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Abstract

Rationale: Exposure of the lung to ionizing radiation, such as during radiotherapy, can result in pulmonary fibrosis (PF), which has few treatment options. PF is characterized by an accumulation of extracellular matrix proteins that form scar tissue, resulting in dyspnea, disruption of gas exchange, and even death. We and others have shown that metabolic reprogramming is a hallmark of idiopathic pulmonary fibrosis (IPF). IPF lung tissue, and lung fibroblasts treated with TGF-β, exhibit increased aerobic glycolysis with increased expression of lactate dehydrogenase A (LDHA) and excess production of lactate, leading to reduced extracellular pH that activates latent TGF-β. **Here, we hypothesized that ionizing radiation would cause aerobic glycolytic metabolic dysregulation in primary human lung fibroblasts.**

Results: Primary non-fibrotic HLFs exposed to irradiation exhibited significant upregulation of Pyruvate Dehydrogenase Kinase (PDK1 (0.5 – 3-fold, $p<0.05$) and LDHA (1.4-fold, $p<0.05$). Cell viability was unaffected by increased radiation dose.

Conclusions: Radiation increased fibroblast expression of genes involved in fibrotic phenotypes (αSMA) and aerobic glycolysis (PDK1 and LDHA), in a similar pattern to that seen in IPF fibroblasts. The metabolic changes are closely associated with creating a profibrotic extracellular environment in IPF by promoting an acidic environment. This phenomenon in fibrotic fibroblasts is similar to observations of the Warburg effect in cancer cells, where aerobic glycolysis occurs despite the presence of oxygen, allowing growth advantages. Our evidence suggests this phenomenon can be driven by radiation in lung fibroblasts and affirm that glycolytic reprogramming may also be a hallmark of radiation-induced fibrosis. Further understanding of the common mechanisms that create this metabolic shift could provide novel therapeutics for fibrosis treatment.

Radiation and Fibrosis

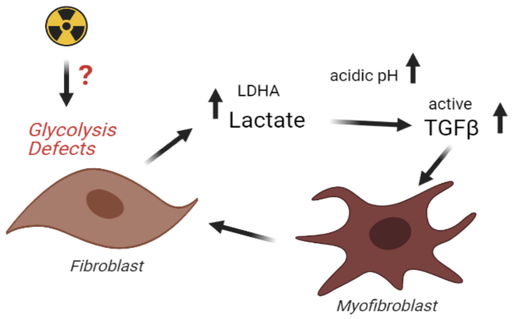


Figure 1. Ionizing radiation presents injury which activates a wound healing response where fibroblasts differentiate into myofibroblasts. Our lab has shown that lactate and LDHA (enzyme that produces lactate) increased post – irradiation. Lactate excretion causes increases in extracellular acidic environment. This activates latent TGFβ (cytokine) which induces myofibroblast differentiation.

Methods

Primary human lung fibroblasts (HLFs) from three non-fibrotic donors were seeded and subjected to either no treatment, TGF-b treatment (500 and 1000 pg/mL), or radiation (3, 5, and 7 Gy). Cell lysates were harvested 2 and 5 days after irradiation for RNA and protein, respectively. Gene and protein expression of metabolic markers were determined by RT-PCR and western blot. TBP (RT-PCR) and GAPDH (western blots) were used as loading controls.

Results

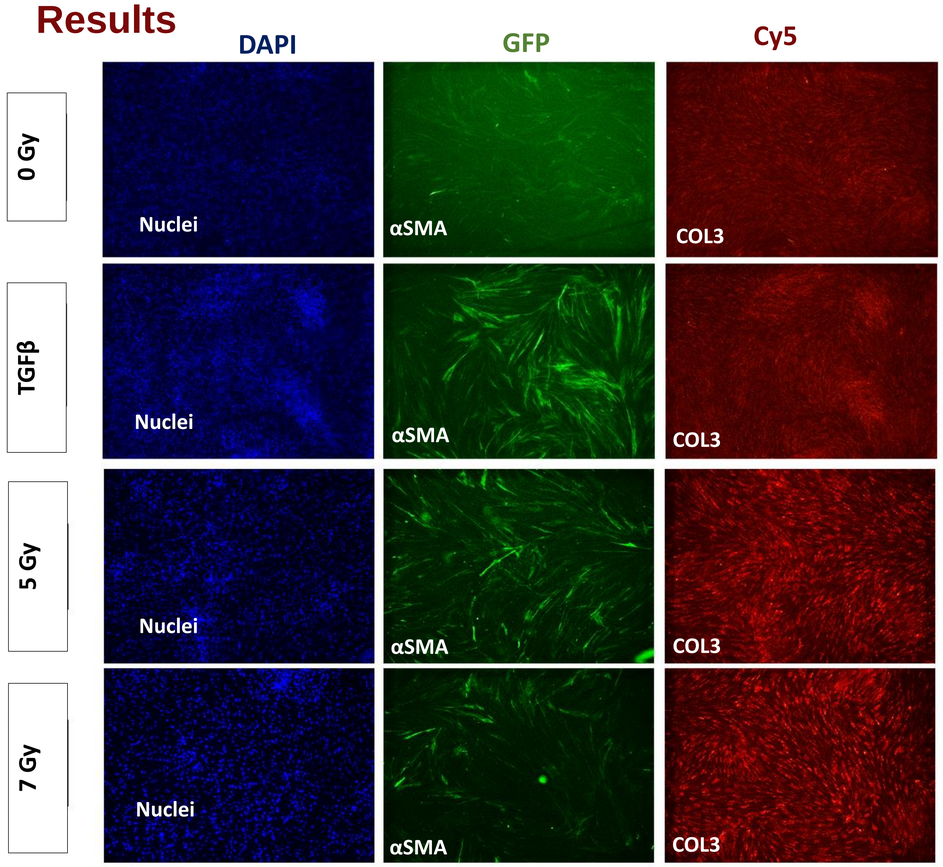


Figure 2. Immunohistochemistry Staining of Profibrotic Markers Non-fibrotic primary human lung fibroblasts were irradiated as described and harvested and stained with DAPI - Nuclei, GFP - αSMA, and Cy5 - Collagen 3 5 days after irradiation. Radiation induced αSMA and collagen 3 protein expression compared to untreated. Radiation promoted profibrotic phenotypic changes.

