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# MCF-7 Dense Breast Cancer Cells Modulate Stress through Senescence

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## Abstract

Breast cancer remains a leading cause of cancer-related mortality among women worldwide, with dense breast tissue being recognized as a significant risk factor for its development. This dense breast tissue, characterized by a higher concentration of mammary glandular tissue, represents additional sites for tumor development. The following study explores how the MCF-7 immortal cell line, representing dense breast cancer, modulates stress responses through cellular senescence. Cellular senescence is a defense mechanism that halts the cell cycle and acts as a tumor suppression mechanism, preventing out-of-control cell proliferation triggered by cytological stress. To simulate these stress conditions, we applied two agents: carboplatin (CBDCA), a platinoid chemotherapeutic medicine that alters the structure of DNA and has downstream effects on a cell's ability to replicate said DNA; and hydrogen peroxide ($H_2O_2$), a common cytological stress indicator which itself can also act as a stressor under high enough concentration and exposure times. This study measured cell proliferation and activity, cytological stress responses, and the senescence response of MCF-7 and Br(EPI) cell lines to the introduced stressors. The results demonstrated that MCF-7 cells exhibited vastly increased cytological stress resistance and proliferation than non-tumorigenic cells. When exposed to CBDCA, the MCF-7 cell lines did not undergo apoptosis, characterized by Caspase-3 activation. Instead, they entered a state of cellular senescence, allowing the cells to adapt and continue proliferating. These findings highlighted critical cellular mechanisms in dense breast cancer, suggesting potential targets for future research. Furthermore, the study underscores the role of cellular senescence in the promotion of metastasis of breast cancer by making the tumor environment more favorable to angiogenesis, immune evasion, and tumor progression. Further research into the specific hormonal, physiological, and cytological changes in dense breast tumor environments may present novel, personalized treatment methods to improve patient outcomes.

**Keywords**

Breast Cancer, Oncology, Cytotoxicity, Chemotherapy, Senescence, Dense Breast Tissue

## 1. Introduction

The Hallmarks of Cancer are a fundamental component of cancer research as they guide researchers on the important aspects of cancer and how these qualities make up a cancer cell [1]. The eleven hallmarks of cancer can demonstrate changes in the cellular behavior of a cancer cell and how a cancer cell can grow uncontrollably in its local tissues and possibly metastasize throughout the body. The eleven hallmarks of cancer include proliferation signaling, evading growth suppressors, resisting cell death (apoptosis), replication of cells, inducing angiogenesis, invasion of healthy tissues and metastasis, energy metabolism reprogramming, and destroying the immune system [1]. These eleven hallmarks of cancer are a fundamental outline for cancer research and how cancer research is conducted throughout various studies on various types of cancer, including the research in this study.

Additionally, recent findings have shown that certain forms of breast cancer, namely triple-negative phenotypes, utilize unique stromal subtypes and their associated proteins to further evade and suppress the immune system, leading to greater malignancy [2]. These systems specifically reduce the number of cytotoxic T-cells in areas where these breast neoplasms have propagated. The most abundant of these stromal components are cancer-associated fibroblasts [3]. These particular cells secrete growth factors and nutrients by way of exosomes and physically alter the extracellular matrix to better suit the needs of the tumor site. This manifests in larger, more rigid, and well-protected tumor masses that are more resistant to the physical entry of drug therapies and immune cells alike. This also forms an isolated body in which tumor cells are able to interact with cytokines to improve their overall malignancy.

The increased density in breast tissue, characterized by higher amounts of glandular tissue, provides more potential sites for tumor development, thereby elevating cancer risk. The risk is approximately a one-to-sixfold increase compared to patients with lower-density breast tissue [4]. The risk of female athletic patients, when compared to the standard female population for breast tumor severity, remains to be seen. Several studies have explored the effects of exercise on patients already diagnosed with breast cancer [5], but an important gap in this knowledge is the impact of endurance exercise on the tumor environment, possibly increasing tumorigenesis by way of raising cytological stress compounds.

To this end, this study aims to stimulate the female athlete under stress by introducing $H_2O_2$ to cells in culture. These cells were examined for their senescence response, particularly in the MCF-7 model cancer cell line compared to a control cell line. These senescent cancer cells were examined for their effects on

their tumor environment. These findings can act as a guide for future research [6].

