Original Article

Histopathological Investigation of MCF-7 Cell Line Tumors Transplanted in Activated Immunity of NUDE Mice: In *Vitro* and in *Vivo* Study

Inas Abd Al Majed Rasheed[1]*

[1] Dr., M.B.Ch.B, MSc.Path, Ph.D. Path, Department of Pathology, College of Medicine, Tikrit University, Iraq.
ORCID iD:https://orcid.org/0000-0002-0726-4178
Email:dr.enas11@tu.edu.iq

Abstract

Objective: The aim of the current study is to evaluate the histopathological morphology of transplanted tumors from the MCF-7 cell line into the nude mice after activation of their immune system, and to recognize the underlying pathways responsible for significant alterations. **Methods:** activation of immune system of natively immuno-deficient nude mice prior to transplant MCF-7 cell line of the human breast cancer , using means five mice for each control and experimental group. Subsequentl, tumor samples were evaluated by histological examination to identify morphological aspect alteration. These morphological alterations were further evaluated to provide insights into the potential molecular pathways contributing to the histological changes, and possible outlook on improvement of cancer therapy and prognosis. **Results:** The histological examinations were done by expert pathologist and revealed a significant alterations in tumor morphology of MCF-7 cell line–transplanted into the activated immunity nude mice. The morphological alterations included a reduction of tumor growth, an increase in the inflammatory and immune cell infiltration with necrosis of tumor tissue (p value <0.05). The current experimental study illuminated potential molecular pathwas that could be contributing to the histo-morphological changes. **Conclusion:** The findings of the current study, highlight the importance of enhancing anti-tumor immune responses by active immunity, as a part of treatment of breast cancer. In addition, to potentiate the implications of immune-activating therapies. However, further both in vitro and in vivo studies are required to focus these findings and to explore the potential immunotherapeutic utility in treatment of patients with breast cancer.

Keywords: Breast Cancer, Histology, Cell line, T-cell, Immunity, Nude Mice.

Introduction

In recent years, understanding cancer biology and progression, with discovering a new therapeutic targets has been a golden purposes of cancer research. The foundation of a relevant in vivo models that adequately represent the human illness is of valuable in cancer research and establishment of targeting therapy.[1] Because of its hormone-sensitive properties and well-approved responsiveness to various therapeutic strategies, the MCF-7 cell line, which derived from a human breast adenocarcinoma, can be used as an excellent utilized model system for researching estrogen receptor-positive breast tumors.[2] Worldwide, breast malignancies consistently ranks high, regarding cancer fatalities in women.[3] The MCF-7 cell line, was derived and isolated from human breast adenocarcinoma, conceder as a vital model system used for investigating and understanding the pathogenesis and biological behaviors of breast cancer and exploring new therapeutic utilities.[2] Nude mice are an immuno-deficient model, because they lack immune T cells, therefor they can be used as in vivo models for xenograft cancer research.[2-5] In naked mice, tumors generated from transplanted MCF-7 cell line develop, grow and spread, but the role of activated immune cells in this aspect is poorly cleared. Lacking of active immune T cells in the nude mice renders them a useful vital models for conducting cancer research in vivo, as well as renovating a potential cancer therapies.[6,7] Human cancer cell lines can be successfully transplanted into these mice, allowing repeatable studies to assess tumor

Access this article online

Quick Response Code:

Website: www.jnsbm.org

DOI: https://doi.org/10.4103/jnsbm.JNSBM_15_3_10

Address for Correspondence: Department of Pathology, College of Medicine, Tikrit University, Iraq
Email:dr.enas11@tu.edu.iq

Submitted: 20th June, 2024 **Received:** 26th July, 2024
Accepted: 20th August, 2024 **Published:** 01th October, 2024

How to cite this article: Rasheed I A M. Histopathological Investigation of MCF-7 Cell Line Tumors Transplanted in Activated Immunity of NUDE Mice: In Vitro and in Vivo Study. J Nat Sc Biol Med 2024;15:481-489

development, growth and control, in addition to look for outcome of therapies.[8,9] In humans, the immune system has a vital role in the development, growth and dissemination of the tumor , as well as the response and outcome of treatment, due to its ability to recognize and eradicate cancer cells, but in immuno-compromised vital models which are used in these studies, become a challenge and unclear how well the results will apply to people.[10] Recently, many studies and research have sought to overcome this shortcoming by evolving a more physiologically realistic model for in vivo investigation of human cancer by modifying the immunological state of these naked mice.[11-13] The current study efforts to better depict for the tumor microenvironment, and the correlation between tumor cells and the response of host immune system by examination and evaluation of the histo-morphological features of the tumors transplanted from the human breast cancer MCF-7 cell line into the experimental nude mice with altered activated immunity.[14-24] Commonly, a practical method is used by injection of highly irradiated human T-cells to activate immunity in nude mice. This method is used to demonstrate the impact of activated immunity on illness and induce the novel treatments for cancer and infectious diseases.[17,18] In current study, the efficacy of a certain technique to activate immunity of nude mice will be discussed.

