Peptide Mediated Macrophage Polarization Enhances Anticancer Efficacies of Chemotherapeutics in Triple Negative Breast Cancer

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Introduction

- Tumor-associated macrophages (TAMs) drive triple-negative breast cancer (TNBC) progression, metastasis, and chemoresistance through their M2-like (anti-inflammatory) phenotype
- Immunomodulatory peptides, also known as host defense peptides (HDPs), activate immune cells, promoting the production of chemokines, cytokines, and antibodies and repolarize macrophages to the M1-like (pro-inflammatory) phenotype.
- Co-culturing M1 macrophages with an anticancer drug (e.g., doxorubicin) enhances immune responses and apoptotic gene activation, leading to more effective TNBC cell death compared to the drug alone.

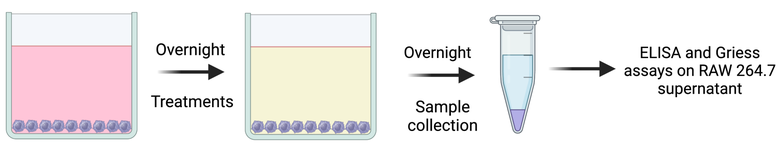
Objectives

- Evaluate the immunomodulatory potential of disulfide-linked macrocyclic peptide c-[CIVIRFKFRC], denoted as 'mCA4', in naïve and TAM-like macrophages using TNBC as a tumor model.
- Assess the anticancer efficacy of 'mCA4' in combination with anticancer drug (e.g., doxorubicin) in a TNBC/macrophage co-culture model.

Methods

1. ELISA and Griess Assay

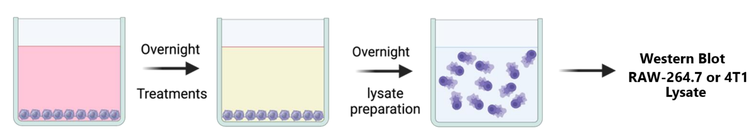
TNF-α, IL-1β, IL-10, TGF-β, IFN-γ, NO



Methods

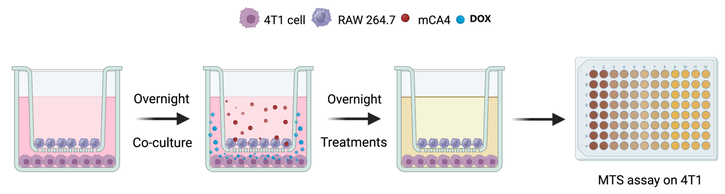
2. Western Blot (WB)

CD-206, CD-86, STAT-1, STAT-3, STAT-6, pro- and cleaved caspase-3, Arginase-1, β-Actin, GAPDH



3. MTS Assay

RAW-264 were seeded in an insert, and 4T1 (TNBCs) were seeded in 0.4 µm TC-inserts



