

Article

Protein Arginine Methyltransferase-5 Inhibition Induces Growth Arrest and Death in Triple-Negative Breast Cancer Cells

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Abstract

Background: PRMT5, or protein arginine methyltransferase 5, is recognized as an epigenetic regulator that suppresses gene transcription through symmetric dimethylation of histone arginine residues, including histone H4 arginine 3 (H4R3me2s) and histone H3 arginine 8 (H3R8me2s), modifications associated with chromatin condensation and transcriptional repression. PRMT5-mediated methylation has been associated with recruitment of polycomb repressive complex 2 (PRC2) and enhancer of zeste homolog 2 (EZH2)-mediated H3K27me3 deposition, contributing to stable repression of tumor suppressor genes and apoptosis-related effectors in breast cancer. **Methods:** The molecular and functional impacts of PRMT5 inhibition were studied in TNBC cell lines with a pharmacological inhibitor (CMP5). Cellular responses were evaluated using a viability assay, qPCR, Western blotting, Annexin V/PI staining, and transwell migration/proliferation assays. **Results:** PRMT5 inhibition substantially reduced TNBC viability in a time- and dose-dependent manner. EZH2 was downregulated, whereas the tumor suppressor retinoblastoma-like protein 2 (RBL2) was induced, concomitant with low expression of Cyclin D1. These changes were accompanied by upregulation of pro-apoptotic effectors (Caspase-3, Caspase-10, death-associated protein 1 (DAP1), and BCL2-associated x protein (BAX) and repression of the pro-survival B-cell lymphoma 2 (BCL2), consistent with apoptosis-associated molecular responses. Functionally, CMP5 treatment was associated with reduced migratory behavior in TNBC cells under the experimental conditions tested. **Conclusions:** These findings suggest that PRMT5 inhibition by CMP5 is associated with reduced TNBC cell viability, impaired migration, increased expression of apoptosis-associated regulators and enhanced apoptotic cell death as measured by Annexin V/PI analysis in vitro. Further mechanistic and in vivo studies are required to clarify the therapeutic relevance of PRMT5 inhibition in TNBC.

Keywords: PRMT5; Caspase-3; Caspase-10; DAP1; BAX; BCL2; PRC2; TNBC; apoptosis; RB-E2F signalling pathway; migration; proliferation



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1. Introduction

Triple-Negative Breast Cancer (TNBC) is recognized as a high-grade BC with the worst clinical outcomes [1]. It is classified as lacking human epidermal growth factor 2 (HER2), estrogen, and progesterone receptors. This subtype constitutes ~15–20% of all BC diagnoses. It also significantly affects young women and individuals of African descent [2].

Due to the absence of estrogen receptor, progesterone receptor, and HER2 expression, TNBC lacks the molecular targets required for endocrine and HER2-targeted therapies, leaving chemotherapy as the principal systemic treatment option [3]. However, TNBC is well-known for early metastasis and high recurrence rates, with poor survival outcomes. This underscores the urgent need to establish a more effective treatment strategy [4].

Recent research has focused on identifying epigenetic regulators that promote tumor development and sustain the malignant characteristics of TNBC. PRMT5 has been identified as a potential molecular target. PRMT5 is a type II protein arginine methyltransferase that regulates gene transcription through symmetric dimethylation of histone arginine residues, including histone H4 arginine 3 (H4R3me2s) and histone H3 arginine 8 (H3R8me2s). These histone modifications are associated with chromatin condensation and transcriptional repression [5–12]. PRMT5 partially mediates gene silencing by interacting with the PRC2 complex. Consequently, this interaction enhances the suppression of tumor suppressor genes and pro-apoptotic factors [13–17].

Previous findings have shown that PRMT5 regulates the expression of apoptotic-inducing genes. Abnormal expression of PRMT5 induces aberrant activation of PRC2 components, particularly EZH2, leading to chromatin compaction and suppression of key apoptotic effectors, including Caspase-10 and DAP1 [13–17].

Importantly, these findings support a coordinated epigenetic framework in which PRMT5 does not function effectively alone but collaborates with the Polycomb machinery to suppress transcription of cell-death programs. Consistent with this idea, recent evidence highlights a crucial interaction between PRMT5 and PRC2 in controlling apoptosis-related genes in TNBC. This crosstalk promotes chromatin condensation and transcriptional silencing [13–16]. Notably, PRMT5 interacts with EZH2 to repress key regulatory genes that control cell-cycle and apoptosis signaling [13–17].

The transcriptional role of PRMT5 appears to be context-dependent and may vary according to tumor type and chromatin environment [7,13,15]. Nevertheless, aberrant PRMT5 activity has consistently been associated with tumor progression, enhanced survival signaling, and aggressive cancer phenotypes across multiple malignancies [7,10,12,15,16].

PRMT5 and EZH2 are frequently overexpressed across multiple tumor types [16,17]. PRMT5 likely suppresses pro-apoptotic genes indirectly by activating the PRC2 complex [13,14]. Beyond its role in Polycomb-mediated transcriptional repression, PRMT5 also contributes to tumor progression through non-histone methylation mechanisms [18–23]. Remarkably, PRMT5 has also been involved in basal-like breast cancer progression through stabilization of oncogenic transcriptional programs, further supporting its role as a potential therapeutic target in breast cancer, highlighting PRMT5 as a potential therapeutic target in BLBC [19].

Beyond its role in breast cancer progression, PRMT5 overexpression has also been reported in other malignancies, including lung cancer (LC) cells [8,18]. Both pharmacological inhibition and shRNA knockdown of PRMT5 notably strengthen the effects of resveratrol. This combination induces apoptosis by activating cell death effector genes, including CASP3 and PARP, in ASTC-a1 and A549 lung cancer cell lines [18]. Furthermore, PRMT5 knockdown markedly decreases phosphorylation of Akt at Thr308 and Ser473, as well as Ser9 on GSK3 β [18]. The cell cycle regulators CCND1 and CCNE1 are also suppressed. These findings support the idea that PRMT5 contributes to LC development and growth by abnormally activating the Akt/GSK3 β signaling pathway [18]. Inhibiting PRMT5 pharmacologically enhances resveratrol's chemosensitivity, suggesting that blocking PRMT5 may reduce the malignant traits of lung cancer and aid in recovery [13,18,19]. Overall, PRMT5 can act as a positive or negative regulator of transcription depending on the cellular context [9,10,12,13].

Previous studies conducted by our research group and other groups have shown that CMP5 (PRT-382) downregulates the PRMT5 enzyme activity by decreasing the levels of H4R3me2s and the total symmetric dimethylarginine (SDMA) levels, indicating the mechanism of PRMT5 activity modulation via an on-target effect. Further ChIP analysis in the TNBC model has also indicated a reduction in H3R8me2s at PRMT5-binding sites upon PRMT5 inhibition [5,12,20]. Therefore, these earlier identified PRMT5-mediated histone methylation modifications constitute evidence of PRMT5 activity inhibition by CMP5.

Given this rationale, the present study investigated the molecular and phenotypic alterations associated with pharmacological PRMT5 inhibition in TNBC cells. RBL2 has been reported as a potential downstream effector of PRMT5-dependent epigenetic repression, especially as part of PRC2-associated signal transduction [13–17]. *CASP10* is an initiator caspase involved in extrinsic apoptotic signals, having been previously reported as a potential target of PRMT5-induced apoptosis in cancer cells [14]. In this study, the effect of PRMT5 inhibition on TNBC cell lines, MDA-MB-231 and MDA-MB-468, was investigated in the presence and absence of the PRMT5 inhibitor compound 5 (CMP5). Target gene expression was evaluated by qPCR and Western blot. TNBC cell growth was assessed by measuring cell proliferation, transwell migration, and programmed cell death.

2. Materials and Methods

2.1. Cell Culture

The human triple-negative breast cancer (TNBC) cell lines MDA-MB-231 (HTB-26™) and MDA-MB-468 (HTB-132™) were obtained from Dr. Saïd Sif's laboratory, Qatar University, Doha, Qatar, and were originally sourced from ATCC (Manassas, VA, USA) were plated in Dulbecco's Modified Eagle Medium (DMEM) with GlutaMAX™ supplement (Gibco, Thermo Fisher Scientific, Waltham, MA, USA; Cat. No. 10564011). supplemented with 2.5% (*v/v*) 7.5% sodium bicarbonate solution (Gibco, Thermo Fisher Scientific, Waltham, MA, USA; Cat. No. 25080094), 1% Penicillin–Streptomycin (10,000 U/mL penicillin and 10,000 µg/mL streptomycin; Gibco, Thermo Fisher Scientific, Waltham, MA, USA; Cat. No. 15140-122), and 10% fetal bovine serum (FBS; Gibco, Thermo Fisher Scientific, Waltham, MA, USA; Cat. No. A5256701). Cells were maintained at 37 °C in a Thermo Scientific™ Heracell™ 150i CO₂ incubator (Thermo Fisher Scientific, Waltham, MA, USA) in a humidified atmosphere containing 5% CO₂.