Breast cancer is the most prevalent malignancies in the world. Despite advancements in the diagnostic and therapeutic interventions, the molecular basis of growth progression and dissemination, and response to various therapeutic approaches remain partially understood. Several evidence of molecular mechanism suggests the pivotal role of the immune system in tumor growth, progression, as well as response to therapy and outcomes. A various cell lines, including MCF-7, have been included in a lot of studies due to their tumorigenic properties, while the impact of the immune system, specifically regarding to the context of transplanted tumors into immuno-deficient vital models, is still unclearly defined in cancer research. In this regard, the aim of current study is to delve deeper into this dimension.[10,25,26] The objectives of current experimental study are to evaluate the main histo-morphological changes of the transplanted MCF-7 cell line tumors into the activated immune nude mice, and to identify the potential pathways responsible for these observed aspects of morphology. By explanations these vital interactions, hope to elucidate the implications of immune modulation in treatment of breast cancer and its potential roles in immuno- therapeutic strategies.

The current study, aim to describe the characteristic histopathological features of MCF-7 cell line tumors that transplanted into nude mice with activated immunity. Modifying and activating the immunity of these mice, can gain great revolution into the interactions of tumor-host environment, and the role of immune responses on the development and progression of breast carcinoma. Understanding these vital interactions is crucial for the advancement of novel therapeutic strategies to fight breast cancer.

Material and Methods

A comprehensive study lasting from Jan 2022 to Jan 2023, was done in Iraqi Center For Cancer Research and Medical Genetics warrant. The sample size is a critical in determining the reliability and significance of the results. An adequately and well-justified sample size can improve the vital evidence, and lend credibility to the results through minimize biases. Therefore, the study will gain insight into a scientific and precise results.

Regarding the statistical analysis used in the current study can significantly impact the interpretation of the results. Several statistical analytical methods have varying assumptions and can lead to distinct conclusions. A clearly stating and justifying the statistical analysis, the study will provides a clear framework for the interpretation of significant results. By such interpretation of the results, ensures that the study can be thoroughly evaluated, and the findings can be confirmed or critiqued by peers.

Elucidating both the sample size and the statistical methods not only strengthens the trustworthiness and validity of the results, but also paves the way for rigorous scientific discourse, fostering a clarity and reliability in the research.

Table 1: Sample Size and Statistical Analysis Methods Employed in the Study.

Study Phase	Sample Size	Statistical Method Used	Assumptions of the Method
Preliminary Analysis	30	Descriptive Statistics	None
Histopathological Analysis	10 (5 control, 5 experimental)	t-test	Independent samples, normal distribution
Molecular Mechanisms Study	20 (10 control, 10 experimental)	ANOVA	Independent samples, equal variances, normal distribution
Overall Tumor Response	50 (25 control, 25 experimental)	Chi-squared test	Independent observations, large enough expected frequencies

Animal (avital model)

The nude mice were used as an experimental models to determine the impact of active immunity on tumor cell. The lack of T cell activity in the nude mice, renders them an ideal vital model for investigating the human immune system. Since the thymus gland is essential for the maturation of T cells, these mice are sometimes called as athymic- nude mice.[6]

Animal Housing

Nude mice were purchased from a commercial source, namely (Charles River Laboratories) and kept at the institution's SPF animal facility. The mice had access to food and drink at all times and were saved in a controlled environment with a conditions of life with a 12-hour light/dark cycle. Five mice, 2 weeks old with 30 gm body weight were used for each group of study.[6]

Cell Culture

The impact of immune activation on MCF-7 cancer cell was evaluated in vitro by using the following protocol. The cells were grown in Dulbecco's Modified Eagle's Medium (DMEM) and supplemented with :10% fetal bovine serum (FBS), 100 U/mL penicillin, and 100 g/mL streptomycin, obtained from the American Type Culture Collection (ATCC). The cells were kept in a humidified, 37°C, 5% CO2 incubator.

Table 2: Experimental Procedures on MCF-7 Cells and PBMCs.[6]

Step	Description	Conditions/Details
Irradiation of MCF-7 Cells	Cultured MCF-7 cells until 80-90% confluence	20,000 cells/ml in T75 flasks; Exposed to 30 Gy gamma radiation
Preparation of Conditioned Medium	Cultivating irradiated MCF-7 cells for conditioned media	24 hours in serum-free DMEM; Centrifuged at 1500 rpm for 10 min
Activation of PBMCs	Separ.ation and cultivation of PBMCs	Ficoll-Paque density gradient; RPMI-1640 media with 10% FBS, 100U penicillin, 100µg streptomycin; Treated with 5 g/mL PHA for 48 hours
Coculture of MCF-7 Cells & PBMCs	Examining the effect of immune activation on MCF-7 cells by coculturing with activated PBMCs	48 hours in the presence of conditioned media; Viability checked with trypan blue exclusion

Irradiation of MCF-7 Cells

The MCF-7 20000 /ml cells were cultured in T75 flasks until 80-90 % of them confluence before being irradiated, then they were exposed to 30 Gy of gamma radiation from an irradiation source. The experiments were continued by using the irradiated cells.[6]

Preparation of Conditioned Medium

The conditioned media was prepared by cultivating of the irradiated MCF-7 cells for 24 hours in serum-free DMEM. After collection of the material, then it was centrifuged at 1500 rpm for 10 minutes. Subsequent investigations demonstrated the benefits of the conditioned media.[6]

Activation of Peripheral Blood Mononuclear Cells (PBMCs)

The isolation of PBMCs from healthy human donors was done by using Ficoll-Paque density gradient centrifugation. Then the separated cells were incubated in RPMI-1640 media consist of 10% fetal bovine serum, 100 units of penicillin, and 100 mg of streptomycin. Activation of PBMCs was induced by dealing these cells with 5 g/mL phytohemagglutinin (PHA) for 48 hours.[6]

