Microbiome and Other Environmental Factors

Dietary Patterns Associated with Increased Abundance of Akkermansia Muciniphila Potentiates Anti-PD-L1 Immune Checkpoint Blockade Response in Triple-Negative Breast Cancer

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Background: Immune checkpoint blockade (ICB) therapies targeting programmed cell death protein 1 pathway (PD-1/PD-L1) have advantageously impacted triple-negative breast cancer (TNBC) patient survival; however, there remains a need to improve responses. Recent studies associated gut Akkermansia muciniphila as an ICB-response related microbe in other cancer types. As diet is a main modifier of the gut microbiome, we investigated whether diet-gut microbiome interactions potentiate ICB response in TNBC.

Methods: Using EMT-6 (n=5-7/group) and E0771 (n=8-10/group) syngeneic models of TNBC, tumor-bearing mice consuming low-fat control, high-fat Western, or a Mediterranean diet were treated with 200 mg of IgG or anti-PD-L1 antibodies and response to therapy was determined by tumor progression. To assess modulation on the gut bacterial microbiome by diet and ICB, 3M read depth metagenomic sequencing was performed on DNA isolated from fecal samples from both models. To further implicate the microbiome, we performed a fecal microbiota transplant (FMT) model (n=8-10/group), where mice consuming a control diet were supplemented via oral gavage with either a control diet-derived FMT, a Western diet-derived FMT, or Mediterranean diet-derived FMT. EMT-6 bearing-mice on each FMT were treated with IgG or PD-L1. Immune response (F4/80 macrophages, granzyme B, and CD8+ cytotoxic T cell infiltrate) in the tumor microenvironment (TME) were examined in residual tumor tissue by immunohistochemistry (n=9/group). Regulation of PD-L1 protein by diet was assessed by Western blot hybridization in tumor sample lysates (n=3/group). Short chain fatty acid (SCFA) analysis was measured in plasma (n=8/group) by LC/MS-MS metabolomics.

Results: In EMT-6 bearing-mice, PD-L1 treatment and consumption of a Western or Mediterranean diet significantly reduced both tumor volume and tumor weight when compared to control diet-fed mice (p<0.05). In E0771 bearing-mice, consumption of a Western diet and PD-L1 treatment resulted in a modest increase in ICB response (56%), with the highest efficacy observed in Mediterranean diet-fed mice (70%), when compared with IgG control animals. Western and Mediterranean-fed mice displayed a 25–45% increase in gut Akkermansia muciniphila proportional abundance. Anti-PD-L1 therapy in mice given an A. muciniphila enriched FMT showed enhanced ICB responsiveness (p<0.05, 70-80%). Mediterranean-diet fed animals treated with anti-PD-L1 antibodies showed elevated plasma butyrate SCFA metabolites. E0771 model results show Western and Mediterranean diet intake regulated expression of PD-L1, macrophages, and cytotoxic T cell function proteins in the TME.

Conclusions: Taken together, these data indicate PD-L1 response in TNBC is potentiated by diet-microbiota interactions and suggests increasing levels of Akkermansia may enhance ICB efficacy.

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