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Dual CD47 SIRPα Blockade Potentiates Monocyte-Driven Clearance of MCF-7 Breast Cancer Cells

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ABSTRACT

Background: Breast cancer is the most common cancer and a major cause of cancer-related death among women worldwide. In Iraq, it is the most prevalent malignancy in females. Breast cancer cells evade immune destruction through overexpression of cluster of differentiation 47 (CD47), a "don't eat me" signal that binds signal regulatory protein α (SIRPα) on monocytes and macrophages to inhibit phagocytosis. This study investigates CD47 inhibition in MCF-7 breast cancer cells and evaluates whether simultaneous SIRPα blockade enhances immune-mediated responses. It aims to assess the potential of treating MCF-7 cells in vitro by blocking the CD47–SIRPα interaction. To our knowledge, this is the first study in Iraq to examine CD47 and SIRPα expression and their therapeutic potential in breast cancer cell lines.

Methods: MCF-7 cells were cultured and treated with anti-CD47 antibodies with or without monocytes preincubated with anti-SIRPα. Cell morphology was assessed using inverted microscopy. CD47 expression in MCF-7 cells and SIRPα in monocytes was confirmed by immunocytochemistry (ICC). Cell viability was measured using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Statistical analysis was performed using analysis of variance with Tukey post hoc test.

Results: ICC confirmed high expression of CD47 in MCF-7 cells and SIRPα in monocytes. Combined anti-CD47 and anti-SIRPα treatment showed a trend toward greater reduction in cell viability compared with single treatments, although the difference was not statistically significant. Anti-CD47 alone reduced cell viability by 47%, likely due to indirect or antibody-mediated effects. Morphological changes included cell shrinkage and loss of adhesion.

Conclusion: Dual blockade of CD47 and SIRPα showed a trend toward enhanced monocyte-mediated reduction in MCF-7 cell viability, suggesting the CD47–SIRPα axis as a potential immunotherapeutic target requiring further validation.

INTRODUCTION

Breast cancer is an important health issue globally because it is common among women everywhere in the world and is the second leading cause of cancer death after lung cancer, as per recent World Health Organization statistics of 185 countries.[1] Iraq is facing a similar challenge; breast cancer is the most prevalent malignancy among the country's 10 major cancers. Notably, females account for nearly the entire burden of the disease with 4922 of 4996 recorded cases (98.5%).[2,3]

The immune system's ability to eliminate tumors through macrophage-mediated phagocytosis depends on its capacity to distinguish "non-self" molecules

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from "self" molecules. When macrophages fail to correctly identify tumor cells, cancer can evade the immune response, a process now recognized as a fundamental hallmark of cancer.[1] Immune-mediated inflammation and epithelial tissues can interact to bring tumor-related histopathological changes, including those of breast cancer.[4,5]

Recent research suggests that phagocytosis is governed by a delicate balance between prophagocytic and antiphagocytic ligands. During the past decade, scientists have identified a wide array of prophagocytic molecules that help the immune system recognize and eradicate tumors. However, the discovery of cluster of differentiation 47 (CD47) has shifted the focus toward antiphagocytic signals.[6] CD47 acts as a "don't eat me" signal by interacting with its receptor, signal regulatory protein α (SIRPα), on the surface of macrophages. By overexpressing CD47, tumor cells can effectively trick the immune system into ignoring them, allowing the malignancy to grow unchecked.[7]

Cell line models have helped us significantly in understanding this CD47–SIRPα checkpoint. Extensive profiling of different molecular subtypes has revealed that the surface expression of CD47 tends to be quite high in breast cancer cell lines.[8] These results have been confirmed by current screening techniques which have shown that deletion of CD47 can enhance phagocytic clearance by 60% to 80%, but that a dual targeting strategy which combats both CD47 and SIRPα gives a slightly greater reduction in cell viability.[9]

Much of the current research in therapeutics focuses on maximizing anti-CD47 antibodies and SIRPα fusion proteins, especially when used with other inhibitors or chemotherapy.[10] To make these models physiologically relevant, scientists are beginning to incorporate patient-derived immune elements and enforce stringent authentication procedures to control genetic drift in these models during long-term cultures.[11]

These advancements have made cell line systems indispensable for decoding the CD47–SIRPα pathway and designing the next generation of immunotherapies.[12] Of them, the MCF-7 cell line is, perhaps, the most important. Established in 1973 at the Michigan Cancer Foundation, MCF-7 was derived from a pleural effusion of a 69-year-old patient with metastatic breast cancer. Her medical history was complex: she had undergone a mastectomy of her right breast for a benign tumor and, 4 years later, a radical mastectomy of her left breast for malignant adenocarcinoma. From cells collected 3 years after her final surgery, this now-legendary cell line was created.[13] For more than 40 years, MCF-7 has remained a cornerstone of breast cancer research, providing a consistent and essential model for scientific discovery worldwide. Although the CD47–SIRPα immune checkpoint has been intensively studied in several tumor types, experimental data assessing this mechanism in breast cancer models residing in regional research environments are still scarce. As a result, this work was conducted to examine the impact of CD47 inhibition in a breast cancer cell line and to produce initial experimental data that can be used as a basis for further research on immune checkpoint inhibitors in breast cancer.