2.2. IC₅₀/IC₇₀ Determination

Cells were cultured in 96-well plates (5×10^3 – 1×10^4 cells/well) and treated with CMP5 (MedChemExpress, Monmouth Junction, NJ, USA; Cat. No. HY-120137). (3, 6, 9, or 12 µM) for 24 h, 48, and 72 h, with Dimethyl sulfoxide (DMSO), sequencing grade (Thermo Scientific Pierce, Thermo Fisher Scientific, Waltham, MA, USA; Cat. No. 20688), was used as the vehicle control. Cell survival was measured using 0.4% Trypan Blue Solution (Gibco, Thermo Fisher Scientific, Waltham, MA, USA; Cat. No. 15250061), and viable and nonviable cells were manually counted using a Bright-Line™ hemocytometer (Hausser Scientific, Horsham, PA, USA). Dose–response curves were generated, and IC₅₀/IC₇₀ values were calculated by nonlinear regression using IBM SPSS Statistics version 31 (IBM Corp., Armonk, NY, USA). Experiments were performed in triplicate.

2.3. cDNA Synthesis and Real-Time PCR

Cells were treated with CMP5 (IC₅₀ and IC₇₀) for 24 h. RNA was extracted using TRIzol™ Reagent (Invitrogen™, Thermo Fisher Scientific, Waltham, MA, USA; Cat. No. 15596026), and cDNA was synthesized using a TaqMan™ MicroRNA Reverse Transcription Kit (Applied Biosystems™, Thermo Fisher Scientific, Waltham, MA, USA; Cat. No. 4366596)

according to the manufacturer's guidelines. qPCR was performed using TaqMan gene expression (TaqMan™ Gene Expression Master Mix, FAM™-MGB (Applied Biosystems™, Thermo Fisher Scientific, Waltham, MA, USA) on QuantStudio™ 5 Real-Time PCR System (Applied Biosystems, Thermo Fisher Scientific, Waltham, MA, USA). Relative gene expression was identified using the $2^{-\Delta\Delta C_t}$ method and normalized to α -Tubulin. α -Tubulin was selected as the internal reference gene because it showed consistent amplification across DMSO- and CMP5-treated samples in the present experimental workflow. Data are presented as fold change relative to DMSO controls.

2.4. Protein Extraction and Western Blotting

Total protein was extracted using RIPA buffer (Thermo Scientific™, Thermo Fisher Scientific, Waltham, MA, USA; Cat. No. 89901, and its concentration was assessed using the BCA assay kit (Pierce™ BCA Protein Assay Kit (Thermo Scientific™, Thermo Fisher Scientific, Waltham, MA, USA; Cat. No. A55864). Following SDS-PAGE, proteins were transferred to a Pierce™ PVDF Transfer Membrane, 0.45 μ m (Thermo Scientific™, Thermo Fisher Scientific, Waltham, MA, USA; Cat. No. 88518) using a wet transfer system and incubated with specific primary antibodies (Thermo Fisher Scientific, Waltham, MA, USA). Membranes were incubated with HRP-conjugated goat anti-rabbit IgG (Cat. No. NA934V) or goat anti-mouse IgG (Cat. No. NA931V) secondary antibodies (Cytiva, Marlborough, MA, USA). Protein bands were visualized using ECL Western Blotting Detection Reagent (Cat. No. RPN2209; Cytiva, Marlborough, MA, USA). Densitometry was achieved with ImageJ software v2.16.0/1.54g (National Institutes of Health, Bethesda, MD, USA) and normalized to β -Actin.

2.5. Wound Healing Assay

90% confluent cell monolayers in a 6-well plate (Corning Inc., Corning, NY, USA) were scratched to create a uniform wound and treated with CMP5 at IC₅₀ or IC₇₀, or DMSO. Wound closure was monitored microscopically using EVOS™ M3000 Imaging System (Thermo Fisher Scientific, Waltham, MA, USA) at indicated time points and quantified using ImageJ relative to the initial wound area.

2.6. Transwell Migration Analyses

Cell migration was measured using transwell cell culture inserts with 8.0- μ m pore polycarbonate membranes (Corning Inc., Corning, NY, USA). Cells were seeded in the upper chamber in serum-free medium, while the lower chamber was filled with medium containing serum. After incubation, migrated cells were fixed, stained with crystal violet (Thermo Scientific Chemicals, Thermo Fisher Scientific, Waltham, MA, USA), and observed under a microscope.

2.7. Apoptosis Assay by Annexin V

Apoptosis was analysed by double staining with Invitrogen™ eBioscience™ Annexin V Apoptosis Detection Kit, FITC/Propidium Iodide (Thermo Fisher Scientific, Waltham, MA, USA; Cat. No. BMS500FI-300) and quantified by BD LSRFortessa™ flow cytometer (BD Biosciences, San Jose, CA, USA). Cells were treated with CMP5 (DMSO, IC₅₀, IC₇₀), stained by standard protocols, and analysed by FlowJo 10.7v software (BD Life Sciences, Ashland, OR, USA). Cell populations were gated to distinguish viable (Annexin V−/PI−), early apoptotic (Annexin V+/PI−), late apoptotic (Annexin V+/PI+), and necrotic (Annexin V−/PI+) cells.

2.8. Cell Cycle Analysis

The cell cycle distribution was analysed by PI staining (Invitrogen™, Thermo Fisher Scientific, Waltham, MA, USA) and flow cytometry. Treated cells were fixed in ethanol, dyed with PI/RNase solution (Invitrogen™, Thermo Fisher Scientific, Waltham, MA, USA), and examined to determine the distribution of cells across cell cycle phases.

2.9. Statistical Analysis

Statistical analysis was performed using IBM SPSS Statistics for Windows, version 31 (IBM Corp., Armonk, NY, USA). Data are presented as mean $\pm$ standard deviation (SD) from three independent biological replicates, each performed in three technical replicates unless otherwise stated.

Comparisons among multiple groups were conducted using one-way analysis of variance (ANOVA) followed by Tukey's post hoc multiple comparison test. For time- and dose-dependent experiments, two-way ANOVA was applied where appropriate. Quantitative PCR (qPCR) data were analysed using the comparative Ct ($2^{-\Delta\Delta C_t}$) method. Fold changes were calculated relative to DMSO-treated controls after normalization to the α -Tubulin gene. Western blot densitometric data were analysed using one-way ANOVA followed by Tukey's post hoc test after normalization to β -Actin. Exact p -values are reported where possible, and statistical significance was defined as ns (not significant), $p > 0.05$, $p < 0.05$ (*), $p < 0.01$ (**), and $p < 0.001$ (***). Given the limited sample size ($n = 3$ biological replicates), formal assessment of normality was not performed. Consequently, the assumptions underlying parametric ANOVA could not be formally verified, and the resulting statistical inferences should therefore be interpreted with appropriate caution. Additional methodological details are provided in the Supplementary Materials and Methods.

3. Results

The results of this research were designed to elucidate the molecular, cellular, and functional effects of PRMT5 inhibition in TNBC.

3.1. PRMT5 Inhibition Is Associated with Altered Expression of RBL2 and Suppresses EZH2 and CCND1

PRMT5 is overexpressed in multiple malignancies, including leukaemia, glioblastoma, melanoma, lymphoma, and non-small cell lung cancer (NSCLC) [7,8,12,21]. Elevated PRMT5 expression has also been observed in breast cancer cell lines and in clinical tumor specimens [19,20]. Our previous observation showed that PRMT5 expression is lower in HMECs than in several TNBC cell lines, including BT549 [20]. The effect of CMP5 treatment on the transcriptional regulation of *EZH2*, *RBL2*, and *CCND1* was therefore evaluated by quantitative PCR, as presented in Figure 1.

PRMT5 expression remains unchanged across all treatments ($p = 0.507$) in MDA-MB-231 and ($p = 0.979$) in MDA-MB-468. *RBL2* expression was significantly induced, while both *EZH2* and *CCND1* were suppressed in the presence of PRMT5 inhibition. *RBL2* mRNA levels increased by ~3.5-fold at IC_{50} and ~13–14-fold ($p = 0.001$) in MDA-MB-231, and ~4.7-fold ($p = 0.002$) at the IC_{50} in MDA-MB-468. Conversely, *EZH2* expression was reduced by 2.3-fold at the IC_{50} and by 142.9-fold at the IC_{70} ($p = 0.001$) in MDA-MB-231. This large reduction in *EZH2* expression likely reflects near-complete transcriptional repression under high concentrations of CMP5. A similar pattern was observed in MDA-MB-468 cells, where *EZH2* mRNA levels decreased by 3.7-fold ($p = 0.001$). *CCND1* followed a similar pattern in both TNBC cell lines, showing a 2.2-fold and 11.8-fold ($p = 0.001$) decrease in MDA-MB-231 cells at IC_{50} and IC_{70} , respectively. In MDA-MB-468, *CCND1* mRNA levels were reduced by 5.3-fold ($p = 0.001$) at IC_{50} (Figure 1A,B). This was confirmed with

WB (Figure 1C–E). Notably, IC_{50} and IC_{70} were analysed for MDA-MB-231 in this study. However, IC_{70} analysis for MDA-MB-468 cells was not feasible because nearly all cells died at this concentration, and repeated attempts yielded the same result.

