Mitigating Radiation Fibrosis with Adipose Derived Stromal Cells

Fei-Fei Liu MD

Chief and Chair Radiation Medicine Program
Princess Margaret Cancer Center, University of Toronto
Senior Scientist, Ontario Cancer Institute

ICTR-PHE

Geneva, Switzerland

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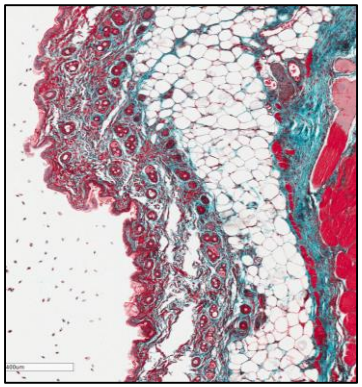
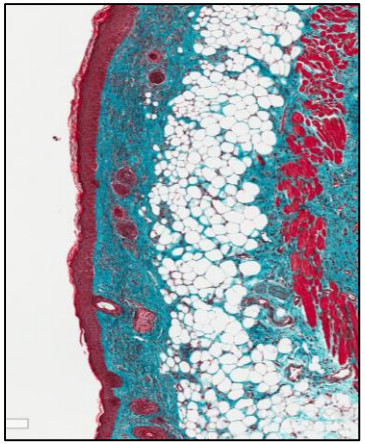
Radiation Oncology
UNIVERSITY OF TORONTO

UHN Princess Margaret Cancer Centre

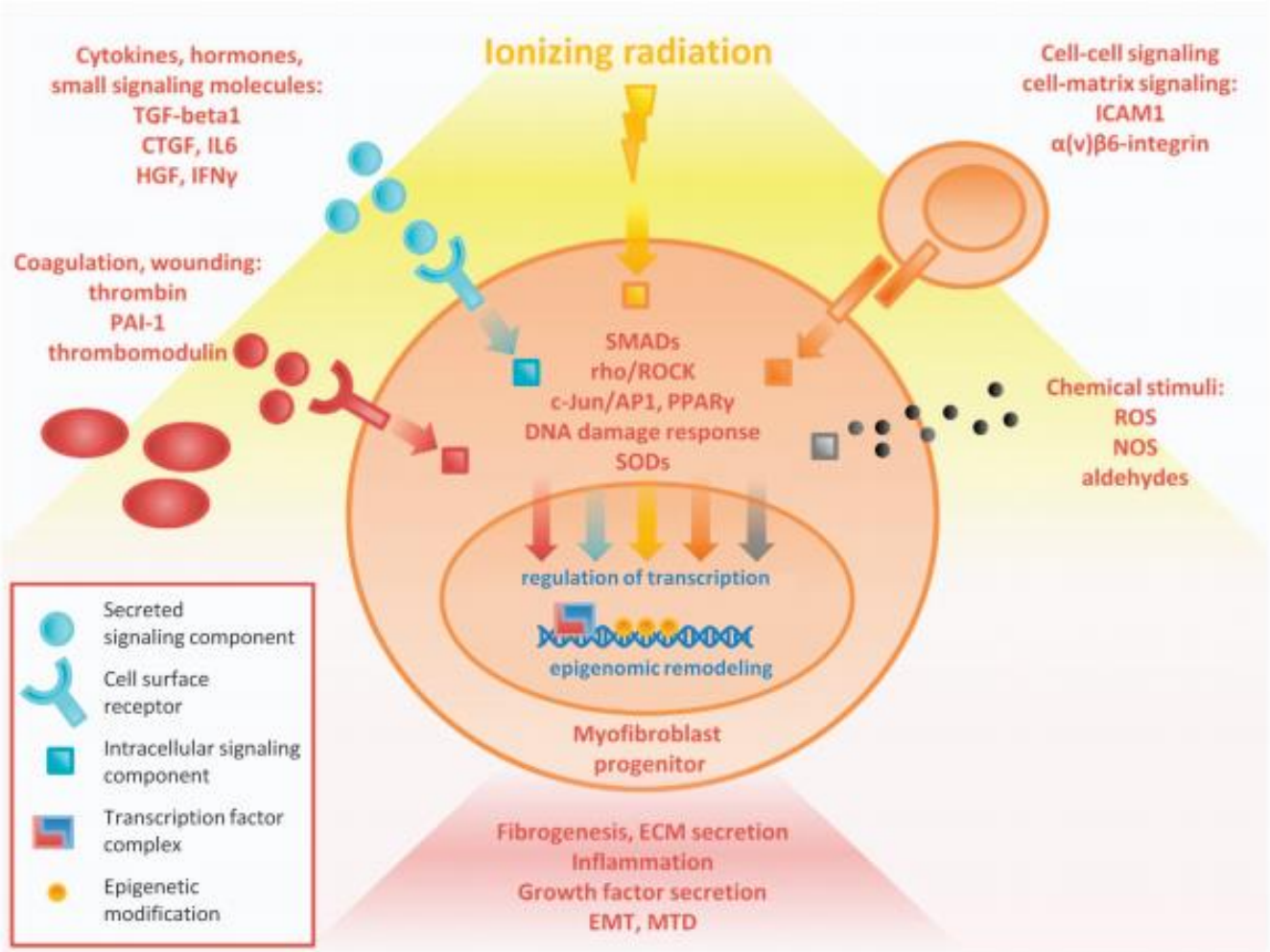
Introduction: Radiation Fibrosis

- Radiation fibrosis is a late side effect of radiotherapy
  - Irreversible
  - Increased Morbidity
  - Poorly understood
  - Poorly treated
- Histologically defined based on excess collagen deposition



