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The Effect of Photobiomodulation on the Conditioned Media of 3T3-L1 Cells in the Treatment of Breast Cancer

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Abstract

Introduction: Breast cancer ranks among the most prevalent malignancies, and its prompt diagnosis significantly amplifies the prospects of successful treatment. Approximately one in seven women will experience a breast cancer diagnosis in their lifetime. Stromal cells and their secreted factors exert various effects on tumor growth, impacting proliferation, invasion, and metastasis. Research has emphasized the significant impact of proteins secreted by adipose tissue on breast cancer proliferation, surpassing the influence of factors released by other cell types. Yet, the specific transcription factors and cofactors involved in adipokine expression in the tumor microenvironment remain enigmatic.

Methods: In this study, adipocyte cells were cultured and exposed to 980 nm and 650 nm Photobiomodulation. The MDA-MD-231 cells (triple negative cancer cell line) were cultured with a conditioned medium from laser-treated cells. The real-time assay was employed to analyze the gene expression level changes involved in apoptosis.

Results: Results showed that the irradiated conditioned medium at 980 nm and 650 nm caused a reduction in cell viability of cancer cells. Conversely, the conditioned medium from the irradiated cells triggered an increase in the expression of *Caspase 3*, *Caspase 9*, and *BAX2* genes, alongside a decrease in *BCL2* gene expression.

Conclusion: The findings highlighted the potential of the laser-treated conditioned medium to induce apoptosis pathways in cancer cells, demonstrating a promising avenue for further research in utilizing low-level laser therapy in breast cancer treatment.

Keywords: Breast cancer; Photobiomodulation; Gene expression; Conditioned medium.

Introduction

Breast cancer is the second fatal cancer among women worldwide. In 2023, an estimated 297 790 new cases were diagnosed, accounting for approximately 31% of all common cancers in women.[1] The survival rate for breast cancer patients varies significantly, with higher rates in developed countries (reaching 80%) and lower rates in developing nations (dropping to less than 40%).[2]

Early diagnosis is crucial for improving patient outcomes and survival. By detecting breast cancer at an early stage, when it is more likely to be localized and treatable, doctors can significantly increase the chances of successful treatment and long-term survival. Therefore, regular breast cancer screenings and self-examinations are essential for early detection and timely intervention.[3] Targeted treatments, radiation therapy, and a variety of surgical and therapeutic methods are employed in patients with breast cancer. In certain cases, the preventive measure of a bilateral mastectomy is considered.[4]

While different treatments have proven effective, the progress in laser therapy and radiotherapy for breast cancer control is undeniable. However, aggressive treatments like surgery can result in physical injuries, and methods such as chemotherapy or radiotherapy come with their own set of side effects.[5] Photothermal therapy and photodynamic therapy (PDT) are rapidly gaining recognition as promising alternatives to invasive surgical methods for cancer treatment.[6-8]

Emerging research suggests a potential link between breast fat and the development of breast cancer.[9,10] Another targeted approach involves investigating the impact of various drugs such as Lunasin, Genistein, and Curcumin on the adipose tissue that feeds breast cancer cells.[11-13]

Photobiomodulation (PBM) utilizes visible light to promote wound healing and tissue regeneration. This therapy offers numerous benefits, including enhanced cell health, anti-inflammatory effects, pain management, and tissue repair.[14] Research suggests that PBM may

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have potential benefits in cancer treatment by affecting the tumor microenvironment, modulating immune responses, and sensitizing cancer cells to other therapies. The ability of PBM to influence cell behavior and gene expression makes it a promising adjunct therapy in oncology. PBM impacts various cell signaling pathways, including those involved in apoptosis, proliferation, and differentiation.[15] By selectively targeting specific signaling cascades, PBM can regulate cellular processes and contribute to overall cellular homeostasis.[16]

Photobiomodulation may stimulate cellular activity and metabolism.[17] In breast cancer, this could potentially affect cancer cell growth and proliferation. Furthermore, Apoptosis induction may help in targeting and reducing breast cancer cell viability.[18] PBM exerts a notable influence on stem cells, which is believed to be mediated by mitochondrial redox signaling pathways. By modulating mitochondrial activity, PBM may affect cancer cell behavior.[19]

Thus, the objective of this study was to explore the effects of radiation at two wavelengths of 980 and 680 nm on the conditioned medium of the 3T3-L1 cell line and further investigate its impact on breast cancer tissue cells.

