Radiation-Induced Neoantigens as Targets for AND-Gate CAR T-Cell Therapy in PDAC

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Pancreatic ductal adenocarcinoma (PDAC) is one of the most lethal cancers, with limited treatment options and poor prognosis. Conventional therapies, including CAR-T cell therapy, have shown minimal efficacy in PDAC due to the immunosuppressive tumor microenvironment (TME) and the lack of highly specific target antigens, increasing the risk of off-target effects. Radiation therapy (RT) is a therapeutic modality with a role in the clinical management of PDAC. Here, we use RT not as a conventional treatment, but as a strategic tool to modulate tumor antigenicity and uncover novel targets for CAR T cell therapy. We hypothesized that RT induces the upregulation of specific surface markers on primary and metastatic PDAC cells, generating a window of neoantigen expression that could be targeted with CAR T cells. To test this, we conducted *in vitro* experiments using the murine pancreatic cancer cell line MH6419, subjected to 8 Gy RT followed by plasma membrane protein purification and proteomic analysis at 24-, 48-, and 72-hours post-irradiation. Our *in vitro* results revealed significant upregulation of plasma membrane proteins, including Muc4 and Muc13, both previously associated with cancer progression and identified as potential tumor-specific antigens. To validate and expand upon these findings, we are currently conducting *in vivo* proteomic analysis on irradiated tumors using two preclinical models: MH6419 cells in immunocompetent C57BL/6 mice and the human PDAC line AsPC-1 in NSG mice. These *in vivo* studies are critical to confirm *in vitro* findings, to identify radiation-induced antigens in a more physiologically relevant TME, and to better approximate clinical settings for future translation. Our goal is to develop a bi-specific AND-gate CAR T cell therapy that selectively targets RT-induced neoantigens, improving tumor specificity while minimizing off-tumor toxicity. By integrating RT as a tool to temporally and spatially regulate tumor antigen expression, this strategy offers a novel and non-invasive approach to enhance CAR T cell efficacy in PDAC and its metastases.

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