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Caffeine as a modulator of redox balance and migration in MDA-MB-231 triple-negative breast cancer cells

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ABSTRACT

Caffeine, a widely consumed stimulant, has demonstrated significant effects on cancer cell behavior, particularly in triple-negative breast cancer (TNBC) cells. This study investigates the impact of caffeine on MDA-MB-231 cells, focusing on cell morphology, viability, antioxidant gene expression, and cell migration. Caffeine at concentrations of 10 mM and above induced notable morphological changes, including cell rounding, detachment, and decreased cell density, indicative of cytotoxic effects and cellular stress responses such as apoptosis. Viability assays revealed a dose-dependent reduction in cell survival, with a substantial decline in total cell count at higher caffeine concentrations. The observed decrease in cell viability is associated with the downregulation of antioxidant genes *SOD2* and *GLO1*, suggesting disrupted redox balance and impaired detoxification systems. A significant positive correlation was noted between SOD2 and GLO1 expression levels, indicating their interdependence in antioxidant defense mechanisms. Additionally, caffeine treatment impaired cell migration, as the wound healing assay shows, with higher concentrations significantly hindering wound closure. This effect on migration, relevant for metastasis, aligns with findings from previous studies on caffeine's influence on cancer cell motility. The results suggest that 10 mM caffeine may serve as an optimal concentration for inducing cellular stress without immediate, widespread cell death, positioning it as a promising candidate for further therapeutic exploration.

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1. Introduction

Triple-negative breast cancer (TNBC) accounts for approximately 15-25% of all breast cancer cases worldwide and represents a particularly aggressive subtype (Almansour, 2022). In a study conducted at Hasan Sadikin Hospital in Indonesia from 2015 to 2019, 628 out of 4,050 breast cancer patients were diagnosed with TNBC, representing approximately 15.5% of the total cases and highlighting the prevalence and clinical significance of TNBC in the region (Gaol et al., 2023).

TNBC is an aggressive subtype of breast cancer that lacks the expression of estrogen receptors (ER), progesterone receptors (PR), and human epidermal growth factor receptor 2 (HER2). This unique molecular profile makes TNBC resistant to targeted therapies that are effective against other breast cancer subtypes, such as hormone receptor or HER2-positive cancers (Gaol et al., 2023). As a result, patients with TNBC face limited treatment options and often experience a poor prognosis, with higher rates of recurrence and metastasis (Kulkarni et al., 2020). This highlights the critical need for identifying novel therapeutic strategies that can effectively target the hallmarks of TNBC, including cell proliferation, survival, and migration. MDA-MB-231 cells are classified as a highly aggressive and invasive TNBC cell line. They also lack ER, PR, and HER2 amplification and exhibit features associated with epithelial-to-mesenchymal transition (EMT), which is crucial for understanding metastasis in TNBC (Huang et al., 2020).

A key challenge in developing such therapies is understanding the mechanisms driving TNBC progression. One important factor is the role of oxidative stress in cancer biology, particularly its impact on tumor development and metastasis (Singh and Manna, 2022). Cancer cells often exhibit high levels of reactive oxygen species (ROS), driven by both increased metabolic activity and distinct cellular respiration patterns. Although TNBC cells rely on glycolysis for energy production (the Warburg effect), they also maintain active mitochondrial respiration, which contributes significantly to ROS generation and affects the redox balance within cells (Pelicano et al., 2014). This unique metabolic reprogramming can promote tumor growth by inducing mutations and activating oncogenic signaling pathways (Aggarwal et al., 2019). However, excessive ROS can also damage cellular components and induce cell death. As a result, cancer cells, including TNBC, must maintain a delicate balance between ROS production and detoxification. This balance

is maintained, in part, by the expression of antioxidant genes that neutralize ROS and protect cells from oxidative damage (Nakamura and Takada, 2021).

Superoxide dismutase 2 (*SOD2*) and glyoxalase I (*GLO1*) have been known as important antioxidant genes. *SOD2* encodes a mitochondrial enzyme that converts superoxide radicals into less harmful molecules, thereby protecting cells from oxidative stress (Zheng et al., 2023). Additionally, *SOD2* is consistently elevated in TNBC and is associated with advanced tumor stages and poor clinical outcomes, making it a relevant target for understanding redox mechanisms in TNBC (Alateyah et al., 2022). *GLO1* is part of the glyoxalase system, which detoxifies methylglyoxal, a byproduct of glycolysis known to cause cellular damage if not properly regulated (He et al., 2020). Both *SOD2* and *GLO1* play critical roles in cellular redox homeostasis and are often dysregulated in cancer cells, where they contribute to tumor survival and resistance to therapy (Morgenstern et al., 2020).