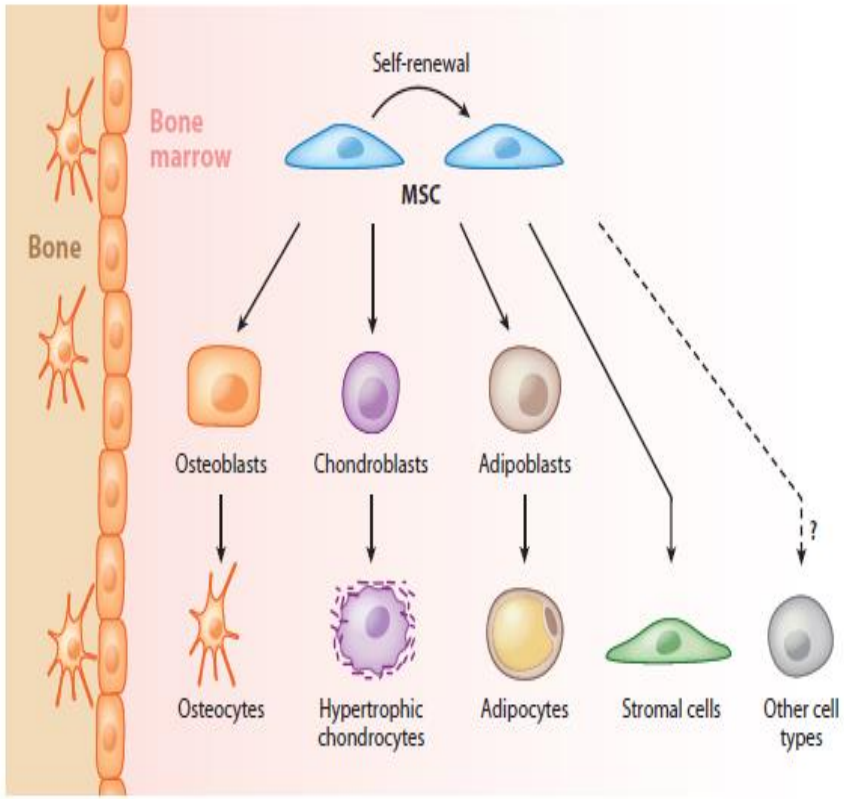


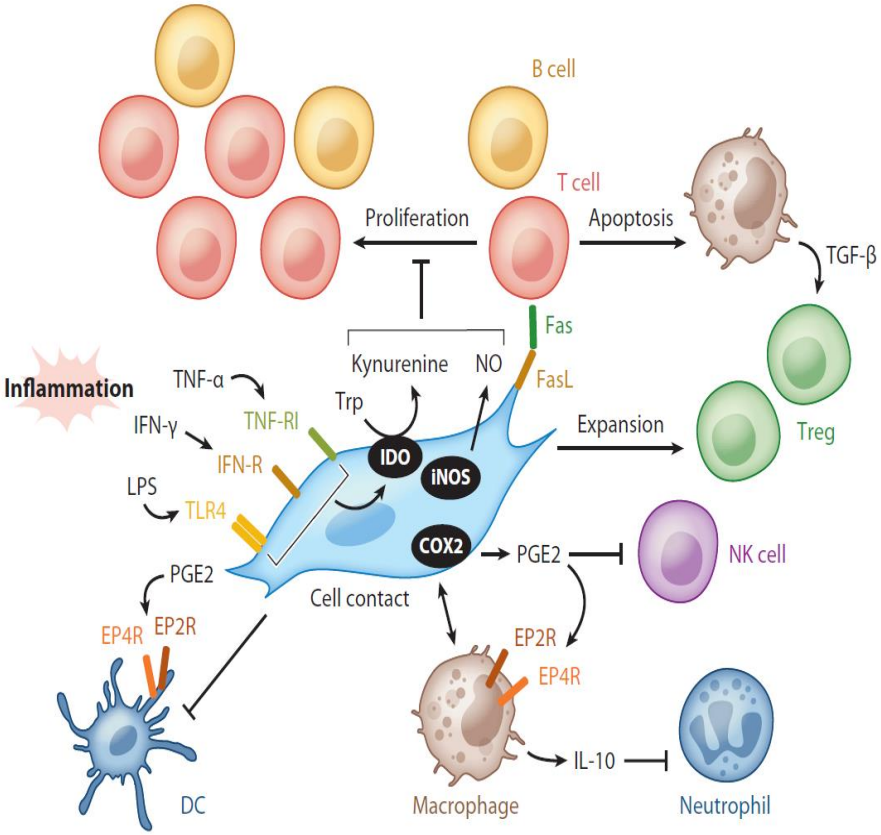
Role of Epigenetic Alterations in the Pathogenesis of Radiation Fibrosis



Weigel *et al; Oncogene* (2014) 1-11

Adipose Derived Stromal Cells (ADSCs)





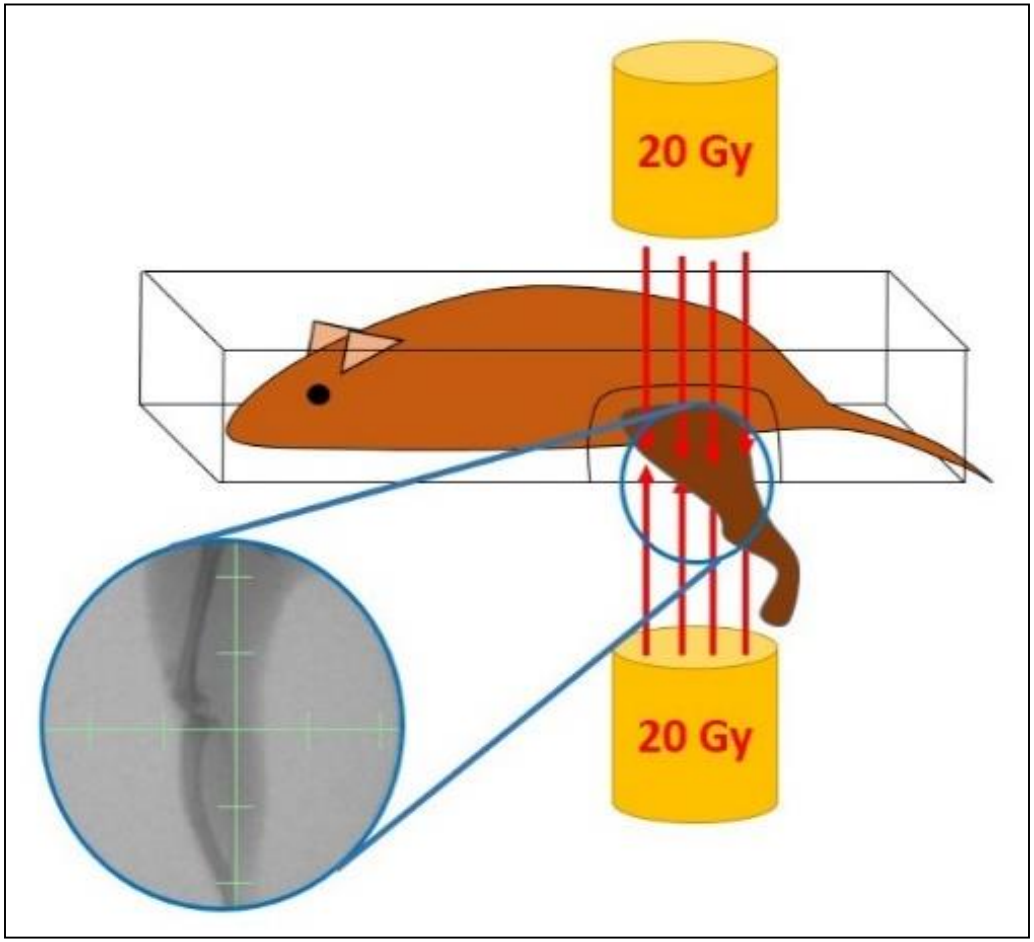
Frenette *et al; Annu Rev Immunol* 2013;31:285

Hypothesis

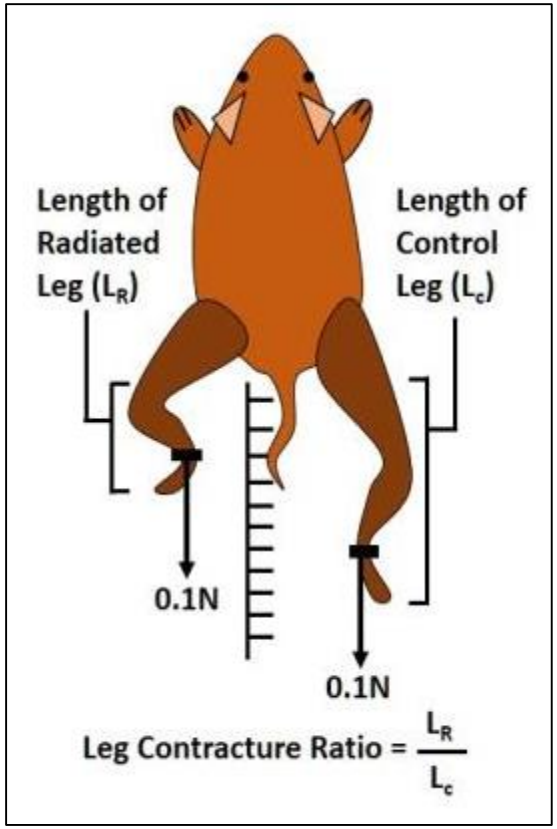
ADSCs may be therapeutically effective in mitigating radiation fibrosis through reversal of methylation aberrations.

