dorsal skin resulting in the development of chronic RIF. Perfusion testing with laser doppler was performed throughout the healing interval. Four weeks following radiation, dorsal skin was harvested and analyzed histologically and biomechanically. Immunofluorescent staining with 4 Hydroxynonenal (4-HNE) antibody was carried out to assess ferroptosis activity and imaged using confocal microscopy. Tissue was collected for single cell RNA sequencing and analyzed using the 10x platform. Cell annotations were ascribed using SingleR to identify fibroblasts and cell-type marker lists were generated using Seurat's native FindMarkers function.

Results: IHC staining showed decreased 4-HNE staining in non-irradiated skin, DFO-treated skin, and ferrostatin-treated skin compared to irradiated untreated skin. Prophylactic treatment with DFO decreased 4-HNE staining and produced histological measures closer to normal skin more so than subsequent DFO treatment. While perfusion levels increased with DFO treatment, they were not affected by ferrostatin treatment. Histological measures of RIF (dermal thickness via Hemotoxylin and Eosin and collagen deposition via Massons Trichrome) were closer to normal skin with both DFO and ferrostatin treatment. Single cell RNA sequencing analysis demonstrated that an expression module of genes associated with ferroptosis decreased in groups treated with DFO compared to irradiated untreated skin.

Conclusion: Ferroptosis occurs more frequently in the dermis of murine skin following irradiation and is decreased by DFO topical treatment. Inhibition of ferroptosis with DFO and Ferrostatin alleviates RIF in murine skin. Single cell analysis indicated DFO treatment decreased transcription of drivers of ferroptosis in irradiated murine skin.

25. FAK Activation Enhances Diabetic Wound Healing In A Murine Model

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Purpose: Diabetes mellitus continues to be a growing healthcare issue. Diabetic individuals experience many complications, including impaired wound healing, subsequent infection, and limb amputation. Few interventions have been shown to successfully improve diabetic wound healing, indicating a clear area of need.

Methods: Using a db/db diabetic mouse model, a splinted excisional wounding technique was used to create full thickness dorsal wounds. Mice were divided into two groups, control and experimental (n = 5 mice per group, 2 wounds per mouse, n = 10 wounds per group). A small molecule Focal Adhesion Kinase (FAK) activator, ZN27 (C23H25F3N3O3), was injected into the wound beds of the experimental group every other day. The control group received a saline injection. Images were taken of the wounds every second day. The wound areas were traced and expressed as a percentage of the original area to generate a wound healing curve. Once all wounds healed, the tissue was harvested, processed, and sectioned for histology (Picrosirius Red collagen staining). In vitro, murine fibroblasts were cultured with and without ZN27 before fixation and IHC analysis.

Results: Wounds treated with ZN27 healed at a significantly faster rate, averaging 14.6 days to wound closure for the ZN27 group vs 19.6 for the control group ($p = 0.0002$). Collagen architecture analysis revealed that healed tissue in the ZN27 group had longer ($p = 0.0239$) and wider ($p = 0.0263$) collagen fibres. Murine fibroblasts cultured with Zn27 were found to express more pFAK Y397.

Conclusion: FAK activation with ZN27 promotes faster wound healing in a diabetic murine model, as well as the formation of longer and thicker collagen fibres. Further analysis of involved molecular pathways will help to strengthen this conclusion. FAK activation could prove to be a future therapeutic target to enhance healing of chronic diabetic wounds in the clinical setting.

26. The Role of Macrophages In Mediating Radiation-induced Fibrosis

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Purpose: Radiation-induced fibrosis (RIF) remains a clinically challenging problem in cancer patients without

effective treatment or prevention. To elucidate the mechanisms of RIF, we investigated the role of macrophages as mediators of fibrosis, and the reciprocal signaling that occurs between macrophages and fibroblasts.

Methods: Bone marrow-derived macrophages and dermal fibroblasts were isolated from C57BL/6 mice. Cells were co-cultured in a three-dimensional collagen gel system and subjected to radiation. Gel contracture was measured over time. Flow cytometry was utilized to analyze macrophage polarization towards inflammatory and anti-inflammatory phenotypes using cell surface markers such as CD38 and CD206, respectively. Macrophages from both radiated and non-radiated co-cultures were subjected to RNA sequencing to investigate radiation induced phenotypic and functional changes.

Results: Co-culturing fibroblasts and macrophages led to pronounced collagen gel contraction compared to fibroblasts alone, highlighting the essential role of macrophages. In addition, radiated macrophages had a significantly increased effect on gel contracture compared to non-radiated controls indicating a changed phenotype promoting contraction. Since macrophage involvement was found to be critical for radiation-induced functional alteration in collagen gels, we investigated macrophage phenotypes in response to radiation. Flow cytometry analysis revealed that radiation-induced alternate activation (M2) of macrophages, as shown by increased CD206 expression. Similarly, RNA sequencing data showed increased expression of interferon response genes such as Ifi206 and chemokines including c-c motif chemokine ligand 7 (ccl7) suggesting a distinct inflammatory response to radiation. Furthermore, we analyzed the impact of radiation on macrophage plasticity. Interestingly, when macrophages were polarized to M2 phenotype and then radiated, their potential to repolarize to M1 was lost, as opposed to non-radiated macrophages suggesting potential loss of macrophage plasticity during radiation.

Conclusion: Our study highlights the crucial role of macrophages in early RIF and their potential as a therapeutic target to mitigate the negative side effects of radiation therapy. We observed that radiation exposure induces an M2 phenotype, upregulates interferon response genes in macrophages, and impairs plasticity. These findings suggest that radiation may contribute to the pro-fibrotic environment by activating macrophages in a physiologically distinct manner. Our study offers insights into the underlying mechanisms of RIF and the role of macrophages in this process.

27. Fibrotic And Regenerative Fibroblasts Emerge In The Lungs To Drive Fibrosis And Repair

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Purpose: Pulmonary fibrosis (PF) represents a pathological outcome of numerous lung injuries and chronic diseases, producing a global clinical burden for over 5,000,000 patients. PF generates a heterogeneous mixture of pro-fibrotic foci and healthy areas within the lungs, and the fibroblast subpopulations that drive these concurrent but opposing regions are not well-understood. To understand the spatially distinct drivers of PF matrix aberration and repair, we performed a multi-omic analysis of fibrotic progression and resolution in a mechanistic mouse model.

Methods: Fibrotic and non-fibrotic matrix architecture was quantified by acquiring >2,500 polarized Picrosirius Red images of bleomycin-induced fibrotic mouse lungs across postoperative day (POD) 0-49, followed by analysis using a machine learning-based algorithm previously developed by our laboratory. Single-cell RNA sequencing (scRNA-seq) was used to identify fibroblast subpopulations enriched at baseline, fibrotic, and reparative timepoints, while single-cell assay for transposase-accessible chromatin with sequencing (scATAC-seq) was used to analyze epigenomic changes in fibroblasts at each stage. Visium spatial transcriptomics was used to identify the anatomical localization of fibroblast subpopulations, and a custom algorithm was applied to identify how these fibroblast subtypes were represented in distinct fibrotic and healthy areas of the lungs.

Results: Matrix architecture evolved from POD 0-49, reaching a peak of fibrosis at POD 28, followed by gradual resolution to baseline by POD 49. ECM-secreting (*Spp1*+) and pro-regenerative (*Pi16*+) fibroblasts emerged at the midpoints of fibrosis (POD 14) and post-fibrotic repair (POD 35), respectively, according to scRNA-seq Further, scATAC-seq revealed that fibroblasts underwent pro-fibrotic activation by genes involved in *JUN* and *TGF-β*