Epigenetic Regulation of ABCB1 Expression in Triple Negative Breast Cancer and its Impact on Taxane Resistance

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Abstract: *Objective*: This research was conducted to examine the part played by epigenetic regulation in up-regulating the expression of ABCB1 (P-glycoprotein) and the role it contributes to the development of taxane resistance in triple-negative breast cancer (TNBC).

Methods: Taxane-sensitive and taxane-resistant TNBC cell lines (e.g., MDA-MB-231) were subjected to the evaluation of the ABCB1 expression with the help of the quantitative PCR (qPCR) and the Western blotting procedures. Bisulfite sequencing, chromatin immunoprecipitation (ChIP), and miRNA profiling were used to study epigenetic alterations such as DNA methylation, changes in histone activity (acetylation), and miRNAs, respectively. To test the reversal of resistance, epigenetic inhibitors were utilized and included 5-aza-2'-deoxycytidine and trichostatin A. Patient-derived TNBC samples were used to make clinical correlations that established the connection between the status of ABCB1 methylation and the response to chemotherapy.

Findings: The expression of ABCB1 in taxane-resistant cells was found to be much higher in comparison to that of sensitive cells, as determined by both mRNA and protein. The DNA analysis of the methylation of the promoter of the ABCB1 showed that it is hypomethylated in the resistant cells, and the histone acetylation of ABCB1 was also significantly elevated in the resistant cells. miR-451 and miR-335, which regulate ABCB1, were suppressed in the resistant cells. ABCB1 overexpression was reversed by treatment with epigenetic inhibitors, and sensitivity to taxanes was restored. It was found that low levels of methylation of the promoter of ABCB1 were linked to ineffective taxane chemotherapy response in patients with TNBC.

Conclusion: Taxane resistance in TNBC occurs with the help of epigenetic regulation of ABCB1. The significant contributors to the overexpression of ABCB1 are DNA hypomethylation, histone acetylation, and miRNA dysregulation. Epigenetic therapies, as an adjunct to the taxane chemotherapy, are one such promising approach that could be used to overcome drug resistance in TNBC, and they should be further explored in clinical trials.

Keywords: Triple-negative breast cancer, taxane resistance, ABCB1, epigenetics, DNA methylation, histone acetylation, miRNA, chemotherapy resistance, epigenetic inhibitors.

1. INTRODUCTION

Triple-Negative Breast Cancer (TNBC) is a very dangerous form of breast cancer that does not have estrogen receptors (ER), progesterone receptors (PR), and human epidermal growth factor receptor 2 (HER2) [1, 2]. This renders TNBC insensitive to hormonal intervention and HER2-targeted therapy that is effective in other types of breast cancer [3, 4]. This causes TNBC patients to have higher chances of recurrence and metastasis, and fewer treatments. The common treatments of TNBC are taxane-based chemotherapy, such as paclitaxel and docetaxel [5]. Nevertheless, irrespective of the early reactions, many patients become resistant to taxane treatment, which results in failure in treatment and low survival rates in the long term. The molecular events underlying the resistance of TNBC to taxanes remain to be fully understood, although it is becoming apparent that the overexpression of the ABCB1 (P-glycoprotein) is a significant contributor to the process [6].

ABCB1 is a member of the ATP-binding cassette (ABC) transporter family, an efflux pump that limits the animal's ability to stay in the cell by actively transporting the chemotherapy drugs, including taxanes, out of the animal cells [7]. The process reduces the cytotoxicity of drugs, which is a contributor to multidrug resistance in cancers [8, 9]. Overexpression of ABCB1 in TNBC has also been linked with poor prognosis because it facilitates the pumping out of the taxanes and other chemotherapeutic agents in the cancer cells, thereby leading to decreased efficacy of the drugs [10, 11]. ABCB1 upregulation has been identified as a major factor in the development of chemotherapy resistance in TNBC [12, 13].

Current breast cancer research indicates that ABCB1-driven drug export plays a crucial role in causing decreased accumulation of taxanes inside cells and multidrug resistance [14]. For TNBCs, this phenomenon holds significant clinical relevance since the availability of few treatments makes the response to chemotherapy a major factor affecting prognosis [2]. It was also recently found that the expression of ABCB1 is regulated via epigenetic means, such as DNA methylation, histone modifications, and microRNAs [15].

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It has been found that epigenetic changes, which alter gene expression without altering the DNA sequence, are important in controlling ABCB1 expression in several cancers, including TNBC [15, 16]. Such changes may include the DNA methylation, histone regulation, and non-coding RNAs (ncRNAs), which regulate the expression of genes. Tumor suppressor genes can be silenced or oncogenes activated by DNA methylation (especially of the promoter regions of genes), resulting in drug resistance [17, 18]. To demonstrate the role of Methylation, it has been demonstrated that the promotion of the ABCB1 gene has been upregulated through hypomethylation of the promoter region in some cancers, such as TNBC [19]. Histone modifications, including acetylation and methylation, may also be able to influence the expression of ABCB1 by changing the chromatin structure, where it specifically enhances or repress transcription [14]. Moreover, the non-coding RNAs, such as miRNAs, are important in the regulation of ABCB1 expression. Abnormal expression of such ncRNAs in TNBC might cause the hyper-expression of the ABCB1, which may predispose to chemotherapy resistance [20].

Previous research had shown the presence of ABC transporters, DNA hypomethylation, histone modifications, and noncoding RNA molecules in cases of drug resistance to chemotherapeutic treatment individually. But very few studies have correlated all of the above-mentioned factors in combination, particularly in taxane resistance of TNBC. The novelty of this research lies in the simultaneous examination of ABCB1 promoter hypomethylation, histone acetylation, and altered levels of miRNA expression in both sensitive and resistant TNBC models.