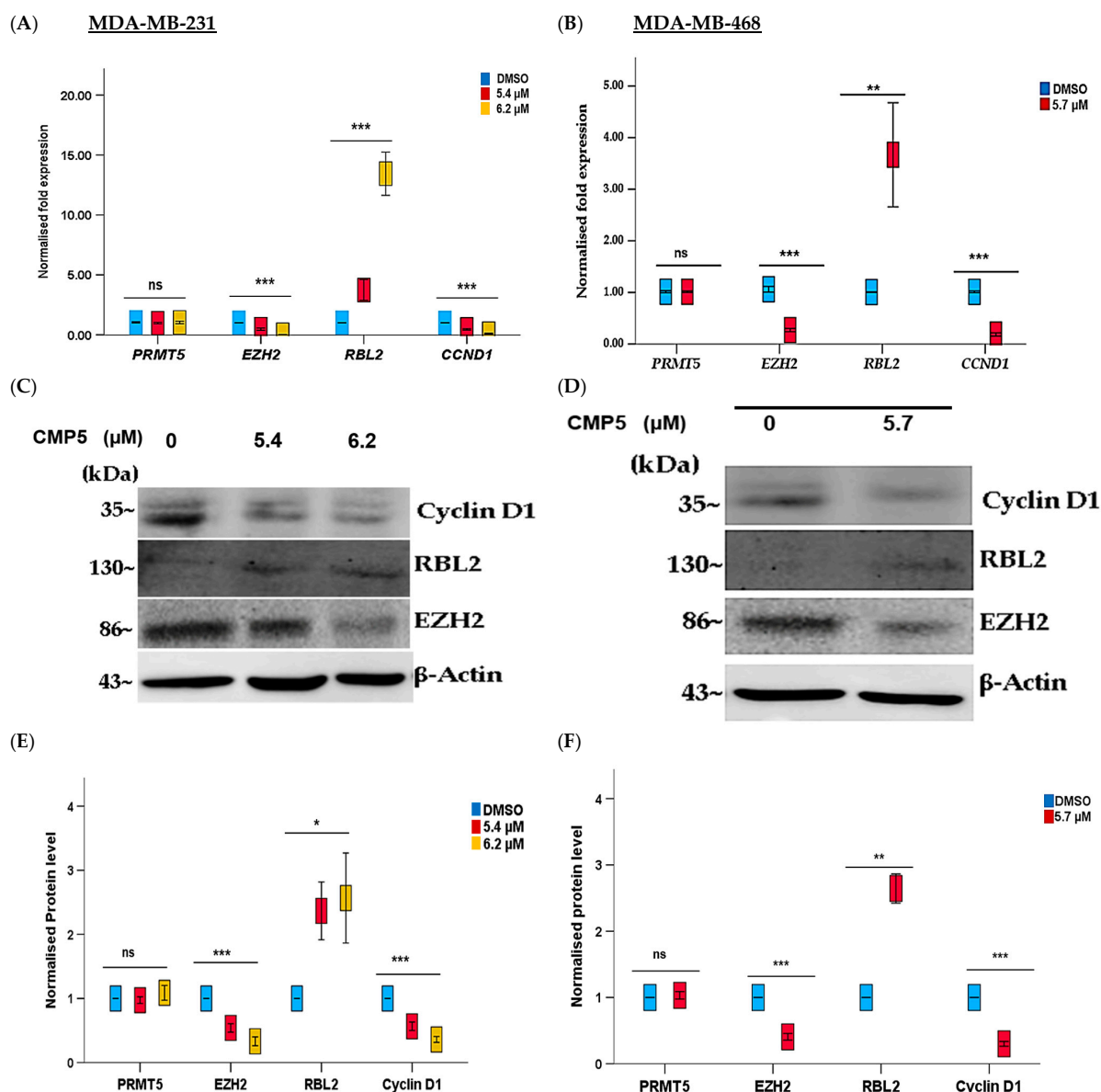


Figure 1. Effect of PRMT5 inhibition on the expression of genes involved in the cell cycle in TNBC cells. (A) Relative mRNA expression of *PRMT5*, *EZH2*, *RBL2*, and *CCND1* in MDA MB 231 treated with DMSO, IC_{50} (5.4 μ M), and IC_{70} (6.2 μ M) CMP5, measured by qPCR. Expression values were normalised to α -Tubulin and reported as fold change relative to the DMSO group. (B) Relative mRNA expression of the same targets in MDA MB 468 treated with DMSO and IC_{50} (5.7 μ M) CMP5, measured by qPCR. Expression levels were normalised to α -Tubulin. (C) Western blot analysis of *PRMT5*, *EZH2*, *RBL2*, and *Cyclin D1* protein abundance in MDA-MB-231 cells with β -Actin as the control. (D) Western blot analysis of the same targets in MDA MB 468 cells. (E) Densitometric quantification of protein bands in MDA-MB-231, normalised to β -Actin and expressed as fold change relative to control. (F) Densitometric quantification of target protein bands in MDA-MB-468, normalised to β -Actin and expressed as fold change relative to control. qPCR data are shown as mean $\pm$ SD from three independent biological experiments ($n = 3$), each measured in three technical replicates. Western blot densitometric data are presented as mean $\pm$ SD from three independent biological experiments ($n = 3$), with densitometric measurements performed in duplicate. Statistical significance was assessed using one-way ANOVA followed by Tukey's post hoc multiple-comparison test. ns, $p \geq 0.05$; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

The pharmacological inhibition of PRMT5 was accompanied by changes in important regulatory proteins related to epigenetics and cell cycle dynamics in MDA-MB-231 cells. The protein levels of PRMT5 did not show significant changes after pharmacological inhibition, with the levels of protein expression close to IC₅₀ and IC₇₀ (ns, $p = 0.220$). EZH2 expression was downregulated by ~2-fold at IC₅₀ and 3-fold at IC₇₀ ($p = 0.001$). Conversely, the tumor suppressor RBL2 was strongly induced by ~1.5–2-fold at IC₅₀ and ~3.9-fold at IC₇₀ ($p = 0.001$) relative to control cells. Consistent with suppression of proliferative signaling, Cyclin D1 expression was decreased by ~2-fold at IC₅₀ and ~3-fold at IC₇₀ ($p = 0.001$).

A similar regulatory pattern was seen in MDA-MB-468 cells following CMP5 treatment, (Figure 1D–F). The protein level of PRMT5 abundance remained unchanged (ns, $p = 0.417$). This observation indicates that CMP5 treatment was not associated with measurable changes in PRMT5 expression levels under the experimental conditions used in this study. However, because PRMT5 catalytic activity and downstream methylation marks were not directly evaluated, no conclusions regarding enzymatic inactivation can be taken from these data alone. While EZH2 protein levels were decreased by ~2-fold relative to DMSO ($p = 0.001$). Concurrently, the tumor suppressor RBL2 was upregulated by ~2.6-fold ($p = 0.01$). In parallel, Cyclin D1 expression was suppressed by ~2.4-fold ($p = 0.001$).

Collectively, these findings demonstrate that CMP5 treatment was associated with altered expression of *RBL2*, *EZH2*, and *CCND1/Cyclin D1* in TNBC cells without significantly changing PRMT5 expression level. However, these results agree with earlier reports from [14] which revealed that PRMT5 inhibition resulted in reactivation of the RB/RBL2 tumor suppressor pathway and in suppression of PRC2-dependent signaling, including EZH2 expression. Although these findings validate our biological hypothesis, no direct mechanistic proof was generated in this study using the TNBC cell system.

3.2. PRMT5 Inhibition Is Associated with Altered Expression of Apoptosis-Related Regulators in TNBC Cells

CMP5 treatment was associated with reduced EZH2 expression and increased RBL2 expression in both TNBC cell lines. Based on these observations, we further examined the expression of apoptosis-associated regulators following CMP5 treatment. PRMT5 transcript and protein levels remained relatively unchanged in both TNBC cell lines, indicating that the PRMT5 transcript and protein levels remained relatively unchanged following CMP5 treatment. Conversely, the expression of several apoptosis-associated genes and proteins increased, whereas the anti-apoptosis protein BCL2 was suppressed. This expression pattern is consistent with a pro-apoptotic molecular profile after PRMT5 inhibition. However, these findings should be interpreted as changes in gene and protein expression rather than direct evidence of biochemical caspase activation. Moreover, treatment of CMP5 was shown to be associated with a significant reduction in the level of EZH2 along with the reappearance of CASP10. Indeed, some earlier reports indicated the involvement of EZH2-induced epigenetic silencing in the inhibition of CASP10, and disruption of this process led to the restoration of CASP10 expression. Hence, the increase in CASP10 levels seen after CMP5 treatment may indicate EZH2-driven inhibition; however, further verification was not within the scope of this study (Figure 2).

