Reshaping the Tumor Microenvironment: New Application of Tamoxifen in Triple-Negative Breast Cancer Immunomodulation

Pharmacology Discovery Services

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Abstract

Tamoxifen (TAM) is a widely known estrogen receptor (ER) modulator commonly employed in adjuvant treatment of ER+ breast cancer for over 30 years. Interestingly, clinical observations reveal that TAM is capable of inducing regression of some tumors lacking ER expression as well as increasing host resistance against cancer in an ER-independent mechanism. These findings suggest that the immunomodulatory effects of TAM may be ER-independent, but little is known about the underlying mechanism and potential clinical implication. Recently, we identfied a novel mechanism by which TAM exerts its DNA-damaging potential to shape an unfavorable tumor microenvironment in breast cancer. A long-term TAM administration induces downregulation of the chromatin "reader" RACK7/ZMYND8, which acts as a suppressor of interferon-stimulated genes (cytokines and chemokines) and CEACAM1 in both ER+ and triple-negative breast cancer (TNBC) cells. To investigate the immunomodulatory effects of TAM in conjunction with RACK7-knockdown, the orthotopic murine TNBC 4T1 model was employed to investigate TAM-mediated cellular modulation in TNBC. The control and RACK7-knockdown 4T1 cells are orthotopically implanted into the mammary fat pad of female BALB/c mice. Peripheral cytokines/chemokines and high-content biomarker studies (multiplex immunoassays, flow cytometry, and CyTOF mass cytometry) are deployed to obtain insghts into the mechanistic rationale behind the immunomodulatory effects of TAM and/or RACK7-knockdown. The TAM-mediated cellular modulation evokes cytokine/chemokine secretion and further induces T-cell infiltration into tumor area. However, the interaction between CEACAM1 and TIM-3, a "check point" receptor expressed in $CD4^+$ and $CD8^+$ T cells, promotes T-cell exhaustion to prevent tumor-killing effects. The expression patterns of CEACAM1 and PD-L1 in 4T1 tumor cells and that of TIM-3 and PD-1 in $CD4^+$ and $CD8^+$ T cells correlate with intra-tumor infiltration of T-cells and tumor cell growth. Altogether, our findings provide direct evidence to support a new therapeutic opportunity by targeting the CEACAM1-TIM-3 interaction in the TAM-mediated tumor immune microenvironment for improving immune checkpoint blockade therapy in breast cancer.

A Working Model for the Tamoxifen–RACK7–Type I Interferons Regulatory Network that Modulates Breast Cancer Immunology and Immunotherapy