METHODS

Michigan Cancer Foundation-7 (MCF-7) is a breast cancer cell line isolated in 1970 from a 69-year-old white woman.[14] The MCF-7 cell line was obtained from the American Type Culture Collection (Manassas, Virginia). Cells were authenticated by short tandem repeat profiling by the supplier and routinely tested to confirm absence of mycoplasma contamination (Cell Technologies Lab., Harthyia, Baghdad, Iraq). The cell lines were grown in Minimum Essential Medium (US Biological) supplemented with 10% (v/v) fetal bovine serum (FBS) (Capricorn Scientific, Germany), 100 IU penicillin, and 100 µg of streptomycin (Capricorn Scientific, Germany). They were then incubated at 37 °C in a humidified environment. Experiments were conducted using exponentially growing cells. Blood samples were obtained from a healthy adult donor at Cell Technologies Lab in Harthyia, Baghdad, following the donor's written informed consent. A single donor was used in this preliminary study to minimize experimental variability and maintain consistency across experiments. Peripheral blood monocytes served as a basic model in this experiment since they constitute the circulating precursors of macrophages and can still differentiate into phagocytic cells. Studying monocytes helps to assess initial immune cell responses in an isolated system even prior to macrophage differentiation and polarization. The blood was collected in a tube containing ethylenediaminetetraacetic acid as an anticoagulant and was stored at room temperature with gentle shaking to prevent clotting.

Isolation of monocytes

The whole blood was first diluted with phosphate-buffered saline (PBS) at a ratio of 1:1 and carefully layered over Ficoll-Paque solution. Next, 4 mL of the diluted blood was gently laid on top of 2 mL of Ficoll-Paque density gradient medium in a sterile 15-mL conical tube. Samples were centrifuged at 400 × *g* for 30 minutes at room temperature with the brake off to allow the layers to be separated

cleanly. After centrifugation, the buffy coat (peripheral blood mononuclear cell [PBMC]) layer was carefully scraped at the interface between the Ficoll medium and the PBS with the help of a sterile pipette. The obtained PBMCs were resuspended in a new tube, rinsed using 10 mL of 1× PBS, and centrifuged once more at 400 × *g* for 6 minutes. This wash step was followed by the removal of the supernatant. Lastly, purified PBMCs were resuspended and cultured in 10 mL of RPMI base medium in tissue culture dishes or flasks. Recombinant human macrophage colony-stimulating factor/colony-stimulating factor 1 protein was added at a final concentration of 50 ng/mL in the flask of PBMCs according to the protocol.[15] PBMCs were isolated by Ficoll density gradient centrifugation. Then, the cells were resuspended in complete culture medium and plated, and were left to incubate for 12 hours at 37 °C in a humidified atmosphere of 5% CO_2 to enable monocytes to attach to the plastic surface. After that, nonadherent cells, which are mainly lymphocytes, were removed by washing the wells with PBS. The remaining adherent cells were considered as a monocyte-enriched population and were used in the subsequent coculture experiments with MCF-7 cells.

Coculture experiments

To evaluate immune cell-mediated effects on tumor cells, the peripheral blood monocytes were combined with MCF-7 breast cancer cells. These monocytes were used directly without differentiation into macrophages. Monocytes were added to tumor cells at an effector-to-target ratio of 5:1. Briefly, MCF-7 cells were seeded in culture plates and allowed to adhere overnight before the addition of monocytes. The coculture system was kept under standard incubation conditions (37 °C, 5% CO_2) for 72 hours of the experiments.

Immunocytochemistry staining protocol

Cells were re-cultured in an 8-well tissue culture slide chamber with RPMI base medium supplemented with 20% FBS and incubated at 37 °C to allow for the formation of a confluent monolayer. After 1 to 2 days, the medium was aspirated, and the cells were fixed with 4% paraformaldehyde for 10 minutes at room temperature. Fixed cells were permeabilized with 0.1% Triton X-100 in PBS for 10 minutes and blocked with 5% bovine serum albumin for 1 hour. The fixed cells were then evaluated for CD47 expression using a primary antibody, which was diluted and applied according to the manufacturer's instructions (Santa Cruz Biotechnology) at a final dilution of 1:100 overnight at 4 °C in a humidified chamber. Subsequently, cells were washed 3 times with PBS and incubated with the appropriate fluorescently labeled secondary antibody for 1 hour at room temperature. Nuclei were counterstained with 4′,6-diamidino-2-phenylindole, and the slides were mounted with antifade medium. Negative controls were included by omitting the primary antibody. Images were acquired using an inverted microscope (magnification 10×) with the suitable excitation and emission filter.

CD47 expression was determined qualitatively by identifying the cells where it was localized and viewed under the inverted microscope (10×) with scale bars = 100 μm for all images. For every sample, 5 microscopic fields were randomly chosen for observation, and the whole set of experiments was repeated 3 times.