Materials and Methods

Cell Culture

The 3T3-L1 and MDA-MB-231 cell lines were cultured in the DMEM medium, supplemented with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin, and maintained at 37 °C in a humidified environment with 5% CO2. Once they reached 80%–90% confluence, the cells were trypsinized and seeded in a 96-well plate (Figure 1).

Laser Treatment

3T3-L1 cells were seeded in a 96-well plate with an empty well between samples to minimize radiation interference. Once fully attached, the cells were exposed to laser radiation in a dark environment. Diode laser radiation was applied at 980 nm wavelength and 5 mW power (P1 Dental Laser, Pioon, China) with a dosage of 4 J/cm^2. Identical conditions were maintained for the 650 nm wavelength, as detailed in Table 1.

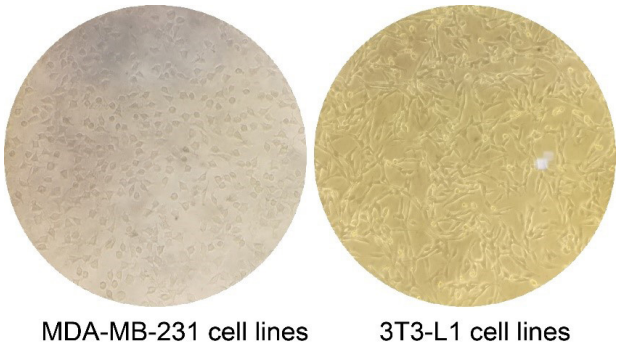

Figure 1. The 3T3-L1 Preadipocytes and MDA-MB-231 Cell Culture Images

MTT Assay

Cell viability was evaluated by using the MTT assay. MDA-MB-231 cells were seeded at a density of 6×10^3 cells per well in a 96-well plate. After exposure to the conditioned medium, the cells were incubated with 0.5 mg/mL MTT solution at 37 °C for 4 hours. The resulting formazan crystals were dissolved in 100 µL DMSO per well, followed by a 10-minute incubation in the dark. Absorbance was measured at 570 nm by using a plate reader. Cell viability was determined relative to untreated control cells for all treatment groups.

Analysis of Gene Expression

The total RNA from each group (control and treated) was isolated by the RNeasy Mini kit (Qiagen, USA) following the manufacturer's guidelines. Subsequently, the quantity and quality of the extracted RNA were assessed by using UV spectrophotometry (Eppendorf, Germany). For cDNA synthesis, 500 ng DNase-treated RNA samples were utilized in conjunction with the QuantiTect Reverse Transcription Kit, employing oligo[20] primers. The PCR reactions utilized specific primers (refer to Table 2). Quantitative polymerase chain reaction (q-PCR) was conducted by utilizing Cyber Green and Primer in an Applied Biosystems StepOne™ thermal cycler (Applied Biosystems, USA). The PCR program commenced with an initial melting cycle lasting for 5 minutes at 95 °C to prime the polymerase, succeeded by 40 cycles involving melting (30 seconds at 95 °C), annealing (30 seconds at 58 °C), and extension (30 seconds at 72 °C). The experiments were performed in triplicate or duplicate. The qPCR analyses were performed with the Livak ($2^{-\Delta\Delta Ct}$) method. The quality of PCR reactions was verified via melting

Table 1. The Irradiation Parameters

Parameters	Value
Wavelength (nm)	650 and 980
Power output (W)	0.05
Energy density (J/cm^2)	4
Time (s)	80
Operation	Continuous wave
Spot size (cm)	1

