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Evaluating the Role of Pharmacological Inhibition for STIM/ORAI Signaling in Cisplatin Treated Breast Cancer Cells

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INTRODUCTION

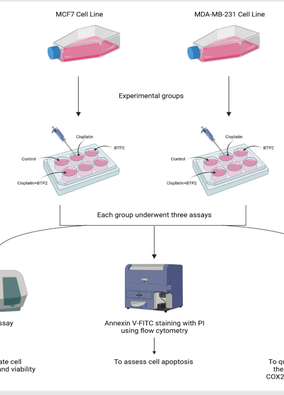
- Chemotherapy is one of the main therapeutic options for breast cancer, but thousands of patients die every year as a result of chemotherapy resistance [1].
- Intracellular calcium (Ca^{2}+) plays a major role in controlling essential cellular functions such as inflammation, proliferation, and migration [2].
- STIM/ORAI signaling pathway mediates the store-operated Ca2+ entry (SOCE) and plays a major role in controlling inflammatory signaling [2].
- STIM/ORAI signaling has been identified as a potential target for cancer therapy.
- Recent evidence suggests critical involvement of STIM/ORAI proteins in the development of breast cancer and other types of malignancies [3].
- According to several studies, activation of STIM/ORAI signaling and SOCE were found to protect ovarian cancer, breast cancer and osteosarcoma cells from undergoing apoptosis in response to cisplatin therapy [4] [5] [6].

OBJECTIVES

- **Hypothesis:** blockade of STIM/ORAI singling pathway sensitizes breast cancer cells to cisplatin via modulating inflammatory response.
- **Aim 1:** assess the influence of the STIM/ORAI signaling blockade on cell viability and cytotoxicity.
- **Aim2:** study the impact of STIM/ORAI signaling blockade on the expression of inflammatory mediators in cisplatin treated breast cancer cells.
- **Long-term goal:** to develop better therapeutic strategies to overcome chemotherapy resistance and to improve treatment outcomes for breast cancer patients.

METHODS

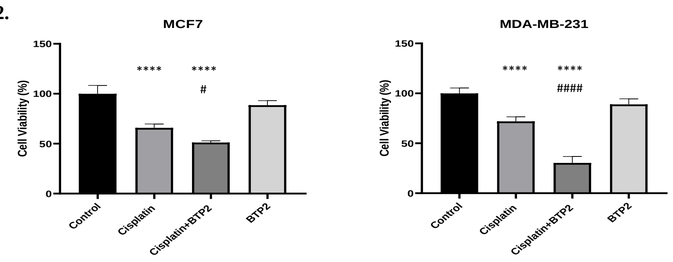
Figure 1. Experimental Design



- Two commercially available human breast cancer cell lines MCF7 (cisplatin sensitive) and MDA-MB-231 (cisplatin resistance) were used.
- The study was divided into four experimental groups as the following: control (untreated), cisplatin, BTP2 (SOCE blocker), and cisplatin plus BTP2.
- MTT assay was used to estimate cell proliferation and viability.
- Annexin V-FITC staining with PI was used to assess cell apoptosis using flow cytometry.
- qPCR was used to quantify the gene expression of the inflammatory mediators COX2, IL-8, and TNF-α as well as STIM1 and ORAI1.
- The difference between the experimental groups was analyzed using one-way ANOVA followed by Tukey's test, and a p-value of <0.05 was considered significant.

RESULTS

Figure 2.

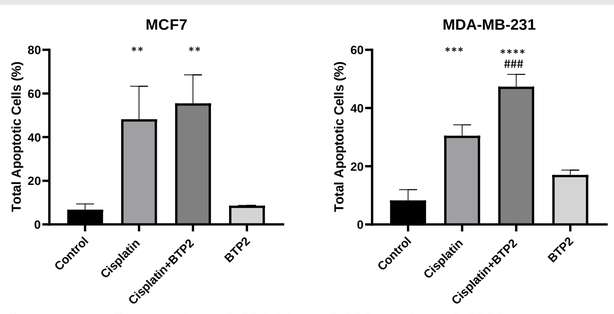


* Significance Compared to Control, **** p<0.00001
\# Significance Cisplatin Compared to Cisplatin+BTP2, # p<0.05 #### p<0.00001

Figure 2: The Impact of STIM/ORAI Signaling Blockade on Cell Viability in Cisplatin Treated Breast Cancer Cells

- Cell viability was significantly reduced in both cisplatin and cisplatin + BTP2 treated cells in MCF7 and MDA-MB-231 cells compared to control group.
- BTP2 in combination with cisplatin significantly reduced the cell viability in MCF7 and MDA-MB-231 cells compared to cisplatin alone group.

Figure 3.