Given the importance of redox balance in cancer cell survival, targeting these antioxidant pathways has become an area of interest in cancer research. Recent studies have identified various organic compounds, including caffeine, as potential anticancer agents due to their ability to influence redox homeostasis and other molecular mechanisms. Caffeine, a widely consumed natural alkaloid, has been shown to significantly reduce melanoma cell viability and migration without affecting non-tumor cells, while also increasing ROS levels in melanoma cells (Manica et al., 2024). In breast cancer, caffeine has been reported to inhibit cell growth and induce cell cycle arrest, particularly in more aggressive subtypes such as TNBC (Rosendahl et al., 2015). Despite this, the full spectrum of caffeine's effects on cancer cells is still being explored, particularly regarding its impact on molecular pathways related to oxidative stress and cellular defense mechanisms.

Although caffeine is known for its anti-cancer effects and the importance of *SOD2* and *GLO1* in maintaining redox balance, there is a significant gap in the literature regarding how caffeine influences the expression of these specific antioxidant genes in TNBC cells. To date, no studies have explored whether caffeine can modulate the expression of *SOD2* and *GLO1*, potentially altering the oxidative stress response in TNBC. This gap presents a unique opportunity to investigate the role of caffeine in regulating antioxidant defenses in aggressive cancer subtypes such as TNBC.

In this study, we hypothesize that caffeine treatment could disrupt the redox balance in MDA-MB-231 cells by altering the expression of key antioxidant genes. Specifically, we aim to investigate the effects of caffeine on cell morphology, viability, and the expression of *SOD2* and *GLO1* in MDA-MB-231 cells. Furthermore, we explore caffeine's impact on cell migration to better understand its role in inhibiting metastatic potential. By addressing these questions, our research seeks to provide novel insights into caffeine's mechanism of action in TNBC and to evaluate its potential as a therapeutic agent targeting oxidative stress pathways.

2. Materials and methods

2.1. Materials

Human Caucasian breast cancer MDA-MB-231 cells were obtained from the Integrated Laboratory, Faculty of Medicine, Universitas Indonesia. Caffeine powder (Cat No. C0750), fetal bovine serum (FBS) (Cat No. F2442), and Dulbecco's Modified Eagle's Medium (Cat No. d6429) were purchased from Sigma-Aldrich (Darmstadt, Germany). Amphotericin B, 250 μg/ml (Cat No. P06-01050), 10.000 U/ml Penicillin, 10 mg/ml Streptomycin (Cat No. P06-07050), Trypsin 0.05%/ EDTA 0.02% (Cat No. P10-023100), β-Mercaptoethanol (Cat No. P07-05100) were purchased from PAN-Biotech (Aidenbach, Germany). Total RNA Mini Kit (Blood/Cultured Cell) (Cat No. RB0100) was purchased from Geneaid (Taiwan). Phosphate buffered saline (PBS) (Cat No. 10010023) and trypan blue solution, 0.4% (Cat No. 15250061) were purchased from Gibco (New York, USA). ReverTra Ace™ qPCR RT Master Mix with gDNA Remover (Cat No. FSQ-301) and KOD SYBR® qPCR Mix (Cat No. QKD-201) were purchased from TOYOBO (Osaka, Japan).

2.2. Methods

2.2.1. Cell culture

MDA-MB-231 cells were cultured and maintained in a 5% CO_2 incubator at 37°C using a complete medium consisting of Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% FBS, penicillin-streptomycin, and amphotericin B. Cell morphology was observed using an inverted light microscope (Nikon Eclipse TS100) at 20X magnification. The medium was replaced 2-3 times per week until the cells reached 70-80% confluence, after which they were prepared for harvesting (Invitrogen, 2020).

2.2.2. Viability assay

MDA-MB-231 cells were seeded in 6-well plates at a density of 1×10^5 cells/well in 2 mL of complete medium. The cells were incubated for 24 hours to allow for attachment and stabilization. After the incubation, the medium was replaced with a fresh complete medium containing various concentrations of caffeine (5, 10, 15, and 20 mM). Caffeine was dissolved in sterile double-distilled water (ddH_2O) and diluted with a culture medium to achieve the desired concentrations. Control wells were treated with a caffeine-free complete medium. The cells were then incubated for 24 hours at 37°C in 5% CO_2. After caffeine treatment, the cells were harvested by trypsinization, and cell suspensions were prepared in 1 mL of complete medium. Trypan Blue dye (0.4%) was added to an aliquot of the cell suspension at a 1:1 ratio, and the mixture was gently pipetted to ensure even distribution of the dye. Live cells (unstained) and dead cells (blue-stained) were counted using a haemocytometer under an inverted microscope (Strober, 2015). The average percentage of viable cells was calculated for each treatment group using the following formula:

$$\text{Cell Viability (\%)} = \frac{\text{Number of viable (unstained) cells}}{\text{Total number of cells}} \text{ x } 100 \quad (1)$$