In this study, the stressors applied were the chemotherapeutic agent carboplatin (CBDCA) and hydrogen peroxide ($H_2O_2$) (manufactured by Sigma-Aldrich). $H_2O_2$ is a common cytological stress indicator alongside nitrite ($NO_2^-$), an oxidized nitric oxide product. $H_2O_2$ was chosen because of its ubiquity as a reactive oxygen species. As a reactive oxygen species, $H_2O_2$ has various downstream effects on overall homeostasis that can cause neurodegeneration [7]. These negative effects even include a worsening of cancer prognosis [8]. CBDCA, a potent chemotherapy agent related to cisplatin, modifies DNA structure and affects DNA synthesis, influencing various cell cycle phases. The assays used in this study were cell proliferation, cell activity, cytological stress levels, and the response to CBDCA. MCF-7 is a commonly used immortal breast cancer cell line first isolated via pleural effusion in 1973. These cells are estrogen receptor-positive (ER+), which historically is a more difficult-to-treat breast cancer phenotype [6]. This is due to estrogen acting as a signaling molecule, promoting the advancement of a cell from its growth (G) phase to its synthesis (S) phase. This causes the cells to reach a mitotic state faster than their ER- counterparts [9]. Br(EPI) cells acted as the control group for this study and represented normal breast cells occurring in the ducts and lobules of the mammary glands [10].

The results of this study demonstrate that MCF-7 cells, representative of dense breast cancer, exhibit increased resilience to cytological stress while proliferating at a significantly higher rate than non-tumorigenic cell lines. Moreover, when exposed to CBDCA, MCF-7 cells did not undergo Caspase 3-induced apoptosis, continuing to proliferate compared to the control group [11]. Instead of apoptotic cell death, these cells entered a state of cellular senescence. These findings reveal critical pathways for further investigation into the treatment and management of dense breast cancer, highlighting potential therapeutic targets. Understanding the hormonal, physiological, and cellular changes in this specific cell population may inform more tailored screening and preventive strategies. Additionally, our study underscores the role of senescence in facilitating metastasis in dense breast cancer by altering the tumor microenvironment, promoting immune evasion, enhancing angiogenesis, and driving tumor progression [12].

## 2. Materials and Methods

### 2.1. Cell Stressors

Hydrogen Peroxide ($H_2O_2$) is a common cytological stressor compound. It was chosen to simulate the possible conditions of prolonged endurance exercise. 10–100 μM of $H_2O_2$ for 72 hours was used to mimic physiological oxidative stress/chronic low-level oxidative damage. Carboplatin (CBDCA) is a common platinoid chemotherapeutic agent used to treat dense breast cancers. It is related to the similar agent cisplatin [13]. All reagents were purchased from Millipore Sigma (Rockville, MD, USA).

### 2.2. Analysis Software

In this study, ImageJ software was used to quantify cellular senescence, cell viability, and proliferation. For senescence detection, stained cells were imaged and analyzed to count beta-galactosidase-positive cells. Additionally, ImageJ's cell counter and intensity analysis tools were employed to quantify cell area, and fluorescence intensity, and to analyze assay images for consistent, reproducible measurements across treatment groups.

### 2.3. Cell Culture

The cell lines used in this study were MCF-7 and Br(EPI) cells. The MCF-7 cell line is an immortal cancerous cell line commonly used in breast cancer studies, and acted as our experimental cells. Br(EPI) cells, short for breast epithelial cells, acted as our non-tumorigenic control group when performing our assays. Br(EPI) was used as the control cell line because they are able to be used as a reference for drug sensitivity when researching cancerous cells and can help to determine when a cell undergoes changes that aid in the progression of tumors. The Br(EPI) cell line was used as a control cell line because it is directly isolated from normal breast tissue and has a limited lifespan. The Br(EPI) cell line is considered non tumorigenic because it is derived from a healthy state and is not able to have the genetic modifications that allow for immediate proliferation under normal circumstances. Both cell lines were cultivated in a DMEM high-glucose solution-based cell culture medium. This medium also had a 10% fetal bovine serum and 1% penicillin/streptomycin content. Our cell lines were incubated at 37˚C in 5% $CO_2$ conditions.

### 2.4. MTT Assay

Cell viability was analyzed by a MTT assay kit (manufactured by Abcam) according to the manufacturer's protocols. A 96-well plate was opened and set up. Using a 10 - 100 microliter micropipette, 50 µL of the selected cell line sample was pipetted into the assigned well along with 50 µL of the MTT reagent. The 96-well plate is then wrapped in foil and placed into the incubator at 37˚C for forty-five minutes. After the incubation period was completed, 150 microliters of the MTT solvent were added to each well. The well plate is then wrapped in foil again and placed on the compact rocker for fifteen minutes. The absorbance of the well plate with the completed samples is then read at 595 nm, and the results are observed and recorded.

