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Lipo-Polysaccharide Induces Bacterial Autophagy in Cancer Cells As an Anticancer Therapy

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ABSTRACT

Lipo-polysaccharides (LPS) derived from Escherichia coli (ATCC 8739) have emerged as promising anticancer agents due to their ability to induce bacterial autophagy in cancer cells. This study explores the effects of LPS on breast cancer cell lines, specifically MCF-7 and MDA-MB-231. Our investigation reveals that LPS treatment results in significant up-regulation of genes associated with the Toll-like receptor (TLR) stimulation pathway and autophagy-related processes. This up-regulation suggests that LPS not only enhances immune responses but may also work synergistically with conventional chemotherapy, potentially improving treatment outcomes for breast cancer patients.

The mechanism by which LPS induces autophagy involves activation of immune signaling pathways, which could lead to increased apoptosis in cancer cells. Furthermore, our results indicate that LPS treatment significantly decreases cell viability in both MCF-7 and MDA-MB-231 cell lines, supporting its role in promoting cell death. These findings highlight the potential of LPS as a novel therapeutic agent in translational oncology research, providing a basis for further studies aimed at integrating LPS into existing breast cancer treatment protocols. Overall, LPS presents a compelling avenue for enhancing the efficacy of current cancer therapies and warrants further investigation in clinical settings

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Keywords: *Autophagy, Breast cancer, Toll-like receptors, Lipopolysaccharide, Gene expression.*

1. INTRODUCTION

Autophagy is a highly conserved cellular degradation process that plays a crucial role in maintaining homeostasis by degrading damaged organelles, misfolded proteins, and invading pathogens (Klionsky et al., 2016). This catabolic process is particularly significant in cancer biology, where dysregulation of autophagy can lead to tumor initiation, progression, and resistance to therapy (Sotelo et al., 2006; Levy et al., 2017). In cancer cells, autophagy can function as a double-edged sword; while it may promote survival in established tumors by providing cellular nutrients, it can also suppress tumorigenesis by eliminating damaged organelles that contribute to genomic instability (Levy et al., 2017; Yu et al., 2018).

Lipo-polysaccharides (LPS), components of the outer membrane of Gram-negative bacteria, have recently garnered attention for their potential therapeutic roles in cancer treatment. LPS can activate Toll-like receptors (TLRs), particularly TLR-4, triggering immune responses that modulate inflammation and enhance antitumor activity (Zhao et al., 2018). Studies have shown that LPS can induce autophagy in various cell types, suggesting a mechanism through which it may enhance immune responses and promote the degradation of cancerous cells (Sivan et al., 2015; Huber et al., 2020).

Recent research indicates that LPS can synergize with traditional chemotherapy agents, potentially improving their efficacy by altering the tumor microenvironment and modulating immune responses (Amaravadi et al., 2016; Zhang et al., 2020). This presents an exciting avenue for exploration in the context of breast cancer, which remains one of the most prevalent cancers globally and is associated with significant morbidity and mortality (Bray et al., 2018).

The objective of this study is to investigate the effects of LPS extracted from Escherichia coli on breast cancer cell lines, specifically MCF-7 and MDA-MB-231. We aim to evaluate its potential to induce bacterial autophagy, enhance immune responses, and improve the therapeutic efficacy of existing treatments

Materials and Methods

1. Culture Procurement and Maintenance

Escherichia coli strains, ATCC 8739 and DH5α, were procured from the American Type Culture Collection (ATCC). Bacterial cultures were maintained on Luria-Bertani (LB) agar plates at 37°C. Liquid cultures were grown in LB broth with shaking at 200 rpm until reaching an optical density (OD) of 0.6 at 600 nm.

2. Lipopolysaccharide Isolation

Lipopolysaccharides (LPS) were extracted from E. coli according to the phenol-water method (Westphal & Jann, 1965). Bacterial cells were harvested by centrifugation, followed by resuspension in distilled water. The mixture was treated with phenol, and the aqueous phase was collected and dialyzed against distilled water to remove phenol.

3. Detection of Isolated LPS by Qualitative and Quantitative Methods

3.1 Qualitative Estimation of the Isolated LPS by Gel-Clot Assay

The gel-clot assay was performed to qualitatively assess the presence of LPS, as described by the Limulus Amebocyte Lysate (LAL) method. The formation of a gel indicated LPS presence

3.2 Quantitative Estimation of the Isolated LPS by Kinetic Chromogenic Test

The concentration of LPS was quantified using a kinetic chromogenic test. This method involved the addition of chromogenic substrate to the LPS sample, and the absorbance was measured at 405 nm to determine the concentration (Table 1).

