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 ^{56}Fe 600 MeV/n and 0.5 Gy ^{1}H 150 MeV/n), euthanized at one and 11 days post-irradiation (IR). To determine the extent of bone loss, tibiae were harvested and cancellous microarchitecture in the proximal tibia quantified ex vivo using microcomputed tomography (microCT). Statistical analysis was performed using Student's t-test. At one day post-IR, expression of FGF18 in skin was significantly greater (3.8X) than sham-irradiated controls, but did not differ at 11 days post IR. Expression levels of other genes associated with antioxidant response (Nfe2l2, FoxO3 and Sod1) and the cell cycle (Trp53, Cdkn1a, Gadd45g) did not significantly differ between the control and IR groups at either time point. Radiation exposure resulted in a 27.0% increase in FGF18-positive hair follicles at one day post-IR and returned to basal levels at 11 days post-IR. A similar trend was observed from FGF18 gene expression analysis of skin. In bone (femora), there was an increase in the expression of the pro-osteoclastogenic cytokine, MCP-1, one day after IR compared to non-irradiated controls. FGF18 expression in skin and MCP-1 expression in bone were found to be positively correlated (P<0.002, r=0.8779). Further, microcomputed tomography analysis of tibia from these animals showed reduced cancellous bone volume (-9.9%) 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 (2Gy) increased expression of MCP-1 in pooled tibial and femoral marrow at 1 day post-irradiation [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

Animal:
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 ^{56}Fe 600 MeV/n and 0.5 Gy ^{1}H 150 MeV/n) at a dose rate of 5 cGy/min (^{56}Fe) , 3 cGy/min (^{1}H) at the NASA Space Radiation Laboratory beamline at Brookhaven National Laboratory (BNL).

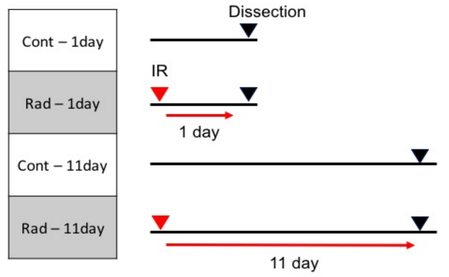
Extraction of RNA:
Total RNA was extracted from skin and femur (flushed of marrow) using Trizol.

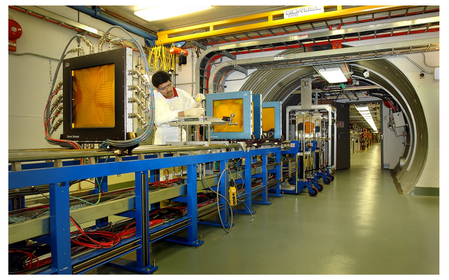
Gene expression analysis:
Quantitative polymerase chain reaction (qPCR) was performed for the following genes:
Cdkn1a, FoxO3, SOD1, Gadd45g, Trp53, FGF18, Nfe2l2 (For skin). MCP-1, Nfe2l2, Rankl (For femur).
Values are normalized to expression levels of L19. [n=5/group]

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). [n=5/group]

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





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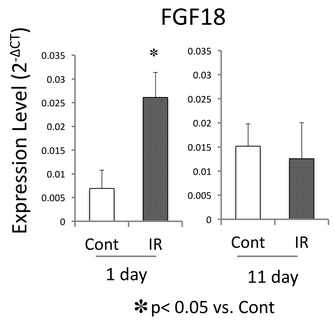
RESULTS

