Iraqi Journal of Biotechnology, 2024, Vol. 23, No. 3, 155-164

The Role of *ABCB1* and *ABCB6* Transporter Genes in Paclitaxel Resistance of Breast Cancer Cells

[1]Dhiya S. AL-Shumary, [2]Marrib N. Rasheed, [3]Ahmed M. AL-Shammari

[1] Department of Pathology, Faculty of Medicine, University of Kufa
[2] Institute of Genetic Engineering and Biotechnology for Postgraduate Studies, University of Baghdad
[3] Department of Experimental Therapy, Iraqi Center for Cancer and Medical Genetic Research, University of Mustansiriyah

Received: October 22, 2023 / Accepted: November 21, 2023 / Published: December 30, 2024

Abstract: Breast cancer (BC) is a highly malignant neoplasm and is fatal in women around the world. The increase in drug dosage does not improve therapeutic results and may potentially result in severe adverse effects. Many cancers in humans are commonly believed to exhibit elevated levels of drug resistance that have been attributed to multidrug resistance genes. The aim of the study to excess expression of ATP-binding cassette transporters (ABC): including ABCB1 and ABCB6, is a primary factor that contributes to the increased effluent of cell-toxic drugs and subsequent resistance to treatment and failure in various types of human BC. Therefore, the current work aims to explore the role of ABCB1 and ABCB6 in chemoresistance activity against paclitaxel in breast cancer cells. The cell was evaluated by a cytotoxic assay and quantitative RT-PCR to measure how much *ABCB* genes are expressed. The study revealed that the breast cancer cell lines AMJ13 and MCF-7 subjected to paclitaxel showed a highly significant resistance (IC50: 175.6µg/ml and 34.73µg/ml, respectively, P-value: 0.0001), that was associated with their overexpression levels of ABCB6, while ABCB1 showed inconsistent expression in both cell lines. It was concluded that the *ABCB6* gene plays an important role in resistance to paclitaxel and, as a result, may provide a possible therapeutic target to help BC patients overcome resistance to paclitaxel.

Keywords: Breast cancer, ATP-binding cassette, ABCB transporters, Paclitaxel, Drug resistance, Drug efflux.

Corresponding author: (Email: alshammari.am@uomustansiriyah.edu.iq)

Introduction

Breast cancer (BC) is the most prevalent neoplastic, is frequently detected, and is the main contributor to cancer-associated deaths among women worldwide. An estimated 2.3 million new cases of BC were reported, resulting in a mortality rate of around 685 thousand related to this clinical disease until 2020. This increase in incidence can be attributed to the growth and age of the global population, as well as the adoption of lifestyles that promote cancer (1,2). Iraqi women also showed a high percentage of new cases according to WHO studies (3).

Paclitaxel (PTX): known by the brand name Taxol, which is also called mitotic spindle inhibitor, is an antineoplastic and antimicrotubule agent used to treat a variety of types of cancer. This includes Kaposi's sarcoma, pancreatic, breast, lung, esophageal, ovarian, and cervical cancer (4). Paclitaxel enhances the assembly of

tubulin into microtubules and inhibits the separation of microtubules which in turn alters the morphogenetic characteristics of growing neurites, preventing mitosis, blocking cell cycle progression, and inhibiting the growth of cancer cells. Taxol has been shown to induce apoptotic cell death, internucleosomal DNA fragmentation, and protein isoprenylation inhibition(5).

The most well-known group of membrane transport proteins is the ATP-binding cassette (ABC) superfamily. In humans, there are 49 ABC transporter subtypes. Based on their amino acid sequences, domain arrangement, and phylogenetic study, these 49 ABCs are further subdivided into 7 subfamilies, ABCA through ABCG (6). ABC subfamily B consists of eleven members (ABCB1 to ABCB11). Among these, the well-characterized transporter related to chemoresistance mechanisms is ABCB1 (P-glycoprotein/P-gp): (7). ABCB1 is a protein that has highly flexible drug binding sites, so it can interact with a wide range of chemical compounds that vary in structure, from anticancer agents and hormones, such as steroids, to hydrophobic toxic peptides(8). ABCB1 is recognized for transporting a large amount of hydrophobic drugs outside of malignant cells, giving chemoresistance to several types of tumors, including lung, breast and pancreatic cancer, as well as neuroblastoma and hepatocellular carcinoma, ultimately resulting in treatment failure and tumor relapse (9). Many anticancer drugs, such as paclitaxel, vincristine, and doxorubicin, as well as tyrosine kinase inhibitors (TKI) such as WYE35459, GSK107091658, and imatinib, have been found to be substrates for P-gp. This suggests that anticancer effects may be diminished when ABCB1 is overexpressed in malignant cells (10).