2.2.3. RNA isolation and quantification

Cells were treated with caffeine as described before and were incubated for 24 hours. Following caffeine treatment, total RNA was isolated using the Total RNA Mini Kit (Blood/Cultured Cell) (Cat No. RB0100) according to the manufacturer's protocol. Cells were lysed directly in the wells using 400 μL of RB buffer, and the lysates were transferred to RNA spin columns. The columns were washed with wash buffer and eluted with RNase-free water to obtain purified RNA. RNA concentration and purity were measured using a NanoDrop spectrophotometer by evaluating absorbance at 260 nm and the 260/280 ratio. To ensure uniform cDNA synthesis across all samples, RNA concentrations were normalized by diluting all RNA samples to the same concentration with RNase-free water prior to reverse transcription.

2.2.4. cDNA synthesis

cDNA synthesis was performed using the ReverTra Ace™ qPCR RT Master Mix with gDNA Remover (Cat No. FSQ-301, TOYOBO) according to the manufacturer's instructions. RNA (1 μg from each sample) was first treated with gDNA Remover to eliminate genomic DNA. The reverse transcription reaction was carried out in a 20 μL total reaction volume containing 4 μL of 5x RT Master Mix, 1 μg of RNA, and RNase-free water. The reaction was incubated at 37°C for

15 minutes, followed by 98°C for 5 minutes to inactivate the enzyme. The cDNA was used directly as a template for qPCR.

2.2.5. qPCR amplification

qPCR was performed using the KOD SYBR® qPCR Mix (Cat No. QKD-201, TOYOBO) to assess the expression of *SOD2* and *GLO1*. cDNA generated from 1 μg of total RNA was used as the template for amplification. Each qPCR reaction was prepared in a 20 μL volume containing 10 μL of KOD SYBR® qPCR Mix, 0.3 μM of each primer for *SOD2* (forward: 5'-CTGGAACCTCACATCAACGC-3'; reverse: 5'-GCTGTAACATCTCCCTTGGC-3'); *GLO1* (forward: 5'-CCCCAGTACCAAGGA TTTTC-3'; reverse: 5'-TCATAAGCCAAGAAG TAGAGTGAA-3'); and *ACTB* (forward: 5'-TGACGTGGACATCCGCAAAG-3'; reverse: 5'-CTGGAAGGTGGACAGCGAGG-3'), 1 μL of cDNA, and RNase-free water to adjust the volume. The reactions were run on a real-time PCR system with cycling conditions as follows: Initial denaturation: 95°C for 2 minutes; followed by 40 cycles of Amplification (Denaturation: 95°C for 10 seconds, Annealing: 60°C for 10 seconds, and Extension: 68°C for 30 seconds). A melting curve analysis confirmed the specificity of the amplification. The relative expression levels of *SOD2* and *GLO1* were calculated using the $2^{(-\Delta\Delta Ct)}$ method, with *ACTB* serve as the housekeeping gene.

2.2.6. Wound healing assay

MDA-MB-231 cells were seeded into a 12-well plate at a density of 3×10^5 cells per well. Cells were cultured in a complete growth medium and allowed to reach confluence (approximately ~90%). After 24 hours, a scratch was made in the monolayer using a sterile 200 μL pipette tip. The scratch was made across the center of each well to ensure uniformity. The wells were washed twice with PBS to remove detached cells and debris (Manica et al., 2023). Cells were then treated with caffeine. After that, cell migration was observed using an inverted microscope (CKX53, Olympus) at 4X magnification, capturing images at 0 and 72 hours. Image analysis was performed using the ImageJ software to measure the gap area. The percentage of wound closure was calculated by the following formula (Zhu et al., 2024):

$$\text{Wound closure percentage (\%)} = \frac{\text{Initial Gap Area} - \text{Final Gap Area}}{\text{Initial Gap Area}} \text{ x } 10 \qquad (2)$$

2.2.7. Data analysis

Statistical analysis was performed using IBM SPSS Statistics 23 and GraphPad Prism 10. The viability and gene expression assays were performed with three replicates to ensure reproducibility. Data were expressed as mean ± SEM. One-way ANOVA followed by Tukey's post hoc test was employed to assess the significance of differences between treatments and the control. Spearman correlation analysis was performed to evaluate the relationship between *SOD2* and *GLO1* expression levels. Statistical significance was considered at $p<0.05$.

