

Mechanoregulation of Proliferation, Differentiation, Senescence and Survival of Bone Marrow Primary Osteoprecursor Cells.

C.M. Juran¹, E.A. Blaber¹, E.A.C. Almeida².

¹University Space Research Association (USRA), Mountain View, CA, USA

²Space Biosciences Division, NASA Ames Research Center, Moffett Field, CA, USA

Cell and animal studies conducted onboard the International Space Station and during the Shuttle program have provided extensive data illustrating bone degenerative responses to mechanical unloading in microgravity. Specifically CDKN1a/p21, an inhibitory modulator of cell cycle progression, is upregulated in osteoprecursor cells of the femur during 15-day spaceflight, suggesting that microgravity can block stem cell-based tissue regenerative process at the level of progenitor proliferation and differentiation. To study a potential role for CDKN1a/p21 in regulating osteogenic mechanosensitivity, we cultured primary bone marrow osteoprogenitor cells from CDKN1a/p21-null (p21-null) and wildtype mice with and without mechanical stimulation, and compared their morphological, proliferative, and in-vitro mineralization responses.

Structural cell alterations due to mechanical stimulation were assessed by fluorescence labeling of f-actin cytoskeleton and focal adhesions. Mechanical stimulation of p21-null cells resulted in more pronounced cytoskeletal alignment with the axis of stretch than for wildtype cells. In addition, p21-null cells subjected to stretch loading also formed significantly more focal adhesions than wildtype cells. **Combined these findings suggest that p21-null cells are structurally more responsive to stretch stimulation than the wildtype cells.** Because osteoprogenitor cells are well known to respond to mechanical stimulation with increased proliferation, we also tested this response in p21-null cells. Results from those experiments show the proliferative capacity of mechanically stimulated p21-null cells far exceeded that of wildtype controls. Specifically, cell counts from 14, and 21 days post mechanical stimulation, show that p21-null cells to have a 4-fold increase in proliferation compared to wildtype. When the p21-null cell differentiation response to mechanical stimulation was evaluated, the p21-null cultures elicited more extensive mineralization at earlier assessed timepoints than control cultures. Specifically, Von Kossa staining for mineralized matrix showed that the p21-null cells produced more than twice the mineralized surface area of wildtype cells, and at an earlier 7-day time point in culture. **Taken together these results suggest that CDKN1a/p21 normally plays a role in negatively regulating osteoprogenitor proliferation and differentiation responses to mechanostimulation in bone.** Findings of CDKN1a/p21's increased expression during spaceflight in microgravity also suggest not only a potential molecular mechanism for arresting regenerative bone growth in space, but potentially also a reduced impact for bone-formation-promoting exercise mechanostimulation. The findings described here constitute a novel role for p21 as a regulator of tissue regeneration in response to mechanical load stimulation, and also suggest a new promising molecular target to promote regenerative health in disuse conditions.