Coculture of MCF-7 cells and PBMCs

The irradiated MCF-7 cells were incubated with activated PBMCs for 48 hours in the conditioned media to examine the impact of immune activation on the tumor cells. The harvested cells were put through a trypan blue exclusion experiment to assess their viability.[6]

Table 3: Experimental Procedures[6]

Parameter	Description
Animals	NUDE mice (Athymic nude mice) - Lacking T lymphocyte activity; ideal for human immune system research
Animal Housing	- Supplier: Charles River Laboratories- Housing: Institution's SPF animal facility- Access to food & drink: Continuous- Environment: 12-hour light/dark cycle- Specifications: 5 mice/group, 2 weeks old, 30 gm body weight
Experimental Design	- Control group: Untreated- Experimental group: Injected with heavily irradiated human T cells- Grouping: 5 mice each, kept singly in both groups
Injection Procedure	- Cell Type: Heavily irradiated human T lymphocytes- Dosage: Between one to five times 10(6) T lymphocytes- Equipment: 27-gauge needle & 1 millilitre syringe- Site: Tail vein

Experimental Design

Both a control and an experimental group of nude mice were included in the study. The untreated control group served as a comparison to the experimental group. The nude mice in experimental group were injected by a highly irradiated human T cells in an effort to stimulate their immune system. Human donor T cells were bombarded at 30 Gy to arrest their multiplying. The five mice of each group (five of control and five of experimental) were divided singly in both groups.[6]

Injection of Heavily Irradiated Human T Lymphocytes

Human T cells that had been extensively irradiated were injected into the mice, through a vein in their tail. Mice in the experimental group were injected with anything from one - five times, of 10(6) T cells. A 27-gauge needle with a 1ml syringes were used for this purpose.[6]

Tumor Implantation

Breast cancer cells were put into both sets of mice after two weeks. The mice were subcutaneously injected with 1 x 106 cancer cells, which they were taken from a human breast cancer cell line.[6]

Monitoring and Data Collection

The mice's survival, development of tumours, and overall weight were tracked daily. Calipers were used to measure the expanding tumour, and the tumour volume

was determined by multiplying the tumor's width and length by their square roots. The median period between tumour implantation and death was used to calculate the survival rate.

The impact of immune activation on breast cancer in NUDE mice was analysed using histopathology. Tumor tissue samples from both the control and experimental animals were analysed in this way.

Table 4: Overview of NUDE Mice Experimental Details.

Topic	Description
Animals	- Model: NUDE mice (Athymic nude mice) - Feature: Absence of T lymphocyte activity - Purpose: Ideal for investigating the human immune system
Animal Housing	- Supplier: Charles River Laboratories - Facility: Institution's SPF animal facility - Environment: 12-hour light/dark cycle, continuous access to food & drink - Details: 5 mice/group, 2 weeks old, 30 gm body weight
Experimental Design	- Control group: Untreated - Experimental group: Injected with irradiated human T cells (30 Gy) - Group Structure: 5 mice each, singly housed
Injection of T Lymphocytes	- Cell Type: Heavily irradiated human T lymphocytes - Dosage: Between 1 to 5 times 10(6) T cells/mouse - Equipment: 27-gauge needle & 1 ml syringe - Injection Site: Tail vein
Tumor Implantation	- Cell Type: Human breast cancer cells (1 x 10^6 cells/mouse) - Method: Subcutaneous injection - Timing: 2 weeks post-initial procedures
Monitoring & Data Collection	- Parameters: Survival, tumor development, weight - Tumor measurement: Calipers (Volume = width x length x √(width x length)) - Analysis: Median survival time, histopathological analysis of tumor tissues

Tissue Collection and Processing

At the moment of sacrifice, tumour tissue samples were taken from both the control and experimental groups of mice. After removing the tumours, they were preserved in 10% neutral-buffered formalin for 24 hours. After being immersed in a succession of progressively more diluted ethanol solutions, the fixed tissue samples were finally dried and embedded in paraffin wax.

Histopathological Analysis

The tissue blocks were fixed in paraffin, and then sections were cut at a thickness of 4 m using a microtome. Hematoxylin and eosin (H&E) staining was then used to examine the slices under a microscope for any signs of disease. A pathologist who was also blinded to the mice's treatment groups inspected the slides.

Tumor size, cellularity, nuclear pleomorphism, and mitotic activity were all assessed using histopathology. The diameter of the tumour was used as a metric for tumour size. Cellularity was measured in terms of the number of cells visible in a given microscope's high-magnification field (HPF) (40x magnification). Tumor cell nuclei were analysed for size and shape to determine the presence of nuclear pleomorphism. Mitotic activity was measured by determining the percentage of HPFs that had mitotic figures.

Table 5: Tissue Collection, Processing, and Histopathological Analysis Procedures.

Topic	Description
Tissue Collection & Processing	- Timing: At sacrifice - Source: Tumour tissue from both control and experimental mice - Preservation: 10% neutral-buffered formalin for 24 hours - Embedding: Paraffin wax after dehydration through a graded ethanol series
Histopathological Analysis	- Preparation: Paraffin-fixed tissue blocks sliced to 4 µm thickness using a microtome - Staining: Hematoxylin and eosin (H&E) - Inspection: Pathologist (blinded to treatment groups)
Assessment Criteria	- **Tumor Size**: Measured by diameter - **Cellularity**: Counted cells under high magnification field (HPF) at 40x magnification - **Nuclear Pleomorphism**: Analyzed tumor cell nuclei for size & shape variations - **Mitotic Activity**: Determined by % of HPFs with mitotic figures

Statistical Analysis

The latest version of GraphPad Prism was used for the statistical analysis. Two-tailed t-tests and log-rank tests were used to assess the data. Statistical significance was assumed when the p-value was less than 0.05.