ABCB6 was previously known as a P-glycoprotein-related protein (PRP): and has a broader range of functions, such as making cells resistant to drugs and toxic metals, improving porphyrin biosynthesis, and protecting cells from stress (11). Some malignancies, including melanoma, prostate cancer, and hepatocellular carcinoma associated with the hepatitis C virus, have been correlated with elevated levels of the mRNA encoding ABCB6. It is possible to hypothesize that higher expression of ABCB6 is related to increased heme production in neoplastic growths (12). In vitro experiments, ABCB6 has been associated with therapeutic resistance to doxorubicin, cisplatin, 5-fluorouracil (SN-38): vincristine, and arsenic trioxide (13).

The current study compared hormone receptor negative AMJ13 breast cancer cells taken from an Iraqi woman with hormone receptor positive MCF-7 breast cancer cells with regard to their resistance to paclitaxel and the relationship between this resistance and the expression of intracellular ABC transporter genes *ABCB1* and *ABCB6*, which may confer multidrug resistance in BC cells. The results of the study suggested that the ABCB1 and ABCB6 transporter proteins have promise as therapeutic targets in individual treatment for BC patients, regardless of the presence or absence of hormone receptor expression.

Materials and methods

Cell culture

In this study, AMJ13 and MCF-7, which were two distinct breast cancer cell lines, were used. The AMJ13 breast cancer cell lines were established using cells obtained from an Iraqi patient. Histological examination verified the presence of ductal carcinomas in the primary tumor of a 70-year-old woman

from Iraq. Immunocytochemical analysis revealed that neither estrogen nor progesterone receptors were expressed. However, a slightly positive outcome was reported with respect to *HER2/neu* gene expression. The passage number for this cell line was 2, and it was deemed nonresponsive to hormone therapy (14). Although MCF-7 cells are distinguished by their positive expression of estrogen and progesterone receptors, making them an adequate model for the study of breast cancer (15).

Tissue culture flasks (T25 cm2; Falcon/United States) were used to culture cells in RPMI 1640 medium (Capricorn/Germany): complemented by 10% serum of fetal bovine (Capricorn/Germany): 100 units/ml of penicillin and 100 µg/ml of streptomycin (16,17). The subculture of cell lines occurred when the monolayers reached confluence. After the cell sheets were removed from the growing medium, they were rinsed twice using a 2 ml solution of trypsin EDTA. After that, the monolayer cell was treated with 1 ml of trypsin again and then the flask was carefully shaken. A few minutes were spent incubating the culture cells at 37°C until they detach; trypsinization was stopped by adding a growth medium; and then the cells were distributed at the desired concentration. Subsequently, the cultured flasks were re-incubated at a temperature of 37 ° C (18). The cell lines provided by the cell bank unit were regularly authenticated by the supplier as standard work.

Paclitaxel cytotoxicity

The WST-8/CCK-8 (Enhanced Cell Counting Kit-8) assay (Santa Cruz Biotechnology/USA) was performed with 96 well-flatted bottoms to measure the cytotoxic effects of Paclitaxel. After 24 hours, a confluent monolayer developed when AMJ13 and MCF-7 cells seeded at an average number of 7,000 cells per well. The experiment was carried out in triplicates. Multiple doses of paclitaxel (1000, 500, 250, 125, 62.5, and 31.2 µg/ml) were used to treat cancer cells and cell viability was assessed by removing the medium after a 72-hour exposure period. Two hours later, in an incubator at 37°C, a volume of 50 ml of solution from the WST-8/CC kit-8 (Elabscience/China) was added to the cells (19). The absorbance was quantified at a wavelength of 450 nm employing a microplate reader, and the procedure was conducted in triple. The proportion for cytotoxicity, or the incidence at which cell proliferation was stopped, was computed using the following formula. When A refers to the OD (optical density) for the control while B refers to the OD for the samples, the inhibition rate is calculated as A - B / A*100 (20).