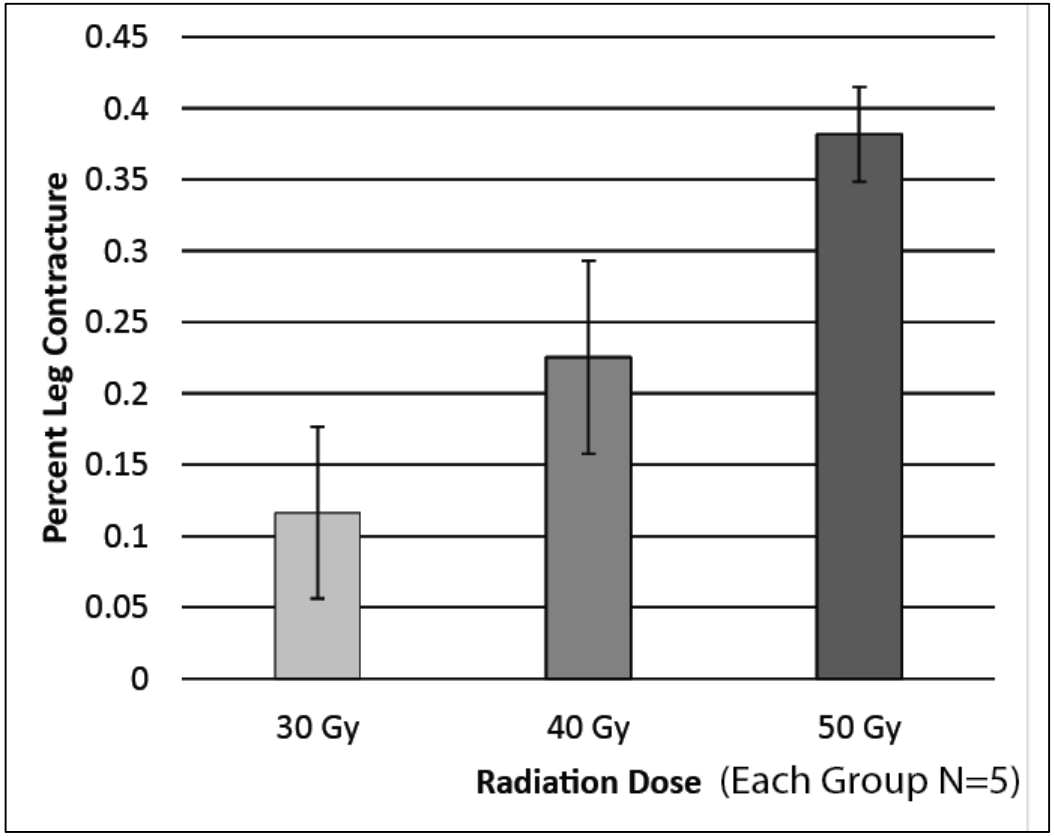
<u>Aim 1</u>: To determine the therapeutic effect of ADSCs