Table 2. Sequence of Primers Used in This Research

Gene		Sequence (5'->3')	Annealing Temperature
GAPDH	F: 5′→3′ R: 5′→3′	GTCTCCTCTGACTTCAACAGCG ACCACCCTGTTGCTGTAGCCAA	60 °C
BCL-2	F: 5′→3′ R: 5′→3′	ATCGCCCTGTGGATGACTGAGT GCCAGGAGAAATCAAACAGAGGC	60 °C
CAS3	F: 5′→3′ R: 5′→3′	GGAAGCGAATCAATGGACTCTGG ACAGTCCGCCTAGAAGCATTTG	60 °C
CAS9	F: 5′→3′ R: 5′→3′	GTTTGAGGACCTTCGACCAGCT CAACGTACCAGGAGCCACTCTT	60 °C
BAX	F: 5′→3′ R: 5′→3′	TCAGGATGCGTCCACCAAGAAG TGTGTCCACGGCGGCAATCATC	60 °C

curve analysis. Moreover, the primer efficiency for each gene was ascertained through a standard curve, involving a logarithmic dilution series of cDNA from each sample.

Statistical Analysis

All data were presented as the mean ± standard deviation (SD) of a minimum of three replicates and were analyzed by using one-way analysis of variance (ANOVA) with a Tukey post hoc test, employing GraphPad Prism version 8 software (GraphPad Software, USA). *P* values below 0.05 were considered statistically significant.

Results

Cell Viability

In this study, the 3T3-L1 cell line was treated with Photobiomodulation. Cell viability decreased over time (Figures 2 and 3). The percentage of cancer cell viability was calculated through the MTT test. This decrease was even more significant at a 650 nm wavelength, as depicted in Figure 2. These results demonstrate that the therapeutic effect is influenced by the wavelength used.

Laser Therapy

In Figure 4, which shows the effect of the conditioned medium under 650 nm radiation on the breast cancer cell line, there was a significant increase in *BAX* gene expression compared to the control group after 24 (*P* value ≤ 0.05) and 48 (*P* value ≤ 0.001) hours. *Caspase 3* gene expression significantly increased compared to the control group only after 48 hours (*P* value ≤ 0.05).

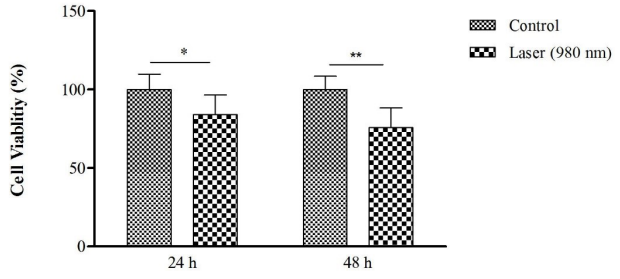

Figure 2. Cell viability percent after 24 hour (P value ≤ 0.05) and 48 hours (*P* value ≤ 0.01) compared to the control group after laser irradiation with 980 nm intensity

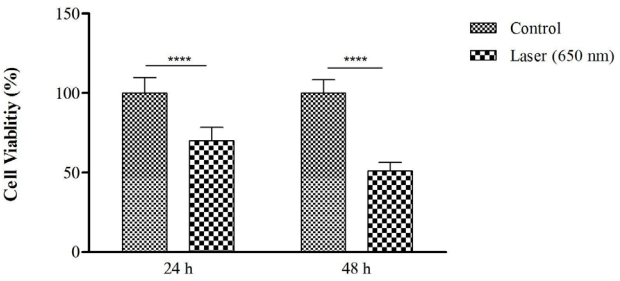

Figure 3. The Cell viability percent after 24 hours (P value ≤ 0.001) and 48 hours (*P* value ≤ 0.001) compared to the control group after laser irradiation with 650 nm intensity

RT-PCR

There was no significant difference between the 24-hour group and the control group. 650 nm laser radiation in the *BCL2* group led to a decrease in the expression of this gene, but this decrease was not statistically significant. Caspase 9 gene expression significantly increased after 48 hours compared to the control group (*P* value ≤ 0.001). Additionally, its expression showed a significant increase after 48 hours compared to 24 hours (*P* value ≤ 0.05). No significant difference was found between the 24-hour group and the control group.

