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

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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 florescence 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 cultuers 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.