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Gut microbiome populations modulate neoadjuvant chemotherapy responsiveness in preclinical triple negative breast cancer murine model

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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. Our group demonstrated increased tumoral Pseudomonas abundance in breast cancer patients receiving neoadjuvant chemotherapy, suggesting that systemic anti-cancer therapy administration may modulate microbiota populations. The purpose of our study was to determine whether gut microbiota populations correlate with chemotherapeutic responsiveness and may be a predictive biomarker of outcome. Moreover, we want to determine the impact of shifting the microbiome on chemotherapy efficacy. To do so, 8-week old female BALB/c mice were injected with 4T1-luciferase 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, or treated with doxorubicin +antibiotics (mixture of streptomycin, ampicillin, and colistin in the drinking water to ablate the microbiome). Tumor size was monitored. Tumors and lungs were collected after the study. Fecal samples were collected at T_0 (before treatment) and T_4 (after treatment). Mice receiving DOX were stratified into DOX-responders or DOX-nonresponders based upon tumor size. Mice from DOX-responders and DOX +antibiotics groups displayed reduced tumor weight and decreased lung metastatic burden. 16S-bacterial sequencing indicates elevated fecal Ruminococcus correlates with DOX-nonresponsiveness and increased abundance of Oscillospira and Bacteroidales are associated with better therapeutic outcome. Protein analysis of tumor tissue indicates a significant increase in apoptosis (cleaved caspase-3) in DOX-responders and DOX +antibiotic groups. In another study to determine whether modulating the gut microbiome influences drug responsiveness, BALB/c mice were stratified into a control group or a group receiving lard diet-derived fecal transplant (LDFT) by oral gavage. Mice were injected with 4T1-luciferase cells into the mammary fat pad. Once tumors reached 100 mm^3, mice were treated with 1x weekly 2.5 mg/kg DOX for 4 weeks. Tumors and lungs were collected at the end of the study. Fecal samples were collected at T_0 (before gavage), T_3 (after 3 weeks of LDFT and before DOX-treatment), and T_7 (at end of study). All tumors from LDFT-group were larger and displayed reduced chemotherapy responsiveness when compared with control animals, and impacted intestinal inflammation as well, suggesting gut microbiome populations can modulate chemotherapy resistance. Taken together, our data demonstrates that chemotherapy efficacy is modulated by gut microbiome, and suggests that modulation of the gut microbiome through dietary or probiotic interventions may affect therapeutic outcomes. Moreover, fecal microbiota populations could be used as a predictive biomarker of chemotherapeutic responsiveness.

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