Hypothesis

The core hypothesis of this investigation is that DNA hypomethylation, histone acetylation, and miRNA downregulation are some of the epigenetic alterations involved in the upregulation of ABCB1 in TNBC, which eventually results in taxane resistance. There is a growing body of evidence to support this hypothesis, which is that epigenetic changes modify drug resistance to chemotherapy and that the ABCB1 plays a crucial role in drug efflux in cancer cells. TNBC cell lines, including MDA-MB-231 and BT-20, were cultured under defined experimental conditions by exposing them to taxanes in order to test this hypothesis and assess the level of epigenetic changes using bisulfite sequencing of DNA methylation, chromatin immunoprecipitation (ChIP) of histone modifications, and miRNA profiling of non-coding RNA regulation. Simultaneously, TNBC samples taken in parallel in patients were assessed to compare the association of the ABCB1-associated epigenetic markers with clinical response to taxane-based chemotherapy. The outcomes of these experiments can be used to gain a mechanistic understanding of how epigenetic regulation of ABCB1 contributes to chemotherapy resistance in TNBC and which epigenetic targets can be used to overcome taxane resistance.

Key Contributions

- This work has revealed the underlying mechanism by which certain epigenetic alterations (DNA methylation, histone acetylation, and miRNA dysregulation) control the expression of ABCB1 in TNBC, resulting in taxane resistance.
- According to the findings, reversing the overexpression of ABCB1 and restoring taxane sensitivity in TNBC may be possible through targeting the epigenetic processes, such as by using DNA methyltransferase or histone deacetylase inhibitors.
- By using *in vitro* TNBC cell lines and patient-derived samples, the research is able to give clinically pertinent findings of epigenetic regulation of the ABCB1, leading to the introduction of personalized treatment plans through epigenetic biomarker-driven treatment strategies.

This research paper explores epigenetic regulation in taxane resistance in Triple-Negative Breast Cancer (TNBC), with respect to the upregulation of ABCB1. Section I is the introduction to TNBC and resistance to taxanes. Section II outlines the procedures, such as cell lines, taxane procedures, and epigenetic analyses. Section III shows the results of DNA hypomethylation, histone acetylation, and miRNA dysregulation in resistant cells. Section IV talks about the impact of epigenetic changes on resistance and possible therapeutic interventions. Section V concludes by suggesting epigenetic inhibitors as a promising approach to overcome drug resistance in TNBC.

2. MATERIALS AND METHODS

2.1. Cell Lines and Taxane Exposure Protocols

The MDA MB 231 cell line and BT 20, of human triple-negative breast cancer (TNBC), were acquired as

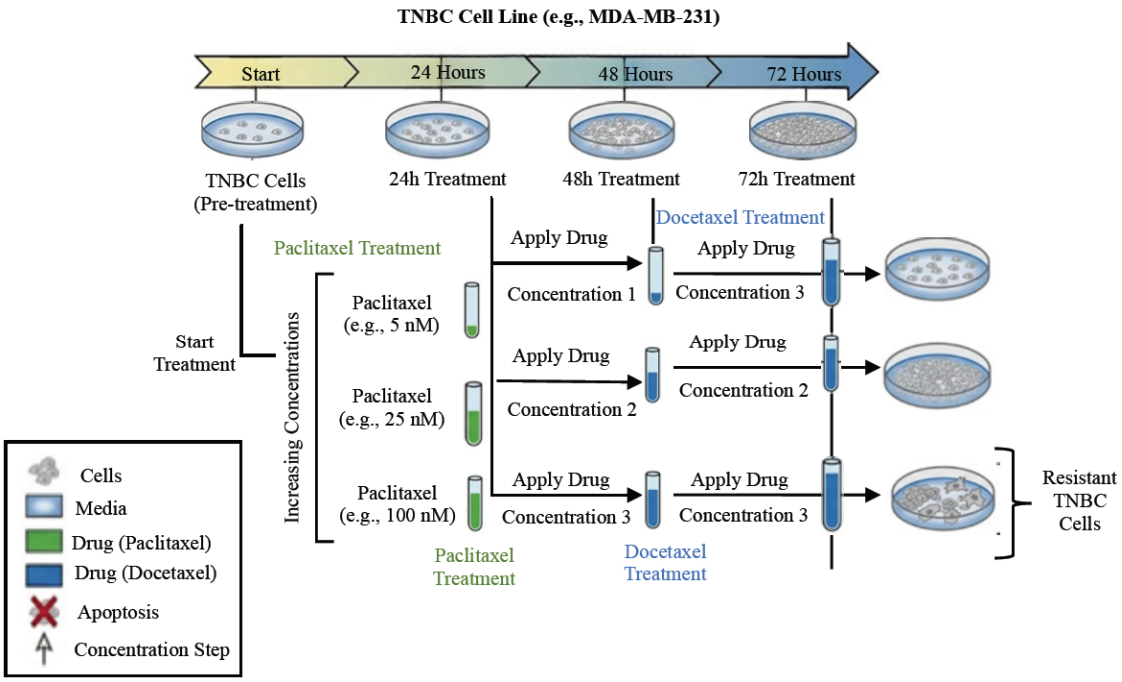

Figure 1: Taxane Exposure Protocol for TNBC Cells.

an authenticated cell bank and cultivated in Dulbecco's Modified Eagle Medium with 10% fetal bovine serum, 1% penicillin streptomycin, and 2 mM L-glutamine at 37°C and in a humidified environment with 5% CO_2. In the case of acute exposure to taxanes, the cells would be sown at the desirable densities and exposed to paclitaxel or docetaxel (5 50 nM) in 24,48 and 72 hours, depending on the initial dose study. Parental MDA MB 231 cells were subjected to stepwise increasing concentration of paclitaxel over 12-16 weeks, beginning with 5 nM and gradually increasing to 100 nM paclitaxel concentration when the cells returned to normal proliferation rate at that dose. The sublines, which were resistant, were kept in a medium of 50 nM paclitaxel and grown on drug-free medium for at least two passages before functional assays.

