ATR-MEDIATED CD47 AND PD-L1 UPRGULATION RESTRICTS RADIOTHERAPY-INDUCED IMMUNE PRIMING AND ABBSCOPAL RESPONSES IN COLORECTAL CANCER

Background: Radiotherapy of colorectal cancer (CRC) can prime adaptive immunity against tumor-associated antigen (TAA)-expressing CRC cells systemically; however, incidences of abscopal tumor remission are extremely rare. We sought to unravel the post-irradiation immune escape mechanisms in CRC.

Methods: Flow cytometry, gene knockdown, RNA and T cell receptor sequencing, and multiple murine syngeneic CRC models were used to interrogate mechanisms of CRC immune evasion following radiotherapy. Comparison of immunohistochemistry staining between pretreatment biopsy and post-irradiation surgical specimens was performed in rectal patients who underwent neoadjuvant radiotherapy with 5 Gy for 5 fractions.

Results: We find that CRC cells utilize a common DNA repair signaling pathway — ATR/Chk1/STAT3 — to upregulate both CD47 and PD-L1 in response to radiotherapy, which through engagement of SIRPa and PD-1 suppresses the capacity of antigen-presenting cells (APCs) to phagocytose them thereby preventing TAA cross-presentation. This post-irradiation CD47 and PD-L1 upregulation can be observed in CRC cells treated with either photon or proton radiotherapy and across a wide variety of human solid tumor cells. Concordantly, rectal cancer patients who responded poorly (tumor regression grade 4–5, n = 10) to neoadjuvant radiotherapy exhibited significantly elevated post-irradiation CD47 levels (P = 0.005). In murine CRC models, the combination of radiotherapy, αSIRPa and αPD-1 (RSP) profoundly enhances TAA uptake, activation of innate immune sensors, and TAA cross-priming across various antigen-presenting myeloid populations in the irradiated tumor microenvironment and facilitates TAA-presenting APC migration to secondary lymphoid organs. Furthermore, we observed robust production of TAA-specific CD8 T cells, functional activation of effector T cells, and increased tumor-infiltrating T cell clonal diversity in mice treated with RSP. Importantly, radiotherapy coupled with phagocytosis checkpoint blockade significantly improves complete response rates in both irradiated and abscopal tumors and prolongs survival in three distinct murine CRC models, including a cecal orthotopic model. In addition, αSIRPa exerts superior tumoricidal efficacy than αCD47 in combination with RT and αPD-1. We find RSP efficacy to be STING dependent as knockout animals lose most benefit of phagocytosis checkpoint blockade.

Conclusion: ATR-mediated CD47 and PD-L1 upregulation restrains radiation-induced immune priming in CRC. Blockade of the phagocytosis checkpoints SIRPa and PD-1 during radiotherapy promotes vigorous anti-CRC immune priming leading to systemic tumor regression.

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Ethics Approval: This study was approved by the Institutional Review Board of Chang Gung Memorial Hospital, Taiwan; approval number: 202001191B0C061.