Morphological study

The treated and untreated cancer cells were stained by adding 100 µl of crystal violet dye per well and incubating the plate at a temperature of 37 ° C for 20 minutes. Following this, the microtiter plate was washed thoroughly. This experiment is designed to examine the morphological changes induced by paclitaxel.

Extracted RNA and performed real-time PCR for quantitative analysis

The breast cancer cell lines AMJ13 and MCF-7 were incubated for 24 hours before being treated with paclitaxel at concentrations of 175.6 µg/ml and 34.73 µg/ml, respectively, representing their IC50 values. After that, 1X PBS (phosphate buffer saline) was used to wash the cells once. Subsequently, 1 ml of TRIzol (Thermo Fisher Scientific/USA) was used to extract total RNA from cellular samples. The quality of the samples for downstream applications was

determined by measuring the amount and stability of the extracted RNA using a Quantus Fluorometer (21).

The qRT-PCR (quantitative real-time polymerase chain reaction) was performed using the GoTaq® 1-Step RT-qPCR system. The *ACTB* (*Beta-actin*) gene, which was supplied by Integrated DNA Technologies (IDT): was utilized as a standard of internal control to normalize the work. The relative formula of the quantified approach (2^-ΔΔCt) was used to figure out the fold change (22). The following primers were used: *ABCB1*: Forward- 5 '-GTCTGGACAAGCACTGAAAGA-3', reverse- 5'-TCTGCTCCTGAGTCAAAGAAAC-3' and *ABCB6*: Forward- 5'-AGTTACGAAGTGGAACGCTATC -3', reverse- 5'-CCAGGTTCTGGGTCTGATTTAG-3'. These primers have been designed by Primer Quest™ Tool and manufactured by Integrated DNA Technologies (IDT) USA. The thermal cycler program is illustrated in table (1).

Table (1): The thermal cycler program

No.	Steps	°C	Minute: Second	Cycle
1.	RT. Enzyme Activation cDNA Synthesis	37	15: 00	1
2.	Initiation of Denaturation	95	10: 00	
3.	Denaturation	95	00: 20	40
4.	Annealing	60,62	00: 20 Acquisition of Green	
5.	Extension	72	00: 20	

The experiment of the RT-PCR reaction for gene expression has been repeated in triplicate, which was adopted with all genes in this study, to achieve the best values (23).

Statistical analysis

Data obtained from the cytotoxicity assay were statistically analyzed, and the IC50 value for paclitaxel was calculated on each cell line using an analysis of variance (ANOVA) multiple comparison test by Graph Pad Prism-9 (Graph Pad Software Inc.; SanDiego/USA). These values are displayed as the mean ± SD for measures performed in triplicate (24).

Result and discussion

***In vitro,* AMJ13 and MCF-7 cell lines showed resistance to paclitaxel**

In this experiment, cell lines AMJ13 and MCF7 were exposed to paclitaxel for a period of 72 hours at varying doses. The CCK-8 assay found that the half maximum of paclitaxel IC50 (inhibitory concentrations) values in AMJ13 and MCF-7 cells were 175.6 µg/ml and 34.73 µg/ml, respectively. The IC50 value of AMJ13 exhibited a greater than 5-fold increase compared to MCF-7 cells, as shown in figure (1A;1B). This confirmed that AMJ13 and MCF-7 were resistant to chemotherapeutic agents, especially paclitaxel. High doses of IC50 exhibited mild lesions and damage in the cells, as illustrated in figure (1C): where the cells stained with crystal violet stain. However, the doses used very high for paclitaxel, which was generally effective at doses of 1 µg/ml in sensitive cell lines. In current cell lines, it used overdoses up to 1000µg/ml to explore the IC50 dose, which was again considered very high compared to sensitive cell lines. The other group of researchers found that the dose of wild type MCF-7 IC50 is 60µg and the dose of MCF-7 docetaxel chemoresistance IC50 is 68 µg while in current study it was found that about 175.6µg/ml which confirms the resistance of the MCF-7

cells used in study which was approximately three times higher (25). Morphological analysis confirmed the results of the cytotoxicity study, which shows that MCF-7 and AMJ13 cells at the IC50 dose have the presence of cell detachment and large viable cells remaining, with fewer cells showing mild lesions such as cell rounding and nuclear condensation. The high dose of 175.6 and 34.73µg/ml respectively, which are the IC50 values, confirmed the chemoresistant nature of the cells. These results were also confirmed by Chen et al (26) who described chemoresistant cells as large cells with cytoplasmic granulation.