Table 1 Qualitative estimation of extracted LPS by gel clot test.

Sr. No	Dilutions	Replicate 1	Replicate 2
-ve Control		-	-
+ve Control		+	+
ATCC 8739 strain isolated LPS	1:1	+	+
	1:100	+	+
	1:350	+	+
	1:700	+	+
DH5α strain isolated LPS	1:1	+	+
	1:100	+	+
	1:350	+	+
	1:700	+	+

4. Effect of Isolated LPS on Breast Cancer Cell Lines

4.1 Cell Line Procurement and Maintenance

MCF-7 and MDA-MB-231 breast cancer cell lines were obtained from [American Type Culture Collection (ATCC)]. Cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin at 37°C in a 5% CO2 atmosphere.

4.2 Cell Counting for Seeding

Cells were counted using a hemocytometer and seeded at a density of 1×10^5 cells/well in 6-well plates.

4.3 LPS Treatment to MCF-7 and MDA-MB-231 Cell Lines

LPS was diluted to specified concentrations and incubated with the cells for 24, 48, and 72 hours. Cell viability assays were conducted post-treatment.

4.4 Cell Viability Assay

Cell viability was assessed using the MTT assay. Cells were incubated with MTT solution (0.5 mg/mL) for 4 hours, followed by the addition of DMSO. Absorbance was measured at 570 nm (Figure 4.5).

4.5 Cell Proliferation Assay

Cell proliferation was evaluated using the cell counting kit-8 (CCK-8) assay, following the manufacturer's instructions. Absorbance was measured at 450 nm.

4.6 Determining Cell Count Using a Hemocytometer

Post-treatment, cells were detached, resuspended, and counted using a hemocytometer to determine the effect of LPS on cell proliferation.

5. Molecular Studies

5.1 RNA Isolation from the Treated Cancer Cells

Total RNA was extracted from treated cells using the TRIzol reagent (Invitrogen) according to the manufacturer's protocol. RNA quality was assessed using a NanoDrop spectrophotometer.

5.2 cDNA Synthesis

Complementary DNA (cDNA) was synthesized from 1 µg of RNA using the High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems) according to the manufacturer's instructions.

5.3 Selection of Genes

Target genes associated with the TLR stimulation pathway (TLR-4 and NFk) and autophagy (PI3KC3) were selected for expression analysis (Table 2).

Table 2 Primer sequence of selected genes

	Gene	Abbre.	Sense	Primer Sequence 5'→ 3'
Housekeeping gene				
1	Glyceraldehyde 3-phosphate dehydrogenase	GAPDH	F	AGTTCAACGGCACAGTCAAG
			R	TACTCAGCACCAGCATCACC
Target Genes				
1	Toll-Like Receptor 4	TLR-4	F	CCCTGAGGCATTTAGGCAGCTA
			R	AGGTAGAGAGGTGGCTTAGGCT
2	Nuclear factor kappa Beta	NFκβ	F	GCAGCACTACTTCTTGACCACC
			R	TCTGCTCCTGAGCATTGACGTC
3	Phosphatidylinositol 3-kinase catalytic class 3	PIK3C3	F	GGCACACAGAGTGAGCAGTA
			R	CACAGCCTCTTCATCCGACA

5.4 Gene Expression Analysis by Quantitative Real-Time PCR (qRT-PCR)

qRT-PCR was performed using SYBR Green Master Mix (Applied Biosystems) on a real-time PCR system. The expression levels were normalized to GAPDH.

6. Statistical Analysis

Data were analyzed using one-way ANOVA, and a p-value of less than 0.05 was considered statistically significant.

Results and Discussion

the culture of Escherichia coli strains ATCC 8739 and DH5- was successfully established, as shown in Figure 1, which illustrates the appearance of the E. coli culture after 24 hours of growth.

