Effects of Low-Dose Radiation on Aging of Human Stem Cells *In Vitro*

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Low-dose radiation (LDR) and radiation protection models

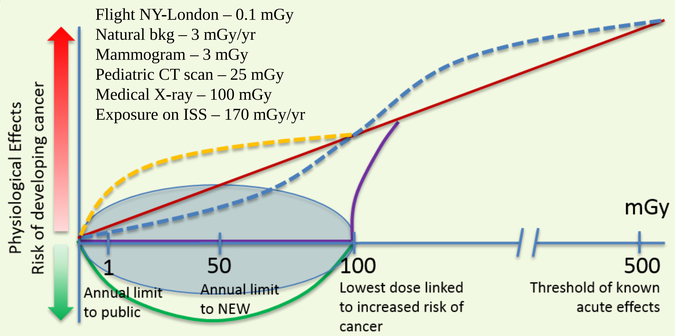
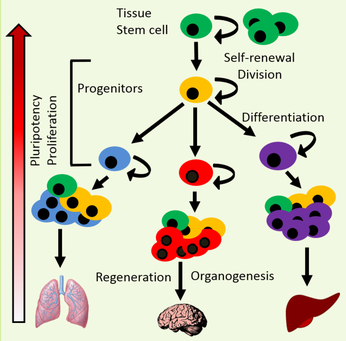


Figure 1: Lack of consensus in risk prediction of competing models of radiation protection. Linear no-threshold (**red**), threshold (**purple**), hormesis (**green**), quadratic (**blue**) and supra-linear (**yellow**) models inconclusively estimate health risks at low doses of radiation exposures (<100 mGy). Low-dose radiation (LDR) has great societal implications due to natural background, occupational, diagnostic/medical and other exposures.

Stem cells (SCs)



1868 – German biologist **Haeckels** coined stamzelle, a cell capable of producing many new types of cells and repairing the body
1909 – Russian scientist **Maximow** proposed the idea of a stem cell in human blood system
1963 – Canadian scientists **McCulloch** and **Till** experimentally demonstrated in mice existence of a blood stem cell
1968 – American immunologist **Good** performs the first allogeneic bone marrow stem cell transplant to treat a patient with severe combined immunodeficiency (SCID) syndrome

Figure 2: Adult tissue stem cells (somatic stem cells), though discovered fairly recently, now occupy a centre stage in biology and medicine. Adult tissue stem cells reside within stem cell niches and are responsible for tissue/organ regeneration and repair[1]. They are multipotent, quiescent cells capable of self-renewal, proliferation and differentiation upon receiving appropriate physiological signals. The functional and proliferative capacity of stem cells decreases with degree of their differentiation and lineage commitment.

Challenges with therapeutic applications[2]

- Stem cells are rare populations - 0.01-0.001% of all somatic cells in a given tissue
- Limited donor availability, requirement for tissue/blood matching
- *Ex-vivo* manipulation and expansion of stem cells results in their pre-mature aging, loss of stemness and regenerative function[3]
- Only 1 stem cell therapy is currently approved in North America – hematopoietic stem cell transplantation using bone marrow and cord blood stem cells

Methods

- Human umbilical cord blood (UCB) derived mesenchymal stem/stromal cells (MSCs)[4-5] and endothelial colony forming cells (endothelial stem cells; ECFCs)[6-7]
- Acute external gamma irradiation at 10 mGy, 50 mGy and 100 mGy total dose
- Passage 4 (p4) – young cells
- Cells aged in culture and monitored with Incucyte – doubling time/proliferation
- Functional capacity of MSCs measured as extent of differentiation to chondorcytes
- Functional capacity of ECFCs measured as extent of cell migration in a scratch wound assay