### 2.5. CCK-8 Assay

Cell viability was analyzed by Cell Counting Kit-8 (manufactured) according to the manufacturer's protocols. Briefly, cells were seeded and cultured at a density of 5000 cells/well in 100 µL of medium into 96-well microplates (Corning, USA). Then, the cells were treated 70 µM of $H_2O_2$ and 60 µM carboplatin. After 72 hours of treatment, 10 µL of CCK-8 reagent was added to each well and then cultured for 2 hours. All experiments were performed in triplicate. The absorbance was analyzed at 450

nm using a microplate reader (Bio-Rad, Hercules, CA, USA) using wells without cells as blanks. The proliferation of cells was expressed by the absorbance.

### 2.6. Griess Reagent Assay

Cellular stress response was analyzed by the Promega Griess Reagent Assay according to the manufacturer's protocols. To perform this assay, a nitrite standard curve was prepared from 1 mL of 100 micromolar nitrite solution through dilution of 0.1 molar of the provided standard. Columns 1 - 3 of the plate used for this experiment were selected for the creation of this curve. In rows B-H, 50 μL of our DMEM culture medium was dispensed to each well in the columns. In row A, the wells were filled with 100 μL of the nitrite standard solution. A serial dilution was then performed, with 50 μL of the first being added to the second, then from the second to the third, etc. The final well had 50 μL of its contents discarded. 50 μL of the test samples were added in triplicate to a series of 4 columns, each representing either Br(EPI) or MCF-7 with or without $H_2O_2$. 50 μL of sulfanilamide was added to each well and mixed thoroughly. The plate was incubated for 10 minutes at room temperature, and shielded from light. The absorbance was then read at a range between 520 and 550 nm.

### 2.7. Caspase-3 Assay

Following the manufacturer's protocol, the cellular stress response related to apoptosis was analyzed using the Caspase-3 Assay Kit (manufactured by Abcam). Cells were placed in a 96-well plate, with sample wells containing 50 μL of cell suspension and background wells containing 50 μL of 2× Reaction Buffer. A Caspase-3 reaction mixture was prepared by combining 50 μL of 2× Reaction Buffer and 0.5 μL of DTT per well in a master mix to ensure consistency. To each sample well, 50 μL of the reaction mixture was added, followed by 5 μL of 4 mM DEVD-p-NA substrate (final concentration: 200 μM). The plate was mixed gently and incubated at 37˚C for 60 minutes. After incubation, the absorbance was measured at a range of 400 and 405 nm using a microplate reader. All samples were run in triplicate, and the average and standard deviation were calculated for each plate.

### 2.8. Senescence Assay

Cellular senescence and stress response were analyzed using the Senescence Detection Assay Kit (manufactured by Abcam) according to the protocol of the manufacturer, using a Br(EPI) non-cancerous epithelial cell line along with an MCF-7 breast cancer cell line. Both cell lines were treated with CBDCA. First, the x-gal solution was prepared by weighing 20 mg of the x-gal and then dissolving it in 1 mL of DMSO solution. The cells were washed once with 1 mL of 1X PBS solution then fixed for 10 min at room temperature. The cells were again washed with 1 mL of 1X PBS. Next, 0.5 mL of the staining solution mix was added to each well. Enough solutions were made for a total of 6 wells of cells, 3 being MCF-7 cells and the other 3 being the Br(EPI) cells. The plate was then covered and incubated at

37˚C for 3 to 7 days. After incubation, the cells were then observed under a microscope for the development of a blue color. Using ImageJ software, the percentage of senescence in each cell line was determined. The presence of senescence was detected by the presence of any blue hue, indicating the presence of SA-$\beta$-gal, an enzyme elevated in senescent cells.

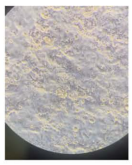
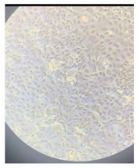
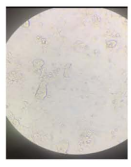
### 2.9. Statistical Analysis

The standard deviation was calculated to measure the variability within each cell type's response, reflecting how much individual data points differ from the mean. P-values and SD were calculated using Excel.