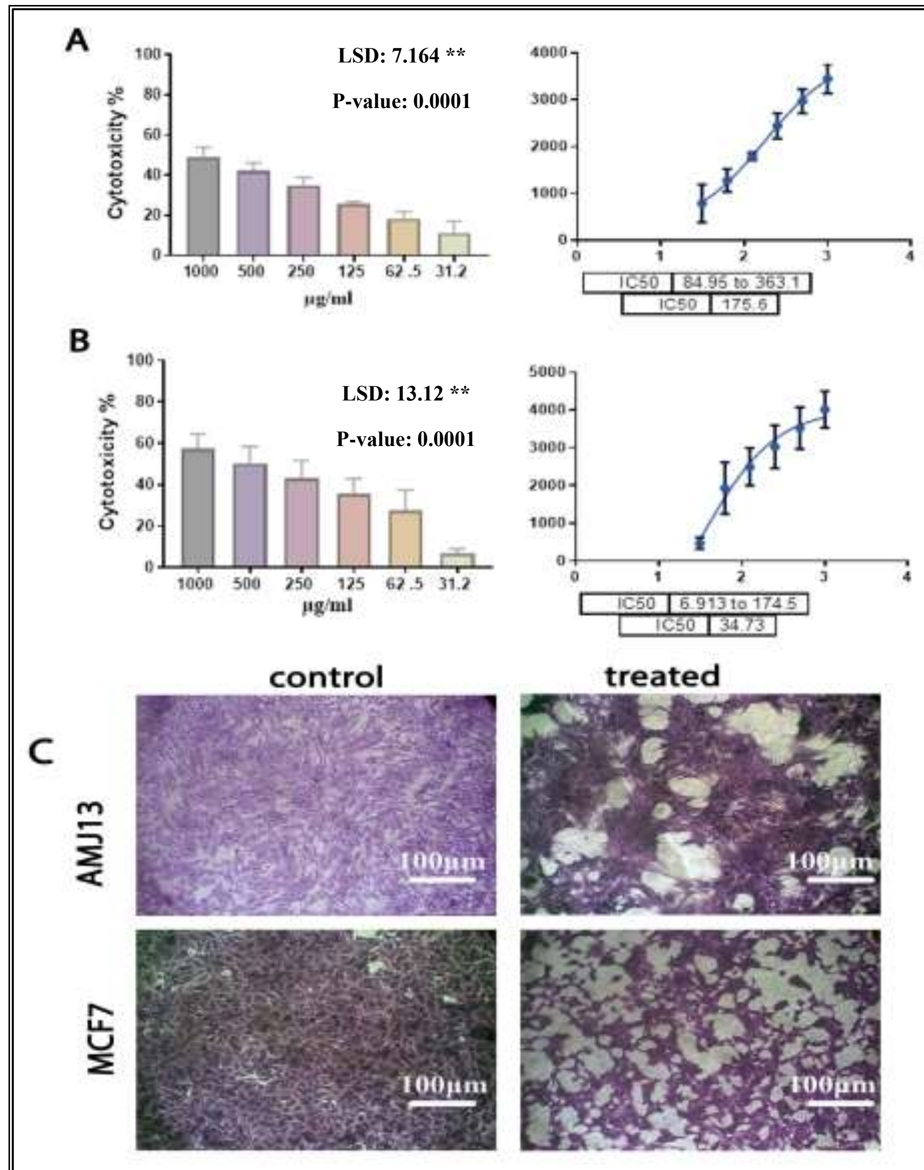

Figure (1): A. Paclitaxel cytotoxicity histogram in AMJ13 and MCF-7 cells (72 hours of incubation), ** (P≤0.01). B. The IC50 value of the Paclitaxel dose. C. Breast cancer cells (AMJ13 and MCF-7) under an inverted microscope after 72h of untreated and treated with paclitaxel, after crystal violet staining, which shows the presence of cell detachment and the remaining viable cells of large size and with fewer cells showing mild lesions such as cell rounding and nuclear condensation. (The size bar: 100µm).

Variable expressions of *ABCB1* and *ABCB6* genes may promotes paclitaxel resistance in AMJ13 and MCF-7 cells.

