Investigating the interplay between diet and the gut microbiome as a potential modulator of anti-PD-L1 responsiveness in triple negative breast cancer

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Triple-negative breast cancer (TNBC), accounting for 10-20% of all invasive breast cancer cases, is one of the most highly aggressive types of the breast cancer. Lacking estrogen and progesterone receptors and having normal expression of HER2, limited the patient population to non-targeted chemotherapy. However, the development of immune checkpoint blockade (ICB) therapies that target the programmed cell death protein 1 pathway (PD-1/PD-L1) have impacted the survival of TNBC patients. Recent studies highlight the importance of the gut microbiome affecting ICB response in melanoma and non-small cell lung cancer. To investigate whether diet and gut microbiome interactions potentiate ICB responsiveness in TNBC, we first explored the effect of diet in PD-L1 responsiveness using the EMT-6 and EO771 syngeneic breast cancer models. Tumor-bearing mice consuming a standard low-fat control diet, a Western diet, or a Mediterranean diet were treated interperitoneally with 200 ug of either IgG control or anti-PD-L1 antibodies every other day for three days. Tumor volume was measured every 2-3 days until the end of the study and tumor wet weight was recorded. 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, indicating an enhanced ICB response. In EO771 bearing-mice, the highest anti-tumor PD-L1 efficacy was observed in mice consuming a Mediterranean diet, with approximately 70% of animals displaying reduced tumor volume and significantly reduced tumor weight when compared with diet alone IgG control animals. Western diet-fed EO771 tumor bearing-mice treated with PD-L1 also displayed a modest increase in ICB responsiveness. IVIS imaging of study subjects supported enhanced therapeutic response with observably smaller tumors in Western and Mediterranean diet PD-L1 responsive animals compared to IgG control and PD-L1 non-responder mice consuming the same diet. These data demonstrate diet modulates PD-L1 responsiveness in TNBC. Metagenomics sequencing was performed on fecal samples to identify shifts in the gut bacterial microbiome mediated by diet and ICB therapy. Enrichment of ICB-responder microbe *Akkermansia muciniphila* was displayed in Western and Mediterranean-fed EMT-6 mice. To further implicate the role of the gut microbiome, we also performed a fecal microbiota transplant (FMT) model, where BALB/c mice fed a control diet were given either a control diet-derived FMT, a Western diet-derived FMT, or a Mediterranean diet-derived FMT by oral gavage. EMT-6 cells were orthotopically injected into the mammary fat pad and following tumor formation, mice on each FMT were given control IgG or PD-L1 antibody treatments. Mice treated with PD-L1 antibody that were given an *Akkermansia muciniphila* enriched FMT showed enhanced anti-tumor ICB responsiveness. Histological examination for immune infiltration was performed on residual tumor tissue to assess CD8+ cytotoxic T cells and granzyme B. Preliminary evidence indicates differential amounts of infiltrating granzyme B positive cells in our Western and Mediterranean diet-fed animals treated with and responsive to PD-L1, supporting enhanced anti-tumor function in these animals. Taken together, these data indicate that PD-L1 ICB therapy responsiveness and infiltrating immune populations are modified by diet and microbiota interactions in TNBC. Moreover, modulating the gut microbiome to increase levels of *Akkermansia muciniphila* and its exerted functions may serve as a method for enhancing anti-PD-L1 ICB therapy efficacy in TNBC.