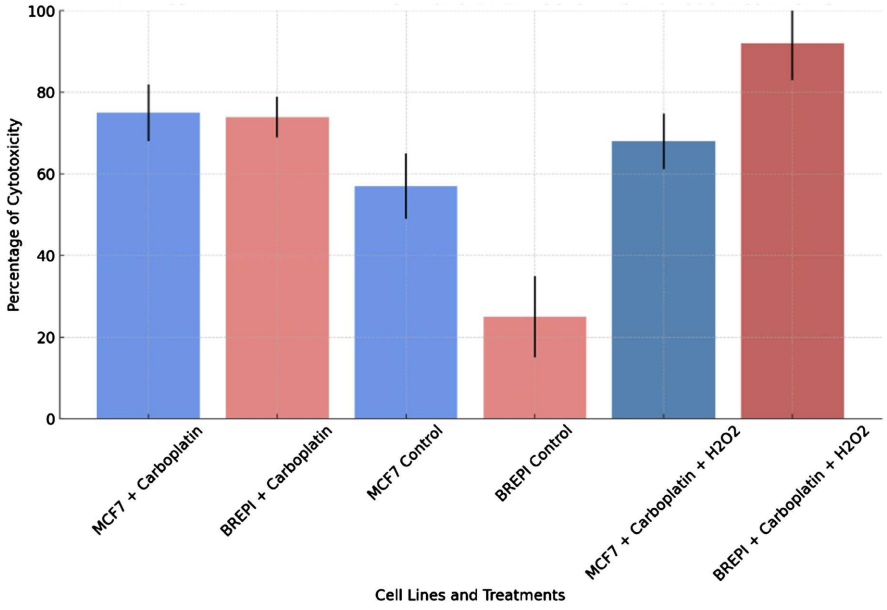
## 3. Results

### 3.1. MTT Assay

Figure 1 shows MTT assay results representing the percentage of cytotoxicity in MCF7 and BREPI cells under various treatments. Both cell types were exposed to carboplatin, $H_2O_2$, and a combination of carboplatin and $H_2O_2$. MCF7 cells showed 75% cytotoxicity under carboplatin alone and 68% under carboplatin + $H_2O_2$ treatment, while BREPI cells showed 74% and 92%, respectively. Controls showed lower cytotoxicity (MCF7: 57%, BREPI: 25%). Error bars represent the standard deviations (7%, 5%, 8%, 10%, 6.8%, and 9%) from three independent replicates. These results highlight the enhanced susceptibility of non-tumorigenic BREPI cells to combined oxidative and chemotherapeutic stress compared to MCF7 cells.

| Name | Image (40x) |
|---|---|
| MCF7+ H2O2 |  |
| MCF7 |  |
| BREpi |  |
| BREpi+H2O2 |  |

(A)




(B)

**Figure 1.** MTT assay: Cytotoxicity of MCF7 and Br(EPI) cells after various treatments.

### 3.2. CCK-8 Assay

**Figure 2** shows cell viability measured by absorbance at 450 nm using the CCK-8 assay after CBDCA and/or $H_2O_2$ treatment. Br(Epi)- CBDCA (blue) represents normal cells untreated with CBDCA, demonstrating high cell viability. MCF7 + $H_2O_2$-CBDCA (orange) and MCF7-$H_2O_2$-CBDCA (gray) represent MCF7 cells treated with oxidative stress alone and in tandem with CBDCA, respectively, indicating reduced cell viability under these conditions. Br(Epi) + CBDCA (yellow) shows a marked decrease in viability in normal cells treated with CBDCA alone. MCF7-$H_2O_2$ + CBDCA (green) and MCF7 + $H_2O_2$ + CBDCA (blue-green) indicate significant viability reduction in MCF7 cells exposed to both oxidative stress and CBDCA. Error bars represent the standard deviation from three independent experiments. These results suggest that MCF7 cells (dense breast cancer) are more sensitive to combined oxidative and chemotherapeutic stress compared to Br(Epi) cells (normal breast epithelial), highlighting potential therapeutic selectivity.

### 3.3. Griess Reagent Assay

The data shown in **Figure 3** demonstrates that overall, MCF-7 has a greater resistance to oxidative stress conditions than their Br(EPI) counterparts. In trial 1 (blue), MCF-7 with the $H_2O_2$ stressor showed a greater oxidative stress response than its counterpart that had no $H_2O_2$ added. Br(EPI) cell lines in trial 1 showed much greater oxidative stress responses than their MCF-7 counterparts. They showed nearly equal stress responses with or without $H_2O_2$ exposure. In trial 2 (orange), MCF-7 cells exposed to $H_2O_2$ showed only marginal increases in their oxidative stress responses compared to the MCF-7 cells not exposed to $H_2O_2$. The

Br(EPI) cells in trial 2 also showed equal oxidative stress responses whether exposed to $H_2O_2$ or not. This oxidative stress response was also greater than their MCF-7 counterparts, though not to as extreme of a degree as in trial 1. The error bars in the study represent a standard deviation between these two trials.

