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Investigating The Role of ORAI Calcium Channels On Lipopolysaccharide-Mediated Inflammatory Signaling

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INTRODUCTION

Several studies found an association between breast cancer progression and chronic inflammation [1]. TLR4 is a major inflammatory receptor which is activated by lipopolysaccharide (LPS) [2]. Treating breast cancer cells with LPS has been shown to enhance the production of inflammatory mediators, oncogenic genes, and migration of breast cancer cells [3,4]. STIM-ORAI pathway facilitate calcium entry through specific calcium channels called ORAI channels and is thought to be essential in inflammatory signaling in immune cells [5]. Previous studies have shown that this pathway can be activated by the drug Thapsigargin and inhibited by the drug BTP2 [6,7].

OBJECTIVES

Hypothesis:

We hypothesized that using pharmacological agents that targets STIM-ORAI pathways will influence LPS-induced inflammatory signaling and the subsequent breast cancer migration.

Aims:

1- Determine the effect of the drug Thapsigargin, which activates STIM-ORAI pathway, on LPS-induced production of inflammatory genes and migration of breast cancer.

2-Investigate the effect of the drug BTP2, which is an inhibitor of STIM-ORAI pathway, on LPS-indued production of inflammatory cytokines in breast cancer cells

METHODS

Experimental design:

Breast cancer cells MDA-MB-231 (estrogen receptor-negative) were treated with:

- Control (vehicle of Serum-free media with DMSO)
- LPS alone (10 ug/ml) for 24 or 48 hours.
- LPS for 24 or 48 hours with either Thapsigargin (100nM) or BTP2 (1uM).
- Real-time PCR was used to determine gene expression of (COX-2, IL-6, and IL-8).
- ELISA was used to measure (PGE2) proteins expression.
- Cell migration was measured using an in-vitro Scratch assay.

STATISTICAL ANALYSIS:

- Each data will represent at least three independent experiments and statistical differences between different groups will be analyzed using a one-way analysis of variance (ANOVA) test followed by Tukey post hoc test. A value of $p < 0.05$ will be considered statistically significant.

RESULTS

Figure1) Thapsigargin significantly enhanced LPS-induced activation of COX-2 /PGE-2 pathway in MDA-MB-231 cells:

A:qRT-PCR analysis for mRNA of COX-2. MDA-MB-231 were treated with vehicle, LPS (10 µg/ml), LPS (10 µg/ml)+TG (100nM) for 24 hours. B: ELISA immunoassay demonstrate the level of prostaglandin E2 (PGE2) in MDA-MB-231 cell.

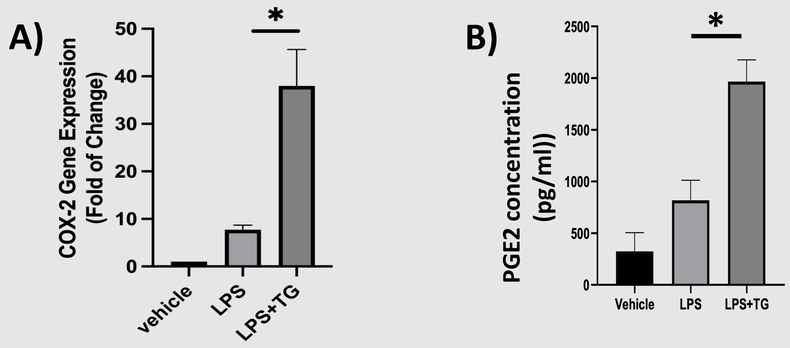


Figure 2) Thapsigargin significantly potentiated LPS-induced production of inflammatory genes in MDA-MB-231 cells

qRT-PCR analysis for mRNA of IL-6(C), IL-8(D).