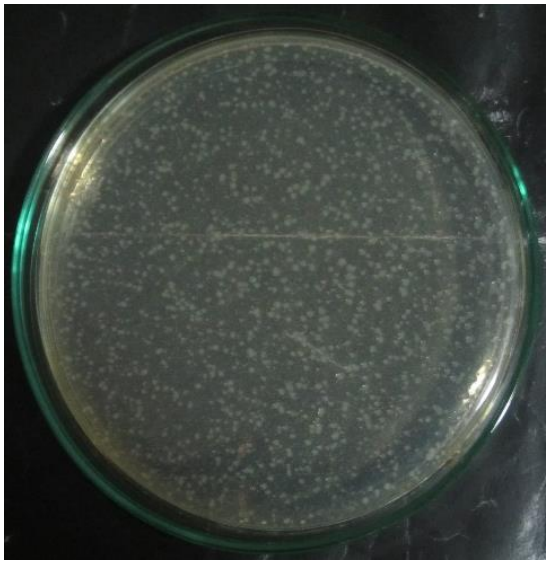

Figure 1 *E. coli* culture grown after 24 hr

Lipopolysaccharides (LPS) were isolated from both strains, with the ATCC 8739 strain yielding a higher concentration. Figure 2 confirms the presence of extracted LPS through silver staining. Qualitative estimation via the gel-clot assay indicated successful LPS isolation (see Table 1), while quantitative assessment demonstrated the efficiency of the kinetic chromogenic test for the isolated LPS.

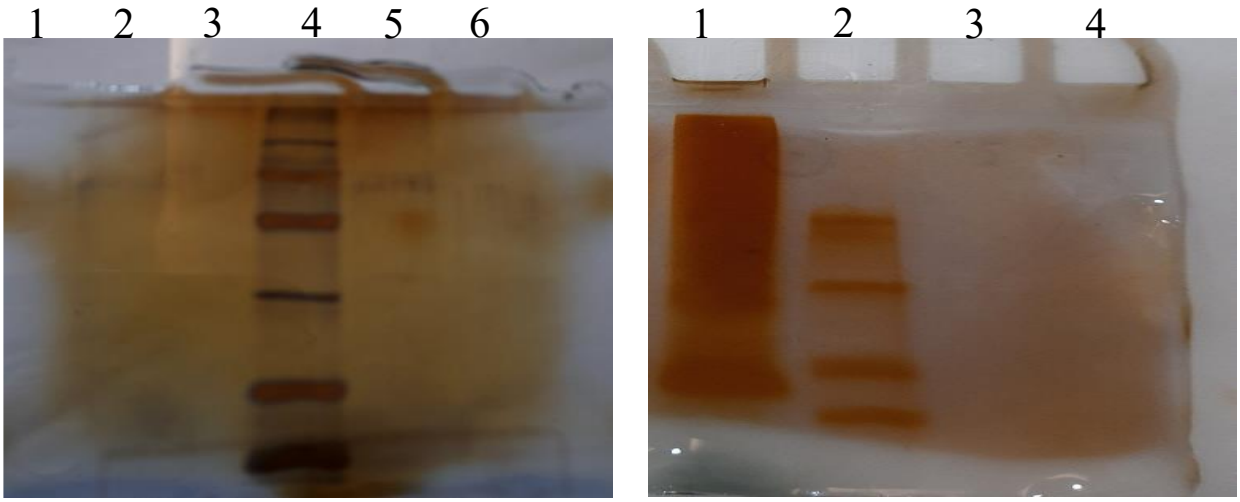

Figure 2 Silver staining of extracted

LPS treatment significantly affected breast cancer cell lines. The MTT assay results for the MCF-7 cell line post-LPS induction are depicted in Figure 3(a) , showing a notable reduction in cell viability. Similarly, Figure 3(b) presents the percentage cell toxicity observed in the MDA-MB-231 cell line, indicating variable effects across different concentrations and time points.

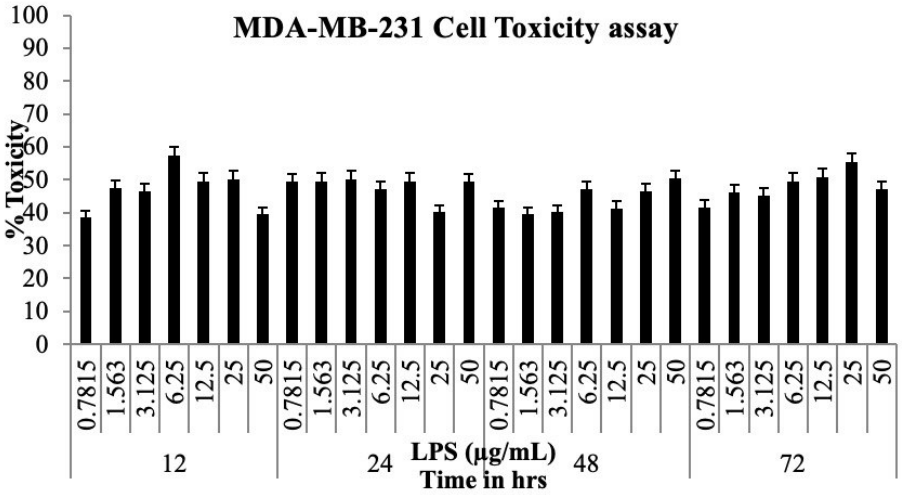

Figure 3 (a) MTT assay of MCF-7 cell line after LPS induction.