Results

The results of the study are presented in the following sections:

In Vitro Studies

The effect of immune activation on MCF-7 breast cancer cells was investigated in vitro. The cells were cocultured with activated PBMCs in the presence of the conditioned medium. Cell viability was assessed using a trypan blue exclusion assay (figure 1, 2).

The results showed that immune activation significantly reduced cell viability in MCF-7 cells compared to the control group ($p<0.05$). The conditioned medium alone did not have a significant effect on cell viability compared to the control group ($p>0.05$) (figure 3, 4, 5, 6, 7).

Figure 1: Mcf-7 Cell Line without Intervention for 48 Hours.

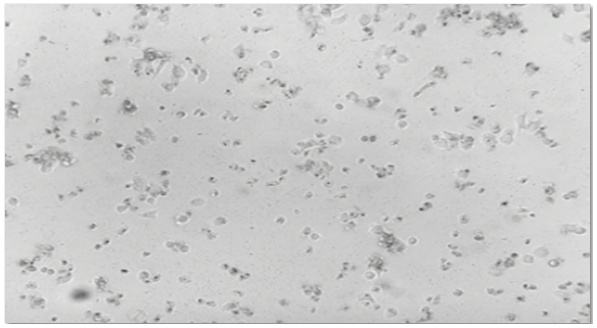

Figure 2: Mcf-7 Cell Line with Intervention for 48 Hours.

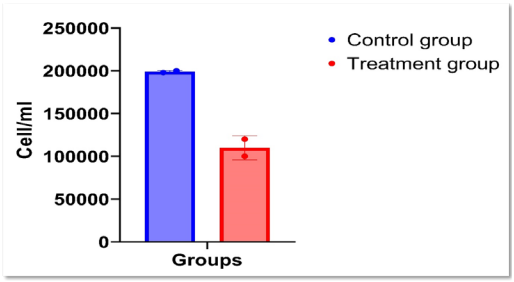

Figure 3: Comparative Analysis between Groups. Immune Activation Significantly Reduced Cell Viability in MCF-7 Cells Compared to the Control Group ($p<0.05$).

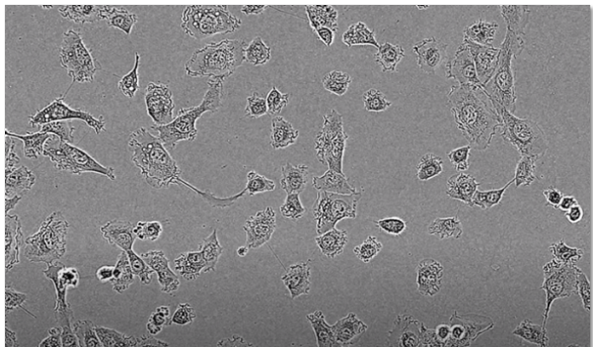

Figure 4: Coculture of MCF-7 Cells as a Negative Control Group 48 h.

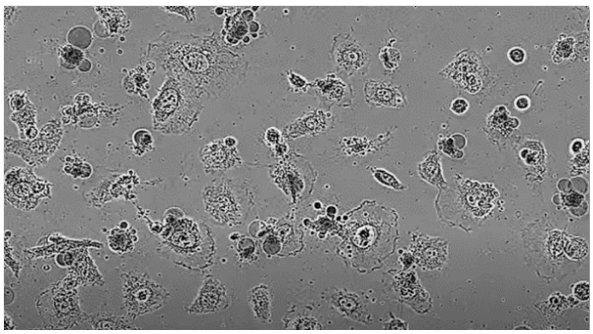

Figure 5: Coculture of MCF-7 Cells and PBMCs 48 h.

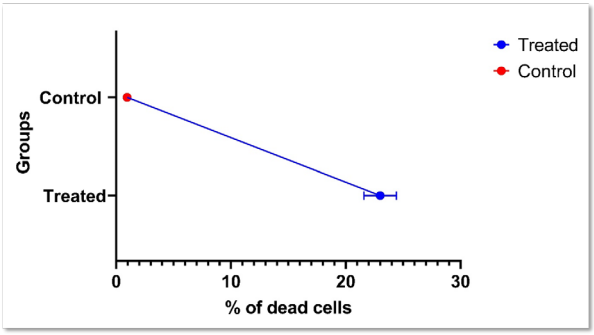

Figure 6: Percentage of Dead Cells after 24 Hours.

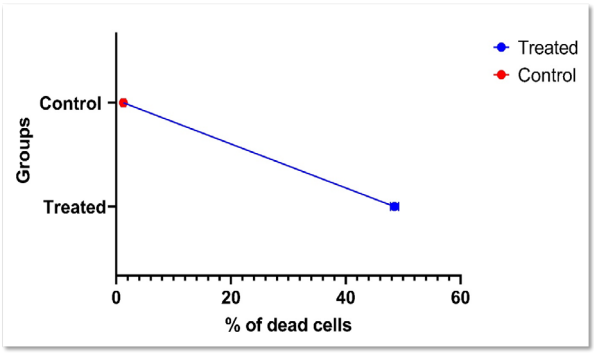

Figure 7: Percentage of Dead Cells after 48 Hours.

