Investigating the Cellular Mechanism of Age-Related Stem Cell Dysfunction

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Purpose: Skeletal stem and progenitor cells (SSPCs) are at the cornerstone of bone maintenance and repair. Previous studies in mice suggest that aging is associated with a decline in SSPC number and function and therefore lending to poor skeletal health, but findings have been inconsistent. We assessed the self-renewal and differentiation potential of young and aged murine and human-derived SSPCs and investigated the molecular mechanisms involved in SSPC dysfunction during aging. We hypothesize that aged SSPCs prematurely differentiate into bone, eventually leading to a loss in number and function of SSPCs in vivo with age.

Methods: Mouse: Bone marrow stromal cells from young 8 to 16-week-old and aged 52 to 82-week-old wild-type (C57BL/6) mice were isolated. After flushing out the bone marrow from femurs and tibiae, cells were plated for CFU-F assays and expanded for functional assays. Human: Bone marrow aspirate concentrate (BMAC) from the iliac crest of consented subjects was used. Red blood cells from collected samples were lysed. Cells were then plated for CFU-F assays and expanded for functional assays. Two-tailed Student t-test was used to determine significant differences between data sets that are normally distributed.

Results: Bone mineral density decreases with age, and thus it is widely thought that SSPC osteogenic potential is also reduced. Unexpectedly, we observed that, while aged SSPCs exhibit a reduced self-renewal capacity compared to their young counterparts, they produce significantly more bone in vitro. To determine the molecular mechanisms that drive this change, we performed RNA sequencing of young and aged SSPCs and identified several components of key osteogenic signaling pathways (BMP and WNT) that are upregulated during aging. However, when young and aged SSPCs are transplanted under the renal capsule in vivo, young SSPCs prove to be more osteogenic.

Conclusion: In vivo, bone formation declines with age. It is largely thought that this is due to a loss of the osteogenic capacity of SSPCs. We show that aged SSPCs in vitro are more osteogenic, whereas young SSPCs are more osteogenic in vivo, suggesting a model whereby native SSPC in vivo functionality is perturbed in vitro. We have identified BMP and WNT signaling pathways as therapeutic interventions to recalibrate the balance between self-renewal and differentiation to replenish the aged SSPC pool and rejuvenate the aged skeleton.

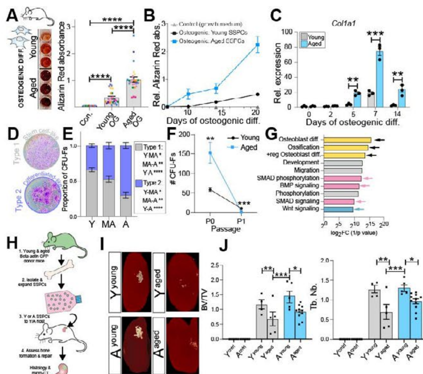

Figure 1. A. Young (12-wk) and aged (110-wk) SSPCs were grown in control growth medium (Con.) or osteogenic differentiation medium (OG) in vitro. Alizarin Red Staining & quantification after 14 days of osteo-induction. Each point represents data from an individual mouse. Colors represent distinct experiments. B. Relative Alizarin Red absorbance of young (12-wk) & aged (110-wk) SSPCs after 10, 14, & 20 days of osteo-induction. C. qRT-PCR time course during osteogenic differentiation of young & aged SSPCs. D. Hilton et al., 2008 defined CFU-Fs of distinct types. Type 1= small, densely packed round stem cell-like cells. Type 2= large, differentiated-like cells with multiple processes. D. Quantification of CFU-F types generated from SSPCs isolated from young (Y: 3-4-wk, n=6), middle-aged (MA: 52-60-wk, n=6), & aged (A: 104-110-wk, n=5) mice. E. SSPCs from young (3-4-wk) & aged (104-110-wk) mice showed a decrease in CFU-F efficiency, indicative of self-renewal capacity after 1 passage that is further reduced with age. n=3. F. Gene ontology (GO) analysis of genes that are significantly upregulated in aged vs. young SSPCs.H. Experimental schematic for renal capsule transplantation of young and aged expanded SSPCs. I. Representative microCT 3D reconstructions of bone grafts in the kidney. J. Bone volume/tissue volume (BV/TV) and trabecular number (Tb.Nb) of bone grafts as quantified using microCT. Nomenclature written as HostDonor. *p <0.05, **p <0.01, ***p <0.005, ***p <0.0001

The FDA has stated that it is the responsibility of the physician to determine the FDA clearance status of each drug or medical device they wish to use in clinical practice.