In MDA-MB-231 cells, PRMT5 was unchanged across all treatments ($p = 0.791$). *CASP3* was induced by 10 to 25-fold ($p = 0.001$), *CASP10* increased by 6 to 13-fold ($p = 0.001$), *DAP1* was enhanced by 2 to 3-fold ($p = 0.036$), and *BAX* rose by 6 to 11-fold ($p = 0.001$) at IC₅₀ and IC₇₀, respectively. Conversely, the expression of the apoptosis-suppressing gene *BCL2* was downregulated by ~3.3-fold ($p = 0.001$) at the IC₅₀ and was undetectable at the IC₇₀ (Figure 2A). A similar pattern was observed in MDA-MB-468 cells, where *CASP3*, *CASP10*, *DAP1*, and *BAX* were induced by ~4.5-fold ($p = 0.001$), ~3-fold ($p = 0.002$), ~2.5 to 3-fold ($p = 0.01$), and ~7-fold ($p = 0.002$), respectively. *BCL2* expression was decreased by 3-fold ($p = 0.001$) (Figure 2B).

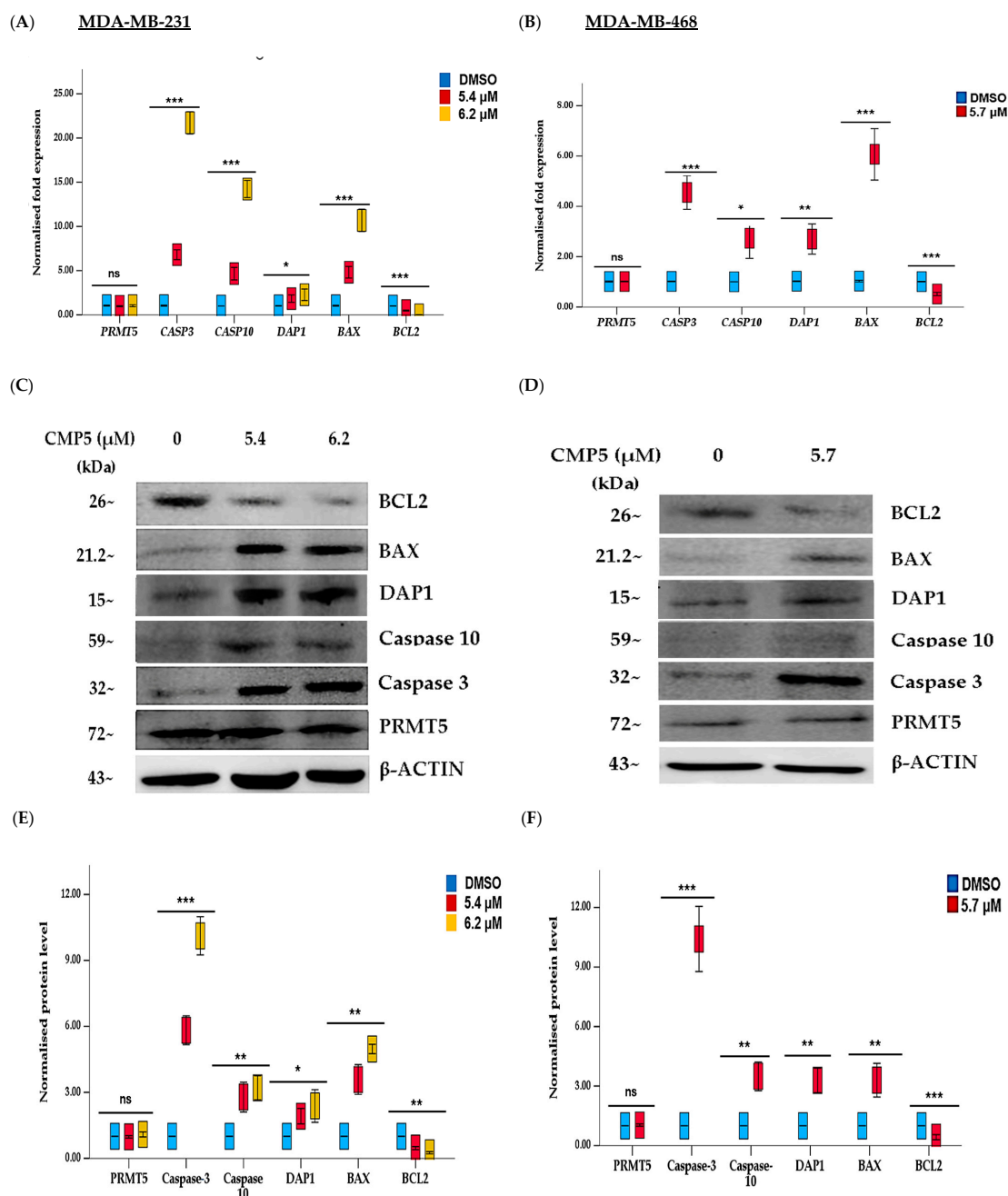


Figure 2. Effect of PRMT5 inhibition on apoptosis-related gene expression in TNBC cells. **(A)** Relative mRNA expression of *CASP3*, *CASP10*, *DAP1*, *BAX*, and *BCL2* in MDA MB 231 cells treated with DMSO, IC₅₀(5.4 μ M), and IC₇₀ (6.2 μ M) CMP5, measured by qPCR. Expression values were normalized to α -Tubulin and shown as fold change compared to the DMSO control. **(B)** Relative mRNA expression of the same targets in MDA MB 468 cells treated with DMSO and IC₅₀ (5.7 μ M) CMP5, assessed by qPCR. Expression levels were normalized to α -Tubulin and presented as fold change relative to the DMSO control. **(C)** Western blot analysis of Caspase-3, Caspase-10, DAP1, BAX, and BCL2 protein levels in MDA MB 231 cells, using β -Actin as an internal control. **(D)** Western blot analysis of the same target proteins in MDA MB 468 cells. **(E)** Densitometric quantification of protein bands in MDA MB 231 cells using ImageJ software, normalized to β -Actin and expressed as fold change relative to control. **(F)** Densitometric quantification of protein bands in MDA MB 468 cells, normalized to β -Actin and expressed as fold change compared to control. qPCR data are shown as mean $\pm$ SD from three independent biological experiments ($n = 3$), each measured in three technical replicates. Western blot densitometric data are presented as mean $\pm$ SD from three independent biological experiments ($n = 3$), with densitometric measurements performed in duplicate. Statistical significance was assessed using one-way ANOVA followed by Tukey's post hoc multiple-comparison test, ns, $p \geq 0.05$; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

To further validate the qPCR findings, WB analysis was performed to assess the gene expression in both TNBC cell lines. In MDA-MB-231, PRMT5 was unchanged ($p = 0.370$), Caspase-3 protein levels increased by ~6-fold at IC₅₀ and ~10-fold ($p < 0.001$) at IC₇₀. Similarly, the levels of Caspase-10, DAP1, and BAX proteins also rose. For Caspase-10, 1.8-fold at IC₅₀ and 2-fold at IC₇₀ ($p = 0.004$). DAP1 1.5-fold at IC₅₀ and 4.8-fold at IC₇₀ ($p = 0.003$). BAX was enhanced by ~4-fold at IC₅₀ and 5-fold at IC₇₀ ($p = 0.009$). BCL2 expression in MDA-MB-231 showed ~2.1-fold decrease at IC₅₀ and a reduction ~3.3-fold ($p = 0.001$) at IC₇₀ (Figure 2C,D). Consistent with the qPCR data, Caspase-3, Caspase-10, DAP1, and BAX protein expression levels were increased following CMP5 treatment. The antibodies used in the present study primarily detected total Caspase-3 and Caspase-10 protein levels rather than specifically distinguishing cleaved active forms. Therefore, the observed increases in Caspase-3 and Caspase-10 protein abundance should not be interpreted as direct evidence of caspase activation but rather as increased expression of apoptosis-associated proteins. In MDA-MB-468, PRMT5 expression also remained unchanged ($p = 0.416$); Caspase-3 was strongly upregulated, showing a 10- to 11-fold ($p = 0.001$) increase, while Caspase-10 and DAP1 were overexpressed by ~3-fold ($p = 0.03$). BAX was induced by 3.5 to 4-fold ($p = 0.001$), and BCL2 was downregulated by 3.5-fold ($p = 0.001$) (Figure 2E,F).