Results

1. Inflammatory Mediator Production Analysis

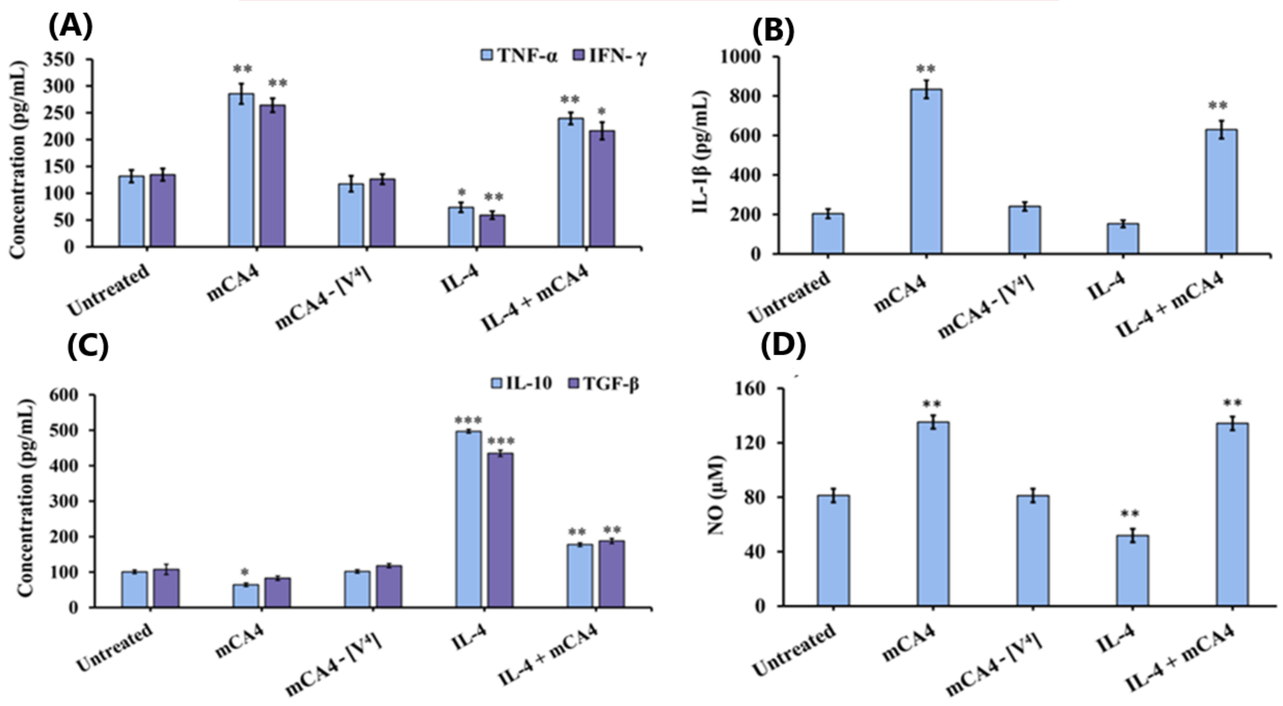


Figure 1. Effect of treatment of mCA4, [V⁴]-mCA4 (inactive analog, control), IL-4, IL-4 followed by mCA4, on RAW-264.7 macrophages by assessing cytokine secretion A) TNF-α and IFN-γ B) IL-1β C) IL-10 and TGF-β D) NO by Griess assay on cell supernatant. Data are presented as the mean ± SD (n = 3). *p < 0.05; **p < 0.01; ***p < 0.001; versus the untreated (control) group.