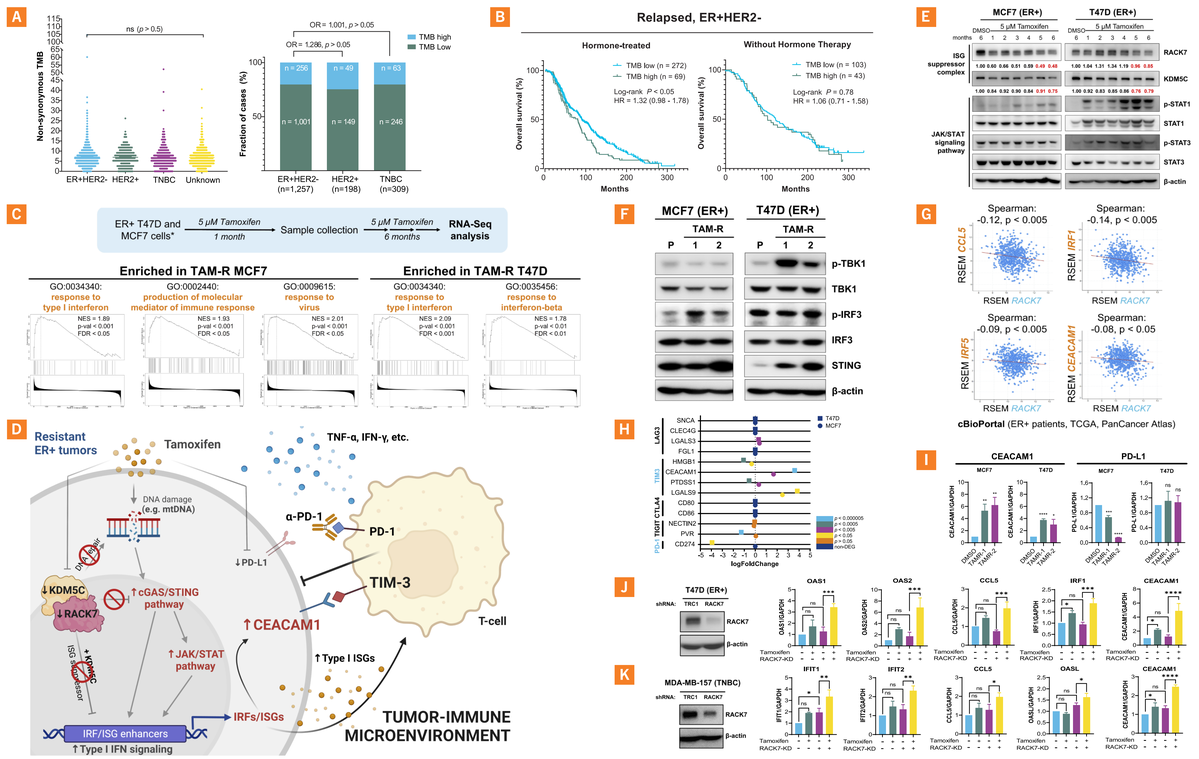

Figure 1. Chronic tamoxifen (TAM) treatment induces breast cancer immunomodulation via regulating the RACK7 • cGAS/STING • IFN • I regulatory axis. A.–B. Distribution of non-synonymous tumor mutational burden (TMB) and proportion of patients with high (TMB>10) and low (TMB≤10) TMB across differnet breast cancer subtypes. C. Top: Scheme for a long-term TAM administration in ER+ breast cancer cell lines (T47D, MCF7). Bottom: GSEA enrichment plots of representative immunomodulatory and IFN-I pathways signigicantly activated in TAM-resistant (TAM-R) T47D and MCF7 cells. D. Proposed model illustrating the molecular basis of TAM in breast cancer immunology and immunotherapy. Created with BioRender.com. E.–F. Immunoblotting showing effects of TAM on the ISG suppressor complex (RACK7-KDM5C), cGAS/STING, and JAK/STAT pathways. G. RACK7 correlation analysis against ISGs (e.g., CCL5, IRF1, IRF5, and CEACAM1) in ER+ breast cancer patients. H. Various T-cell inhibitory ligands differentially expressed between control and TAM-R MCF7 and T47D cells I. RT-qPCR analysis of CEACAM1 and PD-L1 (encoded by CD274) in two independent TAM-R MCF7 and T47D cell clones relative to the DMSO control. J.–K. Left: Analysis of shRNA-mediated RACK7-knockdown (RACK7-KD) in T47D ER+ and MDA-MB-157 triple-negative breast cancer cells. Right: Effects of different combinations of control vehicle, TAM, control-shRNA, and/or RACK7-shRNA on the expression of ISGs and CEACAM1 in T47D and MDA-MB-157 cells.

Effects of Tamoxifen, RACK7-Knockdown, or/and Anti-TIM-3 in Triple-Negative Breast Cancer Growth and Immunomodulation