3.3. Pharmacological Blockade of PRMT5 Leads to a Significant Reduction in TNBC Cell Viability

PRMT5 has been reported to contribute to proliferative signaling in TNBC, and pharmacological inhibition of PRMT5 by CMP5 results in a significant decrease in cell viability, indicating a strong anti-survival effect. Based on PRMT5 expression observed in TNBC models, the impact of CMP5 on cell growth and development was evaluated through dose- and time-dependent viability tests. In the dose-response curve analysis, nonlinear regression revealed a clear reduction in viability with increasing CMP5 concentrations in both cell lines. The fitted curves estimated an IC₅₀ of 5.4 μ M for MDA MB 231 and 5.7 μ M for MDA MB 468. The corresponding IC₇₀ values were 6.2 μ M for MDA-MB-231 cells and 6.9 μ M for MDA-MB-468 cells, and these concentrations were subsequently used in downstream gene expression, migration, apoptosis, and cell-cycle analyses. These results indicate that TNBC cells respond to CMP5 treatment in a dose-dependent manner (Figure 3).

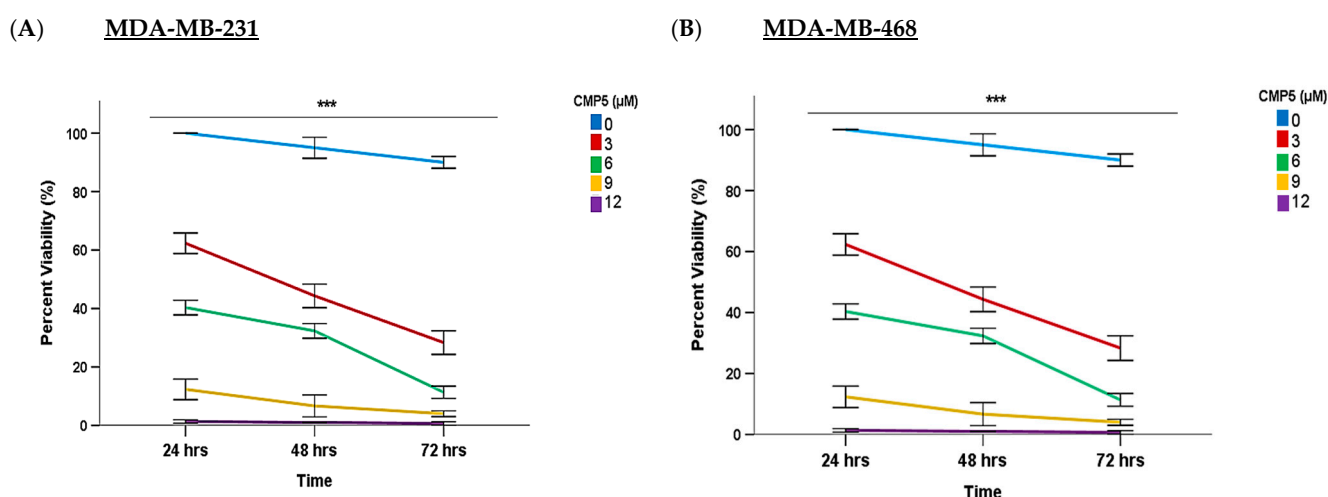


Figure 3. The impact of PRMT5 inhibition on TNBC cell viability. (A) MDA MB 231 and (B) MDA MB 468 cells were treated with increasing concentrations of CMP5 0, 3, 6, 9, and 12 μ M for 24, 48, and 72 h. Data are presented as mean $\pm$ SD from three independent biological experiments ($n = 3$), each measured in three technical replicates. Statistical analysis was performed using two-way ANOVA followed by Tukey's post hoc test in SPSS. *** $p < 0.001$.

To investigate the impact of PRMT5 inhibition on TNBC viability, cells were treated with increasing concentrations of CMP5 (0, 3, 6, 9, and 12) for 24, 48, and 72 h. The trypan blue exclusion assay was used to assess cell membrane integrity, a key indicator of viable cells. Live cells exclude the dye, while dead cells absorb it. After treatment, cell suspensions were mixed with trypan blue, and cells were counted using a hemocytometer. Cell viability was calculated as the ratio of unstained (live) cells to the total number of cells counted.

Using this assay framework, the effect of PRMT5 blocking on TNBC viability was quantitatively assessed, revealing a significant, concentration- and time-dependent cytotoxic response to CMP5 treatment (Figure 3A). Untreated cells maintained 100% viability ($p = 0.92$), whereas at 3 μM CMP5, viability decreased to 66% ($p = 0.001$). Increasing the concentration to 6 μM further reduced viability to 43% ($p = 0.001$), indicating a substantial but slightly less pronounced response than in MDA-MB-468 cells at the same dose. At higher CMP5 concentrations, strong cytotoxic effects were observed, lowering viability to 16% at 9 μM ($p = 0.001$) and to nearly complete cell death (1%) at 12 μM ($p = 0.184$) after 24 h. After 48 h, the control group showed 99% viability. Exposure to 3 μM CMP5 decreased viability to 45% ($p = 0.001$), and a further increase to 6 μM resulted in an additional decline to 27% ($p = 0.001$).

At concentrations of 9 and 12 μM , CMP5 caused a strong cytotoxic effect, reducing viability to complete cell death ($p = 0.001$). After 72 h of drug treatment, the DMSO-treated cells showed 90% viability ($p = 0.406$), while CMP5 decreased viability to 30% at 3 μM , 2% at 6 μM , and 0% at 9 and 12 μM ($p = 0.01$, $p = 0.001$, and $p = 0.001$, respectively). The estimated IC_{50} for MDA-MB-231 cells was 5.4 μM ($p = 0.01$), indicating slightly lower sensitivity to CMP5 compared to MDA-MB-468 cells.

Having established the pronounced, time-dependent reduction in cell viability caused by CMP5 in MDA-MB-231 cells, the viability response in MDA-MB-468 cells was subsequently assessed (Figure 3B). The control group cells showed 100% viability ($p = 0.091$), while at 3 μM CMP5, viability decreased to 66% ($p = 0.001$). Increasing the concentration to 6 μM further reduced viability to 31% ($p = 0.001$), indicating significant loss of membrane integrity and an estimated IC_{50} of 5.7 μM . At higher concentrations, CMP5 caused extensive cytotoxicity, with survival dropping to 10% at 9 μM ($p = 0.001$) and nearly complete cell death (1%) at 12 μM ($p = 0.716$). After 48 h, untreated cells maintained 99% viability ($p = 0.31$), whereas at 3 μM CMP5, the death rate rose to 42% ($p = 0.001$). At 6 μM , viability decreased to 20% ($p = 0.01$). Increasing the dose to 9 and 12 μM resulted in total cell death (0%). After 72 h, the DMSO group showed 88% viability ($p = 0.531$), whereas viability at 3, 6, 9, and 12 μM CMP5 declined to at 72 h, viability at 3, 6, 9, and 12 μM CMP5 declined to approximately 27%, 12%, 3%, and 0%, respectively ($p = 0.001$). The estimated IC_{50} for MDA-MB-468 cells is 5.7 μM .

These outcomes demonstrate that CMP5 noticeably decreases TNBC cell viability in a dose- and time-dependent manner and is associated with reduced cellular survival under the experimental conditions tested. Furthermore, higher concentrations of CMP5 (6–12 μM) induced strong cytotoxic effects, causing extensive membrane damage and near-complete loss of cell viability. The compound may affect cell closure at 48–72 h by inducing cell death.

3.4. PRMT5 Inhibition Is Associated with Altered Growth Characteristics of TNBC Cell Lines

Given the observed alterations in the expression of cell-cycle and apoptosis-related effectors following CMP5 treatment, the effects of PRMT5 inhibition on proliferation, migration, apoptosis, and cell-cycle dynamics in TNBC cells were further investigated. Because CMP5 produced substantial reductions in cell viability, the migration assays were interpreted as exploratory observations and were not designed to distinguish direct effects on cell motility from indirect effects related to reduced proliferation and survival. Wound-

closure assays were therefore conducted in both cell lines following administration with CMP5 at their respective IC_{50} and IC_{70} concentrations (Figure 4).