2. Cell Surface Markers Analysis

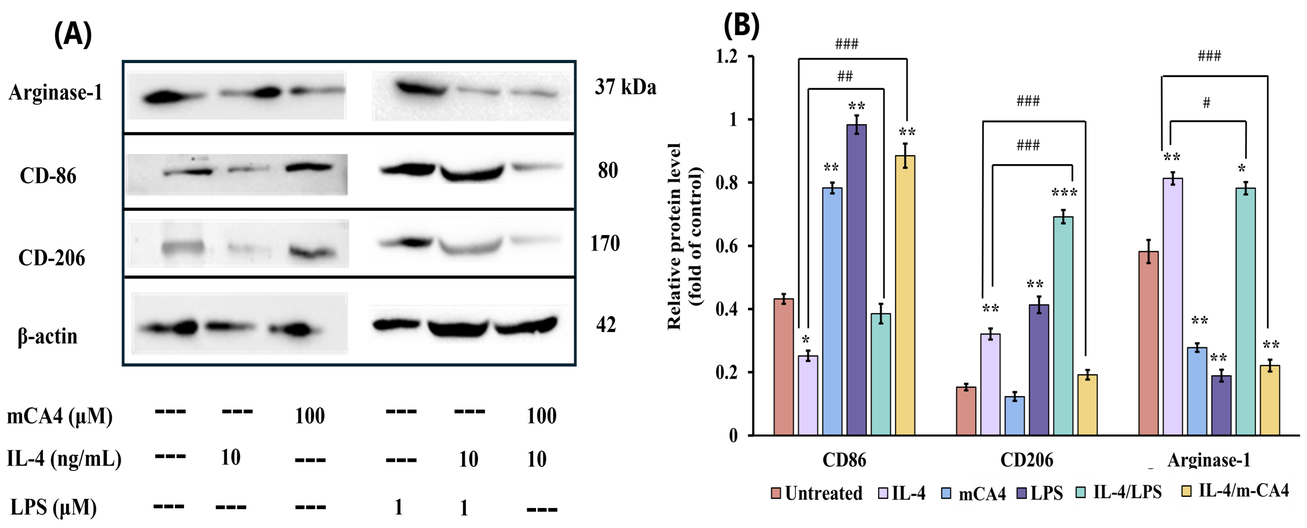


Figure 2: (A) Western blot analysis of RAW-264.7 cells treated with mCA4, using CD-86, CD-206, Arginase-1 as the primary monoclonal antibody. (B) Quantification by Image-J after treatment of RAW-264.7 with IL-4, mCA4, IL-4 followed by mCA4, LPS, IL-4 followed by LPS. Gray-scale value ratios of target proteins to loading protein (β-actin) (C). Data are presented as the mean ± SD (n = 3). *, p < 0.05; **, p < 0.01; ***p < 0.001; versus the untreated (control) group.