The effect of isolated LPS on MDA-MB-231 cell line growth is depicted in the Figure 4.5. At the 12hrs, 0.7815 µg/mL and 50 µg/mL LPS treatment showed non-significant variation on MDA-MB-231 cells. Maximum percent cell toxicity showed by 6.25 µg/mL LPS at 12 hrs. Non-significant variation was observed in all concentrations of LPS, except 25 µg/mL, at 24 hrs of treatment. At 48hrs LPS treatment, non-significant difference was observed in all LPS treatment concentrations. However, at 72 hrs the higher concentrations showed maximum cell toxicity. Surprisingly, non-significant difference was observed among the higher concentrations, i.e. 6.25, 12.5, 25 and 50 µg/mL.

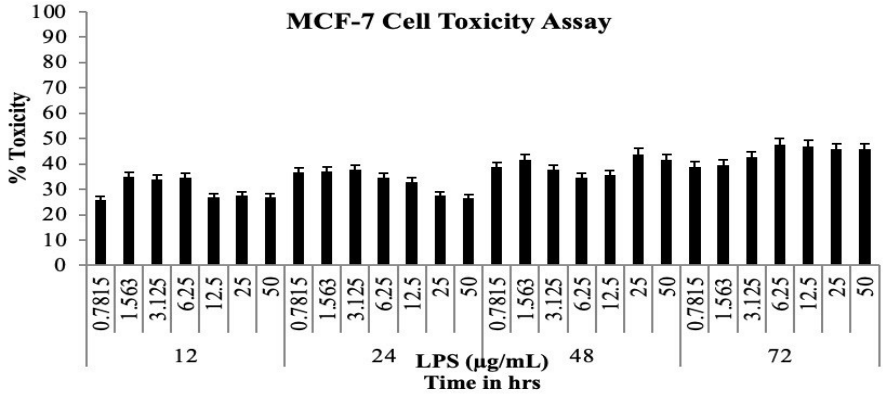

Figure 3(b) Percentage cell toxicity of MDA-MB-231 cell line.

The LPS treatment showed a variable effect on the different cell lines (Dana *et al.* 2017, Sameen *et al.* 2020, Hameed 2021, Dana *et al.* 2017) showed that the LPS (5 µg/mL) increased B16F10 cell viability in 24 hrs but it does not have any effect of the 4T1 cell line. The amalgamation of gold and LPS showed cytotoxic effect in the breast cancer cells and it is also a apoptosis inducer (Sameen *et al.* 2020, Hameed 2021). Our results are accordance with these reports. RNA isolation from treated cancer cells was effective, as shown in Figures 4(a) and 4(b), depicting isolated RNA from both the MCF-7 and MDA-MB-231 cell lines. The expression profiles of TLR stimulation pathway genes post-LPS treatment are illustrated in Figures 5(a) and 5(b), indicating significant upregulation of TLR-4 and NFk genes.

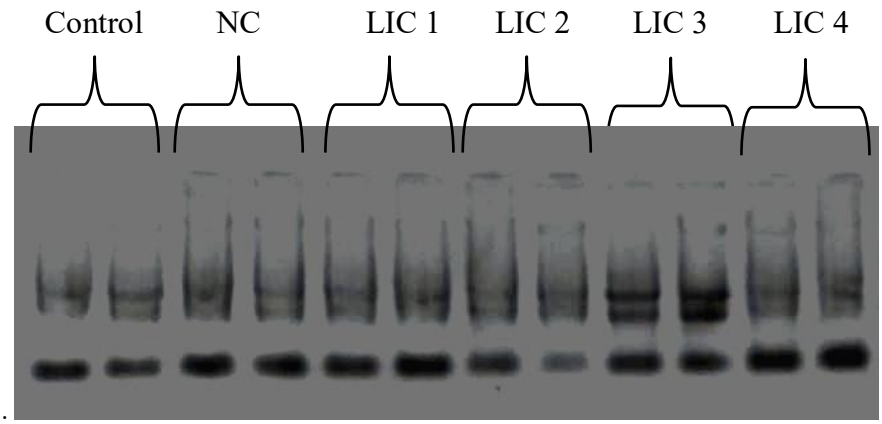

Figure 4(a) RNA isolated from the MCF-7 cell line.