3. Results and discussion

3.1. Caffeine induces morphological changes

The effects of caffeine on the morphology of MDA-MB-231 cells were evaluated at increasing concentrations (0, 5, 10, 15, and 20 mM) and at two-time points (0 hours and 24 hours post-treatment) using an inverted microscope (Fig. 1). After 24 hours of treatment, the effects of caffeine became more pronounced (Fig. 1A'–1E'). The control group maintained a healthy and proliferative monolayer, with no significant changes in cell shape. At 5 mM caffeine, a slight reduction in cell density was observed, with some cells showing rounding and detachment. At 10 mM caffeine, the reduction in cell density was substantial, with many cells appearing rounded and detached from the surface. Treatment with 15 mM caffeine resulted in drastic cell loss, with most cells appearing rounded and detached from the surface. Finally, at the highest concentration (20 mM), almost all cells had detached, and the few remaining displayed significant morphological damage, suggesting extensive cell death (Bode and Dong, 2007).

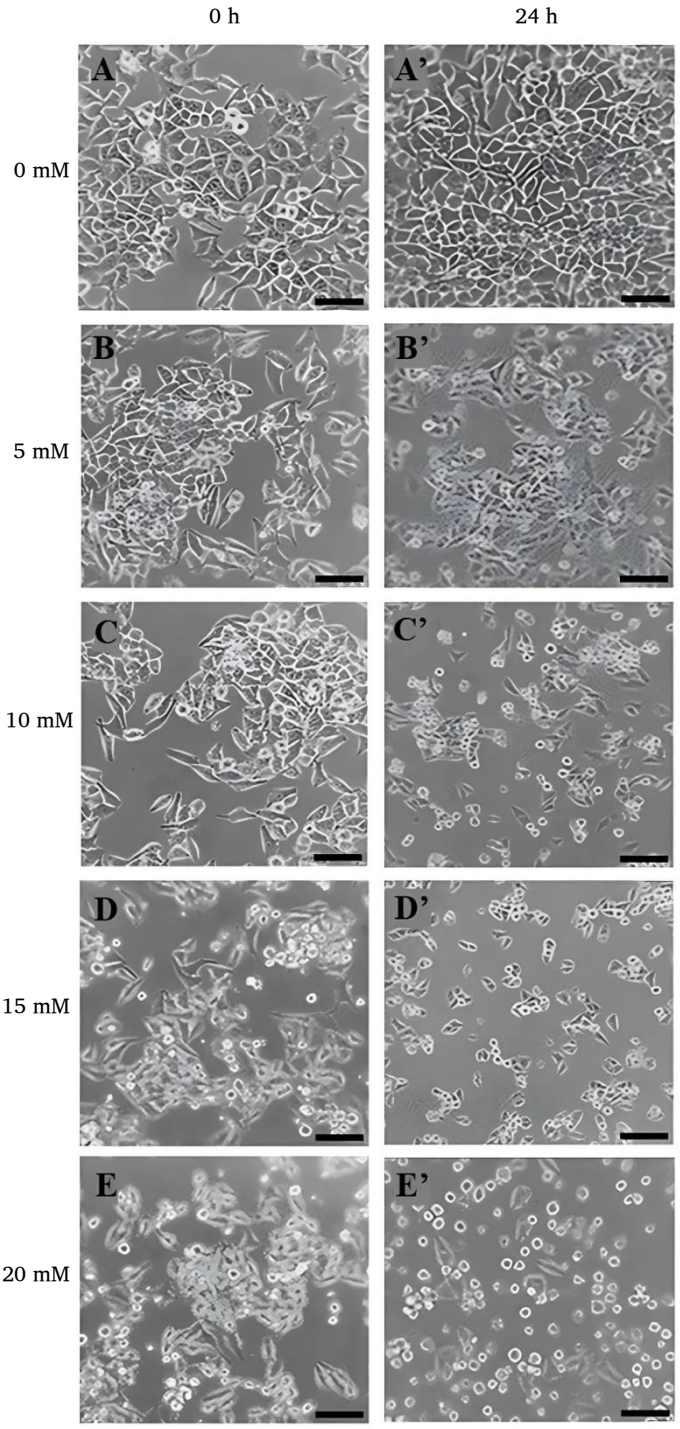

Fig. 1. Morphological changes of MDA-MB-231 cells treated with increasing concentrations of caffeine (0, 5, 10, 15, and 20 mM) at two-time points: A-E) represent images taken at 0 hours, while A'–E') represent images taken 24 hours post-treatment. Images were captured using an inverted microscope at 20X magnification. Scale bar = 25 μm.