Viable cell count

After receiving tissue culture flasks containing cancer cells and decanting the growth medium, the flasks were given 2 washes with 1 mL of trypsin-versene-ethylenediaminetetraacetic acid solution. Then, the flask was filled with 4 to 5 mL of RPMI growth medium (which contains 10% FBS) and 1 mL of trypsin-versene, which was incubated for approximately 3 minutes at 37 °C to allow the cells to separate. In order to identify the live cells, the cells were then counted using a counting chamber under an inverted microscope. For viable cell counts, 5 microscopic fields randomly chosen were examined for each sample. Each experiment was done 3 times, and the average of the values was used for statistical purposes. The number of cells per volume unit (cell/mL) was calculated using the formula below:[16]

$$c = n \times \delta \times 10$$

where c represents the number of viable cells (per milliliter), n represents the total number of viable cells, and δ represents the dilution factor (equal to 10).

MTT assay

Cell viability was assessed using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Anti-CD47 antibody was used at a final concentration of 10 μg/mL, while anti-SIRPα antibody was used at 10 μg/mL. Cells were treated with antibodies for 72 hours at 37 °C in a humidified atmosphere containing 5% CO_2. Cells were seeded at a density of 1×10^4 cells per well in 96-well plates. Subsequently, 20 μL of MTT solution (5 mg/mL) was added to each well and incubated for 4 hours at 37 °C. The resulting formazan crystals were dissolved in 150 μL of dimethyl sulfoxide, and absorbance was measured at 570 nm using a

microplate reader. All experiments were performed in triplicate and repeated 3 times.[17]

Cell viability calculation

Cell viability (%) was calculated using the following formula:

$$\text{Cell viability (\%)} = \frac{\text{OD Control} - \text{OD Sample}}{\text{OD Control}} \times 100$$

where OD Control represents the mean optical density (OD) of untreated control wells and OD Sample represents the optical density of treated wells.

Statistical analysis

Statistical analysis was conducted using GraphPad Prism, version 8.0 (GraphPad Software). The qualitative data from the MTT assay were presented as mean ± standard deviation of 3 independent experiments. One-way analysis of variance with Tukey multiple comparisons test was used to analyze possible differences between the experimental groups. Chi-square test was utilized to compare the percentages among different experimental groups. A P value < 0.05 was regarded as statistically significant.[18]

RESULTS

Immunocytochemical characterization of CD47 expression in MCF-7 cells

The analysis was done using immunocytochemistry (ICC) at Cell Technologies Laboratory (Harthyia, Baghdad, Iraq) to assay the expression of CD47 in the MCF-7 breast cancer cell line. The cells had typical polygonal epithelial morphology, which grew in clusters and incomplete monolayers. Good positive immunoreactivity towards CD47 was also noted as the chromogenic signal (brown staining) was mainly localized in the cell membrane and the cytoplasm. Such intensity of staining reflects the marked overexpression of CD47 in agreement with the classical profiles of breast carcinoma cell lines. Regions of reduced staining may show heterogeneous expression or local areas of reduced antigen-antibody interaction. The negative controls were unstained and confirmed the specificity of the primary antibodies (Figure 1).

Characterization of SIRPα expression in human monocytes

Immunocytochemical techniques were used to confirm the strong expression of SIRPα in cultured human monocytes. Positive SIRPα immunoreactivity was determined as the presence of brown chromogenic precipitates as shown in Figure 2, which is due to the oxidation of the 3,3′-diaminobenzidine substrate by peroxidase. Staining was mostly confined to the plasma membrane and the cytoplasmic compartments, which confirmed the specificity of anti-SIRPα antibody.

Conversely, the nuclei were not stained or had weak counterstaining which indicated the extranuclear localization of the target protein. The slight differences in staining intensity of individual cells indicate some heterogeneity in the levels of SIRPα expression of the monocyte population.