Figure **1** demonstrates the experimental design, which exposes triple -negative breast cancer (TNBC) cells (e.g., MDA-MB-231) to taxanes (Paclitaxel and Docetaxel) during 72 hours. The protocol is split into phases, and the first treatment, then exposure at 24, 48, and 72 hours will follow. The growing doses of Paclitaxel and Docetaxel are administered at every stage of treatment, and the idea is to acquire taxane-resistant TNBC cell lines. The protocol of exposure is meant to simulate chemotherapy therapy and check the development of resistance. Figure **1** is a schematic representation of the taxane exposure protocol and was prepared to illustrate the experimental workflow; it does not represent direct experimental output.

2.2. Validation of Resistant Cell Lines, Controls, and Assay Reproducibility

Taxane-resistant TNBC sublines were first validated prior to epigenetic and functional analysis based on the comparison of their IC_{50} values in response to either paclitaxel or docetaxel in comparison with their parental taxane-sensitive cells using the MTT assay. A taxane-resistant cell line was only considered to be successfully established if it exhibited stable increases in the IC_{50} value after at least two passages without the drugs, together with elevated levels of ABCB1 expression and increased Rhodamine 123/Calcein-AM efflux activities. Parental taxane-sensitive cells, cells that were not treated, cells treated with vehicle, and cells treated only with inhibitors served as control groups in our experiment. In the case of epigenetic inhibitor analysis, samples that contained taxane alone and taxane together with inhibitors were included. Each experiment was repeated in a minimum of three independent biological replicates using technical triplicates in all quantitative experiments.

2.3. ABCB1 Expression Assays

TRIzol RNA was used to extract the total RNA in the taxane-sensitive and taxane-resistant cells in accordance with the manufacturer's guidelines. Reverse transcription of 1 microgram of RNA was performed with random hexamers and reverse transcriptase, followed by quantification of levels of ABCB1 mRNA by quantitative PCR (qPCR), with the

Syngreen chemistry, in a real-time PCR system. As housekeeping genes, GAPDH and β-actin were employed, and the $2^{-\Delta\Delta C_t}$ technique was utilized to determine relative expression.

Whole cell lysates were used in the analysis of proteins in the RIPA buffer in the presence of protease inhibitors, and the protein concentration was calculated through the use of the BCA assay. ABCB1 and GAPDH primary antibodies were used to probe equal quantities of protein (20–30 μg) separated using SDS PAGE, then transferred onto PVDF membranes, and incubated with HRP-conjugated secondary antibodies. Chemiluminescence and densitometry were used to visualize and quantify bands, respectively, where the absorbance of the ABCB1 was divided by GAPDH.

Flow cytometry was used to measure the activity of ABCB1 transporter through the use of Rhodamine 123 or Calcein AM. The fluorescent substrate was incubated with the cells in the presence or absence of a given ABCB1 inhibitor, after which the cells were washed and examined in a flow cytometer. A decrease in the amount of intracellular fluorescence of resistant cells compared with the amount of fluorescence of sensitive cells indicated an effect of increased efflux activity.

The functional role of ABCB1 in Taxane resistance was confirmed by the assay of ABCB1 activity using transport-based validations. Taxane-resistant cells were analyzed for the intracellular retention of Rhodamine 123 and Calcein-AM, both in the presence and absence of the inhibitor of ABCB1. An increase in the accumulation of dyes in the presence of the inhibitor was regarded as reduced ABCB1 activity involved in drug efflux from the cell. Taxane resistance was also analyzed after the inhibition of ABCB1 using the viability assays.

As indicated in Table **1**, the different methods that are used to quantify the expression of ABCB1 in the case of triple-negative breast cancer (TNBC) under taxane treatment are presented. Three major types of assays are shown in the table: qPCR, Western Blotting, and Flow Cytometry, and the methodology is presented for each of them. qPCR will be used to quantify the expression of the ABCB1 mRNA, whereas the protein levels of the ABCB1 are measured using Western Blotting. The activity of the ABCB1 transporter is analyzed using Flow Cytometry with the help of fluorescent dye-based efflux assays. Cumulative analysis of the ABCB1 expression in both mRNA and protein levels, and its active functioning, will be achieved by these methods to further determine its role in taxane resistance in TNBC cells.

2.4. Epigenetic Profiling

A commercial DNA isolation kit and a bisulfite conversion kit were used in the extraction and conversion of genomic DNA, respectively. The degree of methylation at each CpG site was determined by PCR amplification of the ABCB1 gene's promoter region using bisulfite-specific primers, followed by sequencing.

Chromatin immunoprecipitation (ChIP) experiments were used to measure the histone alterations at the ABCB1 gene promoter. Cells cross-linked with formaldehyde, lysed, and chromatin sheared through sonication. The cross-links of the sheared chromatin were immunoprecipitated using the antibodies against acetyl H3 and H3K27me3 or control IgG, which was reversed, and the DNA purified. qPCR was used to enrich the ABCB1 promoter sequences in ChIP DNA and was expressed compared to the input chromatin. Antibody details, dilution ratios, incubation conditions, and ChIP-qPCR primer information were documented to ensure reproducibility of histone modification analysis.