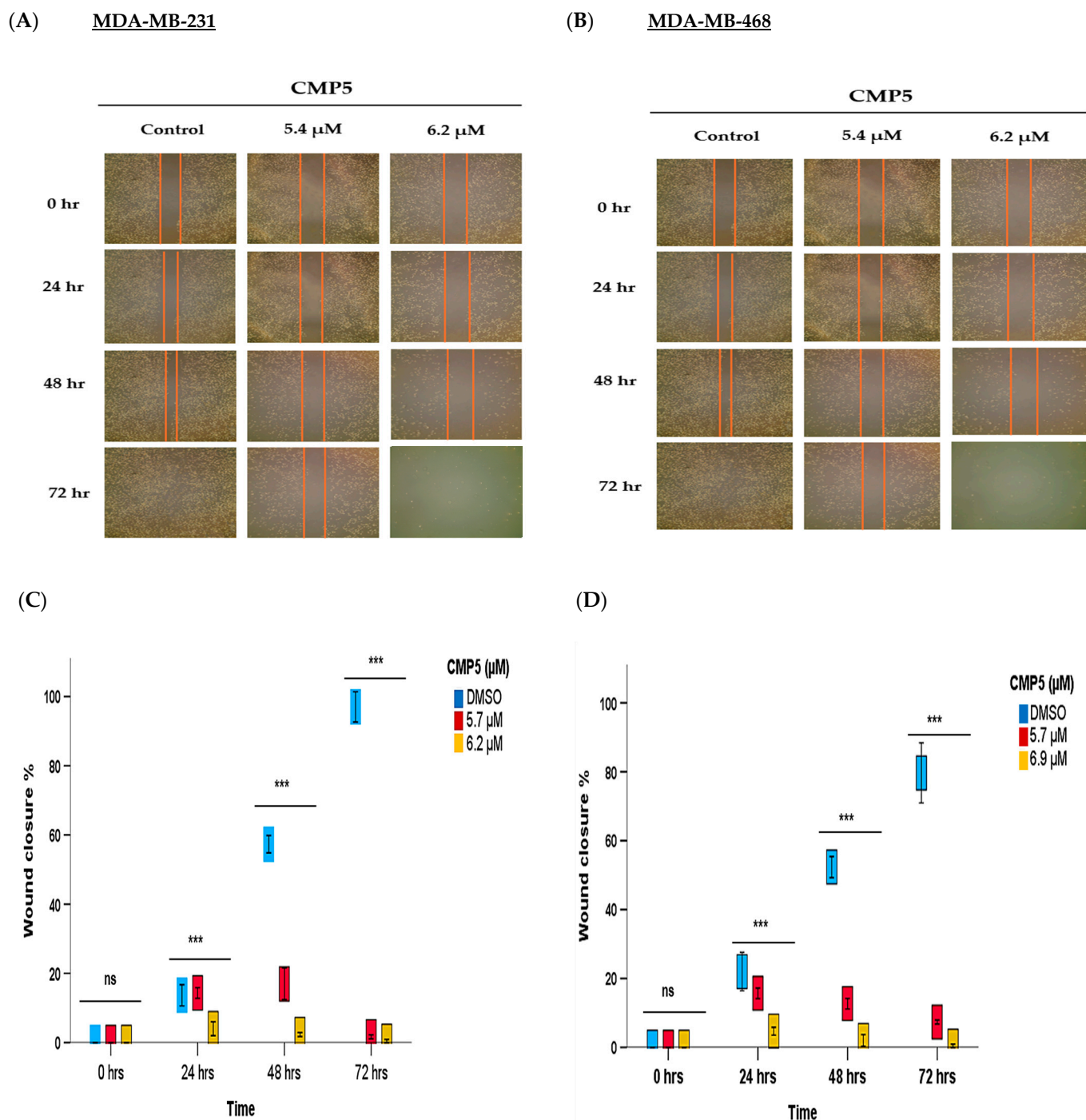


Figure 4. Scratch wound assay illustrating the effect of PRMT5 inhibition on wound closure in TNBC cells. (A) Representative images of wound closure in MDA-MB-231 cells at 0, 24, 48, and 72 h after treatment with DMSO (control), CMP5 at IC_{50} (5.4 μ M), and CMP5 at IC_{70} (6.2 μ M). Wound closure was measured using ImageJ software. (B) Representative images of wound closure in MDA-MB-468 cells treated with DMSO and IC_{50} (5.7 μ M). (C) Quantification of wound closure in MDA-MB-231 cells at the indicated time points relative to control. (D) Quantification of wound closure in MDA-MB-468 cells at 0, 24, 48, and 72 h at the indicated time points relative to control. Data are presented as mean $\pm$ SD from three independent biological experiments ($n = 3$). Statistical analysis was conducted using the general linear model (univariate analysis of variance) in SPSS. ns, *** $p < 0.001$.

Wound closure in control (DMSO) cells progressed steadily, reaching nearly complete closure of around 90–100% after 72 h ($p < 0.001$). In contrast, CMP5 treatment was associated with a significant reduction in wound closure in a dose- and time-dependent manner. (Figure 4A,B) In MDA-MB-231 cells, scratch closure was reduced by ~40 to 50% after 72 h with IC₅₀ ($p = 0.001$) and was almost completely blocked by ~0 to 10% at IC₇₀ ($p = 0.001$). In the MDA-MB-468 model, wound closure was markedly reduced following CMP5 treatment, with an approximately ~50–60% reduction observed after 48 h at IC₅₀ ($p < 0.001$), and near-complete loss of viable cells was observed at the IC₇₀ concentration ($p = 0.0001$). The inhibited wound closure observed at later time points may reflect both impaired migratory behavior and increased cytotoxicity induced by CMP5 treatment.

These results indicate that CMP5 treatment reduces wound closure and migratory capacity in TNBC cells under the experimental conditions. These findings indicate that CMP5 treatment was associated with a marked reduction in TNBC cell migratory capacity in vitro. However, given the substantial reduction in cell viability observed at higher CMP5 concentrations and longer exposure periods, these effects may partially reflect treatment-associated cytotoxicity in addition to potential effects on cellular migration.

Subsequently, the migratory behavior of TNBC was investigated using transwell migration analysis after CMP5 treatment in a dose-dependent manner (Figure 5).

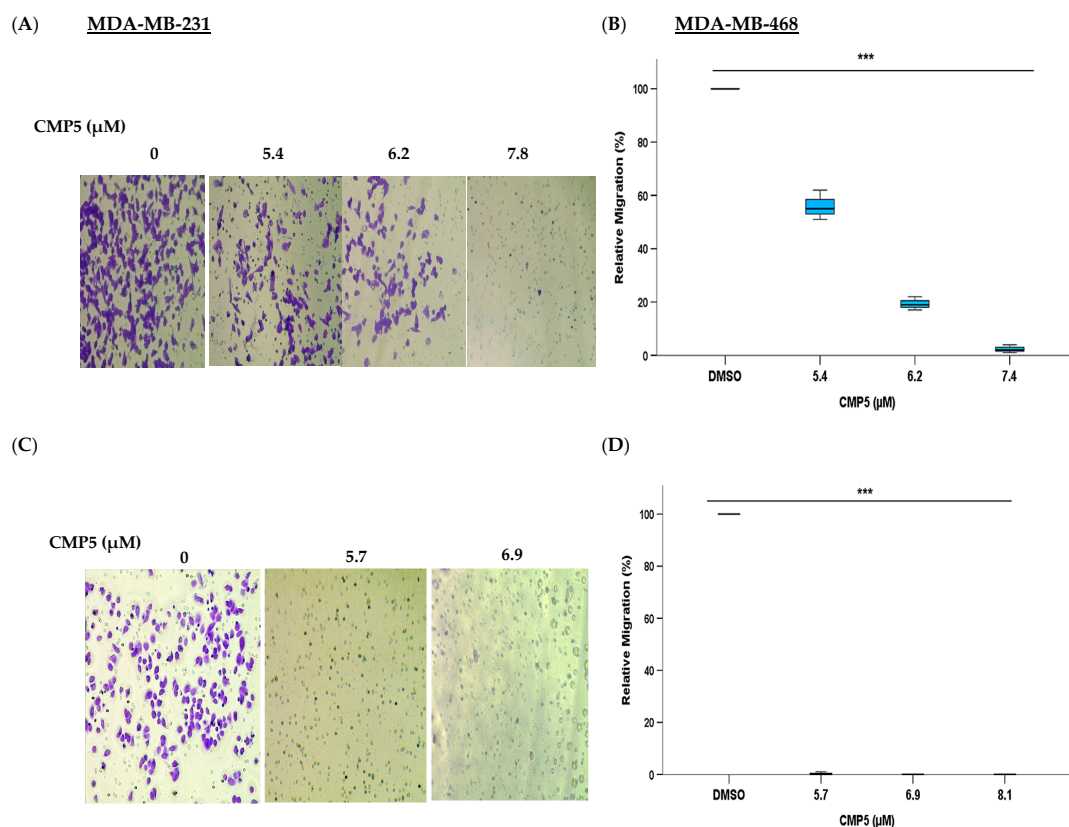


Figure 5. Transwell migration assay demonstrating reduced TNBC cell migration following PRMT5 inhibition. (A) Representative images of migrated MDA MB 231 cells following administration with DMSO control, CMP5 at IC₅₀, IC₇₀, and IC₈₀ after treatment with DMSO, CMP5 at 5.4 µM, 6.2 µM, or 7.8 µM. Migrated cells were fixed with 4% formaldehyde, stained with crystal violet, and visualised by EVOS microscopy. (B) Quantification of migrated MDA MB 231 cells expressed as a ratio relative to control using ImageJ analysis. (C) Representative images of migrated MDA MB 468 cells after treatment with DMSO, CMP5 at 5.7 µM, or 6.9 µM. Migrated cells were processed as described in (A). (D) Quantification of migrated MDA MB 468 cells, normalised to the control. Data are presented as mean ± SD from three independent biological experiments ($n = 3$). Statistical significance was assessed using one-way ANOVA followed by Tukey's post hoc multiple-comparison test. *** $p < 0.001$.