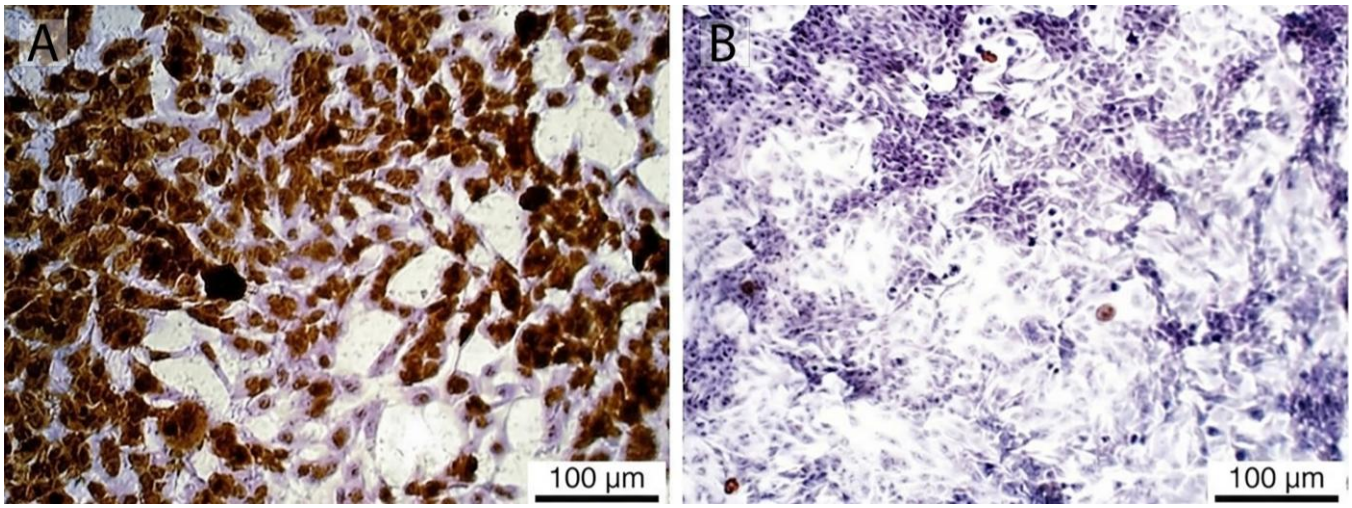

Figure 1. Immunocytochemical Analysis of CD47 Expression in MCF-7 Cells. A, CD47-positive staining (brown chromogenic signal) in MCF-7 breast cancer cells (inverted microscope, 10×; scale bar = 100 µm). B, Negative control prepared by omitting the primary antibody (10×).

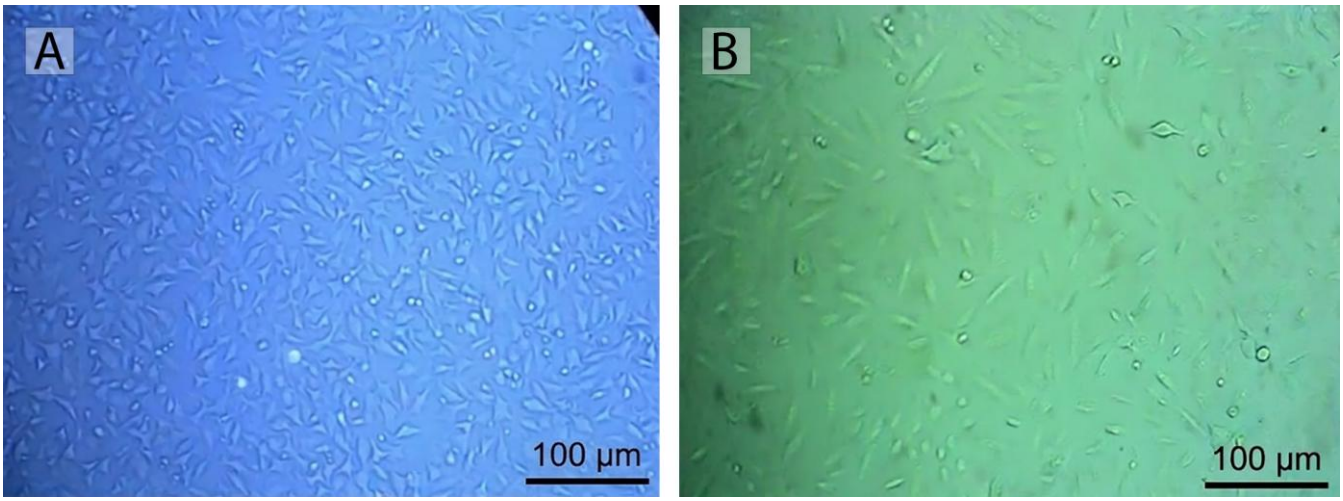

Figure 2. Morphological Analysis of MCF-7 Cells. A, Untreated control MCF-7 cells showing typical epithelial polygonal morphology. B, Morphological changes in MCF-7 cells after treatment with anti-CD47 antibody showing cellular rounding, membrane blebbing, and partial detachment (inverted microscope, 10×; scale bar = 100 µm).

Morphological alterations in MCF-7 cells following anti-CD47 treatment

Untreated control MCF-7 cells had a typical epithelial-like polygonal cell morphology and were able to form adherent monolayers of high confluence. The existence of clear cytoplasm and homogeneous cell size was a sign of a healthy, actively growing culture. Nevertheless, anti-CD47 treatment led to a decrease in cell density and changes in the growth kinetics of the culture. Microscopic analysis showed reduced confluency and fewer adherent cells, suggesting suppressed proliferation and impaired cell viability. Cellular rounding, plasma membrane blebbing, and a decrease in cytoplasmic volume were observed as distinct morphological changes. However, these findings are suggestive and require further investigation to confirm the underlying mechanisms. Also, some cells were partially detached from the growth surface, which was also a sign of decreased cell viability and survival under CD47 suppression (Figure 3).