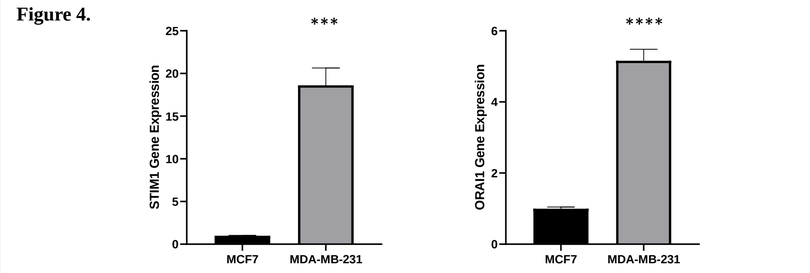
* Significance Compared to Control, ** p<0.001 *** p<0.0001 **** p<0.00001
\# Significance Cisplatin Compared to Cisplatin+BTP2, ### p<0.0001

Figure 3: The Impact of STIM/ORAI Signaling Blockade on Cisplatin induced Cell Apoptosis

- There was no significant differences between cisplatin group and combination group in term of total apoptotic cells.
- On the contrary, BTP2 significantly increased cisplatin induced cell apoptosis compared to cisplatin alone group in MDA-MB-231.

RESULTS

Figure 4.

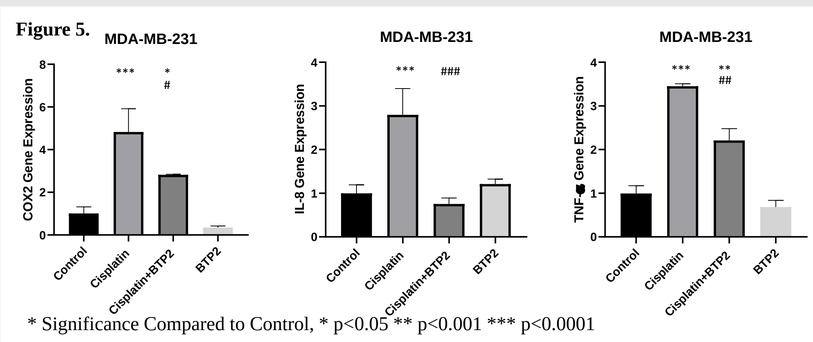


*** p<0.0001 **** p<0.00001

Figure 4: The Gene Expression of STIM1 and ORAI1 in MCF7 and MDA-MB-231 Cells

- STIM1 and ORAI1 genes were highly expressed in cisplatin resistant MDA-MB-231 compared to cisplatin sensitive MCF7, suggesting a role for the overexpression of STIM1 and ORAI1 genes in cisplatin resistance.

Figure 5.



* Significance Compared to Control, * p<0.05 ** p<0.001 *** p<0.0001
\# Significance Cisplatin Compared to Cisplatin+BTP2, # p<0.05 ### p<0.0001

Figure 5: The Impact of STIM/ORAI Signaling Blockade on the Gene Expression of COX2, IL-8, and TNF-α in Cisplatin Treated MDA-MB-231 Cells

- Treatment with cisplatin significantly upregulated the gene expression of COX2, IL-8, and TNF-α compared to control group (untreated).
- BTP2 significantly reduced the gene expression of COX2, IL-8, and TNF-α induced by cisplatin.

DISCUSSION & CONCLUSION

Figure 6.

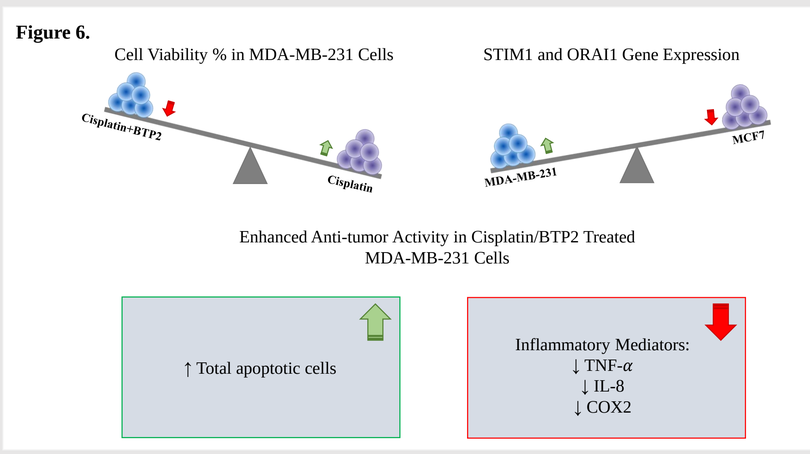


Figure 6: Schematic Summary for the Role of STIM/ORAI Signaling Blockade in Enhancing Cisplatin sensitivity of Breast Cancer Cells.

- We observed an enhanced expression of STIM1 and ORAI1 genes in cisplatin resistance cells compared to cisplatin sensitive cells in which pharmacological inhibition of SOCE enhanced cisplatin sensitivity, suggesting a role for this signaling pathway in chemotherapy resistance.
- We also noticed that pharmacological inhibition of SOCE reduced the gene expression of inflammatory mediators which might indicate a significant role of inflammation in mediating cisplatin resistance via modulating STIM/ORAI signaling.
- The findings of our study are consistent with previous studies which confirmed that activation of STIMI/ORAI singling has a negative impact on chemotherapy.
- In conclusion, inhibition of STIM/ORAI signaling increases the sensitivity of breast cancer cells to cisplatin by modulating inflammatory response. Accordingly, we believe that the findings of our study may contribute to the development of a better therapeutic strategy to overcome chemotherapy resistance.

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