Microbiota-mediated PROTEIN X expression affects Immune Breast Cancer Microenvironment and Overall Survival

A.M.Mozzarelli. [1]*, S.Carloni. [2], D.Abbass. [1], G.Penna. [2], M.Rescigno. [1,2]
*(Alessandro Mauro Mozzarelli) alessandro.mozzarelli@humanitasresearch.it
[1] Humanitas University, Department of Biomedical Sciences, Via Rita Levi Montalcini 4, 20090 Pieve Emanuele – Milan
[2] Humanitas Clinical and Research Center, Via Manzoni 56, 20089 Rozzano – Milan, Italy

Keywords (maximum 8): PROTEIN X, Breast cancer, Microbiota, gram negative, syngeneic, 4T1

The human epidermal growth factor receptor-2 (HER2) is overexpressed in approximately 20% of breast cancers and is associated with poor clinical prognosis and patient survival. An improvement in progression-free survival (PFS) and overall survival (OS), has been obtained by combining target therapies and cytotoxic agents with HER2 targeted Monoclonal antibodies, Trastuzumab and Pertuzumab. The antibody-dependent cytotoxicity (ADCC) of Trastuzumab and Pertuzumab on HER2 is mediated by the presence of immune effector such as NK cells. Nevertheless, progression still occurs in half of the patients in less than two years (20.2 months). Firstly, we previously identified that sera of patients undergoing innate or acquired resistance to HER2 targeted therapy overexpress PROTEIN X (for confidentiality). Moreover, PROTEIN X expression correlates with bad prognosis for breast cancer patients. Consistence with this evidence, we found that PROTEIN X acts as a negative regulator of NK cells and $CD8^+$ T cells cytotoxicity. However, the mechanisms regulating the systemic and intra-tumoral expression of PROTEIN X are still unclear. PROTEIN X is an evolutionary conserved protein that acts as a sensor and a defence mechanism against pathogens in lower organisms. We speculated that it could be modulated by the human gut as well as by tumor associated Microbiota, thus inducing an indirect or a direct effect on the tumor microenvironment and growth. To assess a potential role of the breast cancer associated Microbiota, we used an *in-vitro* assay, exposing cancer cells to supernatant of pathobionts bacteria isolated from the tumor microenvironment. We found that PROTEIN X, produced by breast cancer cells, was upregulated by LPS and by supernatants from manly tumor isolated gram negative bacteria. Conversely, PROTEIN X expression was significantly inhibited by treatment with short chain fatty acids (SCFAs) as butyrate and propionate, two metabolites derived from the anaerobic fermentation of gut symbiotic bacteria. Thus, to assess the potential role of gut microbiota on the intestinal and systemic expression of PROTEIN X, we analysed the colon of AOM/DSS colitis associated cancer mouse model (able to drive also chronic inflammation and microbial dysbiosis). Consistent with *in-vitro* observations, PROTEIN X was induced in colons of AOM/DSS treated mice, and antibiotics treatment significantly reduced PROTEIN X expression. Further, using a syngeneic mouse model harboring 4T1 breast cancer, we assessed the effect of antibiotics against gram negative (Neomycin) and gram positive (Vancomycin) bacteria on the tumor growth and on survival rate. Either a reduction of tumor growth or an increase of survival rate together with a variation in tumor mass immune infiltrates was observed upon Neomycin treatment. We are currently investigating the molecular pathways responsible for PROTEIN X expression modulation. Overall, our findings suggest that microbiota modulation can control PROTEIN X levels. Both microbiota and ProteinX could be used as biomarkers to predict patient's response to HER2 target therapy and theirs modulation could improves treatment efficacy.