Co-targeting PD-L1 and CDK4 benefits plasticizer-associated early-onset breast cancer

Shu-Wei Hu1,2,\*, Ming-Hsin Yeh3,4,\*, Yu-Hao He2,5, Dai-Wei Hu1,2, Ya-Ling Wei1,2, Fang-Ju Cheng2,6, Thanh Kieu Huynh1,2, Bo-Rong Chen1,2,6, Yi-Lun Yeh1,2, Bo-Wei Wang1,2, Der-Yen Lee7, Mei-Chun Lin3, Yi-Hsien Hsieh8,9, Yuan-Man Hsu10, Chih-Hsin Tang1,6,11,12, and Wei-Chien Huang1,2,12,13,14,†

1 Graduate Institute of Biomedical Sciences, China Medical University, Taichung 407, Taiwan

2 Center for Molecular Medicine, China Medical University Hospital, Taichung 407, Taiwan

3 Department of Surgery, Chung Shan Medical University Hospital, Taichung 402, Taiwan

4 Institute of Medicine, School of Medicine, Chung Shan Medical University, Taichung 402, Taiwan

5 Department of Biomedical Imaging and Radiological Science, China Medical University, Taichung 404, Taiwan

6 School of Medicine, China Medical University Taichung 404, Taiwan

7 Institute of Integrated Medicine, China Medical University, 404, Taiwan

8 Institute of Medicine, Chung Shan Medical University, Taichung 402, Taiwan

9Department of Medical Research, Chung Shan Medical University Hospital, Taichung 402, Taiwan

10Department of Animal Science and Biotechnology, Tunghai University, Taichung 407, Taiwan

11Chinese Medicine Research Center, China Medical University, Taichung 40402, Taiwan

12Department of Medical Research, China Medical University Hsinchu Hospital, Hsinchu 302, Taiwan

13 Department of Medical Laboratory Science and Biotechnology, Asia University, Taichung 413, Taiwan

14 Cancer Biology and Precision Therapeutics Center, China Medical University, Taichung 407, Taiwan

**Purpose**

Breast cancer patients diagnosed at the age under 45 years old commonly show poor prognosis and survival rate. Breast tumors from young patients showed higher FOXP3+ tumor-infiltrating lymphocytes (TILs), suggesting a role of dysregulation of immune surveillance in the tumorigenesis of early-onset breast cancer (EOBC). Exposure to plasticizers is one of the potential risk factors of EOBC. The impact of plasticizer exposure on the immune dysregulation for the early onset of breast cancers and the underlying mechanisms are unclear.

**Materials and Methods**

The hair level of DEHP, the most common phthalate plasticizer detectable in human bodies, was measured in GC/Mass analysis, and the levels of immune checkpoint proteins and TILs in human breast tumor tissues were detected in immunohistochemical staining (IHC) analysis. The effects of DEHP on relevant protein expressions in breast epithelial and cancer cells were tested in western blot and flow cytometry analyses. L1000 fireworks display, a transcriptomic signature-based platform, was used to identify potential therapeutic agents against DEHP-exposed cancers. The roles of plasticizers in regulating the expressions of various immune checkpoints, cyclin/CDK pathways, and the cytotoxic activity of T cells were studied using both in vitro cell line models and in vivo mice models. The involved transcription factors and signaling pathways were identified by using various molecular biological analyses.

**Results**

The hair level of DEHP was higher in EOBC patients and positively correlated with the levels of PD-L1 and ERβ expressions but negatively correlated with the tumor infiltration of activated T cells. In mouse models, treatments with DEHP accelerated tumorigenesis and tumor growth and repressed T cell-mediated cytotoxicity due to the upregulation of immune checkpoint PD-L1. The exhaustion of CD8+ T cells induced by DEHP-treated breast cancer cells was reversed by anti-PD-L1 antibody. Our data further demonstrated that DEHP transcriptionally induced PD-L1 expression through ERβ activation even in triple-negative breast cancer (TNBC) cells. Moreover, CDK4/6 inhibitors were predicted as the potential therapeutic agents to reverse DEHP-induced transcriptomic signature. Indeed, DEHP increased CCND1-CDK4 expressions, and CDK4/6 inhibitors prevented DEHP-mediated CD8+ T cell exhaustion. Combination therapy with an anti-PD-1 antibody and palbociclib, a CDK4/6 inhibitor, showed better anti-cancer activity and prevented anti-PD-1 antibody-induced immune-related adverse events (irAEs) in the DEHP-associated 4T1-TNBC syngeneic mice model.

**Conclusion**

Exposure to DEHP suppresses the cytotoxicity of CD8+ T cells and promotes breast cancer onset and growth through the ERβ-dependent upregulation of PD-L1 expression. Additionally, DEHP-induced CDK4 not only drives cancer cell growth but also contributes to T cell exhaustion via PD-L1/PD-1 interactions. Co-targeting PD-1 and CDK4 enhances therapeutic efficacy and reduces immune checkpoint inhibitor (ICI)-related adverse events in plasticizer-associated early-onset breast cancer (EOBC), offering an effective and safe treatment strategy.