



Effects of Radiation on Marrow Cell Differentiation

Susanta Hui, PhD

Kathleen Coghill, BS

Louis Kidder, PhD

Department of Therapeutic Radiology and Radiation Oncology

University of Minnesota, Minneapolis, MN



Purpose

- ❖ The objective of this research project is to understand some of the mechanisms of cancer treatment related bone loss (CTRBL).
- ❖ By improving survival following the diagnosis and treatment of a metastasis, chronic morbidities present significant challenges to the patient and clinician.
- ❖ Since marrow stromal cells are known to express the potential to differentiate toward osteoblastic, adipocytic, or fibroblastic cell types (1), it is possible that they may play a significant role in the etiology of this form of bone loss.
- ❖ We hypothesize that radiation dose and time following irradiation will influence the in vitro phenotypic expression of marrow stromal cells.
- ❖ We anticipate that in vitro radiation doses 0, 2.5, 5, and 10 Gy will result in a linear increase in the appearance of adipocytes with a related decline in osteoblastic cells grown in the presence of ascorbic acid and phosphate.
- ❖ We hypothesize that radiation dose will result in a slower recovery of marrow proliferation and differentiation to an osteoblastic phenotype in these culture conditions.

Background

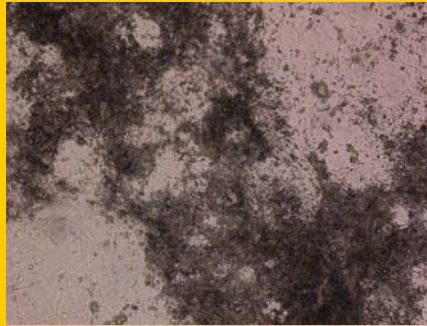
- ❖ Radiation and chemotherapeutic agents are known to increase the adipogenesis of bone marrow (2).
- ❖ We studied the potential for marrow-derived cells to differentiate into osteoblastic, adipocytic, or fibroblastic cell types in particular, since these three are involved in bone remodeling.
- ❖ The main function of fibroblasts is to maintain the structural integrity of connective tissues by continuously secreting precursors of the extracellular matrix.
- ❖ Osteoblasts secrete a collagenous extracellular matrix and mediate its organization and subsequent mineralization.
- ❖ Adipose tissues are composed of adipocytes which specialize in storing energy as fat.
- ❖ Clinically, the decreased bone mass observed in age-related osteoporosis is accompanied by an increase in marrow adipose tissue. (3)
- ❖ This may indicate that there is an inverse relationship between osteoblastic and adipocytic differentiation by marrow cells.

Methods

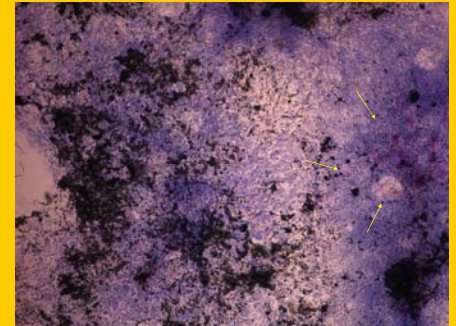
- ❖ Clonal line of marrow stromal cells harvested from p53 knock out transgenic mice cultured at 4×10^5 per flask.
- ❖ After allowing the cells to settle for 12 hours, they were irradiated at various radiation doses: 0 Gy, 2.5 Gy, 5 Gy, and 10 Gy.
- ❖ The cells were fixed and stained into three different time course study groups: two weeks, four weeks, and six weeks.

Results

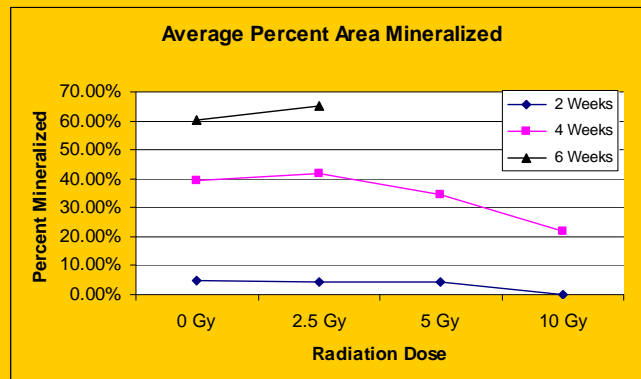
Differentiated cell and tissue areas digitized and measured.



Von Kossa staining reveals any mineralization that has occurred. (Any black/brown color shown.)



Oil Red-O staining reveals any Adipogenic cells that might be present. (The pink color shown.)



Radiation Dose	Avg. Area Mineralized 2 Wks	Avg. Area Mineralized 4 Wks	Avg. Area Mineralized 6 Wks
0 Gy	4.76%	39.32%	60.45%
2.5 Gy	4.41%	41.88%	65.09%
5 Gy	4.25%	34.61%	
10 Gy	0.24%	21.88%	

Summary

- ❖ While 2.5 Gy showed a small increase, there was a decline in mineralized area with increasing radiation.
- ❖ Analysis is ongoing, though we expect there to be an increase in adipogenesis with increasing radiation.
- ❖ These results may aid understanding mechanisms of Cancer Treatment Related Bone Loss.

References

1. Prockop D.J., Science, 276 (5309), pp. 72-74.
2. Kim SH, Lim SK, Hahn JS. Am J Med. 2004; 116: 524-8.
3. Meunier P, Aaron J, Eduard C, Vignon G. Clinical Orthopedic, 1971. 80:147-154.

Acknowledgements

This project continuation is supported by the Undergraduate Research Opportunities Program (UROP), grant number 1K12-HD055887-01 from the National Institute of Child Health and Human Development (NICHD), NIH grant R03 AR055333-02, Academic Health Center, Grant in Aid, and Masonic Cancer Center grant

