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ATTENUATED BACTERIA POTENTIATE THE OUTCOME OF IMMUNOTHERAPY WITH PD-L1 BLOCKADE IN A PRE-CLINICAL MODEL OF COLORECTAL CANCER

by

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Date & Venue

At 12 pm

Thursday, 11 November 2021

Abstract

After more than a century of investigation, it has become evident that cancer development and progression occur at the expense of a dysfunctional immune system. This realization has ushered cancer immunotherapy as a new modality of treatment. In the last decade, the use of immune checkpoint inhibitors to treat cancer patients resulted in unprecedented and durable clinical benefits. However, the response rate in many cancers remains rather modest, with colorectal cancer being at the lower end of the spectrum. Previous work from our laboratory demonstrated the efficacy of using attenuated bacteria as immunomodulatory anti-cancer agents. In the current study, we investigated the potential of utilizing a low dose of attenuated *Salmonella enterica* serovar Typhimurium (henceforth *S. typhimurium*) to enhance the therapeutic outcome of PD-L1 blockade, an immune checkpoint inhibitor, in a preclinical model of colon cancer. In our study, the response of MC38, a murine colon adenocarcinoma, to a suboptimal dose of anti-PD-L1 monoclonal antibody (aPD-L1 mAb; 5mg/kg/dose) was quite variable, with only ~30% of the mice responding to the treatment. The *Salmonella* strain used in this study is a well-characterized, double auxotrophic mutant known as BRD509, with an LD_{50} of $>5x10^6$ CFUs when administered systemically. Utilizing multi-color flow cytometry and immunohistochemistry, we demonstrated that even when used at a very low dose ($5x10^3$/mouse), BRD509 was able to modulate the tumor microenvironment through enhancing the infiltration of $CD4^+$ T cells, increasing the antigen presentation potential of myeloid cells, and decreasing the percentage of tumor-infiltrating lymphocytes that express inhibitory checkpoint ligands PD-1 and LAG-3. Combination treatment with BRD509 and αPD-L1 mAb increased the response rate to 100% and led to ~80% inhibition in tumor growth compared to controls. Mechanistically, the enhanced response correlated with considerable alterations within the tumor microenvironment including (1) an increase in tumor infiltration by $CD45^+$ immune cells including $CD4^+$ and $CD8^+$ T cells, (2) a decrease in the percentage of intratumoral $Ly6G^+$ granulocytic myeloid cells and (3) an increase in the percentage $Ly6G^-$ $Ly6C^{hi}$ macrophages that express MHC class II proteins. These changes were accompanied by a significant increase in apoptotic cells within the tumor parenchyma. Gene expression analysis by real-time PCR of tumor tissues revealed changes in the expression of various chemokines and inflammatory cytokines that were consistent with the observed alterations in the cellular constituents of tumors treated with BRD509 and αPD-L1. Taken altogether, the current study demonstrates that a novel combination treatment utilizing attenuated *Salmonella* and PD-L1 blockade could significantly improve the outcome of immunotherapy for colorectal cancer.

Keywords: Immune checkpoints inhibitors, PD-L1 blockade, *Salmonella typhimurium*, immunotherapy, colorectal cancer, tumor microenvironment.