These findings demonstrate that caffeine induces notable morphological changes in MDA-MB-231 cells, particularly at higher concentrations after 24 hours of incubation. Concentrations of 10

mM and above resulted in significant cell rounding, detachment, and reduced cell density, suggesting that caffeine exerts cytotoxic effects, likely triggering cellular stress responses such as apoptosis or necrosis. These observations are consistent with previous studies, which have reported that caffeine can induce cellular stress responses, including cell rounding and detachment due to cytotoxicity (Bode and Dong, 2007). Importantly, immediate caffeine exposure did not cause significant morphological changes, as cells across all concentrations maintained their normal structure at 0 hours post-treatment (Manica et al., 2024).

3.2. Caffeine decreases the viability of MDA-MB-231 cells

To evaluate the dose-dependent effects of caffeine on cell viability, MDA-MB-231 cells were assessed using a trypan blue exclusion assay after 24 hours of treatment with increasing concentrations of caffeine (Fig. 2). Cell viability remained nearly 100% in the control group, with approximately 1.5 million viable cells/mL. At 5 mM caffeine, cell viability decreased slightly to about 90%, though this reduction was not statistically significant (ns). A more pronounced decline in cell viability was observed at 10 mM caffeine, where viability decreased to around 60% ($p<0.01$). Higher concentrations (15 and 20 mM) led to further reductions, with viability decreasing to approximately 50% ($p<0.001$ for 15 mM and $p<0.0001$ for 20 mM). Despite >50% viability at 15 and 20 mM, this percentage applies only to a much smaller population of surviving cells, as the total cell count decreased from 1.5 million to around 250,000 cells/mL, indicating that most cells were either detached or rendered non-viable.

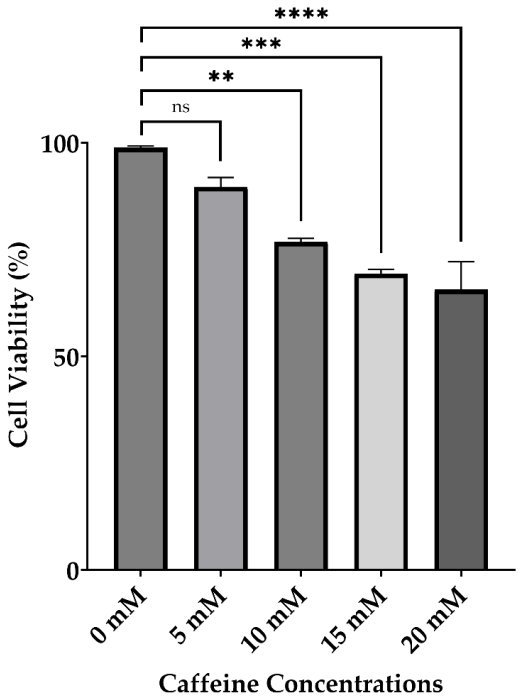

Fig. 2. Effect of caffeine on the viability of MDA-MB-231 cells after 24-hour treatment with increasing caffeine concentrations (0, 5, 10, 15, and 20 mM). A dose-dependent decrease in cell viability was observed, with statistically significant differences indicated as (ns) = not significant, $^{**}p<0.01$, $^{***}p<0.001$, and $^{****}p<0.0001$. Error bars represent the mean ± standard error of the mean (SEM) from three independent replicates.

This dose-dependent reduction in viability corresponds to well-known mechanisms of caffeine-induced cytotoxicity. Caffeine specifically targets deregulated oncogenic pathways in cancer cells, such as the p53 and PI3K/Akt pathways, leading to apoptosis, cell cycle arrest, and oxidative stress (Rodak et al., 2021). Interestingly, caffeine induces apoptosis in cancer cells while generally sparing normal cells. In normal cells, caffeine can induce G0/G1 phase arrest by inhibiting the cyclin D1-cdk4 complex, slowing down their proliferation without causing cell death (Meisaprow et al., 2021). This selective cytotoxicity underscores caffeine's potential as an adjunctive cancer therapy, reducing the viability of cancer cells while remaining relatively safe for normal cells at controlled doses.