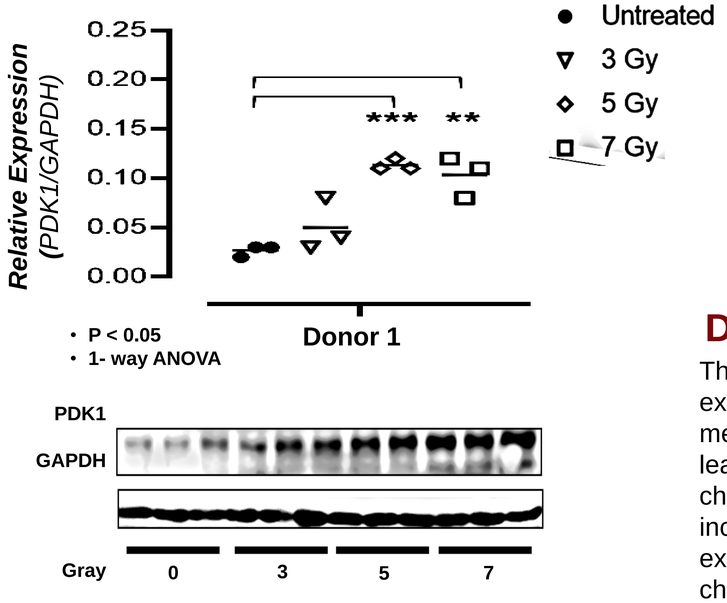


Figure 3. Western Blot of Pyruvate Dehydrogenase Kinase 1 Cells were irradiated as indicated and harvested after 5 days. Irradiation induced significant increases in protein expression of PDK1. Radiation affected normal glycolytic metabolism.

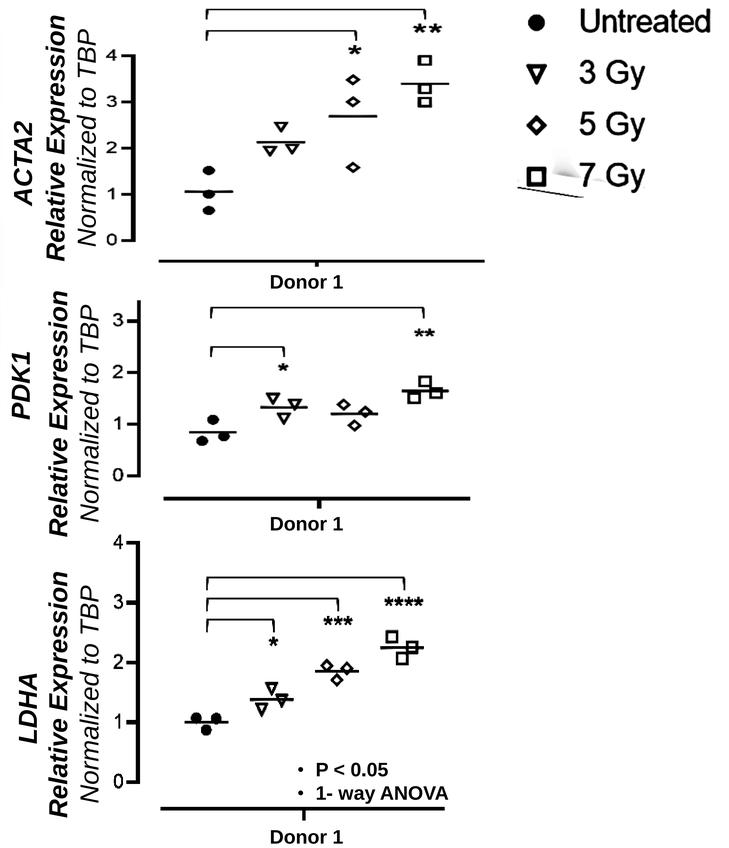
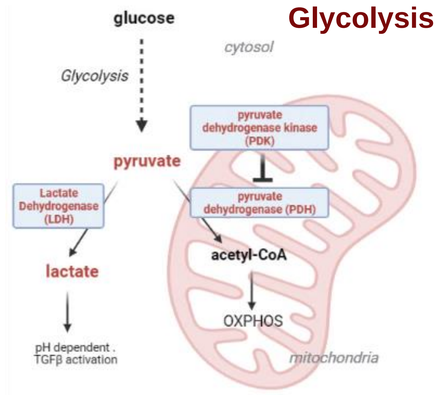


Figure 4. Gene Expression of Metabolic and Profibrotic Markers Cells were irradiated as described. RNA lysates were harvested 2 days followed by RT-PCR analysis. Both profibrotic (ACTA2) and glycolytic (LDHA and PDK1) gene expression increased significantly post-irradiation exposure.



Discussion

Though we have previously demonstrated changes in lactate and LDHA expression post irradiation leading profibrogenic phenotypes, the underlying mechanism remains unclear. Here, the role of precursor glycolytic defects leading as it relates to this phenomenon was investigated. Non-fibrotic HLFs change their metabolism in response to radiation exposure indicated by an increase in LDHA and PDK1 gene expression. Increases in ACTA2 expression after treatment indicated a profibrotic phenotype. Metabolic changes were also observed at the protein level indicated by changes in PDK1 expression.

Support

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