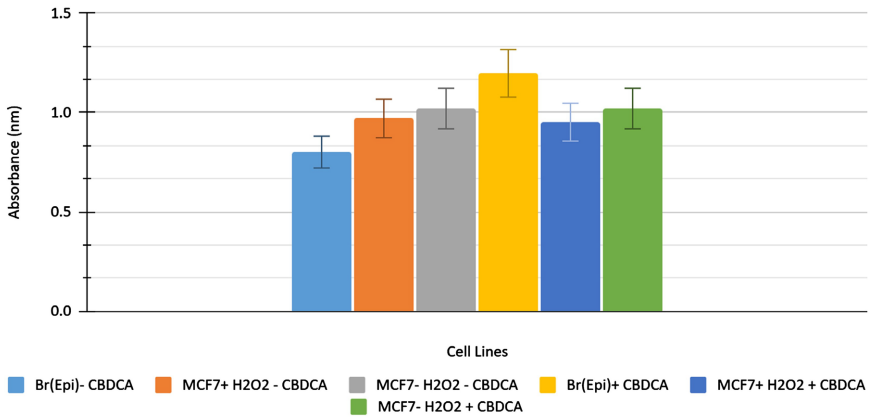



**Figure 2.** Cytotoxicity with and without CBDCA as an additional stressor.

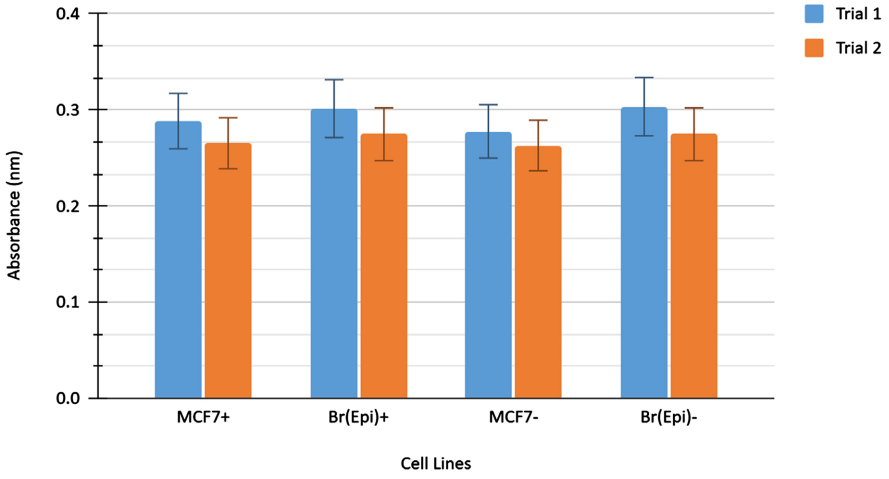



**Figure 3.** Average $NO_2$-Concentration across cell lines representing greater cytological stress.

Cytological Stress Response of Dense Breast Cancer (MCF-7) and Breast Epithelium When Exposed to Oxidative Stressor ($H_2O_2$). **Figure 3** demonstrates the difference in cytological stress response between MCF-7 and Br(EPI) cell lines when exposed to $H_2O_2$. The blue bars represent the data gathered from the first trial of this assay, while the orange bars represent the data gathered from the second trial of this assay.

### 3.4. Caspase-3 Assay

**Figure 4** shows Caspase-3 activity in MCF7 and Br(Epi) cells under stress treatment conditions. The bar graph illustrates Caspase-3 activity, a marker of apoptosis, in MCF7 (dense breast cancer) and Br(Epi) (normal epithelial) cells sub-

jected to various stress treatments. Blue bars represent MCF7 cells, while orange bars represent Br(Epi) cells. Error bars indicate the standard deviations of three independent experiments. Br(Epi) cells exhibit significantly higher Caspase-3 activity ($p = 0.017$) following the combined treatment with $H_2O_2$ and carboplatin, as indicated by the elevated orange bar, suggesting a stronger apoptotic response. In contrast, MCF7 cells show lower Caspase-3 activity, represented by shorter blue bars, indicating greater resistance to the same treatments. These results highlight the differential apoptotic responses of normal and cancerous cells to oxidative and chemotherapeutic stress.