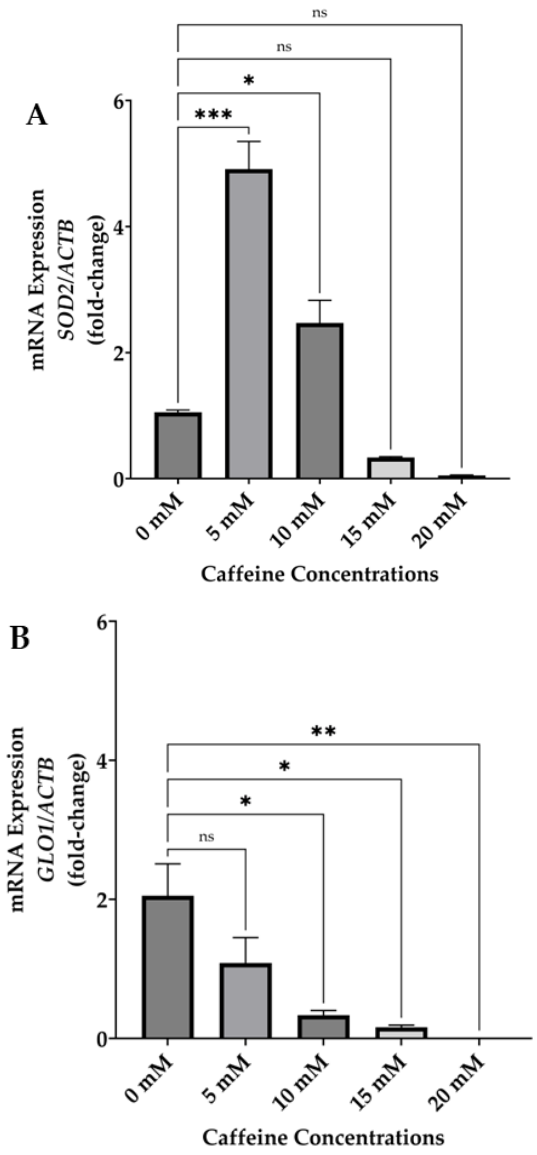

Fig. 3. Effect of caffeine on *SOD2* and *GLO1* gene expression in MDA-MB-231 cells. (A) *SOD2* expression significantly increased at 5 mM ($p<0.001$) but decreased at 10 mM ($p<0.05$) and returned to near baseline levels at higher concentrations (15 and 20 mM). (B) *GLO1* expression remained stable at 5 mM but significantly decreased at 10 mM ($p<0.05$) and further decreased at 15 and 20 mM ($p<0.01$). Spearman's correlation analysis between *SOD2* and *GLO1* expression revealed a significant positive correlation ($\rho=0.705$, $p=0.023$). Data are presented as mean ± SEM, n = 3, $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$, ns = not significant.

3.3. Downregulation of *SOD2* and *GLO1* after caffeine treatment

To investigate the impact of caffeine on the expression of key antioxidant genes, qPCR was used to measure *SOD2* and *GLO1* expression in MDA-MB-231 cells following treatment. As shown in Fig. 3A, *SOD2* expression exhibited a significant dose-dependent effect. At 5 mM caffeine, *SOD2* levels increased approximately 5-fold relative to the control ($p<0.001$), potentially reflecting a compensatory response to moderate oxidative stress. However, at 10 mM, *SOD2* expression significantly decreased ($p<0.05$), though it remained about 2.5-fold higher than in the control. Beyond 10 mM, *SOD2* levels decreased sharply, returning to near-baseline at

15 and 20 mM, indicating a loss of cellular response at higher caffeine concentrations.

Interestingly, *SOD2* expression showed a biphasic pattern, initially spiking at 5 mM before dramatically decreasing at higher concentrations (10-20 mM). This early increase in *SOD2* at 5 mM likely represents a protective response to moderate oxidative stress, where upregulated *SOD2* helps convert superoxide radicals into hydrogen peroxide, helping to manage oxidative damage at tolerable levels. However, as caffeine concentrations rise, *SOD2* levels drop, likely due to excessive ROS production that surpasses the cellular capacity for antioxidant defense, leading to severe oxidative stress and possible cellular damage. This biphasic response underscores *SOD2*'s dual role in cancer cells, functioning both as a tumor suppressor by reducing ROS and as an oncogene by enabling cell survival and proliferation under controlled ROS levels (Kim et al., 2017). In TNBC, *SOD2*'s oncogenic function is linked to high metabolic demands and oxidative stress management, supporting aggressive traits such as migration and proliferation, as seen in metastatic lesions and advanced tumor lines. The observed reduction in *SOD2* expression with high caffeine doses may weaken TNBC cells' ability to balance ROS, resulting in reduced viability and migration (Juliachs et al., 2022)

Similarly, *GLO1* expression followed a progressive decline with increasing caffeine concentration (Fig. 3B). At 10 mM, *GLO1* expression showed a marked reduction ($p<0.05$), and by 20 mM, it reached nearly undetectable levels ($p<0.01$). This suggests that caffeine impairs glyoxalase detoxification mechanisms, contributing to oxidative stress and cellular dysfunction (Tiwari et al., 2014). Additionally, the downregulation of *GLO1*, concurrent with the spike in *SOD2* expression at 5 mM caffeine, suggests a metabolic shift from glycolysis toward mitochondrial ROS management, with cells prioritizing oxidative stress defenses over managing glycolytic byproducts (Fonseca-Sánchez et al., 2012).

