Thymosin α1 Driven CD8⁺ T-Cell Activation and Cytotoxic Reset in Breast Cancer: Integration of in vitro Immunity and Transcriptomic Context

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Abstract

Background: Thymosin α1 (Tα1) is a thymic peptide known to restore immune competence, yet its direct effect on CD8⁺ T-cell effector function and exhaustion reversal in solid tumours remains underexplored. We integrated in vitro cytotoxic assays and transcriptomic profiling to delineate how Tα1 modulates CD8⁺ T-cell function and its relevance in breast cancer immunity.

Methods: CD8⁺ T cells enriched from healthy donor PBMCs (n = 10) were cultured under four conditions: unstimulated, CD3/CD28-activated, Tα1-treated and combined CD3/CD28 + Tα1 or Tα1 rescued exhausted T cells. Functional endpoints included proliferation (CFSE), activation (CD69, CD25, HLA-DR), exhaustion markers (PD-1, TIM-3, LAG-3), cytokines (IL-2, IFN-γ, TNF-α, IL-10), and granzyme B secretion. Cytotoxicity was assessed against MDA-MB-231 breast cancer cells and CD44⁺-enriched cancer stem cells (CSCs) via Annexin V/PI apoptosis and proliferation assays. A compact four-gene Tα1 Response Index (Tα1-RI: TLR9, TLR2, IRF1, NLRC5) was evaluated in TCGA-BRCA and single-cell datasets to contextualize in vitro findings.

Results: Tα1 synergized with CD3/CD28 stimulation to increase CD8⁺ T-cell proliferation, activation, and IL-2/IFN-γ secretion while reducing exhaustion markers in chronically stimulated cells. Functionally, Tα1-treated CD8⁺ T cells induced greater apoptosis and proliferation arrest in both parental tumour and CSC targets, accompanied by enhanced granzyme B release. In silico, Tα1-RI correlated with antigen-presentation and cytotoxic programs and was enriched in CD8-like T-cell clusters, mirroring the functional phenotype observed in vitro.

Conclusions: Convergent experimental and transcriptomic evidence supports Tα1 as a multipronged immunomodulator that reinforces CD8⁺ T-cell activation, restores cytotoxicity in exhausted states, and sensitizes resistant breast cancer cells to immune attack. These findings position Tα1 as a potential adjunct to existing T-cell–based immunotherapies.

Keywords

Thymosin α1, CD8⁺ T cells, T-cell exhaustion, Breast cancer, Cancer stem cells, Cytotoxicity, Transcriptomics.