Idiopathic Pulmonary Fibrosis Causes Imbalance in Pulmonary Cell Viability In Response to Ionizing Radiation

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Introduction

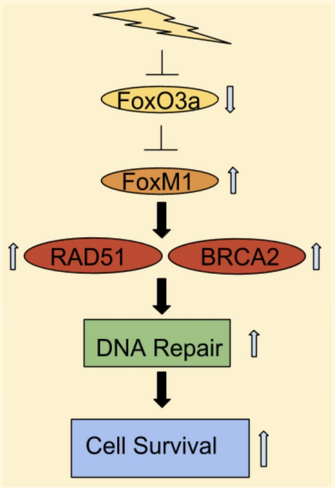
- Idiopathic Pulmonary Fibrosis (IPF) causes irreversible scarring of the lung resulting in breathing complications.
- IPF is characterized by the overproduction of type I collagen rich extracellular matrix and the presence of highly viable fibrotic fibroblasts
- IPF fibroblasts becomes resistant to apoptosis inducing insults including ionizing radiation.

Objectives

- Determine whether the FoxO3a/ FoxM1/ BRCA2& RAD51 DNA repair pathway is involved in apoptosis resistance of IPF fibroblasts following radiation.
- Determine whether radiation will cause an imbalance in viability in IPF fibroblasts, control fibroblasts, and epithelial cells as a result of their differences in radiation sensitivity.
- Determine amount of type I collagen production of IPF fibroblasts, control fibroblasts, and epithelial cells before and after radiation.

Hypothesis

- We hypothesize that BRCA2- RAD51 will be up regulated in IPF fibroblasts due to abnormal expression of FoxM1.



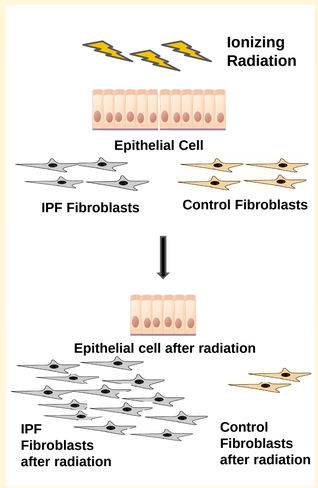


Figure 1 (Left). Proposed mechanism in the proliferative pathway of IPF fibroblasts after ionizing radiation. Ionizing radiation upregulates transcription factor FoxM1 to increase expression of DNA repair proteins BRCA2 and RAD51.

Figure 2 (Right). Proposed model for the progression of lung Fibrosis. After radiation, IPF fibroblasts' resistance to radiation induced cell death will continue to remain viable and produce type I collagen rich extracellular matrix. Epithelial cells and control fibroblasts will accumulate DNA damage and undergo apoptosis

- We predict that epithelial cells will be the most sensitive to radiation induced cell death, followed by control fibroblasts. IPF fibroblasts will remain the most viable.
- We predict that IPF fibroblasts will produce the most type I collagen rich extracellular matrix as compared to control fibroblasts and epithelial cells.

Methods

- Human Bronchiole Epithelial Cell (HBEC-3KT), IPF fibroblasts, and control fibroblasts were plated on pure collagen (PC). Fibroblasts were grown in DMEM media with 10% FCS and 1% antibody. Epithelial cells were grown in airway epithelial cell basal medium.
- Cells were treated with 9 Gy of radiation after 48 hours of initial seeding. Cell lysates and conditioning media were collected at a time point of 1, 6, 12, and 24 hours after radiation. Non-irradiated cells were used as control.
- Cell viability assay was performed 3 days after radiation. Protein levels in conditioning media and lysates were assessed using Western Blot and densitometry analysis.

Results

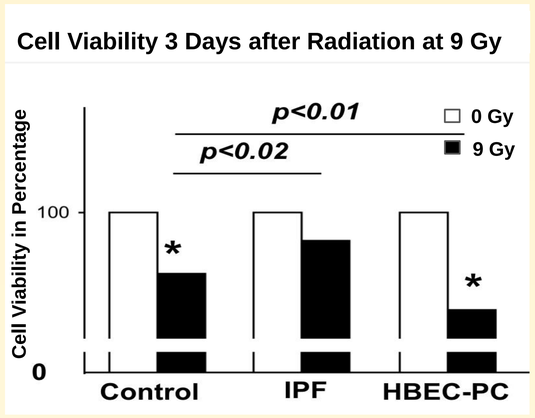


Figure 3. Cell viability assay of control fibroblasts, IPF fibroblasts and HBEC on PC 3 days after irradiation at 9 Gy. Results show IPF fibroblasts remained highly viable while HBEC and non IPF fibroblasts were sensitized to radiation-induced cell death.