TNBC cells often rely on both glycolysis and mitochondrial respiration to meet high metabolic demands. While glycolysis predominates (producing less ROS), mitochondrial respiration remains active and contributes significantly to ROS levels. Reduced *SOD2* levels may lead to mitochondrial superoxide accumulation, increasing ROS and oxidative stress. This disrupts the ROS balance needed by TNBC cells to sustain their aggressive behavior. Lower *GLO1* levels further add to oxidative stress by allowing reactive carbonyl species to accumulate. Downregulating *SOD2* and *GLO1* likely heightens susceptibility to oxidative stress, potentially explaining the dose-dependent decrease in cell viability due to insufficient antioxidant defenses (Pelicano et al., 2014). A concentration of 10 mM appears to be a threshold where antioxidant defenses are impaired, yet the cell's response mechanisms remain active, resulting in significant cytotoxicity without complete loss of gene expression (Kaczmarczyk-Sedlak et al., 2019).

The positive correlation between *SOD2* and *GLO1* across tested concentrations ($\rho=0.705$, $p=0.023$) suggests these genes may be co-regulated or affected by similar mechanisms under caffeine-induced oxidative stress, even with the opposing trends at 5 mM. Both genes are involved in neutralizing oxidative stress, and their coordinated downregulation at higher caffeine doses suggests a disruption in redox balance (Du et al., 2019). The overall reduction in both *SOD2* and *GLO1* expression highlights caffeine's cytotoxic effect in TNBC cells by impairing both superoxide detoxification (via *SOD2*) and glyoxalase pathway clearance (via *GLO1*), further enhancing susceptibility to oxidative stress and reducing cellular viability and migration (Donato et al., 2020; Syed et al., 2023).

3.4. Caffeine has an inhibitory effect on cell migration

To investigate how caffeine affects metastasis, we performed a wound-healing assay to assess its inhibitory effects on MDA-MB-231 cell migration. As illustrated in Fig. 4, caffeine treatment inhibited cell migration in a dose-dependent manner. In the control group (0 mM), the scratch wound nearly closed after 72 hours, reflecting active migration. In contrast, with increasing caffeine concentrations (5 mM to 20 mM), the wound closure rate decreased significantly, particularly at concentrations of 10 mM and higher, where the scratch retained a large gap even after 72 hours.