Morphological characterization of monocytes post-SIRPα blockade

Inverted light microscopy was used to analyze the morphology of human monocytes after SIRPα inhibition. The monocytes (treated and untreated) were predominantly spherical to ovoid morphology and were either suspended or loosely adherent to the culture substrate. These findings are in line with the typical immature monocyte phenotype in vitro. The comparative analysis showed no major structural differences between the study groups, and SIRPα blockade did not trigger any spontaneous activation or differentiation. The absence of cellular elongation and flattened cytoplasmic spreading, both hallmark

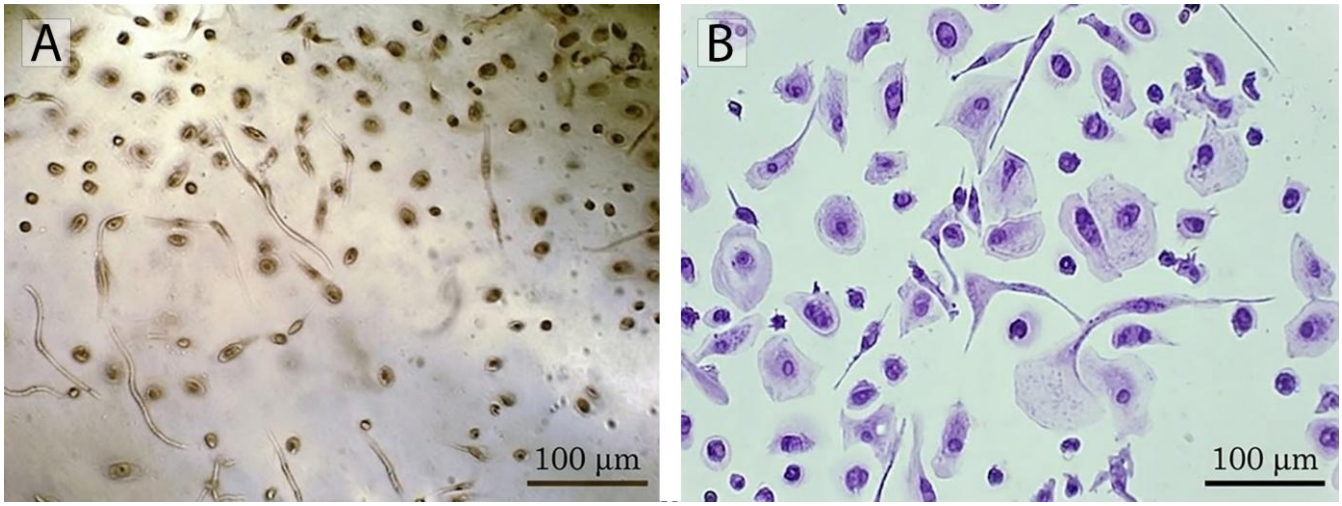

Figure 3. Immunocytochemical Analysis of SIRPα Expression in Monocytes. A, SIRPα-positive staining (brown chromogenic signal) in human monocytes (inverted microscope, 10×; scale bar = 100 µm). B, Negative control prepared by omitting the primary antibody (10×).

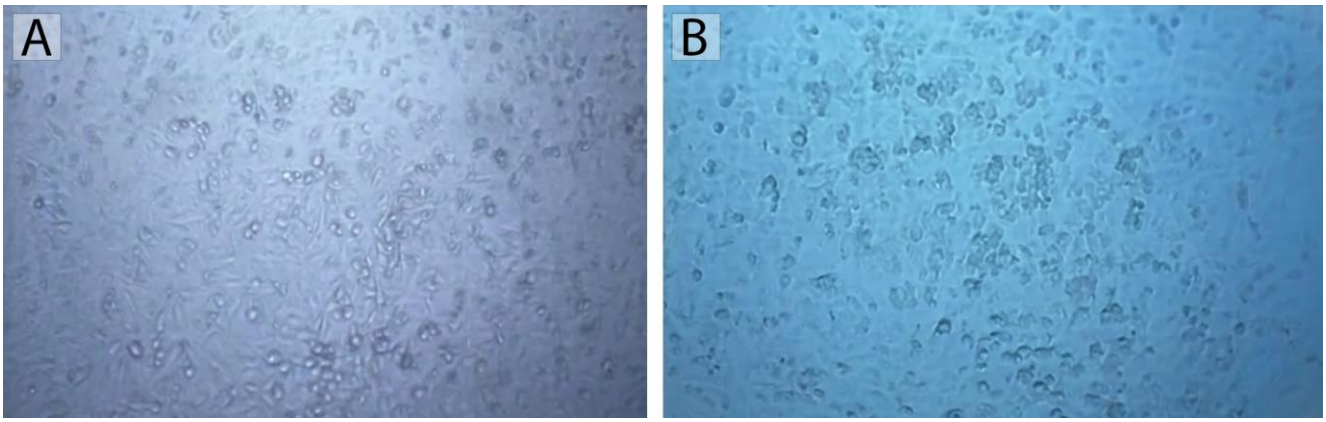

Figure 4. Morphological Analysis of Monocytes. A, Untreated control monocytes showing typical ovoid-to-spherical morphology. B, Monocytes after SIRPα blockade showing predominantly immature morphology without flat cytoplasmic spreading or elongation (inverted microscope, 10×; scale bar = 100 μm).

signs of macrophage differentiation, confirmed this immature state (Figure 4).