Expression of genes involved in apoptosis, in MDA-MB-231 cells that were treated with a conditioned medium of 3T3-L1 cells under 980 nm radiation, revealed a significant increase in *BAX* gene expression after 48 hours of laser irradiation compared to the control group (*P* value ≤ 0.05). However, this relationship was not significant in the group that was exposed to radiation for 24 hours, as indicated in Figure 5.

Discussion

Chemotherapy is a mainstay in treating solid tumors, but its efficacy is often hindered by adverse effects such as nausea, vomiting, and damage to healthy cells, posing significant challenges for patients.[20]

In this study, preadipocyte cells were treated with laser radiation at differing intensities, and their conditioned medium was exposed to breast cancer tissue cells (MDA-MB-231). The cell survival rates, revealing significant increases over 48 hours, yielded *P* values of ≤ 0.001 and ≤ 0.01. Research by Hoseinmardi et al demonstrates

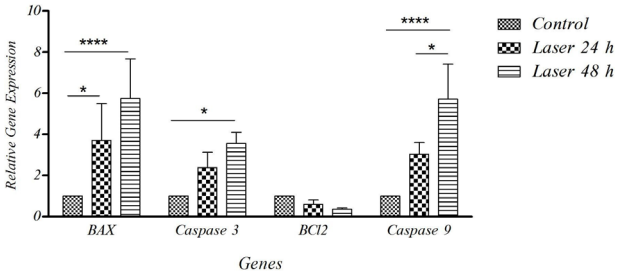

Figure 4. Comparison of Gene Expression in Laser and Control Groups at 24 and 48 hours

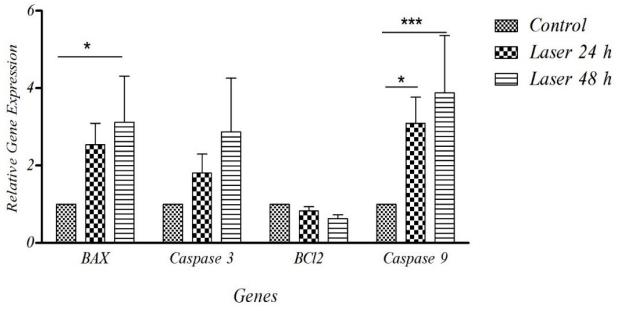

Figure 5. Caspase 9 Gene Expression After 24 Hours (*P* value ≤ 0.05) and 48 hours (*P* value ≤ 0.001)

that photobiomodulation on melanoma cell lines diminishes cancer cell survival.[21]

The bidirectional interaction between normal and cancer cells profoundly influences breast cancer development, metastasis, and treatment resistance. Studies have demonstrated that cancer cells with elevated expression of tumor necrosis factor-α and interleukin-1β can induce neighboring cells to adopt a cancer-promoting phenotype through paracrine signaling, thereby contributing to cancer progression. Consequently, cytokines play a critical role in mediating communication between normal and cancerous cells.[22]

The current investigation reveals that laser radiation at 650 nm and 980 nm affects the survival of adipose tissue fibroblast cell line (3T3-L1), crucial in providing nutrition to cancer cells, resulting in decreased survival rates at 24 and 48 hours. Furthermore, this radiation induces an elevation in the expression of apoptotic genes *BAX*, *Caspase 9*, and *Caspase 3*, indicating an anti-tumor effect of this radiation intensity in breast cancer treatment. Comparative analysis of the experimental outcomes indicates that the 650 nm beam exhibits enhanced efficacy. Apoptosis, a tightly regulated form of cell death dependent on the sequential activation of caspases, plays a pivotal role in numerous physiological and pathological processes, including embryonic development, tissue homeostasis, and the elimination of malignant cells subjected to adverse conditions or cytotoxic therapies such as chemotherapy and radiation therapy.[20]

In the context of photobiomodulation and its potential impact on cellular processes such as apoptosis (via caspase pathways), certain photoreceptors may play a role in transducing light signals into biological responses. Two main classes of photoreceptors commonly associated with light-mediated pathways are flavoproteins and opsins.

