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LIPS

High Salt Diet-Induced Tumor Initiating Stem Cells Mediate Breast Cancer Progression

High salt (sodium chloride) diets have been associated with several chronic inflammatory diseases. While the role of chronic inflammation in cancer is well established, the specific role of a high-salt diet in carcinogenesis is unknown. Previous studies with syngeneic murine breast cancer models, both in our laboratory and others, have shown that high-salt (HS) diets induce tumor regression through inflammatory activation of anti-tumor adaptive immune responses. We tested this counter-intuitive and suspiciously beneficial role of HS diets by performing sequential passage studies in preclinical murine models. Six-week-old mice were placed on a high salt diet for 2 weeks prior to injection with syngeneic 4T1 and Py230 triple negative breast cancer cells (into BALB/c and C57Bl/6J mice, respectively, referred to as passage-1). Tumors were harvested after four weeks of injection. The cancer cells depleted of immune cells were collected from these harvested tumors and reinjected into new non-tumor bearing mice. This cycle was repeated three times. In the designated passage-1 cohort, by day 28, the HS diet induced reduced tumor progression in both 4T1-BALB/c (267 ± 59 mm^3) and Py230-C57Bl/6J (238 ± 54 mm^3) as compared to the regular salt (RS) diet cohort (611 ± 94 mm^3 and 473 ± 69 mm^3, respectively; $p<0.05$ for both). However, in the passage-4 cohort, by day 28, the HS diet induced significantly higher tumor progression in both 4T1-BALB/c (806 ± 91 mm^3) and Py230-C57Bl/6J (743 ± 81 mm^3) models, as compared to the regular salt (RS) diet cohort (577 ± 83 mm^3 and 462 ± 77 mm^3, respectively; $p<0.05$ for both). Cellular analysis by flow cytometry revealed that there was a 12-19 fold increase in tumor initiating stem cells (TISCs) in the passage-4 HS diet cohort compared to the RS diet cohort. Mechanistic studies have demonstrated that there was increased TGFβ expression on TISCs obtained from the passage-4 HS diet cohort which correlated with enhanced exhaustion phenotype ($CTLA4^+$) of tumor infiltrating adaptive immune cells (CD4 and CD8 T cells) in this cohort. Co-treatment with anti-TGFβ and anti-CTLA4 monoclonal antibodies (mAb), in the passage-4 HS diet cohort, significantly reduced tumor progression ($p<0.05$), as compared to treatment with either mAb alone. Taken together, the recorded data demonstrate that a chronic, HS diet likely led to expansion of TGFβ expressing TISCs, which in turn led to host immune exhaustion and tumorigenesis. Importantly, anti-TGFβ mAb exerted a favorable synergistic effect to enhance the anti-tumor efficacy of immune check-point inhibitors.