Combined targeting of dual SIRPα-CD47 blockade on monocyte-mediated clearance

The monocytes exhibited a clear tendency to cluster around the tumor cell when SIRPα-inhibited monocytes were cocultured with CD47-blocked MCF-7 cells. It was observed that monocytes were clustering circumferentially around the cells of MCF-7 with clear morphological evidence of active phagocytic engulfment and internalization. Subsequently, after this contact, the MCF-7 cells experienced an extensive degradation in their phenotype, shifting from their characteristic polygonal adherent morphology to a rounded, condensed, and detached appearance. Concurrent disruption of the inhibitory CD47–SIRPα "don't eat me" signaling pathway restored the phagocytic activity of monocytes, enabling them to interact more actively with breast cancer cells (Figure 5).

Impact of SIRPα and CD47 blockade on MCF-7 viability (MTT assay)

The viability and metabolic activity of the MCF-7 cells at the end of the treatment was examined using the MTT assay (Figure 6). The specific blockade of CD47 was associated with an approximately 47% reduction in cell viability compared to control ($P < 0.001$). This likely represents indirect cellular stress or antibody-mediated effects rather than direct cytotoxicity. Conversely, the use of anti-SIRPα as a treatment alone did not produce a significant change in cell viability ($P = 0.38$) as it is a receptor predominantly expressed on effector cells and not on the tumor targets. The combination of anti-CD47 and anti-SIRPα showed a 58% reduction in cell viability compared with the control ($P < 0.001$). However, when compared with anti-CD47 monotherapy, the difference was not statistically significant ($P = 0.50$). These findings demonstrate that although CD47 blockade reduced MCF-7 cell metabolic activity in this in vitro system, which may reflect indirect cellular stress or antibody-mediated effects rather than a classical immune-mediated phagocytic mechanism, dual blockade in this model may be more effective in providing a strong overall effect on the population of viable cells.

Qualitative assessment of MCF-7 viability via MTT assay

The MTT assay was applied to measure the optical density (OD) and relative metabolic activity of MCF-7 cells after exposure to 4 experimental conditions for 72 hours: untreated control, anti-CD47 monotherapy, anti-SIRPα monotherapy, and combination therapy (Figure 7). Treatment with anti-CD47 resulted in a significant reduction in the

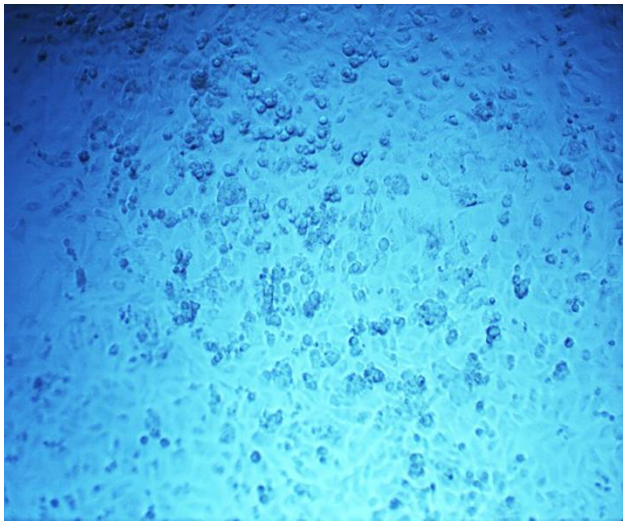

Figure 5. Morphological changes in MCF-7 cells cocultured with monocytes and various treatments. MCF-7 cells show cellular rounding, condensation, and detachment, with monocytes clustering circumferentially around the tumor cells showing active phagocytic engulfment after dual blockade of CD47 and SIRPα (inverted microscope, 10×; scale bar = 100 μm).

metabolic activity of MCF-7 cells compared with the untreated control. On the contrary, although SIRPα inhibition is a key requirement in effector cell-mediated responses, its main therapeutic benefit is achieved in the active interaction of monocytes/macrophages with tumor cells. Therefore, the biological importance of disrupting the CD47–SIRPα signaling pathway will probably be clearer in immune-competent systems. In the current in vitro model that did not include immune effector cells, the combined blocking showed only a numerically greater decrease in cell survival compared to anti-CD47 single monotherapy, and did not reach statistical significance.

As illustrated in the comparative bar graph (Figure 7), the control group that had not been treated showed the highest optical density, which indicated the basal metabolic activity and vibrant growth of MCF-7 cells. Anti-CD47 monotherapy treatment led to significant decrease in optical density ($P < 0.001$), which implies a significant drop in cell viability. This suggests that CD47 acts as an immune evasion signal, and its inhibition may reduce tumor cell viability primarily through indirect or immune-mediated mechanisms rather than direct cytotoxic effects. In turn, anti-SIRPα monotherapy did not demonstrate any significant changes in optical density in comparison with the control. This is in line with the expression profile of SIRPα which is largely confined to myeloid cells rather than the MCF-7 target cells. The most significant viability decrease was seen in the combination therapy (anti-CD47 + anti-SIRPα), which was statistically significant compared to the control ($P < 0.001$). Nevertheless, the difference between the anti-CD47 monotherapy and combination therapy was not statistically significant ($P > 0.05$). This implies that while CD47 blockade reduced cell viability in our system—potentially via indirect cellular stress or antibody-mediated effects rather than classical immune-mediated phagocytosis—SIRPα inhibition provides a significant additive effect that will presumably only achieve its full potential in the presence of immune effectors.

