Effectiveness of siRNA delivery via arginine-rich PEI-based polyplex in metastatic and doxorubicin resistant breast cancer cells, J. Pharmacol. Exp. Ther., (2019).
51. X. Yang, A.K. Iyer, A. Singh, E. Choy, F.J. Hornicek, M.M. Amiji, Z. Duan, MDR1 siRNA loaded hyaluronic acid-based CD44 targeted nanoparticle systems circumvent paclitaxel resistance in ovarian cancer, Sci. Rep., 5 (2015) 1-9.