Establishing Radiation Fibrosis Model



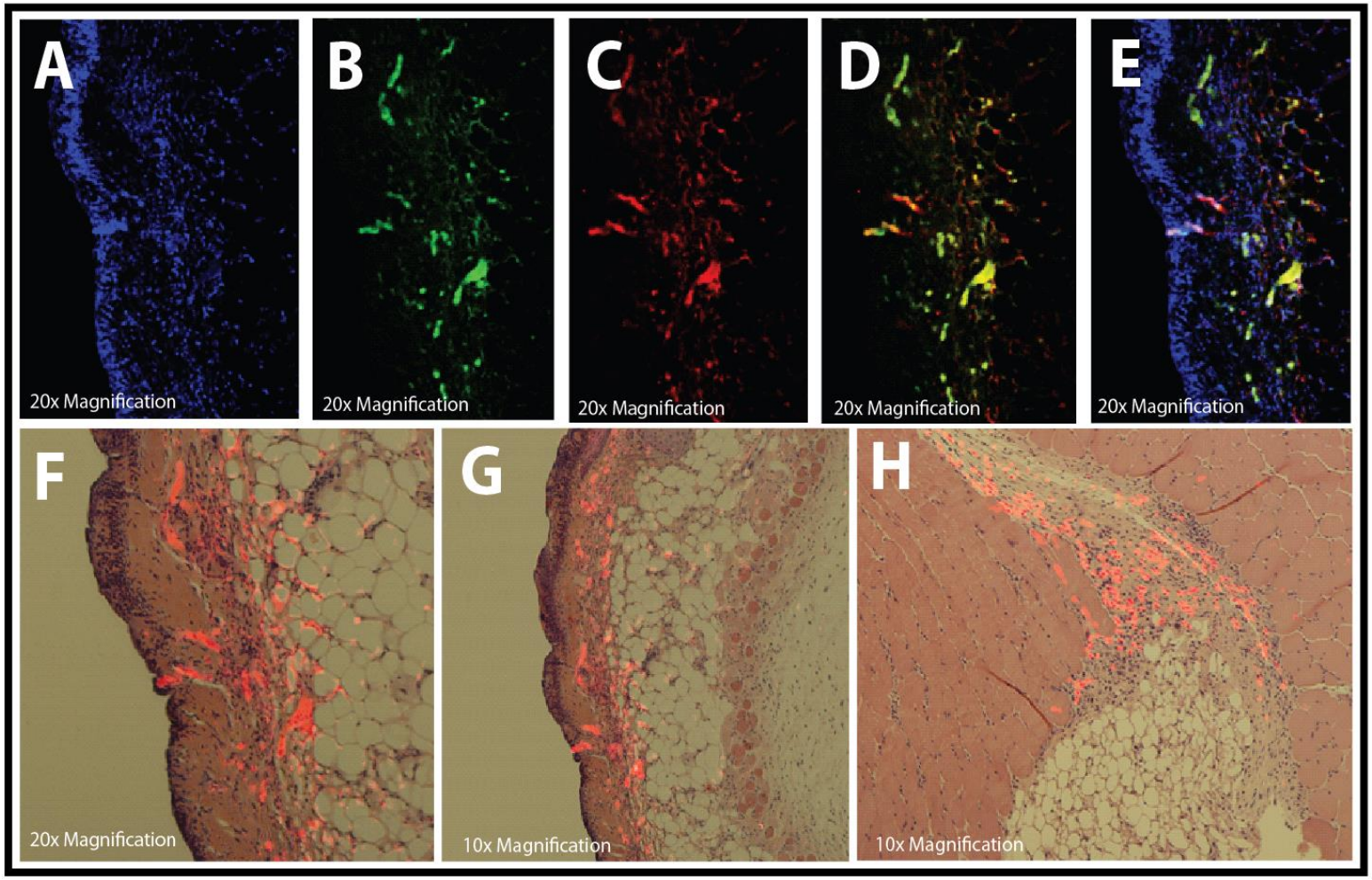
Establishing Radiation Fibrosis Model





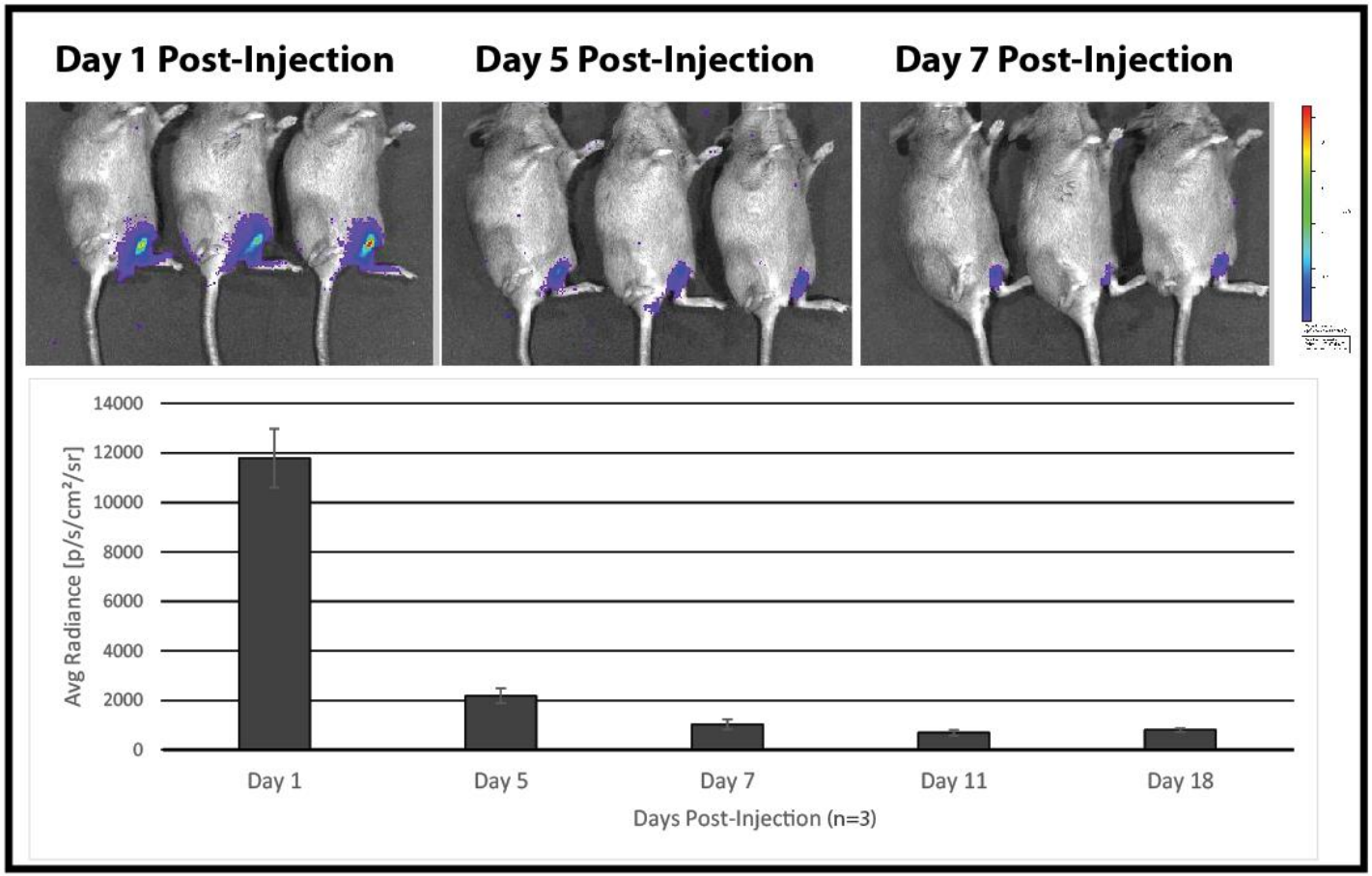
At 6 weeks post-RT

ADSC Localization After Transplantation

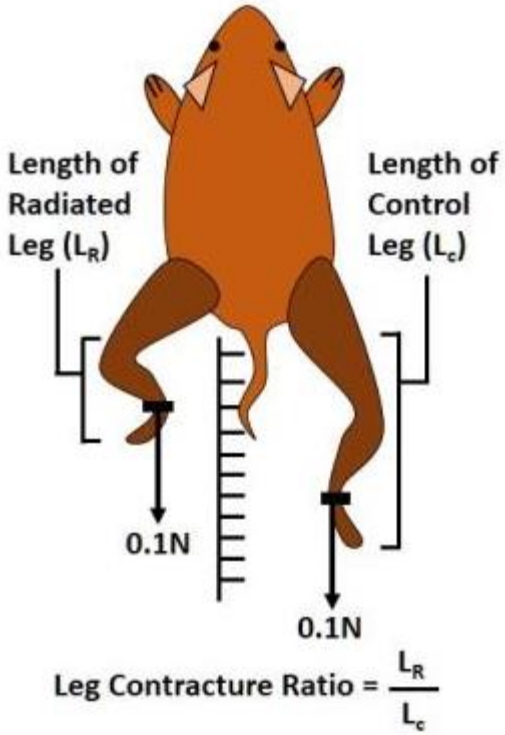


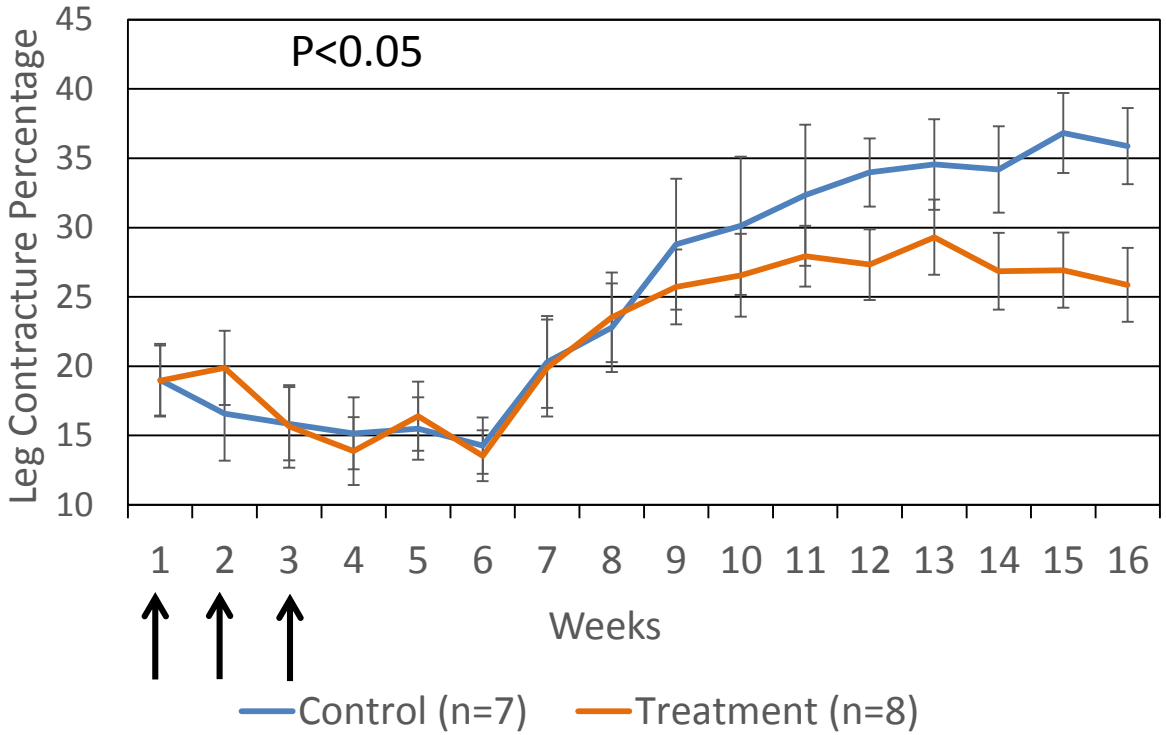
1, and 5-days post-injection

ADSC Survival After Transplantation