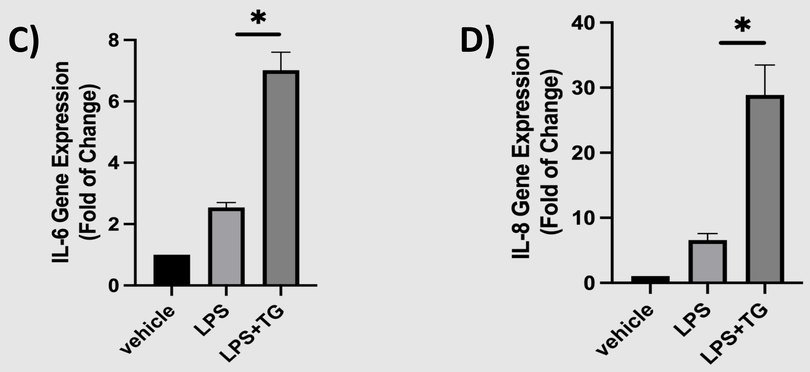


Figure 3) LPS-induced migration of MDA-MB-231 cells is Promoted by Thapsigargin.

(E) Microscopic image of MDA-MB-231 migration after scratch (4x magnification). (F) Quantitative graph of the migrating cells (percentage).

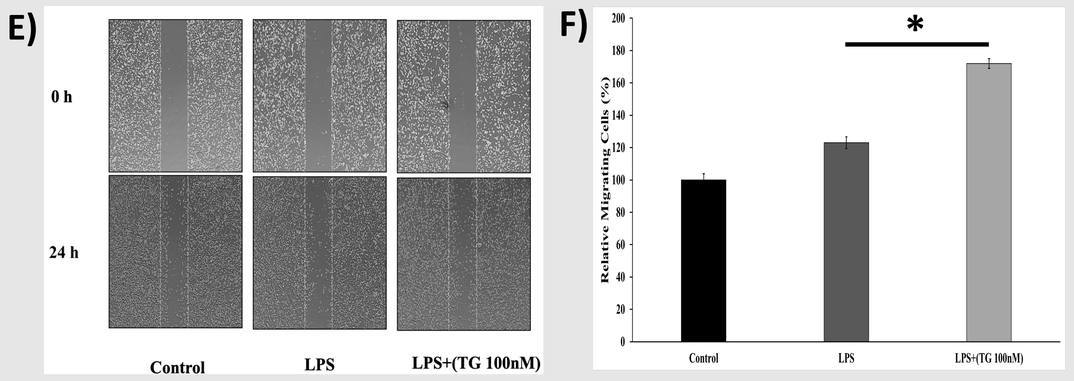
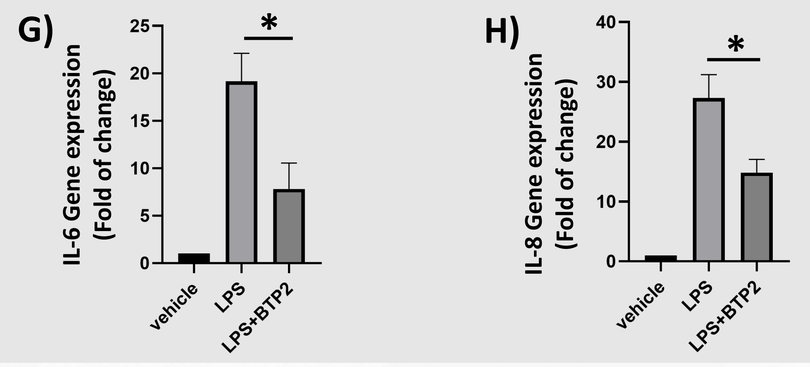


Figure 4) BTP2 significantly inhibit LPS-induced production of inflammatory cytokines in MDA-MB-231 cells:

qRT-PCR analysis for mRNA of IL-6,(G) IL-8(H).



DISSCUTION AND CONCLOSIONS

Collectively, the data obtained from this project suggest that pharmacologically targeting STIM-ORAI pathway using Thapsigargin which is known to activate this pathway enhances LPS-induced inflammation and migration of breast cancer cells. On the other hand, the ORAI channel inhibitor BTP2 blocked LPS-induced inflammatory signaling. Therefore, our data demonstrate that STIM-ORAI pathway could be a potential novel therapeutic target in the treatment of breast cancer.

REFERENCES