The role of CDKN1a/p21 in cellular senescence of bone marrow stem cells under spaceflight stressors

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Abstract

Spaceflight environments and their associated conditions, such as microgravity and space radiation, introduce a collection of variables that cause many biological functions formerly considered to be “standard” to behave in nonstandard ways. Exposure to microgravity has been shown to induce deleterious effects in stem cell-based tissue regeneration, leading to immune system and healing response impairments as well as muscle and bone density loss. Such risks must be mitigated in order for long-term human space exploration to proceed. Thus, our work seeks to explore mechanisms of stem cell-based tissue regeneration that experience changes in spaceflight environments. Cellular senescence can be initiated by various stimuli and is a process by which normal cells stop dividing and adopt a state of permanent cell cycle arrest. This state may be influenced by the cyclin-dependent kinase inhibitor gene Cdkn1a/p21, which is also known to function in osteogenic cell cycle arrest. Under conditions of mechanical unloading, stem cell-based tissue regeneration has shown to be decreased as evidenced by the arrest of several progenitor cell populations. Furthermore, CDKN1a/p21 is up-regulated in expression under conditions of microgravity, suggesting its role in regenerative bone formation arrest in space.

Thus, we hypothesize that CDKN1a/p21 is a mediator of cellular senescence in bone marrow stem cells, and that alterations of p21 activity during spaceflight prevent tissue from repairing and regenerating normally.

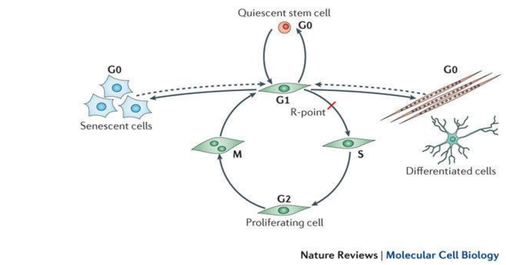

Figure 1: Schematic of stem cell differentiation cycle, showing senescent transition into G0. ***Image credit: Nature Reviews*** *Cheung, T. H., & Rando, T. A. (2013). Molecular regulation of stem cell quiescence. Nature reviews. Molecular cell biology, 14(6).*

Methods

- We performed a series of experiments investigating several stress factors known to be active during spaceflight conditions, including oxidative stress and hypergravity, and their impact on the differentiation capacity of bone marrow mesenchymal stem cells.
- We used mesenchymal stem cells isolated from mouse femur and tibia marrow, cultured for 30 days under osteoblastogenic conditions. We investigated both WT and CDKN1a KO mice to question the role of this molecule in the process
- Stress Tests Performed:

Oxidative Stress
After 6 days of normal culturing, cells were stressed for two hours with 200 µM hydrogen peroxide in osteogenic media. After stressing, cells were returned to osteoblastogenic media for remainder of culturing.

Aging
We cultured cells from both WT and KO animals that were either 16wk or 7mo old.

Hypergravity
After 9 days of normal culturing, cells were treated with 12 hrs of 10G hypergravity using a centrifuge incubator. Treatment occurred every 30 days until cells reached day 30.

- After 30 days of culturing, plates were analyzed via cell count, guava cell cycle analysis, Von Kossa mineralization staining, and senescence detection assay. We also isolated and saved RNA for future gene expression analysis.

Results

OXIDATIVE STRESS

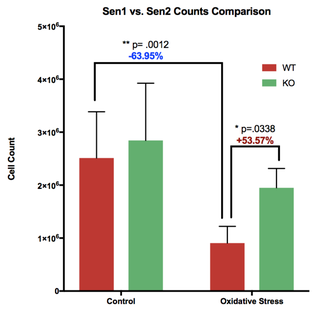

Figure 1: WT and KO cell counts after 30 days culture under either standard conditions or oxidative stress treatment. Significant decrease in WT counts between treatment (-63.95%), but KO samples were able to protect against that deficit.

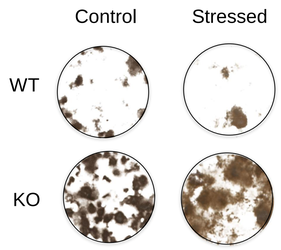

Figure 2: Von Kossa staining for WT and KO samples, both control and stressed. WT samples exposed to stress show decreased mineral production, but KO samples do not experience this decrease.

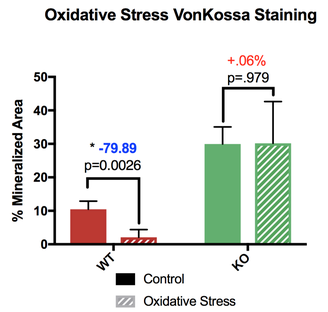

Figure 3: Quantification of Von Kossa staining. Significant decrease in WT mineral production when exposed to stress (-79.89%), but no significant decrease in KO samples.

AGING

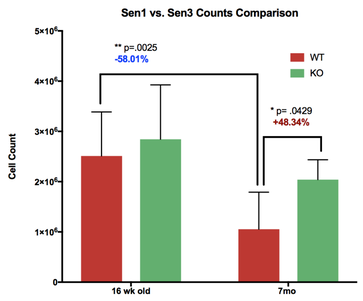

Figure 4: WT and KO cell counts from 16wk and 7mo animals after 30 days culture. Significant decrease in cell count with age, but KO samples decrease at a lower rate than their WT counterparts.