The mRNA gene expression data reveals that the expression level of the cell membrane transporter family demonstrated a different regulation in AMJ13 and MCF-7 cells. ABCB1 showed downregulation in AMJ13 by 0.003, and upregulation in MCF-7 by 1.41 fold changes. Although the greatest increases in expression were detected in ABCB6, which increased by 1.35 and 9.02 times, respectively, compared to the untreated samples with respect to the amplification of the endogenous gene, as shown in figure (2): revealing an overexpression pattern particularly in the *ABCB6* gene as a result of interference with paclitaxel and, consequently, the emergence of drug resistance.

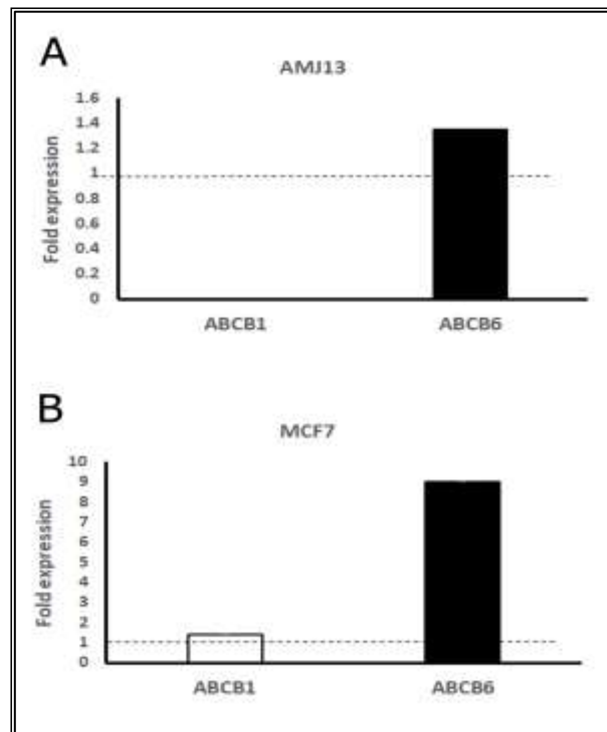

Figure (2): ABCB1 showed downregulation in AMJ13 by 0.003, and upregulation in MCF-7 by 1.41-fold changes. Although the greatest increases in expression were detected in ABCB6, which increased by 1.35 and 9.02 times, respectively, compared to the untreated samples.

It is known that several ABCB transporters play a role in developing drug resistance and cancer progression. One of the most well-known ABC transporters that promote MDR in cancer cells is ABCB1. Chemotherapeutic agents such as paclitaxel, doxorubicin, vincristine, and tyrosine kinase inhibitors were among the anticancer drugs determined to be ABCB1 substrates, indicating that the anticancer effect may be diminished in cancer cells that overexpress the ABCB1 transporter (27). The overexpression results of the present study on human breast cancer cells MCF-7 for ABCB1 / P-gp drug efflux were in accordance with the findings of Ajabnoor et al (28): who reported that, compared to untreated cells, MCF-7

cells demonstrated a progressive up-regulation of the ABCB1 transporter P-glycoprotein with respect to the Ct value of the endogenous *GAPDH* gene during the development of resistance to paclitaxel. Furthermore, they state that the evolution of paclitaxel resistance results in a reduction in apoptosis. To study the mechanisms behind acquired drug resistance using MCF-7 cells that were resistant to docetaxel (MCF-7_{TXT}) have been previously created and compared to the sensitive wild-type parental cell line (MCF-7_{CC}): Wang et al (25): revealed that MCF-7_{TXT} cells were shown to be 10 times more resistant to docetaxel and paclitaxel. These findings were correlated with the extremely elevated expression of the ABCB1 transporter in MCF-7_{TXT} cells. The downregulation of ABCB1 at the level of drug efflux in Iraqi AMJ13 breast cancer cell lines was associated with their triple negative breast cancer (TNBC) status, which is a characteristic feature of this cell. This hypothesis was interpreted by the pathological studies of Delou et al (29): who report that a reduced expression of ABCB1 was determined by immunohistochemistry in a study comprising tissue samples from 712 Brazilian women who underwent mammary surgery, and was correlated with TNBC and a worse prognosis. At the level of genetic factors, significant relationships are present between epigenetic alteration and *ABCB1* expression. For example, it is possible that individuals with breast and ovarian cancer who have CpG island hypermethylation regions that cover the distal promoter of the *ABCB1* gene had longer median overall survival rates (OS) and a lower level of *ABCB1* transcript expression (30). Furthermore, the other reason AMJ13 cells may lose their *ABCB1* gene activity is possibly due to single nucleotide variants that occur in this gene. The ABCB1 locus codes for a highly polymorphic P-gp. There were approximately 66 coding single nucleotide polymorphisms (SNPs) in the *ABCB1* gene. These SNPs have been found to be correlated with altered levels and stability of mRNA, protein change folding, drug efflux, and substrate specificity of P-gp (31,32).