To analyze the microRNA, the total RNA enriched with small RNAs was extracted, and the cDNA was produced through miRNA-specific primers. miR 451, miR 335, and other miRNAs that regulate ABCB1 were measured by using qPCR miRNA assays with U6 snRNA as an internal control. The selection of miR-451 and miR-335 was based on their connections with breast cancer drug response and drug resistance. miR-451 is linked to drug efflux via ABC transporters, while miR-335 plays a role in tumor progression, epithelial to

Table 1: ABCB1 Expression Assays Overview

Assay Type	Methodology	Purpose
qPCR	RNA extraction, cDNA synthesis, PCR amplification	Quantification of ABCB1 mRNA expression
Western Blotting	Protein extraction, SDS-PAGE, transfer to membrane, antibody detection	Detection of ABCB1 protein expression
Flow Cytometry	Fluorescent dye-based efflux assay (Rhodamine 123 or Calcein-AM)	Measurement of ABCB1 transporter activity

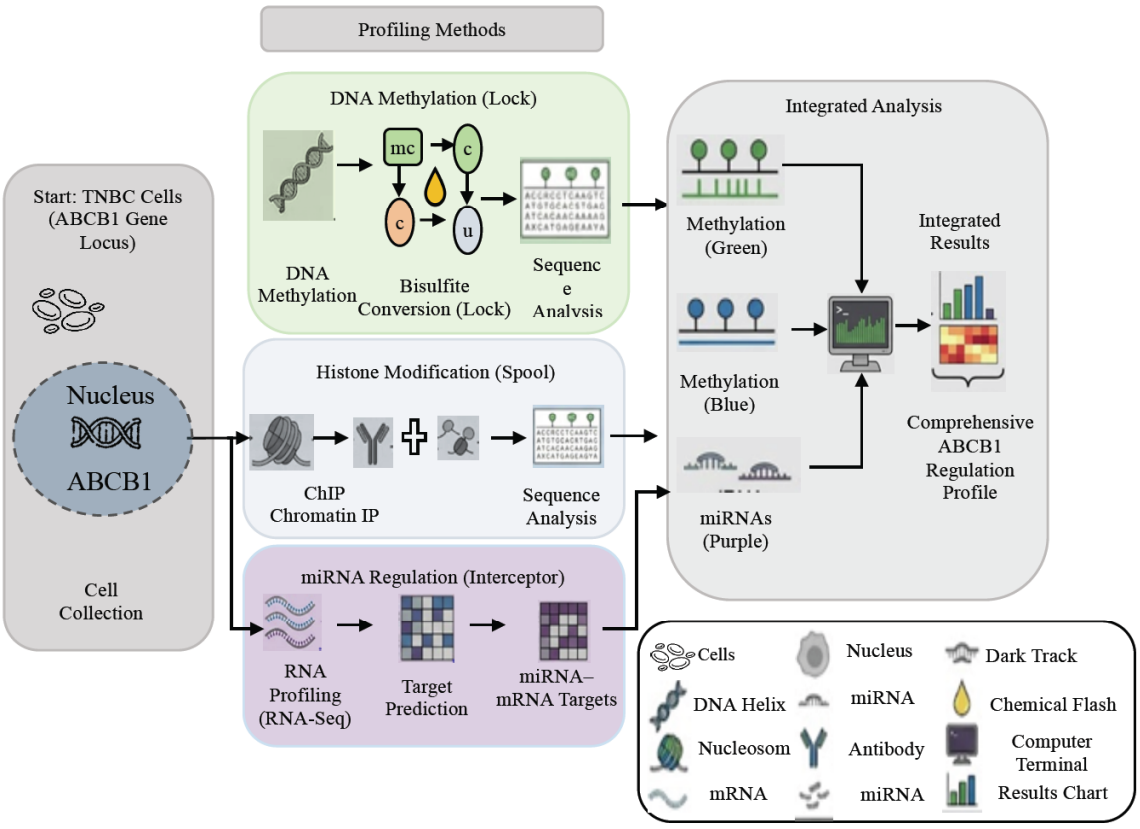

Figure 2: Epigenetic Profiling Workflow.

mesenchymal transformation, and drug response. Lack of these microRNAs could help activate ABCB1 and develop taxane resistance.

The primers, antibodies, reagents, and experimental conditions used were standardized. The primer pair used for ABCB1, GAPDH, beta-actin, miR-451, miR-335, U6 snRNA, and the ABCB1 promoter were also validated before the experiments were carried out. Real-time PCR, western blot analysis, chromatin immunoprecipitation, quantitative PCR, methylation, and inhibition experiments were carried out under standardized conditions.

Figure **2** gives the working process of the analysis of epigenetic regulation of the expression of the ABCB1 gene in TNBC cells. The workflow combines three main profiling techniques, namely, DNA methylation (Lock), histone modification (Spool), and miRNA regulation (Interceptor). The DNA methylation is examined by bisulfite conversion and sequence analysis, the histone modifications are examined by the ChIP assays, and the regulation of miRNA is examined by RNA profiling and prediction of targets. These methods give a global picture of how an epigenetic modification can affect the expression of ABCB1 and cause taxane resistance. The combined study of those factors provides an overall ABCB1 regulation profile, which contributes to the perception of the drug resistance mechanisms. Figure **2** is a schematic workflow prepared to summarise the epigenetic profiling strategy, including DNA methylation, histone modification, and miRNA analysis; it is not an original experimental data output.

2.5. Patient-Derived Samples and Cohorts

Patient-derived tumor samples were collected from 60 confirmed TNBC patients who received taxane-based chemotherapy. The cohort included FFPE and fresh-frozen tumor tissues. TNBC status was confirmed by negative expression of ER, PR, and HER2. Patients were included if they had confirmed primary TNBC, available tumor tissue, complete clinical records, and documented taxane response. Patients with incomplete data, prior experimental therapy, metastatic disease at diagnosis, or poor-quality tissue were excluded.

Clinical data, including age, tumor stage, grade, lymph node status, chemotherapy regimen, response, recurrence, progression-free survival, and overall survival, were collected from records. Patients were grouped as taxane-sensitive or taxane-resistant. The study received Institutional Ethics Committee approval, written informed consent was obtained, and all samples were anonymized.