LDR delayed age-associated decline in SC proliferation

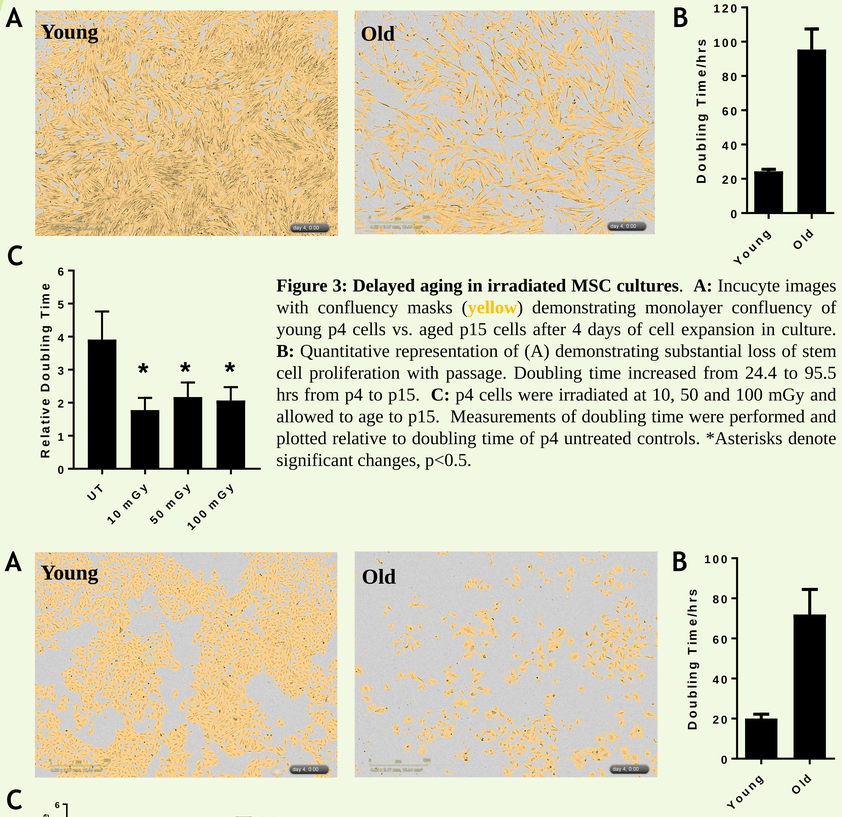


Figure 3: Delayed aging in irradiated MSC cultures. **A:** Incucyte images with confluency masks (**yellow**) demonstrating monolayer confluency of young p4 cells vs. aged p15 cells after 4 days of cell expansion in culture. **B:** Quantitative representation of (A) demonstrating substantial loss of stem cell proliferation with passage. Doubling time increased from 24.4 to 95.5 hrs from p4 to p15. **C:** p4 cells were irradiated at 10, 50 and 100 mGy and allowed to age to p15. Measurements of doubling time were performed and plotted relative to doubling time of p4 untreated controls. *Asterisks denote significant changes, p<0.5.

Figure 4: Delayed aging in irradiated ECFC cultures. **A:** Incucyte images with confluency masks (**yellow**) demonstrating monolayer confluency of young p4 cells vs. aged p8 cells after 4 days of cell expansion in culture. **B:** Quantitative representation of (A) demonstrating substantial loss of stem cell proliferation with passage. Doubling time increased from 20.0 to 71.9 hrs for clone 13. **C:** p4 cells were irradiated at 10, 50 and 100 mGy and allowed to age to p8. Measurements of doubling time were performed and plotted relative to doubling time of p4 untreated controls. *Asterisks denote significant changes, p<0.5.

Conclusions

***In vitro* cultured non-irradiated MSCs and ECFCs aged significantly:**

1) Cell doubling time increased 3.9 fold for MSCs and 3.6 fold for ECFCs
2) Chondrogenic differentiation of MSCs decreased 2.2 fold
3) The migratory capacity of ECFCs decreased by 1.9 fold

LDR treatment at early passage provided lasting effects and delayed aging:

1) Proliferative capacity of aged cells improved 2.2 fold for MSCs and 2.1 fold for ECFCs
2) Chondrogenic differentiation of aged MSCs was completely restored with 50 and 100 mGy treatment and further improved by 30% with 10 mGy exposures
3) Migration of irradiated aged ECFCs improved by 27.1% with 50 mGy exposure

This report provides the first evidence of LDR-mediated delayed aging and improved functional capacity of *in vitro* expanded mesenchymal and endothelial stem cells.