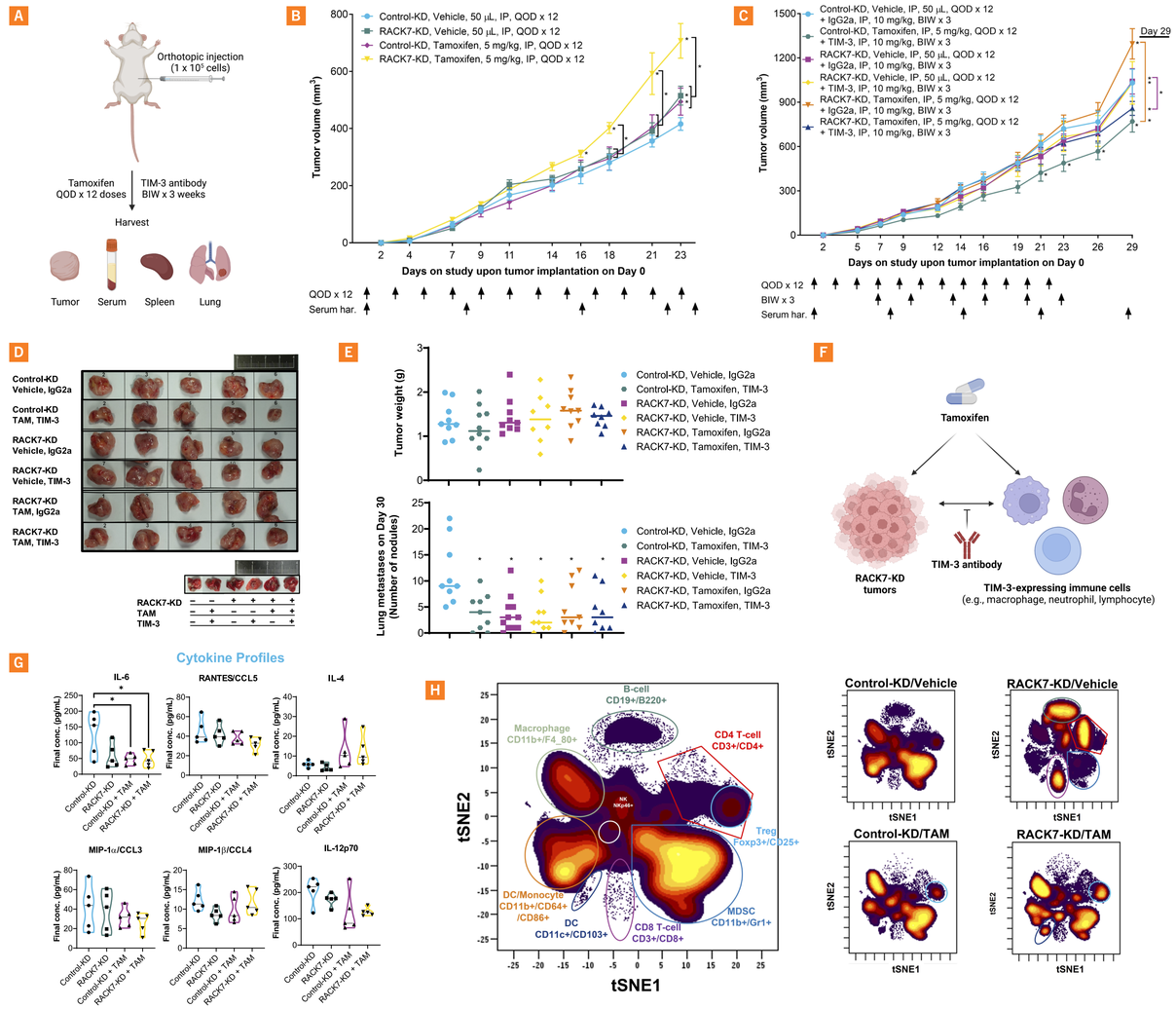

Figure 2. Evaluation of RACK7-knockdown (RACK7-KD) combined with tamoxifen (TAM) treatment in the murine triple-negative breast carcinoma (TNBC) 4T1 orthotopic model. A. Schematic diagram of the TAM and anti-TIM-3 treatment regimen in the murine TNBC 4T1 orthotopic models. B. Tumor growth of control-shRNA (control-KD) or RACK7-shRNA (RACK7-KD) 4T1 cells in female BALB/c mice with or without TAM administration. C. Tumor growth of control-KD or RACK7-KD 4T1 cells in BALB/c mice treated with TAM and TIM-3 antibodies in different combinations as indicated. D.–E. Harvested tumors and lungs (representative examples) from Panel C study. Tumor photo and weights (top panels) and macroscopic examination of metastatic nodules in lungs (bottom panels). F. Proposed model of RACK7-KD, TAM, and TIM-3 antibody potential cooperativity in TNBC cancer immunomodulation. G. Serum cytokine profiles from mice in Panel B study. H. Single-cell CyTOF mass cytometry of tumors harvested from Panel B study. All schematic diagrams were created with BioRender.com.

Summary

1. Tamoxifen (TAM) maintains a chronically activated Type I IFN signaling in TAM-resistant ER+ breast cancer cells.
2. A TAM-RACK7/KDM5C-cGAS/STING regulatory axis induces upregulation of CEACAM1 to establish a "cold" tumor microenvironment.
3. TAM and loss of RACK7 are both indispensable for the expression of CEACAM1 and other IFN-stimulated genes (ISGs).
4. The CEACAM1/TIM-3 blockade may reactivate immune cells for tumor cell killing in the chronic TAM and RACK7 loss-tumor setting.