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How Does PARP-14 Impact the RIG-I Pathway in Mouse Colorectal Cancer Cells?

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Keywords

Poly(ADP-ribose) Polymerase Inhibitors; CRISPR-Associated Protein 9; CRISPR-Cas Systems; Progression-Free Survival; Tumor Microenvironment; Colorectal Neoplasms; Adaptive Immunity; Interferons; Cell Death

Abstract

Colorectal cancer (CRC) is the 3rd most prevalent cancer worldwide. In treating cancer, the tumor microenvironment has a large impact on cell behavior, growth, and tumor progression. Pattern recognition receptors (PRRs) are signaling molecules within the tumor microenvironment that recognize pathogens and trigger an immune response to eliminate them. Targeting PRRs is a potent strategy to enhance immune recognition of tumors. Retinoic acid Induced Gene 1 (RIG-I) is a PRR that identifies viral RNA as non-self, drives cell death, and activates innate and adaptive immune responses. In previous studies, RIG-I activation gene signatures correlated with better progression-free survival in colorectal cancers. One gene in this activation signature, Poly (ADP-ribose) polymerase 14 (PARP-14), is upregulated in response to RIG-I in cell culture. PARPs are a family of DNA repair proteins that include PARP-14, which uniquely combines ADP-ribosyl transferase and hydrolase activities and modulates immune responses to fight infection using interferon pathways. It is unknown how the RIG-I and PARP-14 pathways interact within cancer cells. Based on our observations that RIG-I activates PARP14 and silencing of PARP14 results in increased RIG-I induced cell death, we hypothesize that RIG-I activation and PARP-14 inhibition will enhance anti-tumor immune responses synergistically. To explore the interactions between RIG-I and PARP-14, we generated PARP-14 deleted colorectal cell lines using CRISPR-CAS9 technology. We evaluated two experimental approaches to generate a genetic knockout of PARP-14, which involved electroporation of gRNAs targeting PARP-14 and Cas9 or using a cell-penetrating Cas9 protein, PARP-14 gRNA delivery. Once we validate the deletion efficiency, we will evaluate cellular phenotypes of PARP-14 knockout cells using viability, proliferation and clonogenic survival assays with and without RIG-I activation. We will evaluate molecular phenotypes of these cells using qRT-PCR. The phenotypes of CRISPR deleted PARP-14 cells will be compared to wild-type colorectal cells treated with a small molecule drug that inhibits the ADP-ribosyl transferase function of PARP-14. Expected results include increases in cell death and RIG-I activation signature genes, and a decrease in proliferation. This work has the potential to increase the efficacy of targeting PRRs in CRC by rational combinations with specific PARP inhibitors.