2.6. Epigenetic Inhibitor Treatments

In order to investigate the functional effects of epigenetic modulation on ABCB1 expression and taxane sensitivity, the solution of taxane-resistant cells was treated with the DNA methyltransferase inhibitor 5-aza-2′-deoxycytidine (1–5 μM) and histone deacetylase inhibitor trichostatin A (100 -300 nM) for 48-72 hours. Following the therapy, Western blotting and qPCR were used to measure the amounts of ABCB1 protein and mRNA, respectively, and Rhodamine 123/Calcein AM efflux tests were used to measure the transporter activity. The cytotoxicity of taxanes was measured by using MTT or other cell viability tests in order to estimate the IC_{50} of paclitaxel alone and combined with epigenetic inhibitors.

2.7. Statistical Analysis

All experiments were carried out with a minimum of three independent biological replicates. The quantitative tests, which include quantitative RT-PCR, ChIP-qPCR, miRNA profiling, cell viability, and efflux tests, were conducted using a minimum of technical triplicates. Reproducibility was evaluated prior to any statistical analysis to ensure data consistency among independent experiments. The statistics are in the form of mean standard deviation (SD). Unpaired Student t test was used to test the difference between two groups (e.g., taxane sensitive and taxane resistant), whereas one-way ANOVA followed by the suitable post-hoc tests was used to test the differences among multiple groups. Kaplan-Meier was used to create an S-curve, and the log-rank test was used to compare the curve differences. Pearson or Spearman correlation coefficients were used to examine the correlations between ABCB1 methylation/expression and clinical parameters. GraphPad PRISM and SPSS software were used to do statistical analyses, and p-values less than 0.05 were taken to be statistically significant.

Table **2** outlines the statistical methods and software used for the analysis of experimental and clinical data in the study. It includes three key types of analysis: Comparative Analysis, Survival Analysis, and Epigenetic Inhibition Studies. The Comparative Analysis entails the use of Student's t-test or one-way ANOVA to determine the differences between the taxane-sensitive and taxane-resistant TNBC cells. Kaplan-Meier curves are used to conduct Survival Analysis to determine the relationship between the status of ABCB1 methylation and clinical outcome, e.g., progression-free survival. Finally, Epigenetic Inhibition Studies are based on the statistical testing of the reversal of taxane resistance with the help of DNA methylation or histone modification inhibitors. All these tests will be analyzed with the help of GraphPad Prism or SPSS to ensure the rigor of the analysis and proper interpretation of the findings.

2.8. Ethical Considerations

This research was carried out following approval by the ethics committee of the concerned institution. Written informed consent was acquired from all participants prior to obtaining tumor samples and using their clinical data. Participants' identities and clinical data were de-identified before analysis. Coded samples only were used for molecular assays. All work done on human subjects and their biological samples was carried out in line with the principles of the Helsinki Declaration.

3. RESULTS

3.1. ABCB1 Upregulation in Taxane-Resistant TNBC Cells

TNBC cell lines resistant to taxanes, including MDA-MB-231, displayed a significant enhancement in the expression of ABCB1 when compared with the taxane-sensitive ones. It has been substantiated with the help of both quantitative PCR (qPCR) and Western blot analysis, showing the significant increase of mRNA and protein levels of ABCB1 in resistant cells. In particular, the qPCR results revealed that the level of ABCB1 mRNA in resistant and sensitive cells had increased 3.2-fold. The same pattern was observed in Western blotting, where the concentration of the ABCB1 protein was about 2.8 times as much in resistant cells. On the contrary, cells that are taxane sensitive had significantly lower levels of ABCB1 as they are more

Table 2: Statistical Methods and Software

Analysis Type	Methodology	Software Used
Comparative Analysis	Student's t-test or one-way ANOVA	GraphPad Prism, SPSS
Survival Analysis	Kaplan-Meier curves for progression-free survival	GraphPad Prism, SPSS
Epigenetic Inhibition	Statistical testing for reversal of resistance with epigenetic inhibitors	GraphPad Prism, SPSS

sensitive to the treatment of taxane. This observation supports the theory that one of the factors contributing to treatment resistance in TNBC is ABCB1 overexpression.

These findings suggest that one important mechanism of TNBC cell resistance to taxanes is ABCB1 overexpression. ABCB1 is a drug efflux pump that actively transports chemotherapy agents such as taxanes out of the cells, therefore decreasing the concentration of these chemotherapy agents and decreasing their cytotoxic effect within the cell. The increased levels of ABCB1 in the resistant cells highlight the importance of the gene in the development of resistance to the treatment using taxanes.

Functional Validation of the Role of ABCB1 in Taxane Resistance. Cells that were resistant to taxanes had less retention of Rhodamine 123/Calcein-AM when compared to the cells that were sensitive to taxanes, thus suggesting increased efflux function by ABCB1. With inhibition of ABCB1 function, there was an increase in intracellular retention of dye, as well as lower IC_{50} values for the taxanes among the resistant cells.

Table **3** shows the relative amount of the ABCB1 protein and mRNA expression of the taxane-sensitive and taxane-resistant MDA MB 231 TNBC cells, and it is apparent that the level of the ABCB1 in the taxane-resistant cells has significantly increased.

3.2. Mechanisms of Epigenetic Modification of ABCB1 Upregulation

ABCB1 upregulation in taxane-resistant cells was also examined using the approach of epigenetics. DNA methylation in the ABCB1 gene's promoter region was assessed as one of the most important epigenetic changes utilizing the bisulfite sequencing technique. The findings revealed that the promoter region of the ABCB1 gene was profoundly hypomethylated in taxane-resistant cells, a phenomenon typically associated with gene activation. In particular, there was a significant decrease in DNA methylation of the ABCB1 promoter in the resistant cells, suggesting that the absence of methylation may facilitate ABCB1 transcription.

On the other hand, methylation levels in the ABCB1 gene's promoter region were higher in taxane-sensitive cells, and this was linked to a reduction in gene expression. This result lends credence to the theory that the up-regulation of the ABCB1 gene in taxane-resistant TNBC is largely due to DNA hypomethylation in the promoter region of the gene.