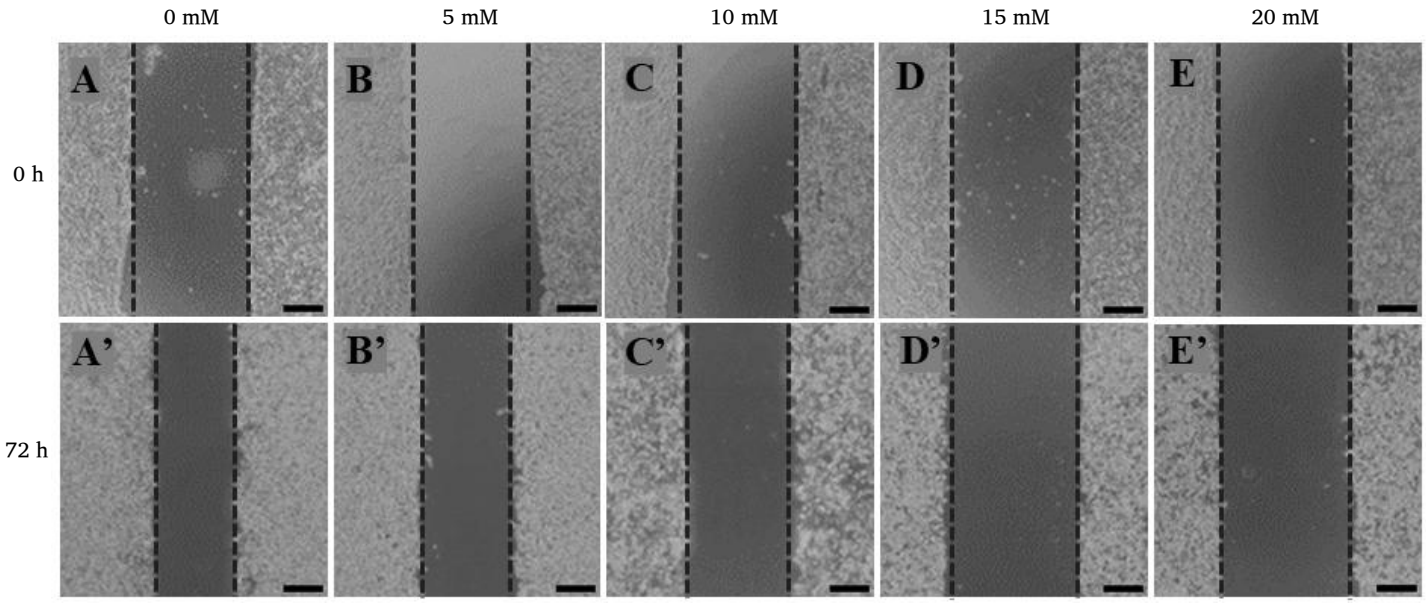

Fig. 4. Scratch wound healing assay of MDA-MB-231 cells treated with varying concentrations of caffeine (0, 5, 10, 15, and 20 mM) at two-time points: A–E represent the initial scratch at 0 hours, showing uniform wound sizes across all treatment groups. A'–E' display the same scratches 72 hours post-treatment, revealing a dose-dependent inhibition of wound closure as caffeine concentration increases. At 10 mM and above, wound closure is significantly impaired, indicating reduced cell migration. Images were taken using an inverted microscope at 4X magnification. Scale bar: 100 μm.

At 0 hours, the initial gap length was consistent across all treatment groups, averaging around 310 μm, confirming uniform starting conditions (Fig. 4A-4E). However, after 72 hours, untreated cells exhibited substantial migration, reducing the gap by 34%. As

the caffeine concentration increased, wound closure gradually decreased. Specifically, at 5 mM, the gap closed by 28%, while at 10 mM, only 7% closure was observed. Furthermore, at 15 and 20 mM, there was no significant closure (Fig. 4A'-4E'). A high caffeine concentration was chosen in this study to evaluate its impact on cellular migration specifically under cytotoxic stress conditions relevant to aggressive cancer cells. This concentration was selected to reveal potential anti-migratory effects that may not be observable at lower doses. In vitro studies have demonstrated that caffeine at concentrations between 0.4 mM and 20 mM can inhibit the proliferation, migration, and invasion of various hepatocellular carcinoma cell lines (Okano et al., 2008; Shan et al., 2024). This inhibitory effect on cell migration suggests that caffeine impairs the migratory capacity of triple-negative breast cancer cells, possibly due to its modulation of the redox balance (Ojeh et al., 2016).

This impaired migratory capacity is particularly concerning in the context of metastasis, a key issue in TNBC. This finding is consistent with previous studies that demonstrated caffeine's ability to inhibit the migration of NCI-H23 cells, emphasizing its role in disrupting a key mechanism involved in lung cancer metastasis (Manica et al., 2024). Moreover, caffeine's impact on redox balance may also play a significant role in its ability to inhibit migration. By influencing oxidative stress levels within cells, caffeine can alter signaling pathways that govern cell motility. This effect is especially relevant in cancer cells, where redox status is often dysregulated (Chen et al., 2014). Consequently, by reducing the ability of cancer cells to migrate, caffeine could potentially slow or prevent the spread of cancer to other parts of the body (Ojeh et al., 2016). In summary, caffeine's ability to impair cell migration highlights its potential as a therapeutic strategy in combating metastasis in TNBC. Continued exploration of caffeine's mechanisms of action could pave the way for novel treatment approaches that leverage its effects on redox balance and cellular dynamics.

4. Conclusion

Our study revealed that caffeine exerts significant dose-dependent effects on MDA-MB-231 cells, influencing cell morphology, viability, gene expression, and migration. At concentrations of 10 mM and higher, caffeine induces notable changes in cell rounding, detachment, and reduced density, indicating cytotoxic effects and cellular stress responses. The reduction in cell viability, along with alterations in *SOD2* and *GLO1* expression, underscores caffeine's potential to disrupt redox balance and cellular functions. Moreover, the inhibition of cell migration highlights caffeine's relevance in metastasis. These findings suggest that 10 mM caffeine may represent an optimal concentration for inducing cellular stress without causing extensive cell death, making it a promising candidate for further exploration as a therapeutic agent. To fully understand its potential, future studies should examine the comprehensive effects of caffeine on apoptosis pathways.

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Conflict of interest

The authors declare no conflict of interest in this research.

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