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Harnessing the microbiome to impact chemotherapeutic responsiveness in triple negative breast cancer

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Triple negative breast cancer (TNBC) is highly aggressive subtype with a 5-year survival rate significantly worse when compared to other breast cancers types. Furthermore, there are no targeted therapy options, limiting these patients to cytotoxic chemotherapy regimens. Increasing evidence show the strong correlation between gut microbiota and the development of many diseases including breast cancer. Moreover, gut microbiota populations are highly plastic, shifting in response to diet changes, drug administration, and geographical location. The purpose of our study was to determine whether chemotherapy modifies the gut microbiota populations and whether ablation of the microbiome modifies drug responsiveness. To do so, 8-week old female BALB/c mice were injected with 4T1-luciferase TNBC cells into the mammary fat pad. Once tumors reached 100 mm^3, mice were either untreated (control group), treated with 1x weekly 2.5 mg/kg doxorubicin (Dox) for 4 weeks, treated with Dox + antibiotics (mixture of streptomycin, ampicillin, and colistin in the drinking water to ablate the microbiome), or treated with antibiotics cocktail alone. DNA was isolated from fecal samples collected at T_0 (before treatment) and T_4 (after treatment) and 3M read depth shotgun metagenomic sequencing was performed. Mice from Dox +antibiotics group displayed reduced tumor weight and decreased lung metastatic burden, suggesting the gut microbiome modifies chemotherapy efficacy. Doxorubicin treatment modulated the diversity and abundance of many bacterial species including increase *in Akkermansia muciniphila, Alistipes shahii, Prevotella copri,* and *Bacteroides vulgatus*. Doxorubicin treatment affected some bacterial species that were used as probiotics including reduced *Bacteroides uniformis*, *Lactobacillus johnsonii* and *increased Bifidobacterium longum* abundance. Doxorubicin treated subjects were stratified into responders and non-responders according to tumor growth over time, and metagenomics data showed elevated proportional abundance in *Alistipes shahii*, *Bacteroides vulgatus* in responders post treatment. On the other side, non-responders showed increase in *Enterococcus faecalis* and reduction in *Bacteroides cellulosilyticus* bacterial species post treatment. These modulations in gut microbiota were associated with intestinal inflammation changes in villi length, muscularis layer thickness and goblet cells counts. Taken together, our data demonstrates that ablation of gut microbiota using antibiotics along with chemotherapy treatment may show better outcomes than using doxorubicin. Moreover, systemic treatment with chemotherapeutic agents can modulate gut microbiota composition suggesting that fecal microbiota populations could be used as a predictive biomarker of chemotherapeutic responsiveness and modulation of the gut microbiota through dietary, antibiotics or probiotic interventions may affect therapeutic efficacy.

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