The results of this study indicate that an important epigenetic mechanism that enhances the overexpression of ABCB1 in taxane-resistant TNBC cells is DNA methylation of the ABCB1 promoter. Epigenetic silencing of DNA methylation seems to inhibit the potential activation of ABCB1 in cells likely to be sensitive, and the absence of epigenetic silencing of DNA in cells with resistance causes ABCB1 to be overexpressed and exhibits drug resistance.

Figures **1** and **2** are schematic representations created to demonstrate the procedure of our experiment and the process of our epigenetics profiling. On the other hand, Figures **3**, **4**, and **5** depict original results generated from methylation profiling, microRNA expression profiling, and clinical correlations derived from patient data, respectively. All figures showing data from experimental results used biological replicates and were properly labeled using statistical notations such as error bars and significance levels.

Figure **3** original experimental results demonstrating the ABCB1 promoter methylation status in Taxane-sensitive and Taxane-resistant TNBC cell lines. Results are expressed as means ± SD from three biological replicates. Significance analysis was done by Student's t-test, where $p < 0.05$ was considered statistically significant.

3.3. Histone Modifications and ABCB1 Expression

The modifications in the histone models were also assessed by ABCB1's epigenetic regulation. Chromatin immunoprecipitation (ChIP) tests were used to quantify the amounts of histone H3 acetylation at the ABCB1 promoter. Its findings showed that taxane-insensitive

Table 3: ABCB1 Expression in Taxane-Sensitive and Taxane-Resistant TNBC Cells

Cell Line	ABCB1 mRNA Expression (Relative Fold Change)	ABCB1 Protein Expression (Relative Intensity)
MDA-MB-231 (Resistant)	3.2	2.8
MDA-MB-231 (Sensitive)	1.0	1.0

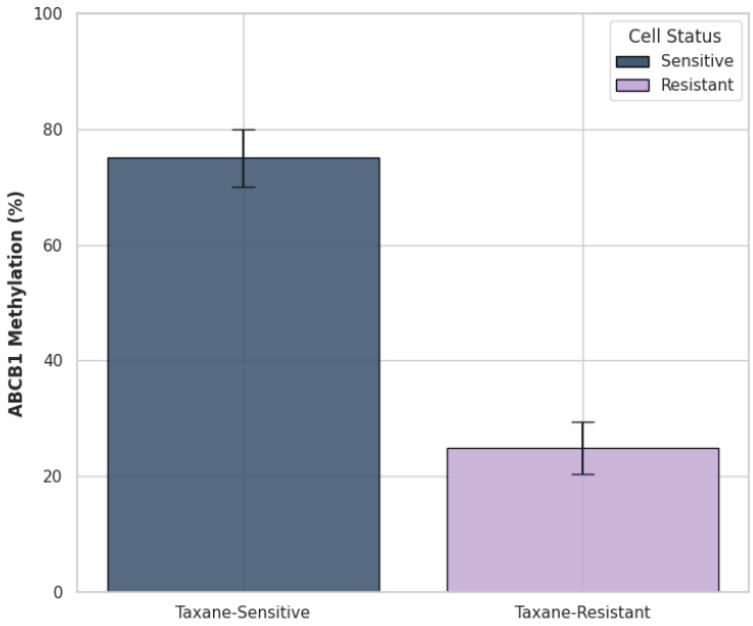

Figure 3: DNA Methylation Status of ABCB1 Promoter in Taxane-Sensitive and Taxane-Resistant TNBC Cells.

cells had much higher levels of histone H3 acetylation at the promoter end of ABCB1 than taxane-sensitive cells. The chromatin structure that is normally linked with the histone acetylation is open, and elevated transcription of the gene. The elevated acetylation in the resistant cells indicates that the ABCB1 promoter is more exposed to transcription in the resistant cells, thereby leading to higher levels of ABCB1 expression.

Also, the histone modifications associated with gene repression were tested. In particular, repressive histone mark H3K27me3 was determined to be low at the ABCB1 promoter in the taxane-resistant cells. This lessening of the repressive signs further confirms the hypothesis that the chromatin structure of resistant cells is less repressive to ABCB1 expression, hence promoting drug efflux and resistance.

The above findings indicate that histone changes, especially acetylation and reduction of the repressive marks at the ABCB1 promoter, are important in the regulation of ABCB1 expression in the taxane-resistant TNBC cells.

Table **4** depicts the relative histone H3 acetylation and H3K27me3 enrichment of the ABCB1 promoter in taxane-sensitive and taxane-resistant TNBC cells, which reveals a more transcription-permissive chromatin status of taxane-resistant cells.

3.4. Non-Coding RNA Regulation of ABCB1

Non-coding RNAs, miRNAs, and their effect on the regulation of ABCB1, namely, binding to the 3' untranslated regions (UTRs) of target mRNAs, resulting in mRNA degradation or translational inhibition, were also examined. It has been shown that several miRNAs, including miR-451 and miR-335, target ABCB1 and suppress its expression.

miRNA profiling showed that these miRNAs were highly downregulated in taxane-resistant cells compared to sensitive cells. In particular, miR-451 and miR-335 were observed to be expressed in lower concentrations in resistant cells, implying that the decrease in their inhibition activity towards ABCB1 could be a reason behind its overexpression in resistant cells. The diminished expression of such miRNAs in cells resistant to taxanes demonstrates that the disruption of post-transcriptional regulation can be one of the factors contributing to the growth of ABCB1.