References

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LDR delayed age-associated loss of MSC chondrogenic differentiation

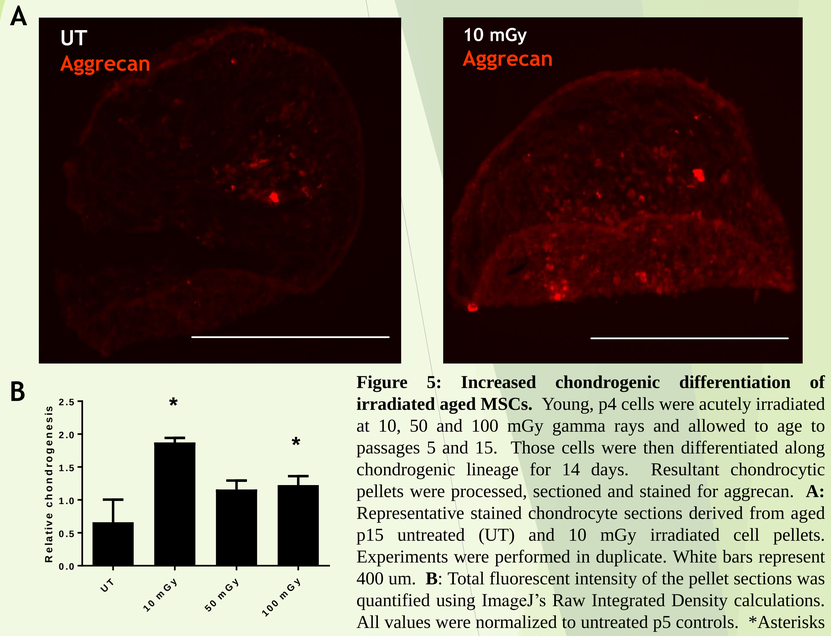

Figure 5: Increased chondrogenic differentiation of irradiated aged MSCs. Young, p4 cells were acutely irradiated at 10, 50 and 100 mGy gamma rays and allowed to age to passages 5 and 15. Those cells were then differentiated along chondrogenic lineage for 14 days. Resultant chondrocytic pellets were processed, sectioned and stained for aggrecan. **A:** Representative stained chondrocyte sections derived from aged p15 untreated (UT) and 10 mGy irradiated cell pellets. Experiments were performed in duplicate. White bars represent 400 um. **B**: Total fluorescent intensity of the pellet sections was quantified using ImageJ's Raw Integrated Density calculations. All values were normalized to untreated p5 controls. *Asterisks denote statistically significant (p<0.05) changes.

LDR delayed age-associated decrease in ECFC migration

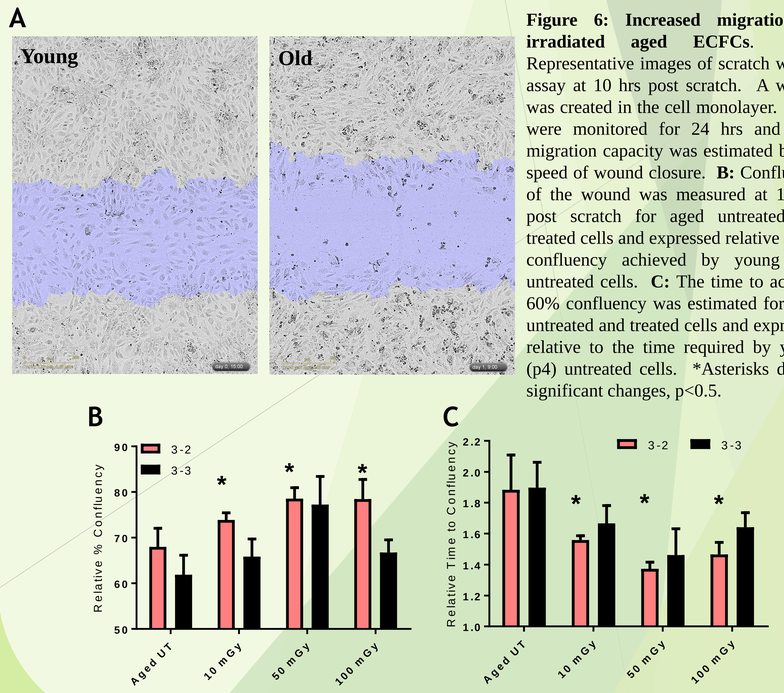

Figure 6: Increased migration of irradiated aged ECFCs. **A:** Representative images of scratch wound assay at 10 hrs post scratch. A wound was created in the cell monolayer. Cells were monitored for 24 hrs and their migration capacity was estimated by the speed of wound closure. **B:** Confluency of the wound was measured at 10 hrs post scratch for aged untreated and treated cells and expressed relative to the confluency achieved by young (p4) untreated cells. **C:** The time to achieve 60% confluency was estimated for aged untreated and treated cells and expressed relative to the time required by young (p4) untreated cells. *Asterisks denote significant changes, p<0.5.