Increased expression of ABCB6 has been associated with resistance to chemotherapeutics in several studies, including resistance to camptothecin in A549 lung cancer cells, paclitaxel in breast cancer, and daunorubicin in acute myeloid leukemia. Furthermore, resistance to cisplatin, paclitaxel, doxorubicin, and vincristine occurs in ovarian cancer cells as a result of up-regulation of ABCB6 (33,34). The data of Mehmetoglu (35): observed a similar increase in *ABCB6* expression, indicating that the transcription of drug-resistant marker genes, such as the ABCB6 protein, was up-regulated in the cisplatin-resistant MCF-7 (MCF-7_{CisR}) breast cancer cell line when exposed to paclitaxel and docetaxel, which increased 2 times compared to normal wild-type parental MCF-7_{CC} cells. Furthermore, they reported that resistant MCF-7 cells showed a decrease in the percentage of cell death compared to normal MCF-7 cells in each case after drug treatment, from 9.9 to 18.1% in MCF-7_{CC} cells, while this range was reduced from 2.2 to 4.1% in MCF-7_{CisR} cells. At the clinical level, the results of this study support the finding obtained by Park et al (36): who report that after receiving weekly doses of neoadjuvant chemotherapy with paclitaxel/FES (F-fluorouracil, epirubicin and cyclophosphamide): it has been shown that the expression of ABCB6 in anticancer drug resistant human breast cancer cells was higher than that in

sensitive cells as determined by microarray analysis.

Conclusion

The present study provides evidence that ABCB6 expression exhibited up-regulation in both cell lines; therefore, it is associated with the development of resistance to paclitaxel in these cells. ABCB1 expression was reduced in AMJ13 but elevated in MCF-7, which excluded this gene from resistance to paclitaxel. These results indicate that targeting the *ABCB6* genes is a potential strategy to reverse resistance to paclitaxel in patients diagnosed with BC.

Acknowledgements

The authors express their gratitude to the Experimental Therapy Department of the Iraqi Center for Cancer and Medical Genetic Research at Mustansiriyah University for their cooperation in providing breast cancer cell lines.

Funding Statement

There is no specific funding received during this study.

Approval

It was approved by the Institute of Genetic Engineering and Biotechnology for Postgraduate Studies, University of Baghdad, Baghdad, Iraq.