Table 4: Histone Modification Analysis at the ABCB1 Promoter

Histone Modification	Taxane-Resistant Cells (Relative Fold Change)	Taxane-Sensitive Cells (Relative Fold Change)
H3 Acetylation	3.5	1.2
H3K27me3	0.5	1.8

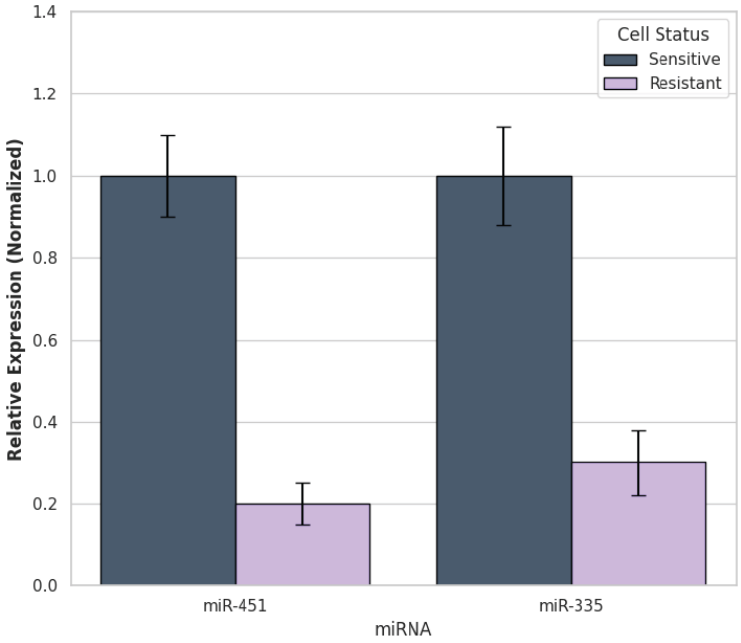

Figure 4: miRNA Expression Profiles in Taxane-Sensitive and Taxane-Resistant Cells.

These findings suggest that miRNA dysregulation, i. e., the downregulation of miRNAs, which target ABCB1, is a contributing factor to the ABCB1 upregulation and consequent drug resistance in TNBC.

Figure **4** raw data representing the expression of miR-451 and miR-335 in TNBC cells that are sensitive and resistant to taxane therapy. Data are shown as mean ± SD of three biological replicates with triplicate measurements per condition. Significance was determined by Student's t-test, where $p < 0.05$ was significant.

3.5. Reversal of ABCB1 Overexpression by Epigenetic Modulation

Experiments have been carried out using epigenetic inhibitors to determine the functional importance of such epigenetic changes. Epigenetic marks at the ABCB1 promoter were regulated by using DNA methyltransferase inhibitor, 5-aza-2'-deoxycytidine, and histone deacetylase inhibitor, trichostatin A. The administration of these inhibitors led to a reduction of expression of ABCB1 at the mRNA and protein levels in taxane-resistant cells. The DNA methylation inhibitor 5-aza-2'-deoxycytidine reduced ABCB1 expression by half and restored the methylation of the ABCB1 promoter. In the same vein, histone acetylation and lowering of the expression of ABCB1 were observed in trichostatin A treatment, which is an inhibitor of the histone deacetylases.

Notably, the sensitivity of the resistant cells to the treatment of taxane was also restored by the treatment with the latter inhibitors. The IC50 values of taxane sensitivity also reduced significantly when treated with the epigenetic inhibitors, which showed that the reversal of the overexpression of the ABCB1 by epigenetic regulation can be used to overcome drug resistance.

Table **5** demonstrates the results of epigenetic inhibitor intervention on ABCB1 mRNA and paclitaxel IC_{50} values in taxane-resistant TNBC cells, which indicates that epigenetic upregulation decreases the levels of ABCB1 and recovers taxane resistance.

Whereas 5-aza-2′-deoxycytidine and trichostatin A administration led to decreased ABCB1 levels and increased taxane sensitivity, it needs to be noted that

Table 5: Effect of Epigenetic Inhibition on ABCB1 Expression and Taxane Sensitivity

Treatment	ABCB1 mRNA Expression (Relative Fold Change)	Taxane Sensitivity (IC_{50})
Control (No Treatment)	3.2	120 nM
5-Aza-2'-deoxycytidine	1.5	60 nM
Trichostatin A	1.2	50 nM

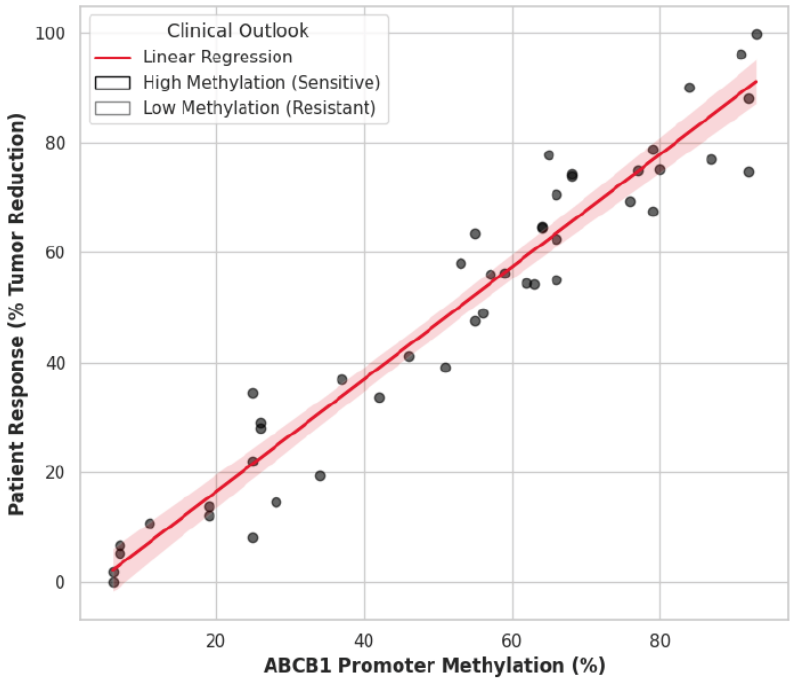

Figure 5: ABCB1 Methylation and Taxane Response in Patient-Derived TNBC Samples.

these observations constitute preliminary experimental data rather than confirmation of therapy itself. Further confirmation, in particular, ABCB1-specific knockdowns, rescue tests, chromatin confirmation after inhibitor application, and *in vivo* testing would be necessary to verify that the sensitization observed is due to epigenetic control by ABCB1 itself.