Gene expression in skin

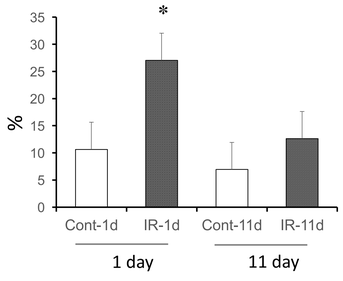


		1 day post-IR		11 days post-IR	
		Average	SD	Average	SD
Nfe2l2	Cont	3.42×10^{-2}	1.05×10^{-2}	4.08×10^{-2}	6.41×10^{-3}
	IR	4.29×10^{-2}	9.87×10^{-3}	3.66×10^{-2}	8.48×10^{-3}
Trp53	Cont	2.52×10^{-2}	7.00×10^{-3}	2.26×10^{-2}	4.24×10^{-3}
	IR	2.11×10^{-2}	5.19×10^{-3}	2.58×10^{-2}	5.55×10^{-3}
Cdkn1a	Cont	1.71×10^{-1}	9.28×10^{-2}	1.43×10^{-1}	3.75×10^{-2}
	IR	2.28×10^{-1}	2.52×10^{-2}	1.86×10^{-1}	4.98×10^{-2}
FoxO3	Cont	4.02×10^{-2}	1.79×10^{-2}	2.12×10^{-2}	5.89×10^{-3}
	IR	3.75×10^{-2}	1.26×10^{-2}	2.82×10^{-2}	1.09×10^{-2}
SOD1	Cont	1.86×10^{-1}	8.34×10^{-2}	2.22×10^{-1}	4.35×10^{-2}
	IR	2.15×10^{-1}	9.26×10^{-2}	1.92×10^{-1}	8.16×10^{-2}
Gadd45g	Cont	1.93×10^{-2}	8.81×10^{-3}	1.11×10^{-2}	2.00×10^{-3}
	IR	1.67×10^{-2}	5.35×10^{-3}	1.05×10^{-2}	2.25×10^{-3}

✻p< 0.05 vs. Cont

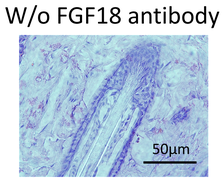
In skin, FGF18 transiently increased 24 hours after irradiation (IR). There was no significant difference in expression levels between IR and Control groups at 11 days post IR. No effects on gene expression were detected 1 day or 11 days post-IR.

Percentage of FGF18 + hair follicles

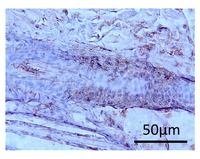


✻p< 0.05 vs. Cont

W/o FGF18 antibody

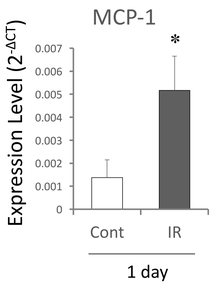


FGF18 + hair follicle



The percentage of FGF18+ hair follicles increased at one day post-IR and returned to basal levels at 11 days post-IR. The changes in gene expression and protein product of FGF18 occurred early after IR.

Gene expression in bone (femur)

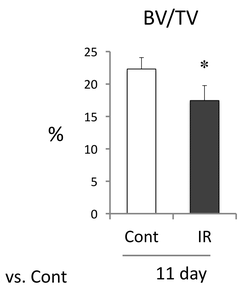


		1 day post-IR	
		Average	SD
Nfe2l2	Cont	8.71×10^{-2}	1.38×10^{-2}
	IR	9.88×10^{-2}	4.86×10^{-3}
Rankl	Cont	1.05×10^{-2}	2.58×10^{-3}
	IR	1.35×10^{-2}	3.76×10^{-3}

✻p< 0.05 vs. Cont

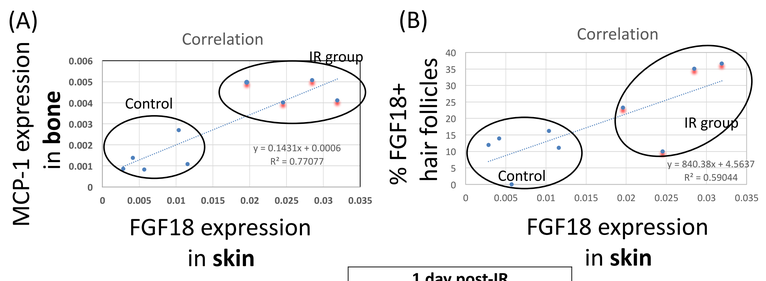
In bone, expression of MCP-1 increased 24 hours after IR.

Bone volume per total volume



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

Correlation plot



		1 day post-IR	
		Correlation coefficient (r)	p value
FGF18 (skin)	MCP1 (bone)	8.78×10^{-1}	1.90×10^{-3}
FGF18 RNA (skin)	% FGF18+ hair follicles	7.68×10^{-1}	1.56×10^{-2}

Correlation of gene expression between skin and bone.
(A) Correlation between skin FGF18 expression and bone MCP-1 expression. (B) Correlation between skin FGF18 expression and % FGF18+ hair follicles.

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 tibiae from animals at a later time (11 days) showed reduced cancellous bone volume/total 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.

ACKNOWLEDGEMENTS

This work is supported by the National Space Biomedical Research Institute through NCC 9-58 (R. Globus), and Space Biology/NASA Space Biology Postdoctoral Fellowship awards to Dr. Terada and Dr. Schreurs.

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