Background Although radiotherapy has been widely adopted in localized colorectal cancer (CRC) treatment, its therapeutic efficacy for distal, non-irradiated tumors remains limited. Ataxia telangiectasia and Rad3-related (ATR) kinase has recently been identified as a key mediator in post-irradiation cancer immune evasion. Here, we aimed to explore the systemic antitumor efficacy of ATR abrogation in combination with radiotherapy and immune checkpoint inhibition.

Methods We assessed the antitumor immune responses of focal radiotherapy, ATR abrogation, and CTLA-4 blockade by using microsatellite instability-high (MSI-H) MC38 and microsatellite stable (MSS) CT26 murine syngeneic CRC models. Flow cytometry, immunoblot, and gene knockdown were adopted to investigate the post-irradiation immune responses.

Results Ionizing radiation triggered the elevation of anti-phagocytic checkpoints, CD47 and PD-L1, in both MC38 and CT26 cells. ATR inhibition or short hairpin RNA knockdown (shATR) prevented RT-induced CD47 and PD-L1 upregulation and sensitized tumor cells to phagocytic clearance by bone marrow-derived antigen-presenting cells (APCs). We observed significantly improved complete response (CR) rates in both irradiated and abscopal tumors and prolonged survival in C57BL/6J mice bearing bilateral MC38-OVA tumors treated with unilateral radiotherapy, an ATR inhibitor, and anti-CTLA-4 antibodies. In the irradiated tumor microenvironment, radiotherapy followed by ATR inhibition and CTLA-4 blockade significantly improved tumor antigen cross-presentation in APCs, increased NK and tumor-specific CD8 T cell infiltration, and decreased regulatory T lymphocyte accumulation. Concordantly, BALB/c mice bearing bilateral shATR-CT26 tumors treated with unilateral fractionated radiotherapy and anti-CTLA-4 antibodies had significantly higher CR rates in both irradiated and abscopal tumors and improved survival, compared with those bearing wild type CT26 tumors. Furthermore, a cecal orthotopic MC38 tumor model consistently demonstrated superior systemic antitumor efficacy of radiotherapy, ATR inhibition, and CTLA-4 blockade combinatorial therapy. Re-implantation of MC38 cells was performed in mice with complete remission of both irradiated and abscopal tumors, and the rechallenged tumors were rejected in all mice in the triple therapy group.

Conclusions Abrogation of ATR potentiates the systemic antitumor immune responses of radiotherapy and CTLA-4 blockade in both MSI-H and MSS murine CRC models.

REFERENCE

Ethics Approval All animal experiments were performed in compliance with the approved protocol (00001378-RN01/ RN02) by the Institutional Animal Care and Use Committee at MD Anderson Cancer Center.