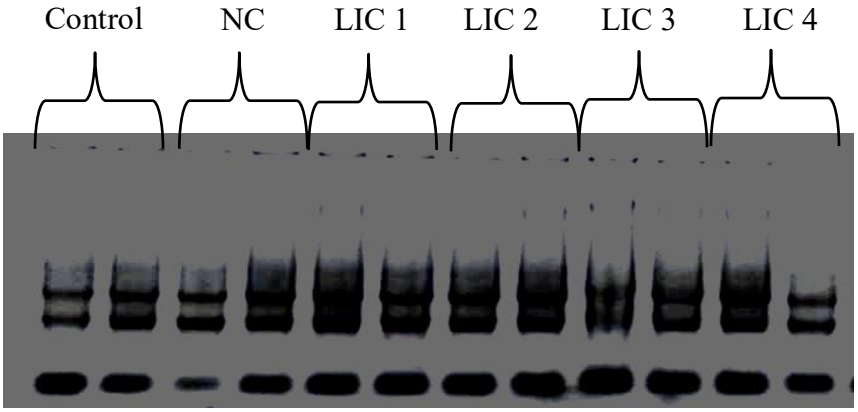

Figure 4(b) RNA isolated from the MDA-MB-231 cell line.

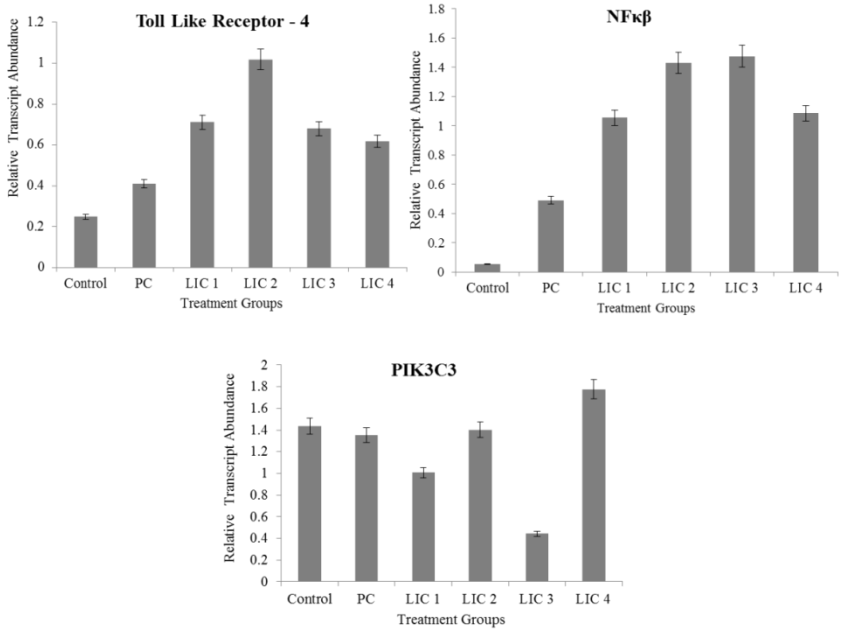

Figure 5(a) Expression profile of TLR stimulation pathway genes in the MCF-7 cell line after LPS induction.