References

1. Sung, H.; Ferlay, J.; Siegel, R. L.; Laversanne, M.; Soerjomataram, I.; Jemal, A. and Bray, F. (2021). Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA: A cancer Journal for Clinicians, 71(3): 209-249.
2. Mohammed, A. R. A. S. (2022). Estimation of IL-21 Gene Expression Associated with Breast Cancer in Iraqi Patients. Iraqi Journal of Biotechnology, 21(2): 245-250
3. Abdulla, K. N.; Abid, S. J. and Al-Darraji, A. T. M. (2022). Epidemiological correlation of breast cancer with cervical cancer. World Bulletin of Public Health, 11, 92-95.
4. Chang, A. E.; Ganz, P. A.; Hayes, D. F.; Kinsella, T.; Pass, H. I.; Schiller, J. H. *et al.*Strecher, V. O. (2007). An Evidence-Based Approach 34.
5. Weaver, B. A. (2014). How Taxol/paclitaxel kills cancer cells. Molecular Biology of the Cell, 25(18): 2677-2681.
6. Dvorak, P.; Pesta, M. and Soucek, P. (2017). ABC gene expression profiles have clinical importance and possibly form a new hallmark of cancer. Tumor Biology, 39(5): 1010428317699800.
7. Dean, M.; Moitra, K.; and Allikmets, R. (2022). The human ATP-binding cassette (ABC) transporter superfamily. Human Mutation, 43(9): 1162-1182.
8. Li, W.; Zhang, H.; Assaraf, Y. G.; Zhao, K.; Xu, X.; Xie, J. *et al.* (2016). Overcoming ABC transporter-mediated multidrug resistance: Molecular mechanisms and novel therapeutic drug strategies. Drug Resistance Updates, 27: 14-29.
9. Karthika, C.; Sureshkumar, R.; Zehravi, M.; Akter, R.; Ali, F.; Ramproshad, S. *et al.* (2022). Multidrug resistance of cancer cells and the vital role of P-glycoprotein. Life, 12(6): 897.
10. Wu, Z. X.; Yang, Y.; Wang, J. Q.; Zhou, W. M.; Chen, J.; Fu, Y. G. *et al.* (2021). Elevated ABCB1 expression confers acquired resistance to aurora kinase inhibitor GSK-1070916 in cancer cells. Frontiers in Pharmacology, 11: 615824.
11. Song, G.; Zhang, S.; Tian, M.; Zhang, L.; Guo, R.; Zhuo, W. *et al.* (2021). Molecular insights into the human ABCB6 transporter. Cell Discovery, 7(1): 55.
12. Tsunedomi, R.; Iizuka, N.; Yoshimura, K.; Iida, M.; Tsutsui, M.; Hashimoto, N. *et al.* (2013). ABCB6 mRNA and DNA methylation levels serve as useful biomarkers for prediction of early intrahepatic recurrence of hepatitis C virus-related hepatocellular carcinoma. International Journal of Oncology, 42(5): 1551-1559.
13. Murakami, M.; Izumi, H.; Kurita, T.; Morimoto, Y. and Yoshino, K. (2020). UBE2L6 is Involved in Cisplatin Resistance by Regulating the Transcription of ABCB6. Anti-Cancer Agents in Medicinal Chemistry (Formerly Current Medicinal Chemistry-Anti-Cancer Agents), 20(12): 1487-1496.
14. Al-Shammari, A.M.; Alshami, M.A.; Umran, M.A.; Almukhtar, A.A.; Yaseen, N.Y.; Raad, K. *et al.* Establishment and characterization of a receptor-negative,