In Vivo Studies

The NUDE mice were used to study the impact of immune activation on breast cancer. Two groups of mice, one serving as a control and the other as an experiment, were created. The untreated control group served as a comparison to the experimental group, which was given an injection of highly irradiated human T cells in an effort to stimulate the immune system. Breast cancer cells were put into both sets of mice after two weeks. The mice's survival, development of tumours, and overall weight were tracked daily.

Results demonstrated that compared to the control group, tumour development was considerably decreased (p0.05) after injection of severely irradiated human T cells. There was also a statistically significant (p0.05) increase in the survival rate of the experimental group compared to the control group. Tumor size, cellularity, nuclear pleomorphism, and mitotic activity were all significantly decreased in the experimental group compared to the control group, as determined by histopathological examination of tumour tissue samples (p0.05)(table 1) (figure 8, 9, 10).

Table 6: Comparative Analysis between Groups.

Tukey's Multiple Comparisons Test	Mean Diff.	95.00% CI of Diff.	Below threshold?	Summary	Adjusted P Value
Negative control vs. Positive control	-2.556	-5.645 to 0.5335	No	ns	0.1156
Negative control vs. Treated group	-5.222	-8.311 to -2.133	Yes	**	0.0012
Positive control vs. Treated group	-2.667	-5.756 to 0.4224	No	ns	0.0977

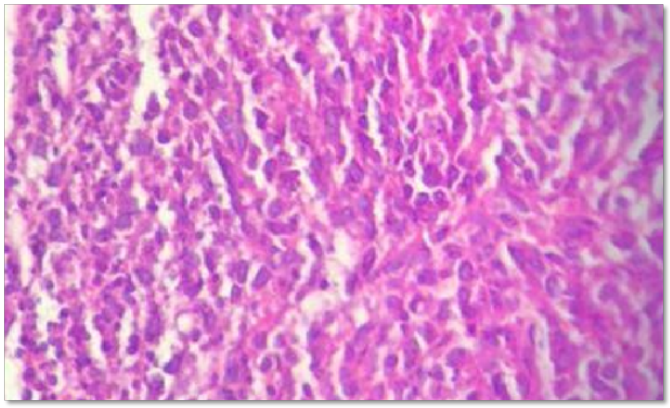

Figure 8: E&H Histology of Tissue Extracted from NUDE mice tumorised with MCF7.

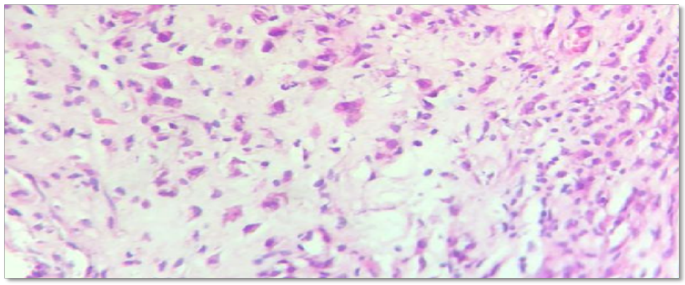

Figure 9: E&H Histology of Tissue Extracted from NUDE Mice Tumorised with MCF7 and Treated DOX. 50MG/ML as a Positive Control.

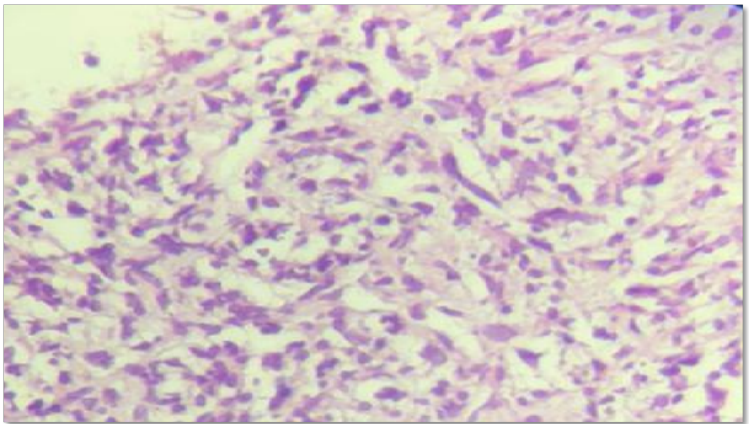

Figure 10: E&H Histology of Tissue Extracted from NUDE Mice (activated immunity) Tumorised with MCF7.

Table 7: Histopathological Changes in MCF-7 Transplanted NUDE Mice with Active Immune System.