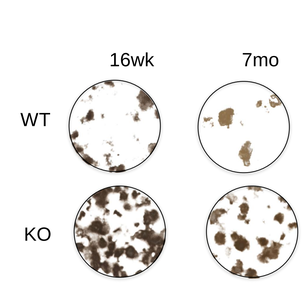

Figure 5: Von Kossa staining for WT and KO samples from 16wk and 7mo samples. Lower mineral in WT vs. KO, but no easily discernable difference across age time points.

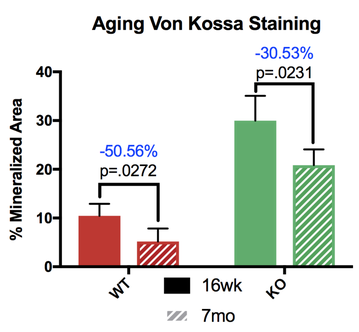

Figure 6: Quantification of Von Kossa staining. No significant difference between age time points for either WT or KO, but a decline in mineral production for older (7mo) samples is seen.

HYPERGRAVITY

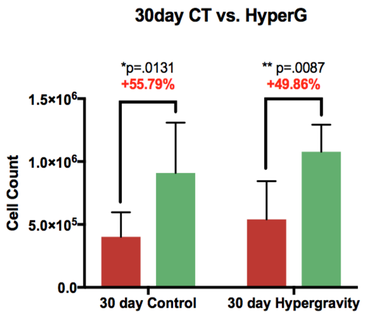

Figure 7: WT and KO cell counts after 30 days culture under either standard conditions or hypergravity treatment. Significant difference between WT and KO for both conditions, but no significant differences due to treatment group.

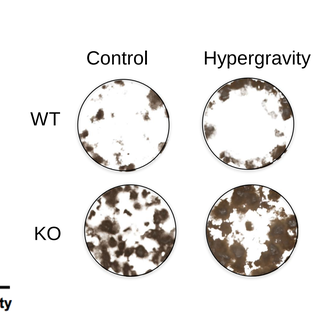

Figure 8: Von Kossa staining for WT and KO samples, both control and stressed. Samples exposed to hypergravity treatment form mineral along edges of the well, rather than in the middle.

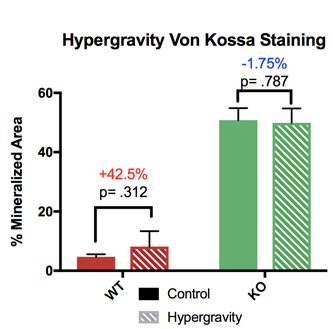

Figure 9: Quantification of Von Kossa staining. Significant difference in mineral production for WT vs. KO samples, but no significant difference due to hypergravity treatment.

Conclusions

OXIDATIVE STRESS FINDINGS

-A significant decrease in cell count between control and stressed WT samples was seen, but KO cells did not experience the same decline. From this data, we can infer that **KO samples show enhanced against damage due to oxidative stress.**

-Significant decline in mineral production for WT cells following stress treatment, but no significant decline for KO cells. From this data, we can infer that **KO samples retain mineral producing functions despite exposure to stress.**

AGING FINDINGS

-Cell count was found to decrease with age for both WT and KO samples, but KO samples experience this decrease in cell count at a lower rate than their WT counterparts. From this data, we can infer that **KO cells experience lower rates of replicative senescence caused by aging.**

-No significant difference was seen in mineral production between WT and KO cells across age time points, however older samples (7mo) show lower mineral production than their 16wk counterparts.

HYPERGRAVITY FINDINGS

-Cell counts show a significant difference between WT and KO samples, but no significant difference between control and hypergravity conditions for either cell type. From this data, we can infer that **Cell proliferation influenced by CDKN1a/p21 is not affected by exposure to hypergravity.**

-No significant difference was seen in the amount of mineral produced between control and hypergravity samples, however, visual images show tendency for hypergravity samples to form minerals along the edges of wells, rather than in the middle.

Preliminary results pose many questions for further exploration. These initial studies opened the door for exploration of The role of CDKN1a/p21 in cellular senescence of bone marrow stem cells under spaceflight stressors

Future Directions

- Adaptation of the B-gal senescence detection protocol by using DAPI to stain for cell distribution will allow more accurate determination of the proportion of senescent cells under each condition group.
- Future qPCR can be conducted using saved RNA sample in order to explore senescence associated gene expression, oxidative stress response, aging, and growth/development.
- Preliminary results are promising, especially for oxidative stress treatment. This experiment can be repeated with larger sample sized in order to explore significance of stress treatment and the role of p21 in stress response. Future continuation of the project will include:
  - Analysis of spaceflight sections for senescence associated b-gal activity in order to assess the role of p21 in induction of senescence in cells that have experienced mechanical unloading in microgravity.
  - Repeat of studies with larger numbers of samples in hopes of expanding upon significant findings.

Acknowledgements

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