

## **Adipocyte Stem Cells Ameliorate Total Body Irradiation Induced Hematopoietic Syndrome and Late Radiation Fibrosis**

**Background/Purpose:** Radiation therapy can result in late radiation fibrosis (RF). Several reports suggest that autologous adipose tissue stem cells (ASCs) can ameliorate RF. We sought to elucidate the cellular and molecular mechanism(s) involved.

**Methods:** In vitro Transwell co-cultures contained a bottom layer of: 1) irradiated human foreskin fibroblasts (HFFs), 2) mouse cell lines derived from fibrosis biopsies; the upper layer contained freshly prepared mouse or human ASCs. We quantitated fibrosis-related gene transcripts in lower layer cells and regulatory cytokines in upper layer cells by quantitative real time (qRT) PCR. Female C57BL/6J mice received 9.25 Gy by TBI, treatment group (n=12) received ASC while control (n=12) received intraperitoneal injection of saline at 24 h. Other female C57BL/6J mice received 35Gy radiation at the right flank. Irradiated and contralateral unirradiated flank tissue was tested for fibrosis related gene transients at day 14 post irradiation (PI). Subgroups had irradiated and control sites injected with ASCs harvested from gender mismatched luciferase+ GFP+ mice. Fibrosis was quantitated by histologic staining for collagen and range of limb motion measurements.

**Results:** Transwell coculture revealed significant down regulation of profibrotic genes (TGF  $\beta$ , and Col 1-4) in irradiated cells. Among the genes expressed in upper layer ASCs, hepatocyte growth factor (HGF) was prominent. Addition of human recombinant HGF to irradiated HFFs significantly down regulated pro-fibrotic gene transcripts. Intraperitoneal injection of 1 million ASCs at 24 hrs after 9.25 Gy total body irradiation significantly increased mouse survival at 30 days ( $p = 0.047$ ). RF was detected in vivo at day 14 and increased by day 28, and confirmed by histological staining for collagen by Masson's Trichrome. At day 28, irradiation-induced a reduction in limb excursion with a range of limb extension of  $11.4^\circ \pm 2.7^\circ$  compared to  $57.0^\circ \pm 2.5^\circ$  ( $p < 0.0001$ ) in the contralateral non-irradiated limb. Furthermore, irradiated limbs that had received an injection of ASCs had significantly increase limb excursion ( $42.5^\circ \pm 2.5^\circ$ ;  $p = 0.0013$ ) at day 28.

**Conclusions:** ASCs ameliorate TBI lethality. HGF secreted by ASCs reduces RF in vitro and in vivo. HGF and other secreted and cell contact regulators in adipose tissue based cell therapy of both the hematopoietic syndrome and radiation fibrosis are being elucidated.