IONIZING RADIATION AFFECTS GENE EXPRESSION IN MOUSE SKIN AND BONE

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ABSTRACT

Future long-duration space exploration beyond low earth orbit will increase human exposure to space radiation and microgravity conditions as well as associated risks to skeletal health. In animal studies, radiation exposure (≥1 Gy) is associated with pathological changes in bone structure, enhanced bone resorption, reduced bone formation and decreased bone mineral density, which can lead to skeletal fragility. Definitive measurements and detection of bone loss typically require large and specialized equipment which can make their application to long duration space missions logistically challenging. Towards the goal of developing non-invasive and less complicated monitoring methods to predict astronauts’ health during spaceflight, we examined whether radiation-induced gene expression changes in skin may be predictive of the responses of skeletal tissue to radiation exposure. We examined oxidative stress and growth arrest pathways in mouse skin and long bones by measuring gene expression levels via quantitative polymerase chain reaction (qPCR) after exposure to total body irradiation (IR). To investigate the effects of irradiation on gene expression, we used skin and femora (cortical shaft) from the following treatment groups: control (normally loaded, sham-irradiated), and IR (0.5 Gy, 1 Gy 600 MeV/n and 0.5 Gy 1 H 150 MeV/n). The changes in gene expression were observed in cancellous tissue, as evidenced by microcomputed tomography (microCT) scanning. The skin was scanned using microcomputed tomography (microCT) for bone volume and total volume (BV/TV). The changes in gene expression were observed in cancellous tissue, as evidenced by microcomputed tomography (microCT) scanning. The skin was scanned using microcomputed tomography (microCT) for bone volume and total volume (BV/TV).

RESULTS

Gene expression in skin

FGF18

Expression level (2-7)

Cont 1 day 11 day IR 1 day 11 day

Cont 1 day 11 day IR 1 day 11 day

In skin, FGF18 transiently increased 24 hours after irradiation (IR). There was no significant difference in expression levels between IR and Control groups at 11 days post-IR. No effects on gene expression were detected 1 day or 11 days post-IR.

Gene expression in bone (femur)

FGF18

Expression level (2-7)

Cont 1 day 11 day IR 1 day 11 day

Cont 1 day 11 day IR 1 day 11 day

In bone, expression of FGF18 increased 24 hours after IR.

Bone volume per total volume

Cont 1 day 11 day IR 1 day 11 day

Cont 1 day 11 day IR 1 day 11 day

IR significantly reduced BV/TV at 11 days post-IR.

CONCLUSION

FGF18 expression in skin and MCP-1 expression in bone were strongly correlated 1 day after exposure to radiation (P<0.002, r=0.8779). Further, microcomputed tomography analysis of tissue from animals at a later time (11 days) showed reduced cancellous bone volume/totale volume (~21.7%) at 11 days post-IR. These results suggest that measurements of early radiation-induced changes in FGF18 gene expression in skin may have value for predicting subsequent loss of cancellous bone mass. Further research may lead to the development of a relatively simple diagnostic tool for bone loss, with the advantage that hair follicles and skin are relatively easy to acquire from human subjects.

INTRODUCTION

Background:

- Simulated space radiation induces bone loss.
- Skin is one of the most radiation-responsive organs.

Previous research:

We performed gene expression analysis on hair follicles from astronauts. FGF18 gene expression levels of hair follicles collected from astronauts on the ISS increased over time [1]. Exposure to gamma radiation (2 Gy) increased expression of MCP-1 in pooled tibial and femoral marrow at 1 day post-IR [5].

Advantages of using hair follicles and skin:

- Hair follicles and skin are relatively easy to acquire from subjects. Bone on the other hand, requires special imaging devices to be analyzed for structure and sampling for biochemical analyses is invasive.

Purpose of this study:

Determine whether skin can be used to predict the responses of bone to simulated microgravity and radiation.

Long-term goal:

Develop a relatively simple diagnostic tool for bone loss by analyzing skin.

Hypothesis:

Changes in skin gene expression may serve as an early radiation biomarker of radiation exposure and may correlate with adverse effects on skeletal tissue.

METHODS

Animals:

Male C57BL/6 mice (Jackson Laboratory, Bar Harbor, ME), 16 weeks of age

Experimental group:

- Control (Cont)
- Radiation exposure (Rad) – 1 day and 11 days after total body irradiation

Mice were exposed to a single dose of radiation consisting of 1 Gy of total body irradiation (0.5 Gy 600 MeV/n and 0.5 Gy 1 H 150 MeV/n) at a dose rate of 5 Gy/min (6.6 Fe), 3 Gy/min (1 H) at the NASA Space Radiation Laboratory beamline at Brookhaven National Laboratory (BNL).

Extraction of RNA:

Total RNA was extracted from skin and femur (flushed of marrow) using Trizol.

Gene expression analysis:

Quantitative polymerase chain reaction (qPCR) was performed for the following genes: Cdkn1a, FoxO3, SOD1, Gadd45g, Trp53, FGF18, Nfe2l2 (for skin). MCP-1, Nfe2l2, Rankl (for femur).

Values are normalized to expression levels of L19, n=5/group.

Microcomputed tomography (microCT):

Tibiae were scanned by microCT. Bone volume per total volume (BV/TV) was calculated. n=6/group.

Immunohistochemistry in skin:

The expression of FGF18 was analyzed using monoclonal antibodies specific for FGF18.

The avidin-biotin immunohistochemical procedure was used for the localization of primary antibody binding according to manufacturer’s instructions (ABC kit, Santa Cruz Biotechnology).

Data shown are means ± S.D. Student T-test was performed, and P<0.05 accepted as significant.

REFERENCES