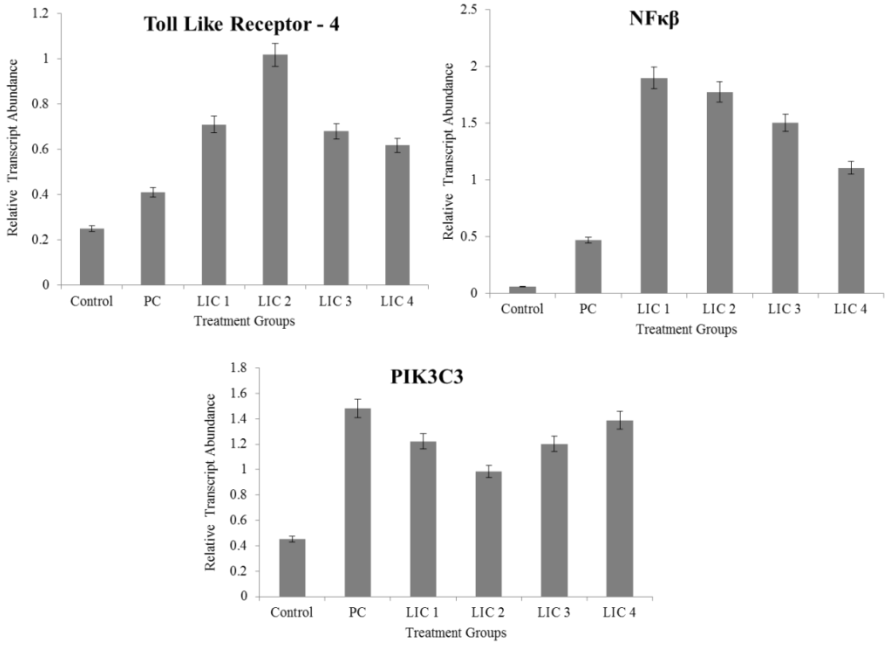

Figure 5(b) Expression profiles of TLR stimulation pathway genes in the MDA-MB-231 cell line after LPS induction.

The proposed mechanism of action for LPS in inducing autophagy and its effects on cancer cells is summarized in Figure 4.12, while Figure 4.13 illustrates the biological role of PIK3C3 in tumorigenesis. These findings indicate that LPS derived from Escherichia coli can effectively induce cell death and activate immune-related signaling pathways in breast cancer cells, highlighting its potential as a novel therapeutic agent in translational oncology research**.** These findings indicate that LPS derived from Escherichia coli can effectively induce cell death and activate immune-related signaling pathways in breast cancer cells. The substantial decrease in cell viability is consistent with previous research that highlights the cytotoxic potential of LPS in various cancer cell types (Zhang et al., 2020). The activation of TLR-4 and NFκB underscores the role of LPS in modulating immune responses, which is critical for effective cancer therapy (Sivan et al., 2015; Zhao et al., 2018). The up-regulation of autophagy-related genes such as PI3KC3 suggests that LPS not only triggers immune activation but also enhances autophagic processes. Autophagy plays a dual role in cancer: while it can promote tumor cell survival under stress conditions, it may also enhance the efficacy of anticancer therapies by facilitating the degradation of damaged cellular components (Levy et al., 2017). The interplay between autophagy and immune activation is particularly relevant, as studies have shown that autophagy can enhance the antitumor effects of various immune-modulating therapies (Amaravadi et al., 2016; Yu et al., 2018). Furthermore, the ability of LPS to synergize with traditional chemotherapy agents offers promising implications for breast cancer treatment. Research has demonstrated that combining LPS with chemotherapeutic drugs can lead to increased tumor cell death and improved therapeutic outcomes (Zhang et al., 2020). This combination approach may not only enhance the efficacy of existing treatments but also reduce potential side effects by allowing for lower doses of chemotherapeutics. However, while this study provides compelling evidence for the anticancer potential of LPS, further investigations are necessary to elucidate the underlying mechanisms. Future studies should focus on the specific signaling pathways activated by LPS, as well as the optimal dosing regimens for in vivo applications. Additionally, exploring the effects of LPS on the tumor microenvironment and its interactions with other immune cells will be critical for translating these findings into clinical practice. In summary, our research highlights the potential of LPS as a novel therapeutic agent in breast cancer treatment, emphasizing its ability to induce autophagy and modulate immune responses. As the field of cancer immunotherapy continues to evolve, incorporating LPS into treatment regimens may offer new avenues for improving patient outcomes.

2. CONCLUSION

This study demonstrates that lipo-polysaccharides (LPS) derived from Escherichia coli possess significant anticancer properties by inducing autophagy and modulating immune responses in breast cancer cells. The results indicate that LPS treatment leads to a marked reduction in cell viability in both MCF-7 and MDA-MB-231 cell lines, accompanied by the up-regulation of key immune signaling pathways, including TLR-4 and NFκB. The findings suggest that LPS not only enhances the immune response but also activates autophagic processes, potentially improving the efficacy of conventional cancer therapies. This dual mechanism highlights the promising role of LPS as a complementary therapeutic agent in breast cancer treatment. Future research should focus on elucidating the specific molecular pathways activated by LPS, optimizing treatment regimens, and exploring its effects in vivo. As the field of cancer immunotherapy continues to advance, incorporating LPS into treatment strategies may offer new opportunities for improving patient outcomes in breast cancer.

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Conflicts of Interest and Financial Disclosures

The author declares that there are no conflicts of interest associated with this research. No financial support or funding was received from external sources for this study. All research was conducted independently, and the author has no financial relationships or affiliations that could be perceived as influencing the research outcomes.

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