Background: A driving factor in PDAC treatment resistance is the tumor microenvironment, which is highly immunosuppressive. One potent immunological adjuvant is radiation therapy. Radiation, however, has also been shown to induce infiltration of immunosuppressive populations, which can contribute to tumor progression. Another negative effect of radiation is that it can contribute to the formation of fibrotic stroma within the tumor microenvironment. To gain benefit from the immunogenic effects of radiation and obtain a durable tumor response, it must be rationally combined with targets aimed at mitigating the influx of immunosuppressive cells and fibrosis. One such target is ephrinB2, which is overexpressed in PDAC and correlates negatively with prognosis.

Material and Methods: Immunocompetent C57BL/6 and immune compromised athymic nude mice were injected subcutaneously in the right flank with either a patient derived xenograft (PDX), PANC 272, or a mouse pancreata derived cell line (FC1242). Treatment groups involved 5-7 mice which were randomized to receive B11 (an inhibitor of EFN2 and EphB4 ligand pair), PBS, RT and combinations thereof. Mechanistic studies involved mice receiving the same treatments, but tumors were harvested after 72 hours and processed for flow cytometric analysis. After sacrifice, their tumors were fixed in paraffin and tissue slices were stained with primary antibodies.

Results: Based upon previous studies of ephrinB2 ligand-EphB4 receptor signaling, we hypothesized that inhibition of ephrinB2-EphB4 combined with radiation would regulate the microenvironment response post radiation, leading to increased tumor control in PDAC. Our data show this treatment regimen significantly reduces regulatory T-cell and neutrophil infiltration (33-44% and 54-60% respectively), TGFβ-1 secretion (71-79% less staining in B11+RT compared to other groups), and stromal fibrosis (B11 mitigation of 1.6-2.6 fibrosis increase seen in RT group), enhancing effector T-cell activation (RT increased CD8+IFNγ+ and CD4+TNFα+ infiltration by 2 fold and CD8+TNFα+ and CD4+IFNγ+ by 4 fold, which was sustained or enhanced by B11+RT) and decreasing tumor growth (fold changes: B11+RT 2.298±0.84 vs control 13.11±3.4811, B11 alone 9.557±3.070, and RT alone 5.239±3.851). Further, our data show that depletion of regulatory T-cells in combination with radiation reduces tumor growth (424.4±25.07 mm³ in IgG±RT vs αCD25+RT 211.2±25.17 mm³ in αCD25+RT) and fibrosis (2.2 to 1.4 reduction in fibrosis). These are the first findings to suggest that in PDAC, ephrinB2-EphB4 interaction has a profibrotic, pro-tumorigenic role, presenting a novel and promising therapeutic target.

Conclusions: Our study provides strong evidence of Tregs contributing to PDAC resistance to RT and a potential method for reduction in Tregs as well as fibrosis, two areas of clinical importance in PDAC, via EFN2-EphB4 blockade. The effects of targeting EFN2-EphB4 are not limited to Tregs and fibrosis, but also include reduced angiogenesis and neutrophil infiltration and increased Teff activation. The ability to target multiple tumorigenic pathways is a strength of targeting this signaling pair. Our findings are supported by clinical data and have translational potential to enhance the therapeutic efficacy of RT in patients with PDAC.

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