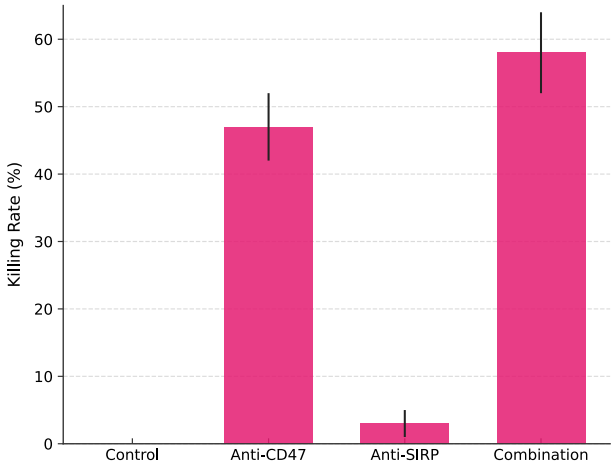

Figure 6. Morphological changes in MCF-7 cells cocultured with monocytes and various treatments. MCF-7 cells show cellular rounding, condensation, and detachment, with monocytes clustering circumferentially around the tumor cells showing active phagocytic engulfment after dual blockade of CD47 and SIRPα (inverted microscope, 10×; scale bar = 100 µm).

DISCUSSION

Cell line culture is still one of the most essential instruments in the study of immunotherapy and it is used to recreate the intricate interactions between immune elements and cancerous cells in a controlled setting.[19] The MCF-7 cell line, which is described as having stable growth parameters and well-documented in vitro behavior, is a powerful model that has been used to study human breast cancer.[20] The CD47–SIRPα immune checkpoint axis has recently been a center of oncological interest as a result of its critical role in enabling tumor cells to survive macrophage-mediated surveillance.[21]

The current study demonstrates the crucial involvement of this pathway in immune evasion. CD47 is mainly recognized as a “don’t eat me” signal that prevents phagocytosis through its interaction with SIRPα on myeloid cells.[22] Hence, the decrease in tumor cell viability following the blockade of CD47 is most likely attributable to indirect or antibody-mediated mechanisms rather than a direct cytotoxic effect. While growth arrest of the target cell

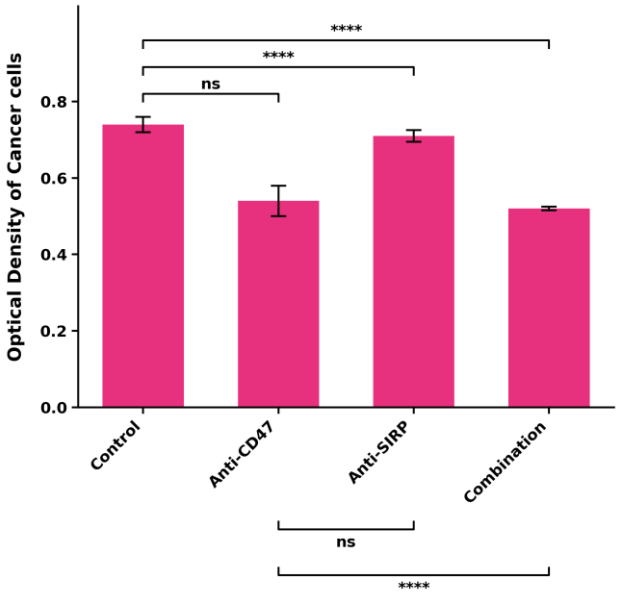

Figure 7. Optical density (OD) values at 570 nm from the MTT assay showing cell viability of MCF-7 cells after 72 hours of treatment with anti-CD47, anti-SIRPα, or combined therapy. ns indicates not significant.

is often applied in effector–target assays to reduce confounding effects of tumor cell proliferation on viability-based readout, it was not implemented in this study. Instead, a non-arrested coculture system was purposefully employed to better sustain dynamic tumor–immune interactions and to simulate a more physiologically appropriate in vitro environment. We recognize that the absence of growth arrest may present a complicating factor in MTT-based viability assessment; nonetheless, the experimental design was aimed at capturing the combined effects of immunological interaction and antibody administration over a defined incubation period. As a result, this strategy strikes a methodological compromise between physiological relevance and experimental control, and future research using growth-arrested target cells is needed to further confirm immune-mediated effects. In our results, we have shown that interference with this axis plays a significant role in improving the phagocytic clearance of MCF-7 cells. Through the MCF-7 model, this study can be used to design new generations of macrophage-mediated immunotherapies and can offer a preliminary framework to assess the proposed CD47-targeting and SIRPα-modulating approaches.