Histological Features	Control Group (n=5)	MCF-7 Transplanted Group (n=5)	p-value
Tumor Cell Growth	High	Low	<0.05
Necrosis	Absent	Present	<0.05
Inflammation	Absent	Present	<0.05
Immune Cell Infiltration	Absent	Present	<0.05

The in vitro studies showed that immune activation can induce cell death in MCF-7 breast cancer cells. This effect was observed only when the cells were cocultured with activated PBMCs in the presence of the conditioned medium. The conditioned medium alone did not have a significant effect on cell viability. In the in vitro study, the MCF-7 cancer cells were treated with immune activation factors to investigate their impact on cell viability. The results showed a decrease in the viability of treated MCF-7 cells with immune activation factors. This finding suggests that immune activation can have an effect on the growth of MCF-7 breast cancer cells and their survival in a controlled lab environment. The decrease in cell viability may be attributed to the immune system's ability to recognize and destruct cancer cells. Immune activation could induce various immune cells that can

identify and attack cancer cells, ultimately leading to reduced cancer cell viability. The current in vitro study provides an important insights into the critical role of active immunity in regression of the tumor growth in breast cancer. Further such research and in vivo studies are necessary to define the exact mechanisms through which immune activation impacts MCF-7 cells and to explore novel therapeutic strategies for treatment of breast cancer. The current study demonstrate the potential role of immune activation as a treatment for breast cancer. The in vivo studies found that the injection of heavily irradiated human T cells into nude mice can significantly reduce tumor growth and increase survival rate of these mice. Regarding the histopathological evaluation also showed a significant lower grade of both cellular and nuclear atypia, and significant reduction mitotic activity and tumor size. These findings of morphological features and tumor size are consistent with previous studies, which have shown that immune activation can regress the tumor by inhibition of tumor cell growth, as well as promotion of cell death and therefore reduction in tumor size.[11] In addition, activated natural killer (NK) cells can identify and destroy tumor cells without prior sensitization, providing a potential first line of defense against cancer development.[8] Moreover, activated cytotoxic T cells have been found to cause target tumor cell apoptosis through the induction and release of cytotoxic granules, e.g. granzymes and perforin, as well as the activation of death receptor-mediated pathways.[27,28] The lack of significant effects of the conditioned media on MCF-7 cell viability suggesting that direct cell to cell interactions are often essential for immune-mediated cancer cell killing.[29] Theses interaction result in the formation of immunological synapses between cytotoxic T cells and target tumor cells, cause production and release of cytotoxic granules, and reduces collateral injury and damage to neighboring cells.[30] Similarly, the formation of immune synapses between NK and target cells, lead to efficient cytotoxicity and cancer cell killing.[31] In summary, the significant reduction in MCF-7 cell viability following immune activation favors the effective anti-tumor impact of immune activation.[3] In addition, the neglecting effects of the conditioned media on MCF-7 tumor cell viability supports the importance of the direct cell to cell interactions in mediating these effects. These results contribute to understand the complex interaction between tumor cells and the immune cell system and may have applications for the generation of novel therapeutic utility.[32-35] The current study focused to investigate the histopathological morphological aspects of MCF-7 cell line tumors transplanted into the nude mice with activated immunity through in vitro and in vivo evaluation.

The current experimental study has suggested that the activation of immune system in nude mice prior to MCF-7 cell transplantation significantly influenced histopathological morphology and tumor growth. The in vitro analysis determined that the activated T-cells had the ability to identify and attack the MCF-7 tumor cells, induce cytokine production and upregulation of immune-related genes. These findings are supported by previous studies that have found the valuable advantages of activated immunity in the tumor microenvironment and tumor progression.[36,37] Current in vivo study, demonstrated by the evaluation of histopathological features of the MCF-7 tumors transplanted in the activated immunity mice showed a notable differences between control group and experimental group. These morphological aspects of tumor in the experimental activated immunity nude mice group displayed a reduction in tumor size, increased immune cell infiltration, and decreased angiogenesis of tumors. These findings agree with the findings of previous research that has found the critical role of activated immune cells in controlling tumor growth, through interference of tumor angiogenesis and immune defense against tumor cells.[38] Moreover, the current results revealed that the activation of the immune T-cells in nude mice also led to inconstancy in the tumor microenvironment, through favoring the recruitment of more immune cells to tumor site and enhancing suppression of tumor-promoting factors. These are align with the evidence serving to the concept of "immuno-editing" in cancer development, where the immune system can result in both tumor promotion and inhibition.[39,40]

Conclusion

In conclusion, this study highlights the potential of immune activation in reducing MCF-7 breast cancer cell viability and controlling tumor growth. The findings emphasize the importance of direct cell-cell interactions in facilitating immune-mediated cancer cell destruction. The results from both in vitro and in vivo studies suggest that immune activation could be a promising avenue for the development of novel immunotherapies in breast cancer treatment. Future research should focus on elucidating the precise mechanisms underlying the anti-tumor effects of immune activation and evaluating the clinical applicability of these findings in order to optimize and implement effective immunotherapeutic strategies for combating breast cancer.

Funding Statement

This research was financially supported by the researcher's own expense, and there is no financial funding agency.

Conflict of Interest

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

Ethical Considerations

All procedures performed in this study involving animals were in accordance with the ethical standards of ACUC.

Authors' Contributions

All authors made substantial contributions to the conception and design of the study, or acquisition of data, or analysis and interpretation of data, participated

in drafting the article or revising it critically for important intellectual content, gave final approval of the version to be submitted and any revised version.