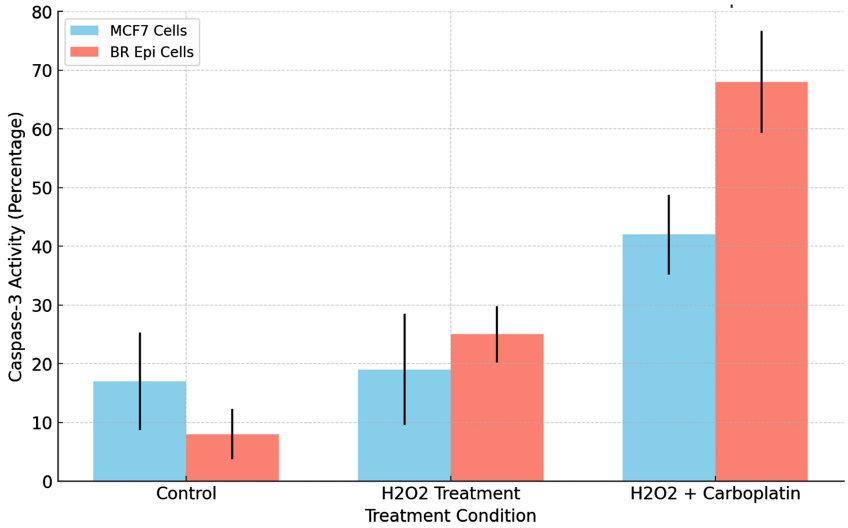



**Figure 4.** Average $NO_2$-Concentration across cell lines representing greater cytological stress.

Caspase-3 Activity in MCF7 and Br Epi Cells when exposed to $H_2O_2$ and Carboplatin as stressors. **Figure 4** shows the differences between $H_2O_2$ treatment and $H_2O_2$ with carboplatin. Activity was measured based on the amount of caspase-3 present in each group. The blue bars demonstrate MCF7, and the red bars represent BR Epi Cells.

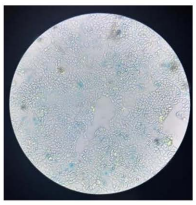
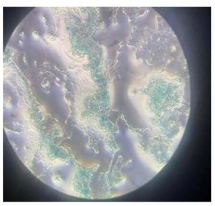
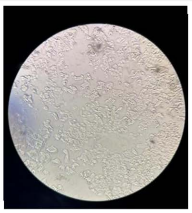
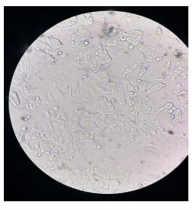
### 3.5. Senescence Assay

The results of this assay kit showed that the MCF-7 cells showed a much higher detection of senescence in comparison to the Br(EPI) cells when treated with carboplatin. This is seen in **Figure 5(A)**, as the MCF-7 cells showed a greater hue, indicating a positive result for SA-$\beta$-gal. Using ImageJ software, the percent senescence was determined for each cell line. These results, shown in **Figure 5(B)**, highlight the contrast between MCF-7 cells and Br(EPI) cells in response to the chemotherapy treatment from CBPT, as the MCF-7 cells showed 33.3% senescence while the Br(EPI) cells showed less than 5% senescence. The standard deviation was calculated at 7.49 for the MCF-7 cells and 5.8 for the Br(EPI) cells, with the error bars in the graph suggesting moderate variability in the re-

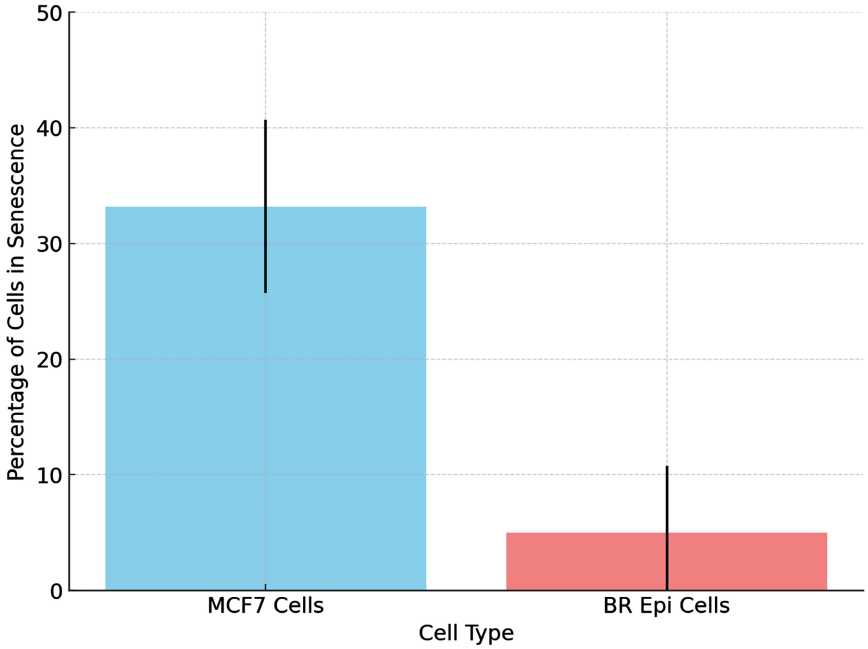
sponse among the cell populations, possibly due to heterogeneity within the cell types.