Upregulation of CD47 in most malignancies is highly significant and is a highly advanced form of innate immune escape in which it sends a "don't eat me" signal to SIRPα, inhibiting phagocytosis. Targeting this axis is, therefore, a potential approach for cancer immunotherapy.[23]

Validation of expression and mechanistic insights

The observation that CD47 is present in MCF-7 breast cancer cells and SIRPα in human monocytes formed the molecular basis of the immune evasion that we observed. SIRPα on the monocytes/macrophages binding to CD47 on the tumor surface activates an inhibitory signaling cascade to inhibit phagocytosis. We established that the inhibition of CD47 was significantly associated with a reduction in MCF-7 cell viability, implying that the inhibition of this pathway may decrease tumor cell viability through modulation of immune-related or antibody-mediated processes rather than direct cytotoxicity. These findings are consistent with previous studies[24] which showed that SIRPα inhibition enhances the activity of antibody-drug conjugates in breast cancer models.

To the best of our knowledge, this is the first attempt in Iraq to assess the expression and therapeutic potential of CD47 and SIRPα in breast cancer cell lines. An interesting result of our research was that anti-SIRPα monotherapy did not cause any serious reduction in cell viability in the MTT assay. This is likely because the culture system contained no immune effector cells. Since SIRPα is primarily expressed on myeloid cells, its inhibition does not directly affect tumor cell viability in the absence of monocytes or macrophages. Nonetheless, the combination of CD47 and SIRPα inhibition led to a slightly greater reduction in cell viability, although the difference was not statistically significant. This finding is supported by recent studies, which have demonstrated that concurrent blockade of the CD47–SIRPα pathway enhances phagocytosis and inhibits tumor growth in vitro.[25,26]

The experimental efficacy of CD47 blockade spreads to numerous cancer models. Our results are aligned with other studies on phagocytosis kinetics[27], which indicated that inhibition of CD47 enhanced macrophage-mediated activity against MCF-7 cells. Moreover, in vivo experiments involving metastatic models have demonstrated that anti-CD47 treatment can result in up to a 50% reduction in tumor volume and weight with a large increase in macrophage infiltration into the tumor microenvironment.[28]

Such success has also been seen in colorectal cancer where CD47 inhibition enabled macrophages to eliminate colorectal cancer stem cells and the resulting differentiated progeny.[29] This indicates that CD47 targeting is applicable to solid tumors. In addition to the myeloid lineage, more recent evidence[30] indicates that natural killer (NK) cells also have SIRPα expression and that CD47 inhibition may potentially enhance NK cell-mediated immune responses against tumor cells. This complex immunological response explains why CD47 inhibition may become an effective primary or relapse treatment.[31]

Interference with the CD47–SIRPα axis has also been confirmed to be effective on aggressive brain tumors, including gliomas, in which it inhibited tumor progression and extended survival in both in vitro and in vivo models.[32] Moreover, the CD47 knockdown has been demonstrated to have a strong antiproliferative effect on breast cancer cells and to decrease the size of tumors in animal models.[33]

Combination of CD47 inhibition with other tumor-targeting antibodies may represent a potential therapeutic approach. It increases antibody-dependent cellular phagocytosis (ADCP) by decreasing the amount of "don't eat me" signals while increasing the amount of "eat me" signals via the Fc domains of the antibodies.[34] This is essential since enhanced ADCP has the ability to elevate antigen cross-presentation, thus triggering an escalation of adaptive immune response in T cells.[35]

Limitations

One limitation of this study is that peripheral blood monocytes have been used instead of

differentiated macrophages. While monocytes offer a simplistic model for immune clearance research, they fall short in depicting the complexity of the tumor microenvironment. Hence, subsequent studies should involve the use of macrophage differentiation and polarization models for the assessment of responses to CD47–SIRPα blockade more realistically. Another limitation of this study is the lack of growth arrest in target tumor cells in the coculture system. This could have impacted the interpretation of immune-mediated cytotoxicity, as continuous tumor cell proliferation can cause variability in MTT-based viability assays.

CONCLUSION

This study shows that the CD47–SIRPα pathway may serve as a potential target to enhance immune-mediated clearance of MCF-7 breast cancer cells. The results show that SIRPα inhibition does not significantly affect cell viability when used alone without immune effectors, whereas CD47 blockade alone is associated with a reduction in cell viability. The most notable observation was seen with the combination therapy, which indicated a higher trend without statistical significance between the neutralization of the tumor's "don't eat me" signal and the deactivation of the monocyte break switch. As the first study of its kind in Iraq, the study provides a basis for further in vitro investigation to explore potential immunotherapeutic strategies in breast cancer.

ETHICAL CONSIDERATIONS

Ethical approval was issued from Scientific Research Committee College of Science, University of Baghdad (Ref CSEC/1124/0089, Date November 8, 2024) signed by Prof Harith Jabbar Fahad Al-Mathkhury, Head of College of Science Research Ethics Committee.

DATA AVAILABILITY

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

CONFLICT OF INTERESTS

The authors declare no competing interests.

FUNDING

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None.

AI DISCLOSURE

No artificial intelligence (AI) tools were used to create or modify any images in this manuscript.

AUTHOR CONTRIBUTION

ISK: Conceptualization, Methodology, Investigation, Data curation, Formal analysis, Writing – original draft, Writing – review & editing; BJM: Conceptualization, Methodology, Writing – review & editing.

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