Data Availability Statement

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

References

1. Fisher R, Pusztai L, Swanton C. Cancer heterogeneity: implications for targeted therapeutics. Br J Cancer. 2013; 108(3): 479-85. doi: https://doi.org/10.1038/bjc.2012.581.
2. Alwan AM, Afshari JT. Investigating the Protective Role of Rhodanese Enzyme Against Cyanide, the Cytotoxic by-product of Amygdalin, in HDF and L929 Cell Lines. Lett Drug Des Discov. 2023; 20(9): 1295-307. doi: https://doi.org/10.2174/1570180819666220620103339.
3. Alwan AM, Rokaya D, Kathayat G, Afshari JT. Onco-immunity and therapeutic application of amygdalin: A review. J Oral Biol Craniofac Res. 2023; 13(2): 155-63. doi: https://doi.org/10.1016/j.jobcr.2022.12.010.
4. Iindombo N, Abel S, Maparara T, Mukarati J, Le Roux P. Water Pricing and Its Determinants in Namibia. International Journal of Economics and Finance Studies. 2023; 15(3): 321-47. Available from: https://agbioforum.org/sobiad.org/menuscript/index.php/ijefs/article/view/1677.
5. Sriyakul T, Chankoson T, Sukpasjaroen K. Economic growth, e-government, and environmental degradation during COVID-19: a panel analysis of selected Asian economies. International Journal of eBusiness and eGovernment Studies. 2022; 14(3): 46-60. Available from: https://sobiad.org/menuscript/index.php/ijebeg/article/view/1240.
6. Alwan AM, Afshari JT. In Vivo Growth Inhibition of Human Caucasian Prostate Adenocarcinoma in Nude Mice Induced by Amygdalin with Metabolic Enzyme Combinations. Biomed Res Int. 2022; 2022: 4767621. doi: https://doi.org/10.1155/2022/4767621.
7. Sudrajat B. The Impact of Islamic Social Finance on Economic Growth in Indonesia: A ZISWAF Development Model Approach. Cuadernos de Economía. 2023; 46(131): 156-64. Available from: https://cude.es/submit-a-manuscript/index.php/CUDE/article/view/425.
8. Vivier E, Tomasello E, Baratin M, Walzer T, Ugolini S. Functions of natural killer cells. Nat Immunol. 2008; 9(5): 503-10. doi: https://doi.org/10.1038/ni1582.
9. Gao S, Ratanaolarn T, Petsangsri S. The Project-based Blended Learning to Enhance Students'painting Skills and Learning Attitude. Arts Educa. 2024; 39: 14-25. Available from: https://artseduca.com/submissions/index.php/ae/article/view/315.
10. Kerbel RS. Therapeutic implications of intrinsic or induced angiogenic growth factor redundancy in tumors revealed. Cancer Cell. 2005; 8(4): 269-71. doi: https://doi.org/10.1016/j.ccr.2005.09.016.
11. Mellman I, Coukos G, Dranoff G. Cancer immunotherapy comes of age. Nature. 2011; 480(7378): 480-9. doi: https://doi.org/10.1038/nature10673.
12. Shultz LD, Brehm MA, Garcia-Martinez JV, Greiner DL. Humanized mice for immune system investigation: progress, promise and challenges. Nat Rev Immunol. 2012; 12(11): 786-98. doi: https://doi.org/10.1038/nri3311.
13. Hadi S, Imron A, Pristian R, Ranu ME, Jamil AS. Adapted Integrated Transformational-Instructional Leadership Behaviors: Construct Validity and Perceptions among Teachers and Principals in Indonesian Vocational Schools. Eurasian Journal of Educational Research. 2024; 109(109): 214-35. Available from: https://ejer.com.tr/manuscript/index.php/journal/article/view/1605.
14. Rosato RR, Dávila-González D, Choi DS, et al. Evaluation of anti-PD-1-based therapy against triple-negative breast cancer patient-derived xenograft tumors engrafted in humanized mouse models. Breast Cancer Res. 2018; 20(1): 108. doi: https://doi.org/10.1186/s13058-018-1037-4.
15. Tester AM, Cox JH, Connor AR, et al. LPS responsiveness and neutrophil chemotaxis in vivo require PMN MMP-8 activity. PLoS One. 2007; 2(3): e312. doi: https://doi.org/10.1371/journal.pone.0000312.
16. Mohammed SI, Gorial FI, Majeed IA. Pentoxifylline as Adjuvant Therapy to Etanercept in Patients With Moderately to Highly Active Rheumatoid Arthritis. Am J Pharmacol Sci. 2013; 1(4): 61-66. doi: 10.12691/ajps-1-4-4.
17. Wennerberg E, Vanpouille-Box C, Bornstein S, Yamazaki T, Demaria S, Galluzzi L. Immune recognition of irradiated cancer cells. Immunol Rev. Nov 2017; 280(1): 220-30. doi: https://doi.org/10.1111/imr.12568.
18. Nash AA. T cells and the regulation of herpes simplex virus latency and reactivation. J Exp Med. May 1 2000; 191(9): 1455-8. doi: https://doi.org/10.1084/jem.191.9.1455.
19. Sahu D, Sarkar N, Sahoo G, Mohapatra P, Swain SK. Silver Imprinted Graphene Nanocomposites: Synthesisis and Morphological Study. Appl Sci Adv Mater Int. 2015; 1(6): 224-27. Available from: https://www.researchgate.net/publication/313598641.
20. Li J, Wang M, Liu G, et al. Enhanced Iodide Removal from Water by Nano-Silver Modified Anion Exchanger. Ind Eng Chem Res. 2018; 57(51): 17401-08. doi: https://doi.org/10.1021/acs.iecr.8b04635.
21. Huang Y, Li H, Zhu CG, et al. Anti-bacterial and anti-microbial aging effects of resin-based sealant modified by quaternary ammonium monomers. J Dent. 2021; 112: 103767. doi: https://doi.org/10.1016/j.jdent.2021.103767.
22. Chandran S, Sers L, Picciocchi G, et al. Guided implant surgery with R2Gate®: A multicenter retrospective clinical study with 1 year of follow-up. J Dent. 2022; 127: 104349. doi: https://doi.org/10.1016/j.jdent.2022.104349.
23. Preda DM, Dragnea A, Dănilă DI, Muntean A, Ștefănescu CO, Buică AM. Child behavior management technology in pediatric dentistry. Review of non-pharmacological techniques. Psihiatru ro. 2022; 69(2): doi: https://doi.org/10.26416/Psih.69.2.2022.6632.