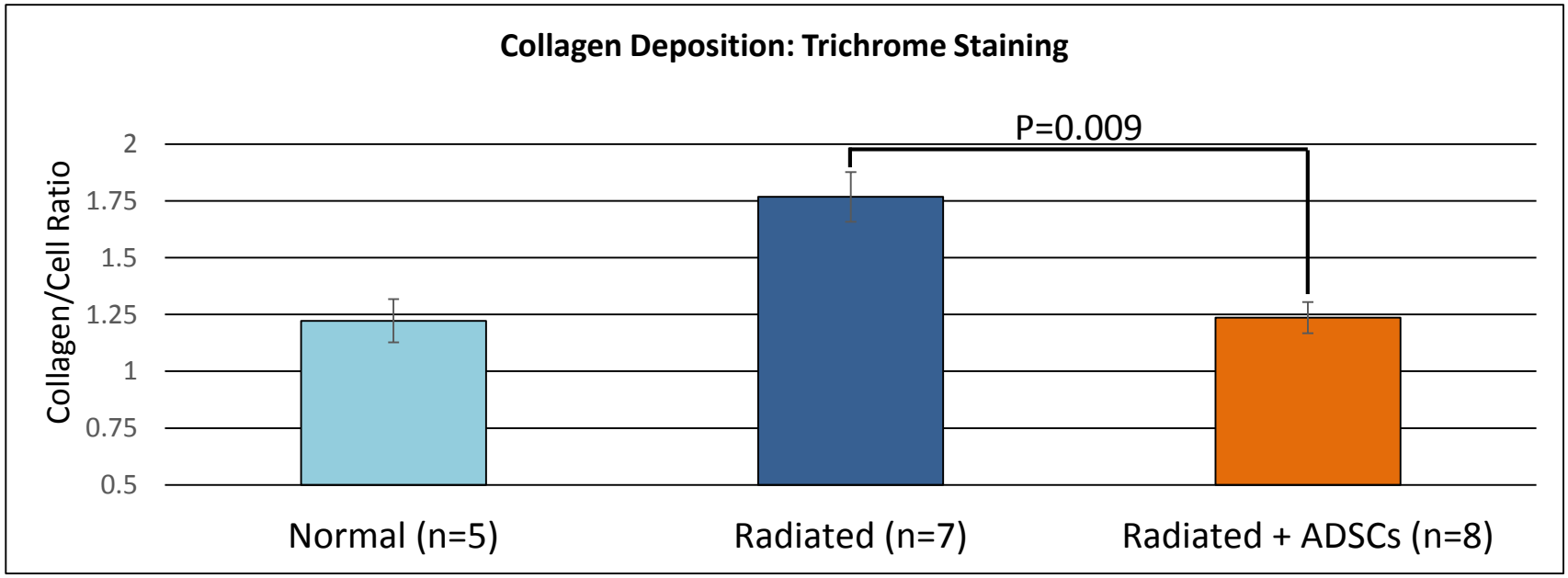


ADSC Improves Radiation Fibrosis Leg Contracture

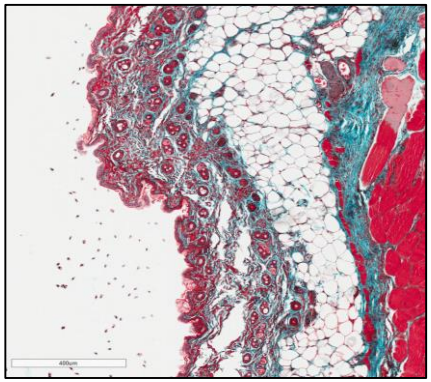


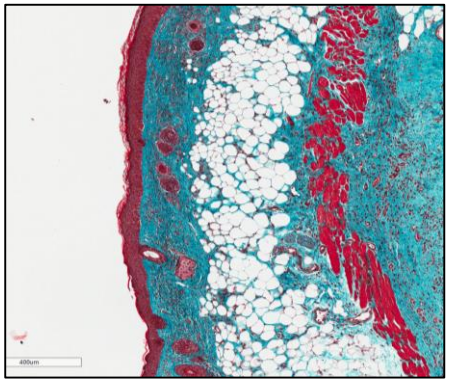


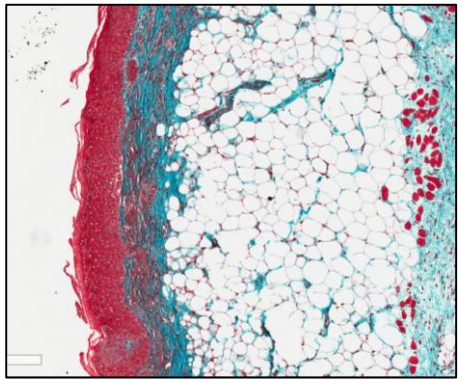
ADSCs Decreases Collagen Deposition *in vivo*



Representative Sections at 10x Magnification







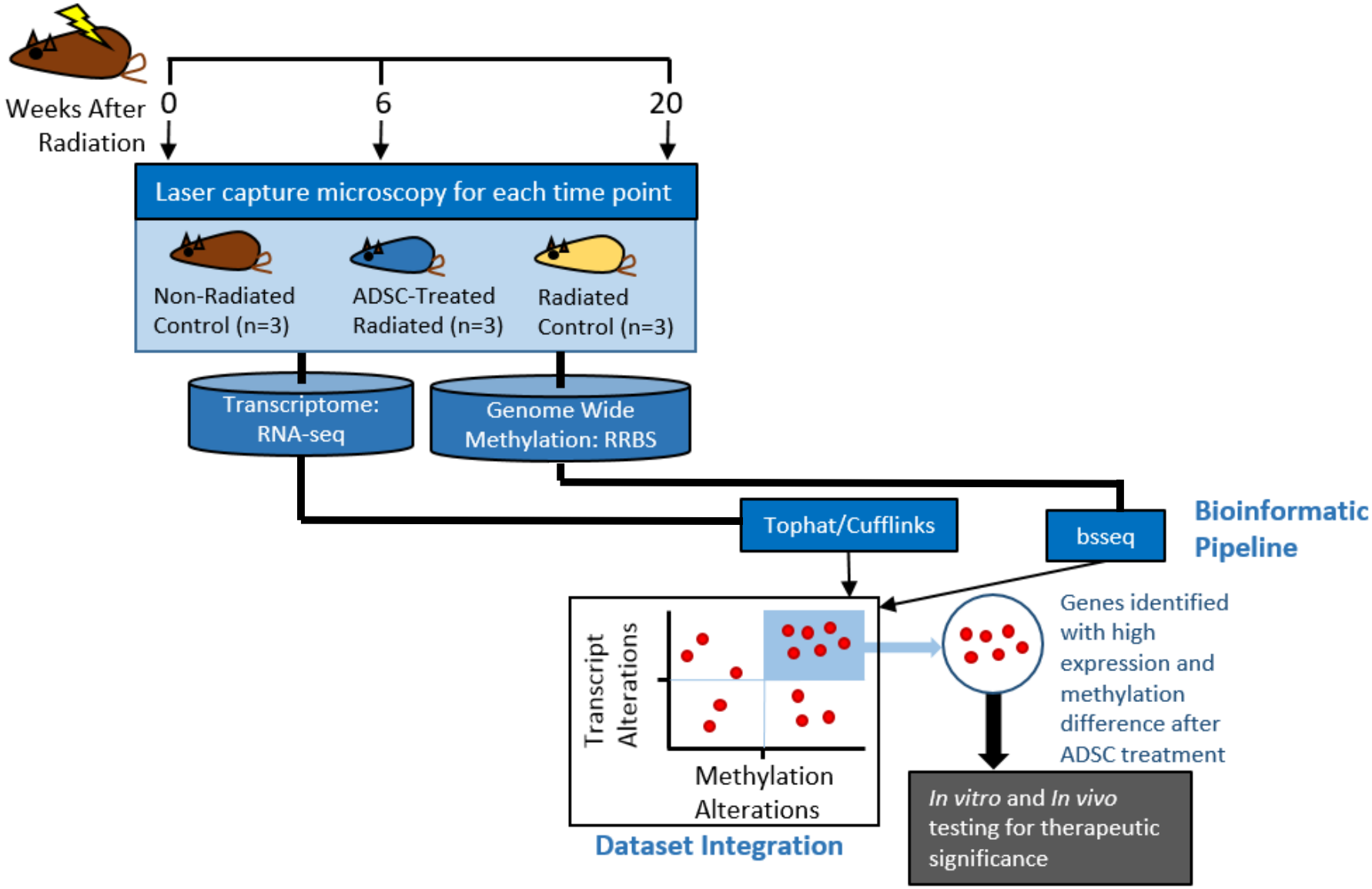
Assessment at 16 weeks post-ADSC transplantation

Aim 2:

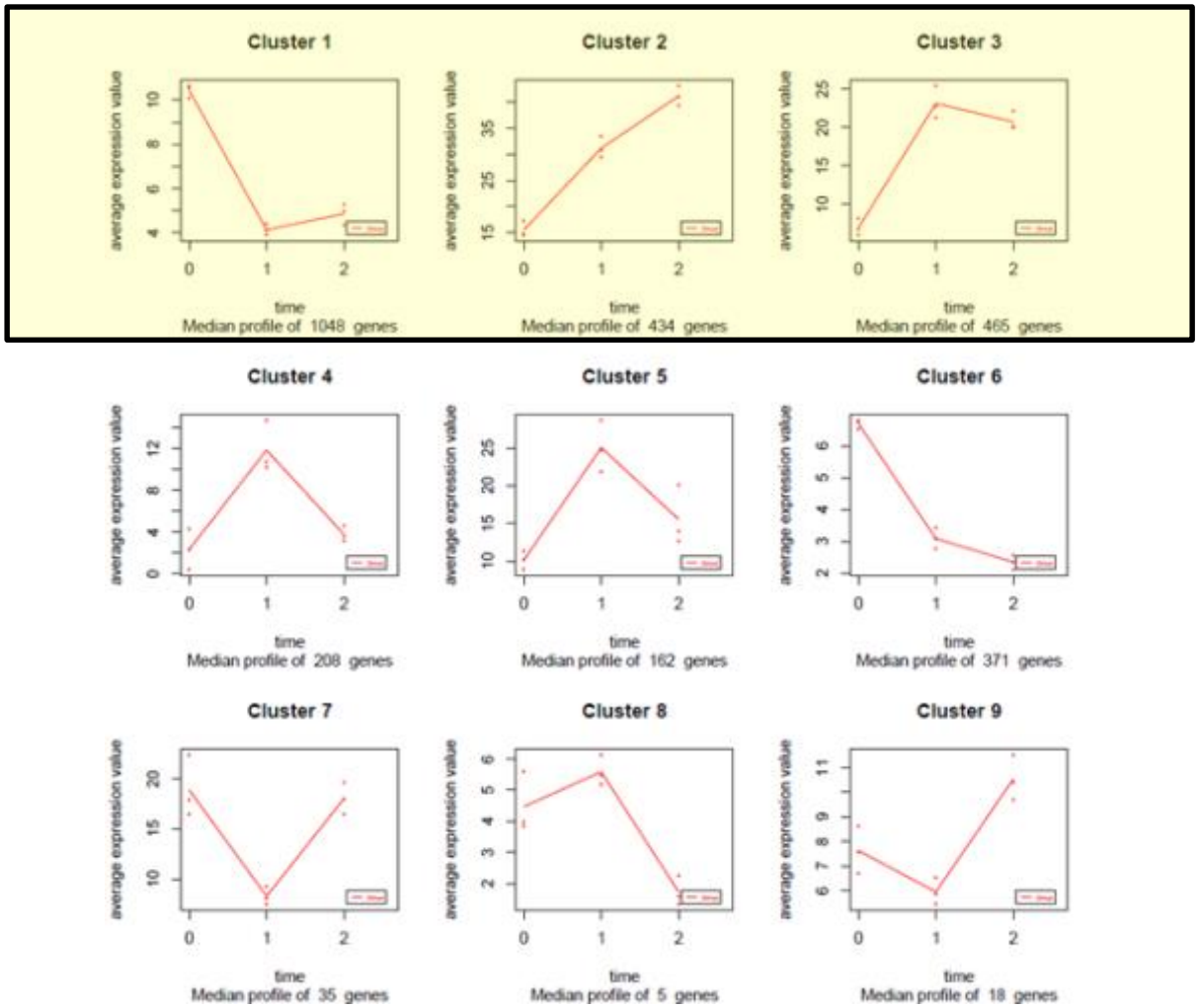
To interrogate the transcriptomic and genome wide methylation alterations that occur at different time points after ADSC treatment

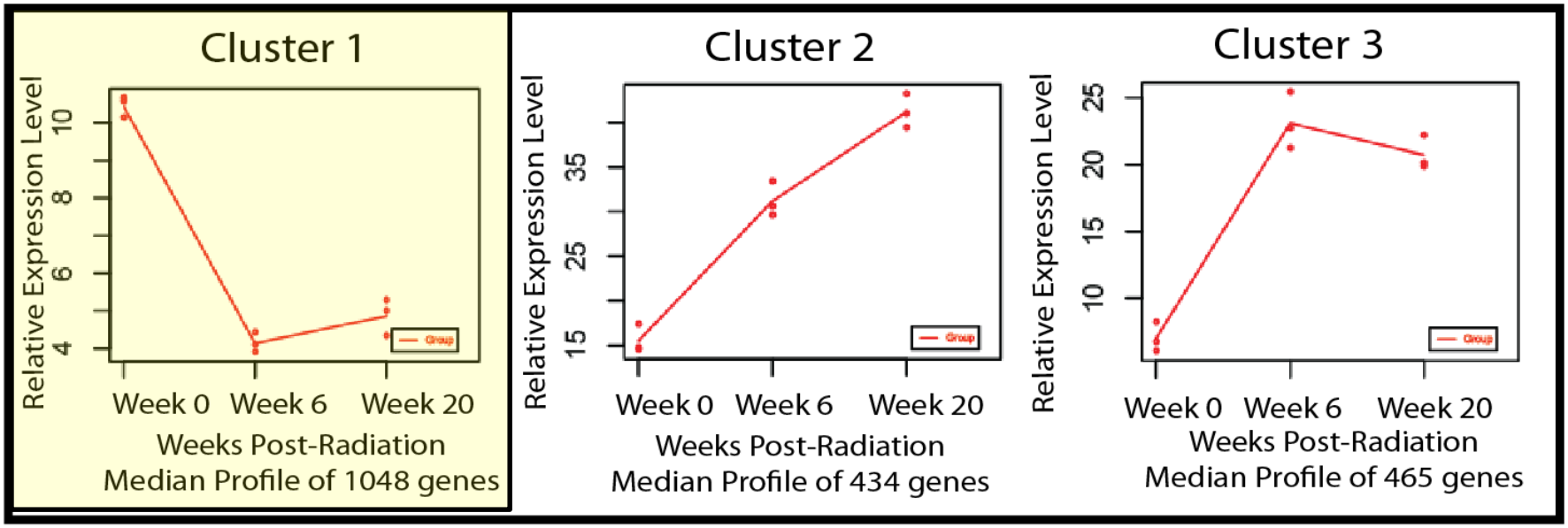
a. **Genome wide methylation and transcriptome assessment at 0, 6, and 20 weeks post-radiation injury**
b. **Establish an *in vitro* fibrosis model to examine mechanisms by which ADSCs exert their therapeutic effect**

Work Flow


Weeks After Radiation
0
6
20
Laser capture microscopy for each time point
Non-Radiated Control (n=3)
ADSC-Treated Radiated (n=3)
Radiated Control (n=3)
Transcriptome: RNA-seq
Genome Wide Methylation: RRBS
Tophat/Cufflinks
bsseq
Bioinformatic Pipeline
Transcript Alterations
Methylation Alterations
Dataset Integration
Genes identified with high expression and methylation difference after ADSC treatment
In vitro and In vivo testing for therapeutic significance

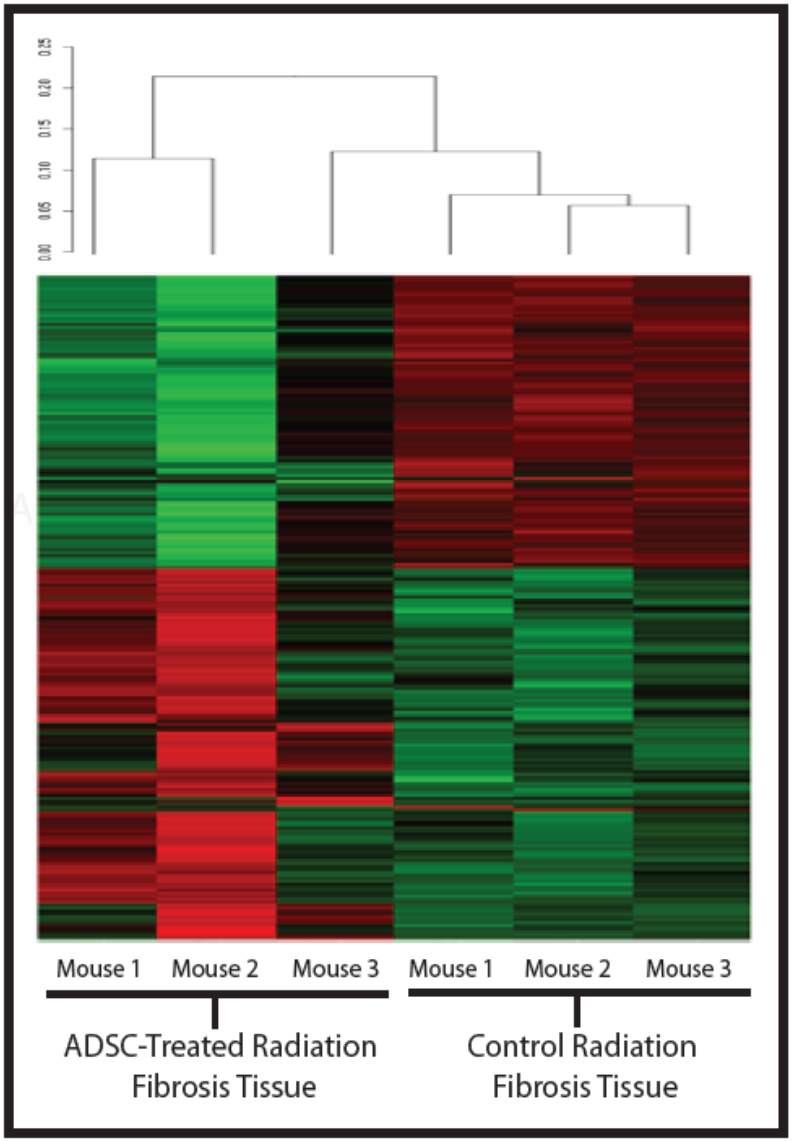
Clustering Radiation Fibrosis Transcript Levels Based on Temporal Patterns