MCF-7 cells exhibit increased resilience to cytological stress and proliferate at a higher rate than non-tumorigenic cell lines. After CBDCA treatment, MCF-7 cells did not undergo apoptosis as expected but instead entered a state of cellular senescence after CBDCA. The MTT assay showed that CBDCA induces high cytotoxicity in both cell types and the BREPI cell line is more susceptible to combined $H_2O_2$ and CBDCA. The Griess Reagent assay showed that the MCF-7 cell line yielded lower nitrite levels, reducing the oxidative response and enhancing stress resilience. The CCK-8 assay showed that the MCF-7 cell line had a greater survival under stress due to resistance to apoptosis. The Caspase-3 assay showed that the Br(EPI) cell lines had higher apoptosis rates, and the MCF-7 cell lines had low Caspase-3 activity, which yielded adaptive resistance and tumor persistence. The senescence assay showed that the MCF-7 cells had high senescence levels that evaded apoptosis (33.2%) and promoted tumor progression.

A bar graph showing the percentage of cells in senescence after carboplatin treatment. These results highlight the contrast between MCF7 cells (33.2% senescence) and Br(EPI) cells (less than 5% senescence).

| Name | Image (40x) |
|---|---|
| MCF7+ CBPT |  |
| MCF7 + CBPT |  |
| Br(EPI) + CBPT |  |
| Br(EPI) + CBPT |  |

(A)




(B)

**Figure 5.** (A) Selected images of cells undergoing senescence; (B) Percentage of cells in senescence after carboplatin treatment.

## 4. Discussion

The MTT assay assesses cell viability based on metabolic activity. In the context of dense breast cancer, it was expected that a decrease in MTT reduction would indicate reduced cell viability under stress conditions [14]. If stress conditions lead to significant reductions in cell viability, this could indicate that the cells are undergoing senescence rather than apoptosis. An increase in senescent cells may correlate with decreased metabolic activity, suggesting that stress contributes to senescence in dense breast cancer tissue [15].

The Cell Counting Kit-8 assay [16] measures cell proliferation and viability based on the reduction of WST-8 by viable cells. In the context of dense breast cancer, it was expected that reduced CCK-8 activity would indicate decreased cell proliferation. Persistent stress could lead to a significant reduction in proliferation rates, contributing to the accumulation of senescent cells. If the assay shows reduced proliferation alongside markers of senescence, it strengthens the connection between stress and senescence in dense breast cancer.

A discrepancy between these results was expressed in that the MTT assay's CBDCA-treated Br(EPI) cell line showed a greater amount of cytotoxicity, the most among that assay in fact. Conversely, the CCK-8 assay showed that this cell line had the lowest incidence of cytotoxicity. We suggest that the most likely explanation is that the CBDCA-treated Br(EPI) cell line in the CCK-8 assay entered a precancerous state. The reason for this is unknown, but we hypothesize that the Br(EPI) sample entered this state in response to the stressors it was exposed to, however, further research will need to be performed to explore this reaction more

deeply utilizing more cytotoxicity assays along with follow-up Griess Reagent Assays and Caspase 3 Assays on this cell line to explore how this specific combination of stressors may have caused this reaction.

The Greiss Reagent System [17] measures nitric oxide (NO) production, which can be indicative of inflammation and cellular stress. In the context of dense breast cancer, it was expected that increased NO levels would indicate higher levels of inflammatory response, often associated with cellular stress. Elevated NO production may contribute to oxidative stress, leading to DNA damage and triggering senescence. Correlating NO levels with senescence markers can help elucidate the inflammatory response's role in promoting senescence in dense breast cancer. For example, suppose further experimentation shows a correlation between heightened inflammatory conditions and senescence. In that case, introducing anti-inflammatory compounds such as corticosteroid medications is an advisable next step for research [18].

The Caspase-3 assay is used to measure apoptosis [11]. When a cell becomes cancerous, it evades apoptosis, as outlined in the Hallmarks of Cancer [1]. Looking at the Caspase-3 activity can show us if cells are undergoing apoptosis and may be entering into a senescence state [11]. Senescence and apoptosis are two different cellular responses to stress. While apoptosis eliminates damaged or stressed cells, senescence puts cells into a state of permanent growth arrest without killing them. These states are often mutually exclusive. If cells do not show significant Caspase-3 activity in response to stress or treatment, it suggests they are not undergoing apoptosis [11]. Instead, they might be entering senescence as an alternative protective mechanism. Senescent cells avoid apoptosis, but they remain metabolically active, often secreting inflammatory signals that can alter the surrounding tissue environment [12]. Looking directly at the caspase-3 activity can help to show the effect that inducing stress will have on the response that dense breast cancer cells can have. This is because it has been shown that women with dense breasts have a four to five times higher risk of developing breast cancer compared to those with more fatty tissue [19]. The greater amount of glandular tissue in dense breasts increases the potential sites for tumor development, making detection more challenging and giving more opportunity to cancer cells to avoid apoptosis [20]. Caspase-3, which would indicate heightened apoptosis in response to cellular stress. If stress induces apoptosis, it may prevent the accumulation of senescent cells. Conversely, low Caspase 3 activity may suggest that cells are escaping apoptosis and entering a senescent state, contributing to tumor progression. Analyzing these results can help determine whether stress leads to cell death or senescence [11].