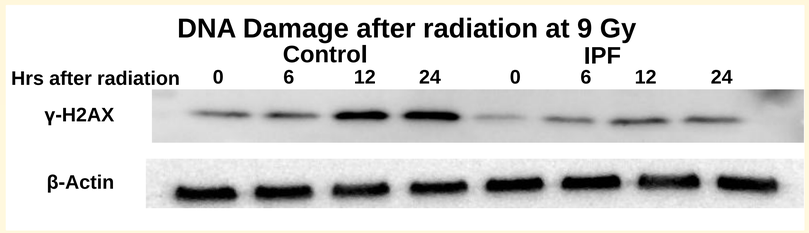


Figure 4. Western blot analysis of DNA damage at a time point of 6, 12, and 24 hours after radiation at 9 Gy. 0 hour samples were not treated with radiation. γH2AX protein is an indirect indicator of DNA damage. Housekeeping gene, β-Actin, was used as a control.

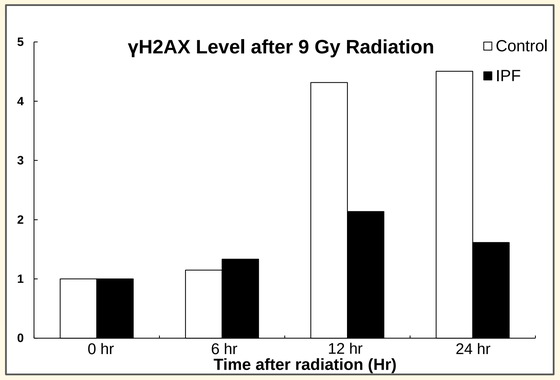


Figure 5. Densitometry analysis of γH2AX. Values are normalized by β-Actin and folds are calculated to make a direct comparison between the control fibroblasts and IPF fibroblasts. The change in abundance of γH2AX before and after radiation can be observed in this figure. Control fibroblasts show more DNA damage than IPF fibroblasts.

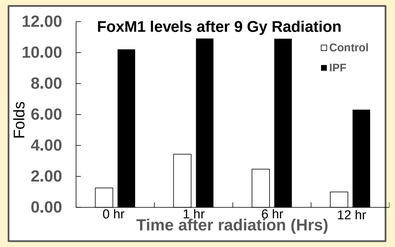


Figure 6. Western blot densitometry analysis for protein abundance of FoxM1. Control and IPF fibroblasts were treated with 9 Gy radiation and lysates were collected at 1, 6, and 12 hours after radiation. 0 hours sample serves as control and was not treated with radiation. From the results, it is seen that FoxM1 were much more abundant in IPF fibroblasts.

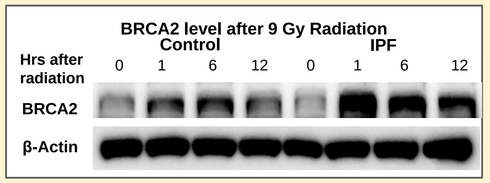


Figure 7. Western blot of BRCA2 protein levels in control and IPF fibroblasts after radiation at 9 Gy. Lysates were collected at 1, 6, and 12 hours after the radiation treatment. 0 Gy is the control sample and was not treated with radiation. It can be seen that BRCA2 is upregulated in IPF fibroblasts.

Conclusion

- The results from the cell viability assay in *Figure 3* supports our hypothesis that IPF fibroblasts become resistant to radiation induced cell death due to the hyper-activation of FoxM1 dependent DNA repair proteins, RAD51 and BRCA2.
- Non-IPF (control) and HBEC are highly sensitive to radiation-induced cell death.
- The presence of apoptosis resistant IPF fibroblasts can contribute to the progression of lung fibrosis.
- Our results support our proposed mechanism that the alteration of FoxO3a/FoxoM1/BRCA2-RAD51 in IPF fibroblasts protects them from radiation induced cell death, leading to the progression of lung fibrosis. .

Future Directions

- Study the FoxO3a/ FoxM1/ BRCA2& Rad51 axis in epithelial cells and compare it to control and IPF fibroblasts.
- Examine type I collagen rich extracellular matrix in IPF fibroblasts, control fibroblasts, and epithelial cells before and after radiation.
- Measure total RNA levels of COL1A1, the pro-collagen gene, in IPF fibroblasts, control fibroblasts, and epithelial cells by real time PCR.

References

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