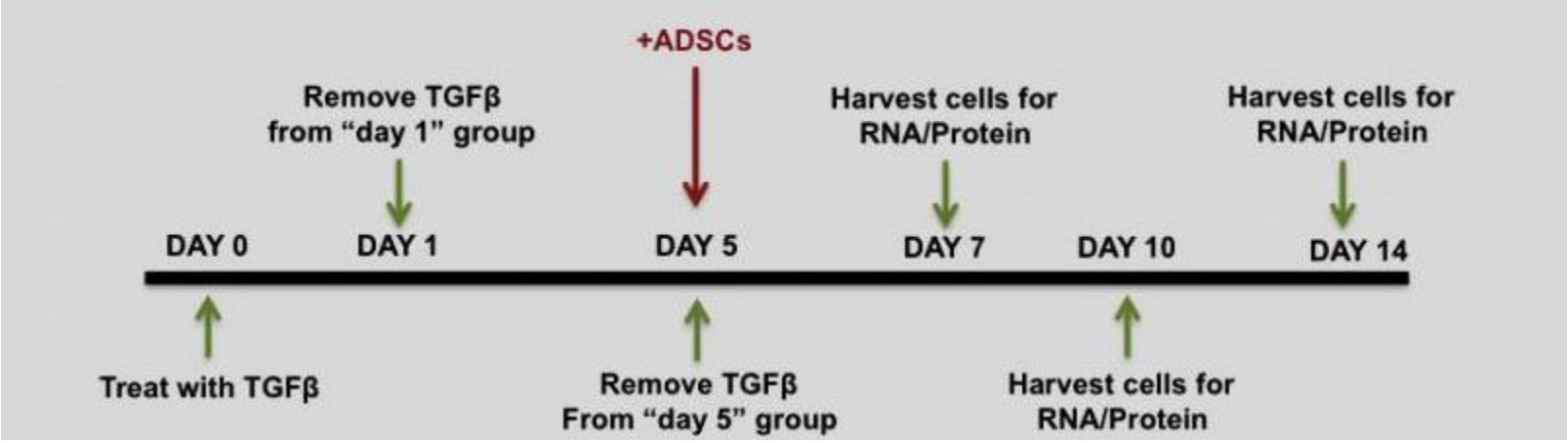
Pathway	Source
Salivary secretion	KEGG
XPodNet - protein-protein interactions in the podocyte expanded by STRING	Wikipathways
Cholinergic synapse	KEGG
Synthesis of bile acids and bile salts via 24-hydroxycholesterol	Reactome
Fatty acid degradation	KEGG
Adipogenesis	Wikipathways
PPAR signaling pathway	KEGG
Phosphatidylinositol signaling system	KEGG
PPAR signaling pathway	Wikipathways

Transcriptome Data RNA-seq



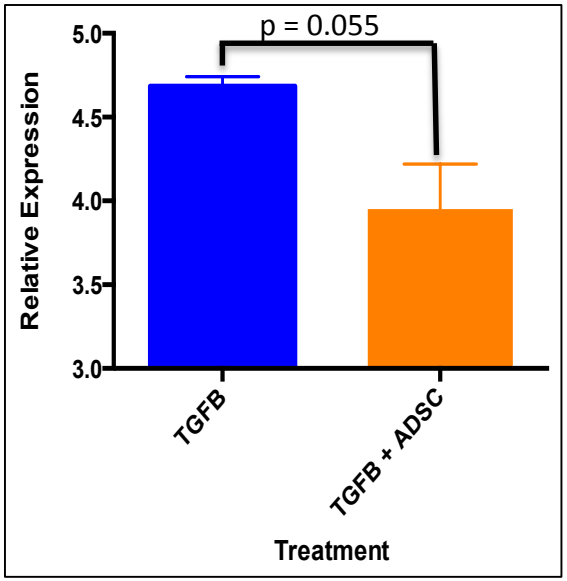
Pathway	Source
Metabolism	Reactome
Adipogenesis	Wikipathways
Metabolism of lipids and lipoproteins	Reactome
Biological oxidations	Reactome
Transmembrane transport of small molecules	Reactome
Metabolism of xenobiotics by cytochrome P450	KEGG
Ovarian steroidogenesis	KEGG
PPAR signaling pathway	KEGG
PPAR signaling pathway	Wikipathways
SLC-mediated transmembrane transport	Reactome
Vascular smooth muscle contraction	KEGG
Chemical carcinogenesis	KEGG
glutathione-mediated detoxification	MouseCyc
Phase 1 - Functionalization of compounds	Reactome

Co-Culture of ADSCs with TGF-B Treated Primary Human Fibroblasts

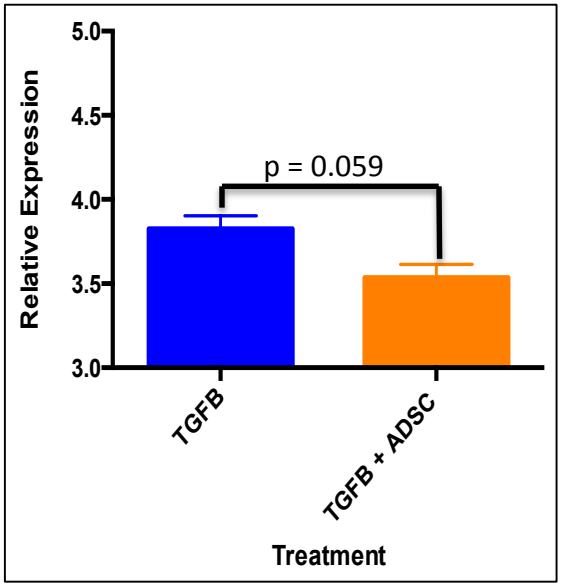


ADSCs Co-Culture Results in Less Collagen I Gene Expression and Collagen I Secretion

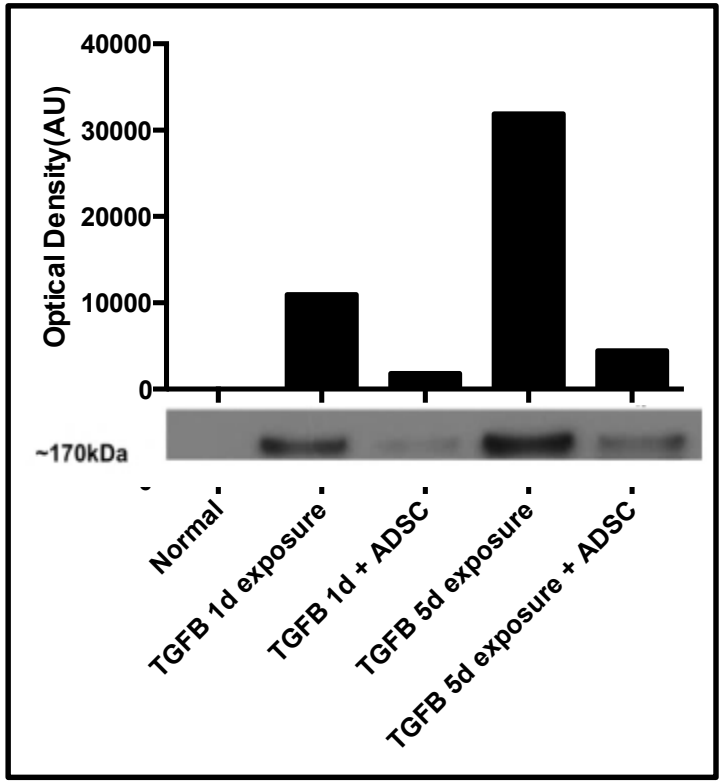
Assessment at 5 Days Post ADSC Co-culture