Flavins, such as flavin mononucleotide and flavin adenine dinucleotide, can absorb light in the blue and ultraviolet spectrum. Upon light absorption, flavoproteins may undergo conformational changes or generate reactive oxygen species, potentially influencing downstream signaling pathways, including those involving caspases. Opsins (light-sensitive proteins found in photoreceptor cells of the retina), especially melanopsin (a non-visual opsin), have been implicated in non-image-forming functions such as circadian rhythms and light-induced biological effects.[23,24]

Caspase 3 (CASP3) occupies a central position in the execution of apoptosis, and many cancer cells are believed to employ CASP3 deactivation as a resistance mechanism against cytotoxic agents. Furthermore, CASP3 exerts a regulatory influence over stress-responsive immunomodulatory pathways, including the secretion of type I interferons. In a study conducted by da Lima et al, which examined the effects of low-level lasers and LEDs on human breast cancer cells, photobiomodulation induced by low-level red laser and blue LED did not alter cell viability or migration but did result in reduced cell invasion in breast cancer cells. These findings are consistent with contemporary research in the field.[25]

Karimi et al. studied the effect of red laser radiation and ajwain essential oil on two-dimensional and three-dimensional breast cancer cell culture models of MDA-MB-231. The results showed that laser therapy increases the anti-cancer effect of AEO and has a positive effect on inhibiting the growth of cancer cells.[26] Additionally, Javani et al investigated the effects of PDT, encompassing both blue laser and zinc oxide QDs, on MDA-MB-231 cancer cells. Their findings demonstrated the inhibition of cancer markers and the induction of apoptosis.[7]

In vitro studies examining the effects of PBM on breast cancer cell lines and 3T3-L1 cells have shown promising results in terms of cytotoxicity, apoptosis induction, and modulation of cellular pathways.[27,28]

Clinical trials and in vivo investigations have established the safety of photobiomodulation therapy (PBMT) with respect to tumor growth, while also highlighting its benefits in preventing and treating specific complications associated with cancer therapy. Human studies, corroborated by animal research, provide evidence for the safety of PBMT when employed within recommended clinical parameters, particularly in the context of head and neck cancer. Notably, PBMT has demonstrated potential for improving overall survival outcomes in cancer patients.[18] PBMT may act as a radiosensitizer and increase the sensitivity of cancer cells to chemotherapy. The literature suggests that PBMT is safe and effective and has potential benefits for patient outcomes.[14,29]

The results of this study indicate that laser radiation stimulates adipose tissue fibroblast cells to secrete mediators, whose contents effectively influence the expression of apoptosis-related genes, potentially aiding in the control and treatment of breast cancer.

Conclusion

Upon analyzing the findings of our study, it became evident that radiation at 650 nm and 980 nm intensities holds the potential to influence the secretions of adipose tissue fibroblast cells. Subsequently, when breast cancer cells were treated with the conditioned medium from these cells, there was a notable increase in the expression of apoptotic genes such as *Caspase 3*, *Caspase 9*, and *BAX*, whereas the expression of the anti-apoptotic *Bcl-2* gene decreased. Considering these collective outcomes, it can be suggested that irradiation of 3T3-L1 cells and the utilization of cell surface secretions could represent a novel treatment approach in addition to the existing modalities.

Authors' Contribution

Conceptualization: Maryam Sinaei.
Data curation: Maryam Sinaei.

Formal analysis: Saba Sekhavat.
Funding acquisition: Maryam Sinaei.
Investigation: Saba Sekhavat.
Methodology: Saba Sekhavat.
Project administration: Atousa Moradzadegan.
Resources: Maryam Sinaei.
Software: Maryam Sinaei.
Supervision: Atousa Moradzadegan.
Validation: Jaber Zafari.
Visualization: Maryam Sinaei.
Writing–original draft: Maryam Sinaei.
Writing–review & editing: Maryam Sinaei.

Competing Interests

The authors declare no competing interests.

Ethical Approval

This research was approved by the research ethics committee of Islamic Azad University of Science (IR.IAU.D.REC.1401.040).

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