In MDA-MB-231 cells, migration was significantly decreased, achieving ~58% inhibition at 5.4 μ M, ~87% at 6.2 μ M, and almost total blockage (99%) at 7.8 μ M compared to control DMSO ($p = 0.001$) (Figure 5A,B). MDA-MB-468 cells showed a great response to PRMT5 inhibition, with >98% decrease at 5.7 μ M and >99% inhibition at 6.9 μ M ($p = 0.001$), indicating more sensitivity than in MDA-MB-231 (Figure 5C,D). Together, these findings indicate that CMP5 treatment was associated with reduced wound closure and transwell migration under the experimental conditions tested. However, because CMP5 also produced substantial reductions in cell viability, these observations should be interpreted cautiously and do not establish a direct anti-migratory effect of PRMT5 inhibition.

Although increased Caspase-3 and Caspase-10 expression was observed following CMP5 treatment, cleaved active caspase forms were not specifically evaluated in the current study. Thus, the apoptosis-related findings should be interpreted in combination with Annexin V/PI analysis and transcriptional alterations rather than as definitive biochemical confirmation of caspase activation.

3.5. PRMT5 Inhibition Triggers Programmed Cell Death and Alters Cell Cycle Transit in TNBC

Having shown that PRMT5 inhibition was associated with reduced TNBC cell proliferation and migration-related phenotypes under the experimental conditions tested, its impact on apoptosis signalling and cell-cycle progression was further investigated (Figure 6).

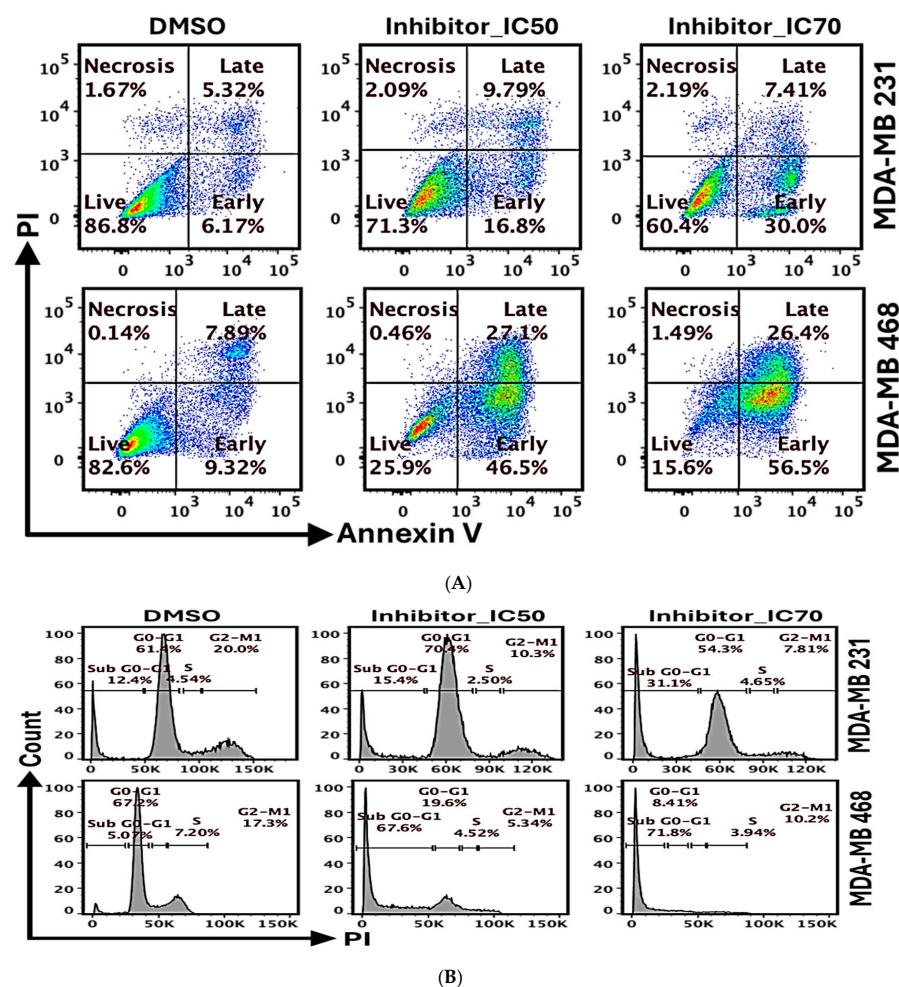


Figure 6. PRMT5 inhibition induces apoptosis and alters cell-cycle distribution in TNBC cultures, as assessed by flow cytometry. (A) MDA-MB-231 and MDA-MB-468 cells were treated with DMSO or CMP5 (IC₅₀ or IC₇₀) as identified. The quantity of dead cells was determined by FACS analysis after co-staining cells

with Annexin V/FITC and propidium iodide (PI). **(B)** A representative histogram of cell-cycle distribution was analysed by flow cytometry following propidium iodide (PI) staining. MDA-MB-231 and MDA-MB-468 cells were treated with DMSO (control), CMP5 at IC₅₀ (5.4 µM and 5.7 µM, respectively), or CMP5 at IC₇₀ (6.2 µM and 6.9 µM) for 48 h, and the cell cycle entry phase distribution was normalised to control (DMSO). Data are presented as mean ± SD from three independent biological experiments (*n* = 3). Apoptotic cell populations were quantified by Annexin V/PI flow cytometry, whereas cell-cycle distribution was determined by flow cytometry following propidium iodide (PI) staining. Statistical significance was assessed using one-way ANOVA followed by Tukey's post hoc multiple-comparison test.

Annexin V/PI FACS analysis revealed that PRMT5 inhibition by CMP5 significantly increased apoptosis in both TNBC cells in a dose-dependent way after 48 h. MDA-MB-231 DMSO-treated controls were largely viable (86.8%), with only 6.17% in early apoptosis and 5.32% in late apoptosis. Treatment with CMP5 at IC₅₀ (5.4 µM) reduced viability to 71.3% while increasing early and late apoptosis to 16.8% and 9.79%, respectively, and at IC₇₀ (6.2 µM), viability further declined to 60.4% with early apoptosis rising sharply to 30.0% (*p* = 0.0001) (Figure 6A). MDA-MB-468, a more substantial effect was observed: viability decreased from 82.6% in controls to 25.9% at IC₅₀ (5.7 µM) and 15.6% at IC₇₀ (6.9 µM), while early apoptosis increased from 9.32% to 46.5% and 56.5%, and late apoptosis rose from 7.89% to 27.1% and 26.4%, respectively (*p* = 0.0036). In both lines, necrosis remained low (<2%), indicating that programmed cell death was the primary mode of cell death (Figure 6A). Consistently, the cell cycle assay validates these results. In MDA-MB-231, the Sub-G0 apoptotic fraction increased from 12.4% in controls to 15.4% at IC₅₀ and 31.1% at IC₇₀, while CMP5 at IC₅₀ also induced G0 to G1 arrest (70.4%) (*p* = 0.0001) with reduced S and G2/M phases (Figure 6B).

In MDA-MB-468, however, blocking PRMT5 significantly increases cell death. The Sub-G0 level rises from 5.07% in controls to 71.8% at IC₅₀ and 67.6% at IC₇₀ (*p* = 0.016). This results in notable decreases in the G0/G1, S, and G2/M phases (Figure 6B, lower panel). Overall, these findings show that PRMT5 inhibition mainly triggers programmed cell death in the TNBC model; MDA-MB-231 cells initially experience G0/G1 arrest before apoptosis, while MDA-MB-468 cells quickly accumulate in Sub-G0, confirming apoptosis as the primary outcome of PRMT5 treatment.

4. Discussion

TNBC is still classified as a life-threatening tumor and a hard-to-treat subtype of breast cancer, characterised by rapid mitotic division, widespread metastasis, and limited targeted therapeutic intervention [1,3]. In recent years, several signalling and epigenetic effectors have emerged, including PARP, immune checkpoint inhibitors, and antibody-drug conjugates [4]. Clinically, improvements and near-recovery cases have been observed in some patients selected for study when these inhibitory drugs were used [4]. However, the chemotherapeutic regimen remains the preferred choice of treatment for many patients with TNBC [1–4]. This points out the urgent need for more efficient, minimal cytotoxic side effects targeted therapeutics [1–4].

CMP5 is a first-generation, cell-permeable, SAM-competitive, selective inhibitor of PRMT5/MEP50, thereby reducing histone marks such as H4R3me2s and H3R8me2s. It was created primarily for mechanistic and preclinical research rather than as a candidate for clinical use [5,20,22]. Moreover, it is frequently employed to study PRMT5 biology across various pathways, including Wnt/β-Catenin, ERK1/2 signaling, oncogenic transformation, hematologic malignancies, and immune cell regulation [14,20,23–27].