1 Day TGF-B Exposure



5 Day TGF-B Exposure



Collagen 1 Protein Secretion

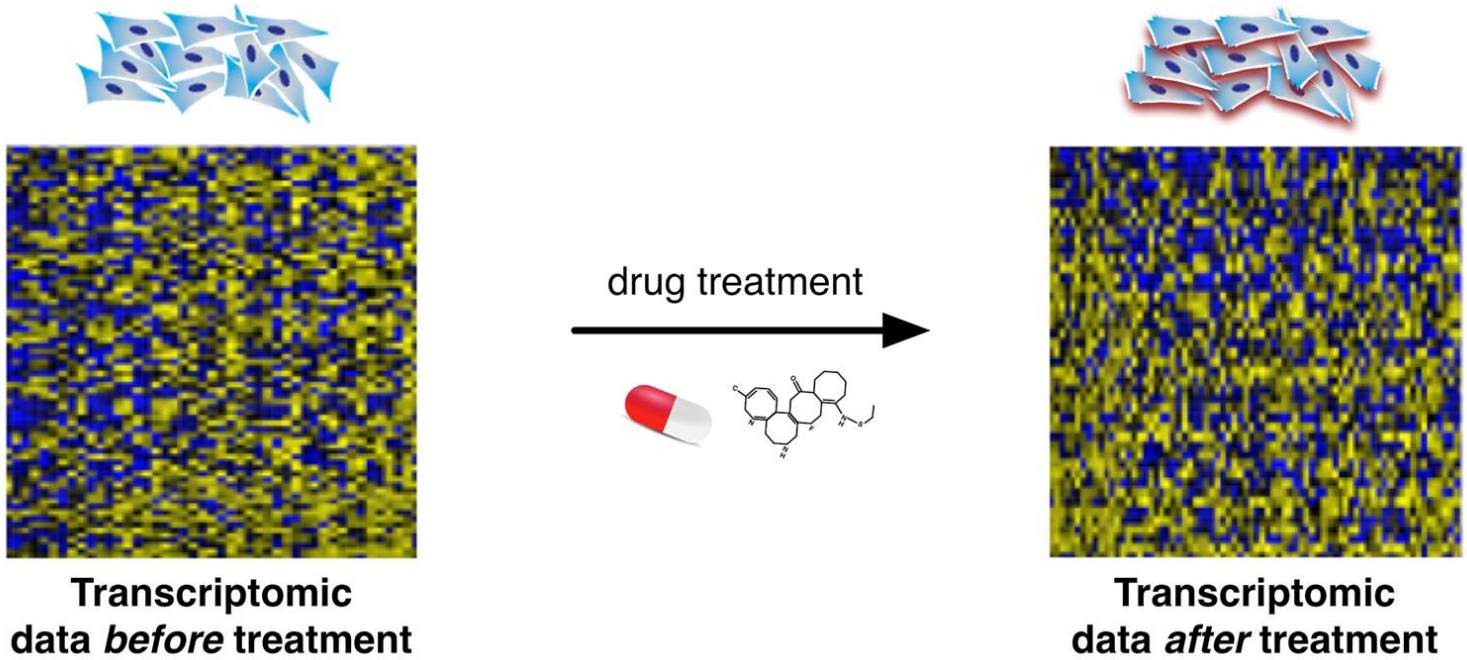
Aim 3:

Utilize ADSC-mediated transcriptome and methylation alterations to identify candidate drugs to be repurposed for the treatment of RF.

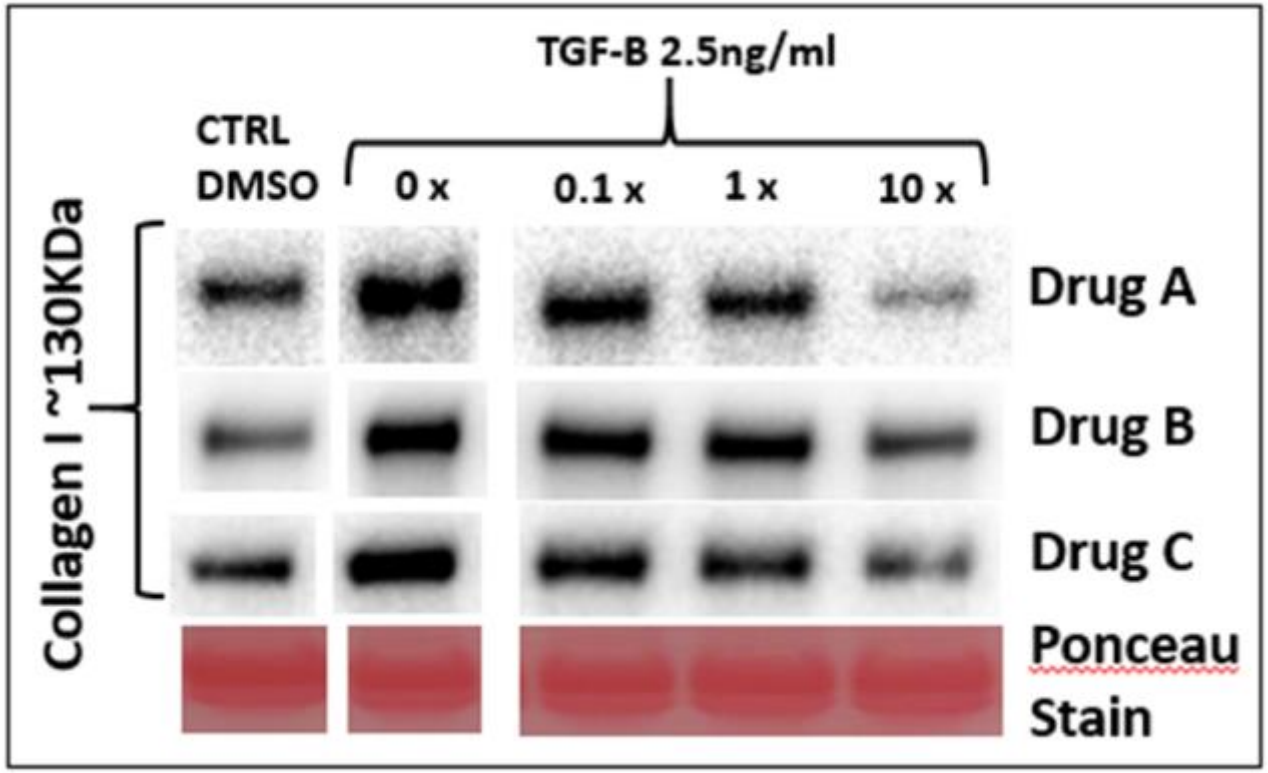
a. **Pharmacogenomics utilizing two databases of drug-mediated transcriptome alterations (CMAP, L1000)**
b. **Testing of potential drugs *in vitro* and *in vivo***

L1000 and CMAP Pharmacogenomic Databases

CMAP and L1000 are both large databases charactering drug-mediated transcriptome alterations in cell lines



Preliminary Data with 3 Candidate Drugs



Conclusions

1. ADSC transplantation has a beneficial effect on radiation fibrosis
   i. Leg Contracture
   ii. *In vivo* collagen deposition

2. Whole transcriptome profiling at across multiple time points in RF tissue:
   i. Variations of temporal pattern of pathways altered by ionizing radiation
   ii. ADSC treatment reverses the gene expression pattern of RF

Conclusions

3. **Using a pharmacogenomics approach; candidate drugs are being identified.**

CIHR IRSC

JOE'S TEAM

Canadian Cancer Society / Société canadienne du cancer

Laurie Ailles
Daniel De Carvalho
Benjamin Haibe-Kains

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