3. Cell Cytotoxicity and Apoptosis Analysis

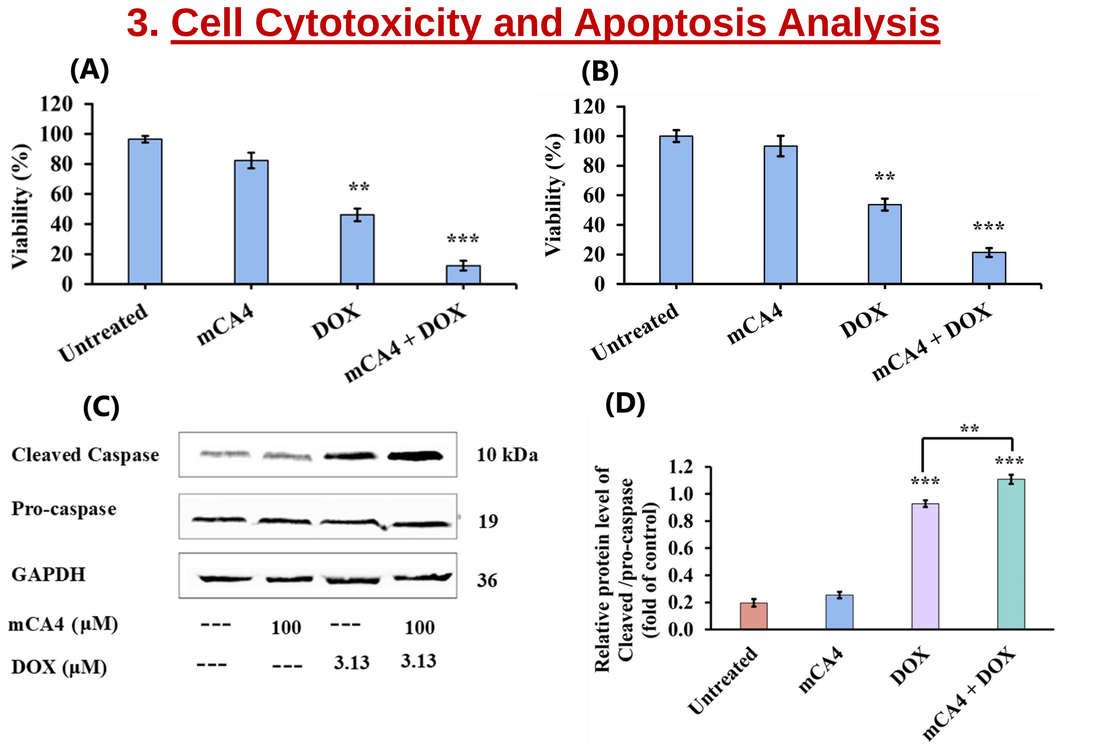


Figure 2. Cytotoxicity of TNBCs after co-culture with RAW-264.7 (A) MTS on 4T1 (B) Supernatant Transfer from RAW-264.7 to 4T1 post- mC4 treatment and cytotoxicity analysis (C), western blot analysis on 4T1 lysate after co-culture with RAW-264.7 and evaluation of caspase activation. (D) Gray-scale value ratios of target proteins to loading protein (GAPDH) Data are presented as the mean ± SD (n = 3). **, p < 0.01; ;***p < 0.001; versus the untreated (control) group.

4. Macrophage/TNBC Co-Culture

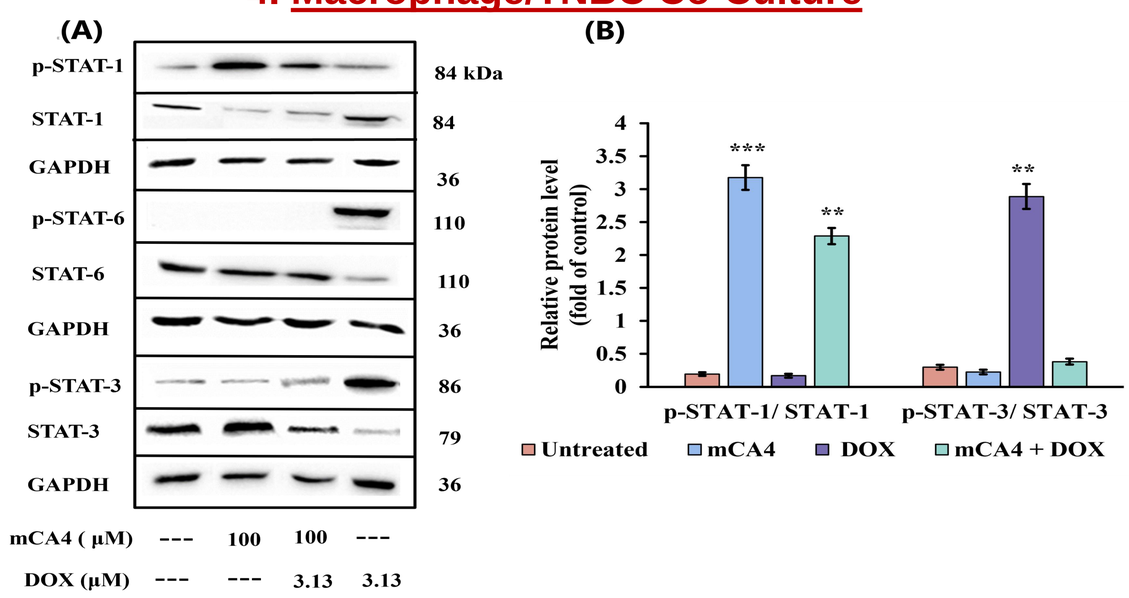


Figure 4. (A) Western blot analysis on RAW-264.7 lysate after co-culture with 4T1 cells using STAT-1, 3, and 6 signaling pathways using the primary monoclonal antibody. (B)Gray-scale value ratios of target proteins to loading protein (GAPDH). Data are presented as the mean ± SD (n = 3). **p < 0.01; ***p < 0.001; versus the untreated (control) group.

Conclusion

- In this study, co-culture of pro-inflammatory macrophages (induced by mCA4 peptide) with TNBCs suppressed anti-inflammatory pathways, promoted pro-inflammatory mediators profuction, and activated apoptotic genes, resulting in more effective TNBCs killing than doxorubicin alone.

Reference

Todrick, C., Ma, J., & Singh, M. (2024). Targeting Macrophages as a Novel Therapy to Treat Triple-Negative Breast Cancer: A Literature Review. Undergraduate Research in Natural and Clinical Science and Technology Journal, 1-9.

Acknowledgment

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