The senescence assay measures the presence of senescence-associated $\beta$-galactosidase (SA-$\beta$-gal), a hallmark of cellular senescence [21]. It was expected that increased SA-$\beta$-gal activity would confirm the presence of senescent cells in response to stress. A higher number of senescent cells in stressed samples compared to control samples would suggest that stress modulates the senescence pathway in

dense breast cancer. This accumulation can contribute to the pro-tumorigenic environment through SASP factors, which include different proteins and other factors secreted by senescent cells that help promote tissue repair [22]. One specific component is interleukin-6, a cytokine that accelerates cancer cell proliferation. Studies have found that components, along with other SASP factors, can create an immunosuppressive environment that promotes tumor growth. Furthermore, senescent cells can drive angiogenesis by secreting vascular endothelial growth factor, a signaling protein crucial for the formation of new blood vessels. The creation of new blood vessels then promotes metastasis, as it facilitates the movement of cancer cells to another site apart from the primary site of growth [23].

The results from these assays highlight the response of cancerous and non-cancerous cells to stress. Increased Caspase 3 activity could indicate a shift toward apoptosis under severe stress, while lower activity may imply a preference for senescence [11]. Decreased viability in MTT and CCK-8 assays can correlate with increased senescence, suggesting that stress leads to senescence rather than proliferation [24]. Alternatively, the unexpected proliferation of normal cells under chemotherapeutic conditions indicates the need for alternate treatment options other than platinoid agents [25]. The higher proliferation of Br(EPI) cells could also be a result of increased baseline ROS levels of the MCF-7 cell lines, or the Warburg Effect, causing metabolic differences more susceptible to cytotoxicity [26]. High SA-$\beta$-gal activity directly confirms the presence of senescent cells, linking stress to senescence [24]. Increased NO production from the Greiss assay could further support the idea that stress-induced inflammation contributes to both senescence and the tumor microenvironment in dense breast tissue [23].

Our findings highlight the potential of therapeutic strategies that focus on overcoming apoptotic resistance in dense breast cancer cells. Exploring agents such as senolytic drugs that selectively target senescent cells or that sensitize resistant breast cancer cells to apoptosis could enhance the efficacy of standard treatments and potentially improve outcomes for patients with dense breast tissue [27].

## 5. Future Research

Targeting apoptotic and senescence pathways may improve treatment efficacy. Exploring agents that selectively target senescent cells or sensitize resistant dense breast cancer cells to apoptosis may be of particular interest. Developing treatments that are tailored to overcoming resistance in dense breast cancer cells can help reduce metastasis in breast cancer patients. Investigating the cellular markers and pathways, such as phosphoinositide 3-kinase (PI3K) and mTOR pathways, to counter survival mechanisms [28].

A novel strategy for targeting the senescence pathway, particularly in triple-negative breast cancers (TNBC) that are highly prone to relapse, involves combining the senescence-inducing agent palbociclib with the senolytic drug navitoclax [29]. This combination was shown to reduce both tumor growth and metastasis. However, navitoclax's broad effectiveness in eliminating senescent cells also leads

to off-target side effects. To address this, Estepa-Fernandez *et al.* developed a galacto-conjugated version of navitoclax, termed nav-Gal, which is selectively activated in senescent tumors. This specificity is achieved because senescent cells, particularly in neoplasms, overexpress $\beta$-galactosidase, an enzyme that cleaves the galacto-conjugate and activates the drug.

Cancer cells in dense breast tissue often rely on the PI3K/mTOR pathways to manage metabolic demands, especially during oxidative stress. Targeting these pathways disrupts the energy supply needed for tumor growth and progression. Clinically, drugs like alpelisib (a PI3K inhibitor) and everolimus (an mTOR inhibitor) are already in use or under investigation for breast cancer treatment [28]. Focusing on these pathways in dense breast cancer could lead to more effective, tailored therapies by reducing survival mechanisms that drive tumor progression. Conducting trials on patients in vivo to assess CBDCA and oxidative stress effects on nitric oxide levels can also further dense breast cancer research. One other method that is of note is investigating $H_2O_2$ exposure's effects on other forms of cancer treatments, as seen in a 2024 study by Zaher *et al.* [30]. Advocating for the importance of the use of ultrasounds to accompany MRI testing screenings can help further investigate the effects of dense breast tissue on the number of dense breast cancer diagnoses per year.

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## Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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