Background Triple-negative breast cancer (TNBC) stands as a malignancy with a high unmet medical need, especially given its notably low response rate to emerging immunotherapies that have been drawing attention in recent years. An abundance of tumor-associated macrophages (TAMs) within TNBC contribute to the malignant progression of cancer by establishing an immunosuppressive tumor microenvironment. In this study, we demonstrate the potential of enhancing the cross-presentation capacity of TAMs in TNBC through toll-like receptor 4 (TLR4) dependent signaling via Paclitaxel (PTX), thereby promoting tumor infiltration by CD8⁺ T cells. Furthermore, we elucidate a novel anticancer immunological mechanism of PTX, which explains why a combinatorial strategy of PTX and PD-1 blockade could prove beneficial in clinical settings.

Methods We analyzed the mRNA and protein expression profiles of PTX-treated and untreated macrophages via Nanostring and Western blot. In vitro experiments involved stimulating PTX-pretreated macrophages with Ovalbumin peptide and evaluating MHC-1 peptide binding using flow cytometry. The macrophages were co-cultured with T cells to validate the enhanced cross-priming potential by PTX treatment, and interferon-gamma (IFNg) release was measured using ELISA. The in vivo efficacy of PTX monotherapy was evaluated in a murine TNBC model through flow cytometry and immunohistochemistry analysis of tumor tissue. Furthermore, we investigated the combinatorial effect of PTX with anti-PD-1. The TLR4 dependency of PTX was determined by pretreating macrophages with a TLR4 antagonist before PTX incubation in vitro. In the case of in vivo experiment, TLR4 dependency was assessed by monitoring tumor burden growth in TLR4 knockout mice treated with PTX.

Results Our mRNA and protein expression analyses revealed that PTX treatment upregulates antigen presentation-related factors in macrophages, including TPN, b2M, NCF2, TAPBP, TAP1 and TAP2. In vitro experiments demonstrated that PTX enhances the antigen cross-presentation potential of macrophages by 8.8-fold, and this increase was abolished when the TLR4 antagonist was incubated in advance. Co-culture of PTX-treated macrophages with T cells showed an 8.5-fold increase in IFNg release. In the TNBC mouse model, PTX monotherapy resulted in a 32% reduction in tumor growth and a significant increase in MHC-1 expression on TAMs. Combination therapy with PTX and anti-PD-1 improved the tumor growth reduction by 72%.

Conclusions This study demonstrates that PTX can modulate TAMs into dendritic cell-like functioning cells through TLR-4 signaling in aspect to the cytosolic cross-presentation pathway of tumor antigens. These findings provide a rationale for the potential immunotherapeutic application of a PTX-based regimen to enhance antitumor immunity.

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