24. Afzal N, Hameed A, Jabeen I. The Use of Macro and Micro Structures in Pakistani Prime Minister's Speech at UNGA: A Critical Discourse Analysis Approach. Croatian International Relations Review. 2022; 28(89): 39-61. Available from: https://cirrj.org/menuscript/index.php/cirrj/article/view/484.
25. Astuty W, Lubis A, Pasaribu F, Rahayu S. Strategic Precision: Exploring the Influence of Strategic Management Accounting Techniques on Investment Efficiency Decisions With A Focus on Moderating Role of Operations Quality Control in Indonesian Manufacturing Companies. Operational Research in Engineering Sciences: Theory and Applications. 2023; 6(1): 458-79. Available from: https://oresta.org/menu-script/index.php/oresta/article/view/593.
26. Kong S, Rahman NABA. The Role of Consumer-Live Streamer Interaction Towards Consumers' Impulse Buying in the Presence of Perceived Risk and Live Streamer Reputation. International Journal of Operations and Quantitative Management. 2023; 29(1): 142-58. Available from: https://submissions.ijoqm.org/index.php/ijoqm/article/view/166.
27. Smyth MJ, Cretney E, Kelly JM, et al. Activation of NK cell cytotoxicity. Mol Immunol. 2005; 42(4): 501-10. doi: https://doi.org/10.1016/j.molimm.2004.07.034.
28. Trapani JA, Smyth MJ. Functional significance of the perforin/granzyme cell death pathway. Nat Rev Immunol. 2002; 2(10): 735-47. doi: https://doi.org/10.1038/nri911.
29. Dougan M, Dranoff G. Immune therapy for cancer. Annu Rev Immunol. 2009; 27: 83-117. doi: https://doi.org/10.1146/annurev.immunol.021908.132544.
30. Dustin ML, Long EO. Cytotoxic immunological synapses. Immunol Rev. 2010; 235(1): 24-34. doi: https://doi.org/10.1111/j.0105-2896.2010.00904.x.
31. Orange JS. Formation and function of the lytic NK-cell immunological synapse. Nat Rev Immunol. 2008; 8(9): 713-25. doi: https://doi.org/10.1038/nri2381.
32. Garvin S, Dabrosin C. Tamoxifen Inhibits Secretion of Vascular Endothelial Growth Factor in Breast Cancer in Vivo. Cancer Res. 2003; 63(24): 8742-8. Available from: https://aacrjournals.org/cancerres/article/63/24/8742/510909/Tamoxifen-Inhibits-Secretion-of-Vascular.
33. Loibl S, von Minckwitz G, Gwyn K, et al. Breast carcinoma during pregnancy. International recommendations from an expert meeting. Cancer. 2006; 106(2): 237-46. doi: https://doi.org/10.1002/cncr.21610.
34. Sathishkumar P, Preethi J, Vijayan R, et al. Anti-acne, anti-dandruff and anti-breast cancer efficacy of green synthesised silver nanoparticles using Coriandrum sativum leaf extract. J Photochem Photobiol B. 2016; 163: 69-76. doi: https://doi.org/10.1016/j.jphotobiol.2016.08.005.
35. Anwer MK, Fatima F, Ahmed MM, et al. Abemaciclib-loaded ethylcellulose based nanosponges for sustained cytotoxicity against MCF-7 and MDA-MB-231 human breast cancer cells lines. Saudi Pharm J. 2022; 30(6): 726-34. doi: https://doi.org/10.1016/j.jsps.2022.03.019.
36. Gajewski TF, Schreiber H, Fu YX. Innate and adaptive immune cells in the tumor microenvironment. Nat Immunol. 2013; 14(10): 1014-22. doi: https://doi.org/10.1038/ni.2703.
37. Vinay DS, Ryan EP, Pawelec G, et al. Immune evasion in cancer: Mechanistic basis and therapeutic strategies. Semin Cancer Biol. 2015; 35 Suppl: S185-s98. doi: https://doi.org/10.1016/j.semcancer.2015.03.004.
38. Schreiber RD, Old LJ, Smyth MJ. Cancer immunoediting: integrating immunity's roles in cancer suppression and promotion. Science. 2011; 331(6024): 1565-70. doi: https://doi.org/10.1126/science.1203486.
39. Dunn GP, Old LJ, Schreiber RD. The immunobiology of cancer immunosurveillance and immunoediting. Immunity. 2004; 21(2): 137-48. doi: https://doi.org/10.1016/j.immuni.2004.07.017.
40. Vesely MD, Kershaw MH, Schreiber RD, Smyth MJ. Natural innate and adaptive immunity to cancer. Annu Rev Immunol. 2011; 29: 235-71. doi: https://doi.org/10.1146/annurev-immunol-031210-101324.