3.6. Clinical Correlations

The patient-derived TNBC samples were utilized to measure the clinical relevance of ABCB1 methylation as a status. A better response to taxane chemotherapy was linked to high levels of methylation of the ABCB1 promoter. On the contrary, patients who had low ABCB1 promoter methylation, which is associated with increased ABCB1 expression, had a poor clinical outcome and resistance to the taxane therapy. These findings suggest that the ABCB1 promoter's methylation status could be utilized as a predictive biomarker for taxane response in TNBC patients.

Figure **5** original patient-derived clinical correlation data relating ABCB1 promoter methylation and taxane sensitivity in TNBC tissue specimens. Each data point corresponds to one specimen from an individual patient. The regression curve shows the trend line for the correlation, while the gray area is the 95% confidence interval. Correlation analyses were carried out using either Pearson or Spearman tests; $p < 0.05$ was deemed statistically significant.

4. DISCUSSION

The outcomes of this study provide valuable insights into the role of epigenetic regulation in mediating taxane resistance in TNBC. The hypomethylation of the ABCB1 promoter in taxane-resistant cells suggests that reduced promoter methylation may contribute to increased ABCB1 expression, although further mechanistic validation is required to confirm a direct causal relationship. Such activation leads to a reduction in the efficacy of medications and elevation of the ABCB1 protein that regulates drug efflux. The hypothesis that epigenetic changes elevate ABCB1 and cause drug resistance is also evidenced by the fact that the histone H3 level of the ABCB1 promoter is high, and the levels of miRNAs in the ABCB1 promoter, including miR-451, miR-335, etc., have dropped. These results indicate that epigenetic processes may participate in the regulation of ABCB1 expression and may provide useful targets for further investigation into chemotherapy resistance in TNBC. The transporter inhibition and efflux assay findings strengthen the mechanistic interpretation by showing that ABCB1 activity directly contributes to reduced intracellular drug retention and taxane resistance in TNBC cells.

This emphasis on miR-451 and miR-335 is biologically reasonable since these two miRNAs are involved in breast cancer progression and responsiveness to chemotherapy. The downregulation of these miRNAs in TNBC-resistant cells might be

responsible for the loss of their repressive effects on ABCB1-dependent multidrug efflux from tumor cells, leading to low intracellular taxane accumulation and subsequent survival of chemoresistant cells. In other words, this result indicates that besides promoter hypomethylation and histone acetylation, miRNA deregulation contributes to ABCB1 resistance.

The findings are in agreement with what is available in the literature on epigenetic alterations in the development of drug resistance, compared to the existing literature. Previous studies revealed that the process of DNA methylation and histone acetylation contributes to the increase in the expression of ABCB1 in various cancers, including breast cancer. The present research adds TNBC-focused evidence suggesting that specific epigenetic changes are associated with ABCB1 upregulation and taxane-resistant behavior. The fact that the *in vitro* models have been used in this study is a weakness, but the results provide a good mechanistic understanding. Future research that utilizes patient-derived samples or *in vivo* models will be instrumental in establishing these findings and establishing the generalizability of the research findings in the clinical environment.

These results may have potential therapeutic implications, but they should be interpreted cautiously. Epigenetic modulation may provide a potential approach for reducing taxane resistance in TNBC; however, further mechanistic validation is required before drawing strong therapeutic conclusions. DNA methyltransferase and histone deacetylase inhibitors may be explored as adjunctive strategies to restore taxane sensitivity, but their therapeutic relevance should be confirmed through ABCB1-specific functional assays, rescue experiments, toxicity assessment, and *in vivo* models. More so, miRNA-based therapies to correct miR-451 and miR-335 expression may also be used to lower the level of ABCB1 and increase the efficacy of taxanes. Future studies using ABCB1-specific knockdown, rescue experiments, *in vivo* models, toxicity assessment, and larger patient cohorts are needed before clinical translation can be considered.

In summary, the evidence presented indicates a correlation among increased expression of ABCB1, changes at the epigenetic level, and taxane resistance in TNBC. Nevertheless, the findings must not be taken as conclusive evidence of clinical effectiveness. The evidence from the inhibitors provides initial evidence for function, but mechanistic proof and more research involving ABCB1 is necessary.

5. CONCLUSION

To sum up, this paper is able to conclude that epigenetic regulation of ABCB1 is an essential process that contributes to taxane resistance in TNBC. The DNA hypomethylation, hyperacetylation of histones, and down-regulation of important miRNAs observed enhance the up-regulation of the ABCB1 that influences the drug efflux and limited chemotherapy effect. These results give a new understanding of the importance of alterations in the epigenetic changes in resistance and the possibility of manipulating epigenetic alterations as a treatment approach to surmounting drug resistance in TNBC. The discovery of epigenetic alterations as the cause of ABCB1 overexpression presents numerous opportunities in prospective studies and clinical use. The results indicate that the use of epigenetic regulation can increase taxane sensitivity, but more studies on the mechanisms involved are required before using the combination of taxane and DNMT or HDAC inhibitors in TNBC patients. Moreover, the reinstatement of the expression of the major miRNAs, such as miR-451 and miR-335, could also provide another solution to control the level of expression of ABCB1 and reverse resistance. The future clinical trials will be important in determining the effectiveness of these therapeutic measures and the use of epigenetic markers as biomarkers for predicting taxane resistance. The final objective is to establish personalized treatment strategies combining epigenetic alterations with standard chemotherapy and enhance the patient outcomes of TNBC, as well as to give fresh hope to overcoming chemotherapy resistance in the present type of aggressive cancer.

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