Moreover, CMP5 is highly potent in cultured cells, is used as a standard reagent to validate PRMT5-driven phenotypes, and clarifies downstream methylation-triggering networks in malignancies and immunology models [5,20,22]. A pharmacologic inhibitor of

PRMT5, combined with CMP5 in our research, reproducibly reprogrammed TNBC cells away from survival and migration towards apoptosis. These findings reinforce the concept that PRMT5 is more than an epigenetic modifier, positioning it as a key oncogenic regulator of transcription and signalling in TNBC.

At the molecular level, PRMT5 inhibition disrupted the key oncogenic regulators. Both transcript and protein analysis consistently revealed downregulation of Cyclin D1 and EZH2, two critical drivers of cell cycle progression and tumor growth. Simultaneously, the repressive E2F pathway regulator RBL2 was strongly induced, indicating a mechanistic disruption to the PRMT5-PRC2-RB/E2F oncogenic signalling pathway. Notably, this research has observed the induction of several apoptotic mediators: Caspase-3, Caspase-10, DAP1, and BAX were substantially upregulated, whereas BCL2, a classical anti-apoptotic factor, was significantly repressed. These findings provide additional evidence that PRMT5 normally functions to maintain cell survival and repress apoptotic signalling. Its inhibition shuts down this process, thereby allowing apoptosis to proceed. The result also showed that PRMT5 expression in TNBC was unaffected, indicating that the inhibition affects PRMT5 activity and suggesting an operational rather than a quantitative mechanism of action.

Furthermore, functional assays showed strong results, confirming a link with earlier observations regarding phenotype. Annexin V/PI staining indicated dose-dependent apoptosis in TNBC. In MDA-MB-231 cells, CMP5 reduced viability, accompanied by an increase in early apoptosis. In contrast, in MDA-MB-468, apoptosis was much more pronounced: nearly three-quarters of the population was apoptotic at the IC₅₀. This difference suggests potential subtype-specific vulnerabilities within TNBC. Cell cycle analysis confirmed these findings. In MDA-MB-231, CMP5 initially caused G0/G1 arrest, indicating early anti-proliferative effects, but at higher doses, it progressed to a Sub-G0 phase. While the flow cytometric analysis of Annexin V/PI and sub-G0 accumulation provided strong evidence for the induction of apoptosis by CMP5, no other complementary apoptosis assay, such as the detection of PARP cleavage and caspase activity analysis, was undertaken in this study. Further studies using these other methods will be required to provide validation of CMP5-induced apoptosis signaling. Conversely, MDA-MB-468 cells bypassed the intermediate arrest, undergoing direct and extensive Sub-G0 accumulation, which indicates rapid apoptotic commitment. Collectively, these results strongly suggest that apoptosis is the main fate of TNBC cells following PRMT5 inhibition, with MDA-MB-468 cells showing greater sensitivity than MDA-MB-231. In addition to affecting survival, CMP5 treatment was also found to result in reduced wound healing and transwell migration in both TNBC cell lines. Nevertheless, these results must be interpreted cautiously because marked decreases in cell viability have also been noted. Considering the lack of proliferation-independent controls like mitomycin C in these experiments, the current results cannot discriminate between direct inhibition of cell motility and indirect inhibition secondary to decreased cell viability/proliferation. Thus, the current results regarding migration are merely suggestive and hypothesis generating. In MDA-MB-231 cells, migration steadily decreased, nearly ceasing at IC₇₀, while the MDA-MB-468 model was more responsive, with migration almost completely inhibited even at IC₅₀.

Mechanistically, the observed effects of CMP5 treatment were accompanied by changes in the expression of apoptosis- and proliferation-related effectors, supporting a potential association between PRMT5 inhibition and attenuation of malignant TNBC phenotypes *in vitro*. The noticeable sensitivity of MDA-MB-468 cells to PRMT5 inhibition suggests that TNBC subtypes vary in their dependency on PRMT5 for aggressiveness and survival. Comparatively, MDA-MB-231 cells exhibited a modest apoptotic response, indicating resistance and highlighting the heterogeneity of PRMT5 dependency among TNBC subtypes *in vitro*.

Based on the current findings, PRMT5 plays a role in triggering proliferative and migratory phenotypes in TNBC cells. Inhibition of PRMT5 by CMP5 was associated with reduced cell viability, diminished migratory capacity, and induction of apoptosis in both TNBC cell lines. Nevertheless, additional mechanistic and *in vivo* studies are required to further support these results and interpret the functional role of PRMT5 in TNBC tumorigenesis.

5. Limitations of the Study

There are several limitations present in this study. First, the migration and wound healing studies were carried out at the IC₅₀ and IC₇₀ concentrations of CMP5, which could also be highly toxic for the cells, especially when the duration of exposure is long. Hence, the decrease in the ability to migrate and wound closure may also be due to the reduction in cell viability along with migration impairment. Furthermore, because proliferation-independent controls such as mitomycin C were not employed, the current study cannot conclusively separate direct effects on cell migration from indirect effects caused by reduced viability and proliferation. Future research should focus on conducting experiments using a lower concentration of CMP5 that would not be cytotoxic to the cells to separate its anti-malignant properties from other cytotoxic activities. Moreover, in the current research, the approach used was mainly pharmacological inhibition of PRMT5, and hence, genetic knockout would be necessary.

Even though CMP5 was previously known to act as an inhibitor of PRMT5 due to its capability of reducing PRMT5-mediated histone methylation signals, including H4R3me2s, H3R8me2s, and SDMA, such pharmacodynamic measurements were not conducted in this study. Moreover, no genetic methods to assess PRMT5's role were utilized. Consequently, care needs to be taken in interpreting such results as possible off-target effects may not be ruled out at higher concentrations of CMP5. Further investigations involving PRMT5 activity and genetic analysis will need to be conducted for a more definitive confirmation of PRMT5 dependency in TNBC cells.

6. Conclusions

PRMT5 inhibition tips the TNBC cells toward growth arrest and apoptotic cell death and was associated with reduced wound closure and transwell migration under the experimental conditions used. Further studies employing proliferation-independent migration assays will be required to determine whether PRMT5 directly regulates TNBC cell motility *in vitro*. CMP5 treatment led to downregulation of EZH2 and Cyclin D1 and an upregulation of RBL2 protein levels. The pro-apoptotic regulators Caspase-10, Caspase-3, DAP1, and BAX were upregulated, whereas the anti-apoptotic factor BCL2 was reduced. Notably, MDA-MB-468 cells were more susceptible to PRMT5 inhibition compared to MDA-MB-231 cells, supporting cell-line-dependent sensitivity to CMP5.

Based on the findings above, it can be concluded that PRMT5 enzyme activity may play a role in stimulating TNBC survival and migration; therefore, more mechanistic and *in vivo* research will be imperative to validate the clinical significance of targeting PRMT5 inhibition in breast cancer, especially when considering the combination of genetic knock-down approaches together with pharmacological inhibition, to determine whether PRMT5 inhibition is responsible for decreased viability, migration, EZH2, Cyclin D1 inhibition, and cell sensitivity differences.

From a future perspective, these findings point out the key role of PRMT5 inhibition in triggering apoptosis and reducing migration in TNBC. Several important questions still need answers. First, future studies should investigate how PRMT5 activity, chromatin remodeling, and EMT regulation are interconnected. This will reveal how PRMT5 regulates cellular processes to promote transitions between survival and metastasis. Second, *in vivo*

models using patient-derived or xenograft TNBC will be vital for assessing the therapeutic potential of CMP5. Evaluating CMP5's therapeutic value will be key in determining its approval, safety, and pharmacokinetic profiles. Third, owing to the biological complexity of TNBC, combination treatments that incorporate PRMT5 inhibition with chemotherapy, immune checkpoint blockade, or targeted epigenetic therapies such as EZH2 inhibitors may exhibit synergistic effects, improving clinical outcomes. Finally, the different apoptotic responses observed between the two TNBC cell lines highlight the need for further research into molecular subtype-specific vulnerabilities.

This could lead to biomarker-guided patient stratification in clinical trials. These conclusions indicate that PRMT5 inhibition could serve as a biomarker to facilitate epigenetic therapeutic avenues in TNBC, with potential application across multiple cancers characterized by PRMT5 overexpression.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/futurepharmacol6030040/s1>, Supplementary Materials and Methods.

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Conflicts of Interest: Fareed Ahmad is an employee of Hamad Medical Corporation. His employment did not influence the design, conduct, analysis, interpretation, or reporting of this study. Hamad Medical Corporation had no role in the study design, funding, data collection, data analysis, data interpretation, manuscript preparation, or the decision to publish the results. Fareed Ahmad contributed to the analysis of the apoptosis and cell cycle flow cytometry (FACS) data as a co-author. All other authors declare that they have no financial or non-financial conflicts of interest related to this work.

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