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# Cannabidiol promotes immunogenic cell death and controls breast tumor development

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**Introduction:** Cannabidiol (CBD) therapy has emerged as a promising anticancer drug in many types of cancer models. However, the molecular mechanisms underlying the contribution of antitumor immunity to the therapeutic efficacy of CBD remain unclear. **Objective:** This study aimed to provide strong evidence that CBD is a *bona fide* immunogenic cell death inducer in breast cancer model. **Methods:** Breast tumor (4T1) cells were treated with CBD and assessed for cell proliferation, as well as HMGB1 and ATP release. CBD-treated 4T1 cells were co-cultured with splenocytes, and the cytokine profile was analyzed using flow cytometry and enzyme-linked immunosorbent assay (ELISA). Additionally, wild-type mice were injected subcutaneously with 4T1 cells, and tumor growth was monitored, followed by the characterization of tumor-infiltrating immune cells. **Results:** CBD administration decreased tumor growth in different models (4T1 and 65NR) of breast cancer. By using immunocompromised mice, it was possible to show that adaptive antitumor immunity is essential for efficient tumor control in CBD therapy. Indeed, CBD treatment resulted in the modification of the tumor microenvironment, up-regulating antitumoral proinflammatory cytokines, associated with activation of dendritic cells and enhanced CD8+ T cell effector function. Mechanistically, CBD treatment induced a stress response in 4T1 tumor cells by reactive oxygen species (ROS) accumulation and upregulation of nitric oxide synthase 2 (NOS2), that leads to unfolded protein response activation, resulting in increased immunogenicity and impaired cell growth and proliferation. CBD efficiently promoted immunogenic cell death hallmarks and enhanced expression of antigen-presenting molecules on the surface of breast cancer cells. In a co-culture system with spleen cells, CBD-treated tumor cells stimulated the production of pro-inflammatory chemokines and cytokines and induced activation of dendritic cells and T cells. Combination therapy revealed that CBD enhanced efficacy of anti-PD-L1 in breast cancer model. Vaccination protocol with CBD-induced dying cells efficiently protected against breast tumor progression. **Conclusion:** The findings revealed CBD as immunogenic cell death inducer anticancer drug and open a new avenue of opportunities in cancer therapy against breast cancer which aims the establishment long-lasting antitumor immunity.

**Keywords:** cannabidiol; immunogenic cell death; breast cancer; tumor.