hormone-nonresponsive breast cancer cell line from an Iraqi patient. Breast Cancer: Targets Ther. 2015; 7:223-230.
15. Soule, H. D.; Vazquez, J.; Long, A.; Albert, S. and Brennan, M. (1973). A human cell line from a pleural effusion derived from a breast carcinoma. Journal of the National Cancer Institute, 51(5): 1409-1416.
16. Sun, B.; Hu, N.; Han, L.; Pi, Y.; Gao, Y. and Chen, K. (2019). Anticancer activity of green synthesised gold nanoparticles from Marsdenia tenacissima inhibits A549 cell proliferation through the apoptotic pathway. Artificial cells, Nanomedicine, and Biotechnology, 47(1): 4012-4019.
17. Mohammed, H. A.; Almahmoud, S. A.; El-Ghaly, E. S. M.; Khan, F. A.; Emwas, A. H.; Jaremko, M. *et al*. (2022). Comparative anticancer potentials of taxifolin and quercetin methylated derivatives against HCT-116 cell lines: effects of O-methylation on taxifolin and quercetin as preliminary natural leads. ACS Omega, 7(50): 46629-46639.
18. Al-Ziaydi, A. G.; Al-Shammari, A. M.; Hamzah, M. I. and Jabir, M. S. (2020). Hexokinase inhibition using D-Mannoheptulose enhances oncolytic newcastle disease virus-mediated killing of breast cancer cells. Cancer Cell International, 20(1): 1-10.
19. Kano, A.; Kamita, M. K.; Iwasaki, T. and Shindo, M. (2017). Bongkrekic acid induces selective cytotoxicity in tumor cells, revealed by CCK-8.
20. Freshney, R. I. (1994). The culture environment: substrate, gas phase, medium and temperature. Culture of animal cells: a manual of basic technique. New York: Wiley-Liss, 71.
21. Simms, D.; Cizdziel, P. E. and Chomczynski, P. (1993). TRIzol: A new reagent for optimal single-step isolation of RNA. Focus, 15(4): 532-535.
22. Livak, K. J. and Schmittgen, T. D. (2001). Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta CT}$ method. *methods*, 25(4): 402-408.
23. Al-Shammari, A. M.; Abo-Altemen, R. A. and Shawkat, M. S. (2021). Cyperus rotundus L. alkaloid extracts enhance oncolytic Newcastle disease virus against digestive system neoplasms. South African Journal of Botany, 143: 266-273.
24. Mavrevski, R. A. D. O. S. L. A. V.; Traykov, M. E. T. O. D. I.; Trenchev, I. V. A. N. and Trencheva, M. I. G. L. E. N. A. (2018). Approaches to modeling of biological experimental data with GraphPad Prism software. WSEAS Trans. Syst. Control, 13, 242-247.
25. Wang, H.; Hajar, A.; Li, S.; Chen, X.; Parissenti, A. M.; Brindley, D. N. and Wang, Z. (2014). Multiple mechanisms underlying acquired resistance to taxanes in selected docetaxel-resistant MCF-7 breast cancer cells. BMC cancer, 14(1): 1-15.
26. Chen, S. Y.; Hu, S. S.; Dong, Q.; Cai, J. X.; Zhang, W. P.; Sun, J. Y. *et al*. (2013). Establishment of paclitaxel-resistant breast cancer cell line and nude mice models, and underlying multidrug resistance mechanisms in vitro and in vivo. Asian Pacific Journal of Cancer Prevention, 14(10): 6135-6140.
27. Wu, Z. X.; Yang, Y.; Wang, J. Q.; Zhou, W. M.; Chen, J.; Fu, Y. G. *et al*. (2021). Elevated ABCB1 expression confers acquired resistance to aurora kinase inhibitor GSK-1070916 in cancer cells. Frontiers in Pharmacology, 11: 615824.
28. Ajabnoor, G. M. A.; Crook, T. and Coley, H. M. (2012). Paclitaxel resistance is associated with switch from apoptotic to autophagic cell death in MCF-7 breast cancer cells. Cell Death and Disease, 3(1): e260-e260.
29. Delou, J. M. D. A.; Vignal, G. M.; Índio-do-Brasil, V.; Accioly, M. T. D. S.; da Silva, T. S. L.; Piranda, D. N. *et al*. (2017). Loss of constitutive ABCB1 expression in breast cancer associated with worse prognosis. Breast Cancer: Targets and Therapy, 415-428.
30. Vaclavikova, R.; Klajic, J.; Brynychova, V.; Elsnerova, K.; Alnaes, G. I.; Tost, J. *et al.* (2019). Development of high-resolution melting analysis for ABCB1 promoter methylation: Clinical consequences in breast and ovarian carcinoma. Oncology Reports, 42(2): 763-774.
31. Wolf, S. J.; Bachtiar, M.; Wang, J.; Sim, T. S.; Chong, S. S. and Lee, C. G. L. (2011). An update on ABCB1 pharmacogenetics: insights from a 3D model into the location and evolutionary conservation of residues corresponding to SNPs associated with drug pharmacokinetics. The Pharmacogenomics Journal, 11(5): 315-325.
32. Tulsyan, S.; Mittal, R. D. and Mittal, B. (2016). The effect of ABCB1 polymorphisms on the outcome of breast cancer treatment. Pharmacogenomics and Personalized Medicine, 47-58.

33. Varatharajan, S.; Abraham, A.; Karathedath, S.; Ganesan, S.; Lakshmi, K. M.; Arthur, N. *et al*. (2017). ATP-binding casette transporter expression in acute myeloid leukemia: association with in vitro cytotoxicity and prognostic markers. Pharmacogenomics, 18(3): 235-244.
34. Lafta, M.; AL-Amili, W. A.; Alwan, A. F. and Khalaf, I. A. H. (2022). Relationship Between the Drug Responsiveness of Acute Myeloid Leukemia Iraqi Patients and Gene Expression of Drug Resistance ABCB1 and ABCG2 Genes. Iraqi Journal of Biotechnology, 21(1).
35. Mehmetoglu, T. (2010). Genomic and proteomic analysis of chemoresistance in breast cancer cell lines: MCF-7 and MDA-MB-231 (Doctoral dissertation).
36. Park, S.; Shimizu, C.; Shimoyama, T.; Takeda, M. and o, M.; Kohno, T. *et al*. (2006). Gene expression profiling of ATP-binding cassette (ABC) transporters as a predictor of the pathologic response to neoadjuvant chemotherapy in breast cancer patients. Breast Cancer Research and Treatment, 99: 9-17.