Impact of Senescent Osteocyte SASPs on Bone Marrow Stromal Cells: A Mechanobiological Perspective on Bone Aging

Chanul Kim[1] and Maryam Tilton[2*]

[1] Department of Biomedical Engineering, The University of Texas at Austin, Austin, TX, USA, 78712

[2] Walker Department of Mechanical Engineering, The University of Texas at Austin, Austin, TX, USA, 78712

Email of the presenter: chanul.kim@utexas.edu. **PI:** M Tilton. **Disclosure**: all authors disclose no conflicts of interest.

INTRODUCTION: Cellular senescence, a hallmark of aging, is characterized by stable cell-cycle arrest and a hypersecretory state. The accumulation of senescent cells (SnCs) and their secretory phenotypes (SASPs) is one of the key drivers of bone loss, a pillar of osteoporosis. With the global aging population, the economic burden of age-related fragility fractures is projected to exceed $25 billion by 2025. This underscores the critical need to understand the mechanobiological mechanisms underlying age-related bone loss. Osteocytes, the most abundant bone cells, in bones play several essential roles including regulation of bone homeostasis and mechanotransduction. Osteocyte response to mechanical loading declines with aging, resulting in impaired bone remodeling. The cytoskeleton, composed of gel-like actin, microtubule, and intermediate filaments and their crosslinkers, plays a key role in osteocyte mechanosensation. Accumulation of SnCs, including senescent osteocytes, can damage their local and distant tissues due in part to their SASP factors. Despite their recognized impact, the mechanisms by which senescent osteocytes influence local healthy primary osteocytes and the bone marrow microenvironment remain largely unexplored. Here, we investigate the local and systemic effects of senescent osteocyte accumulation on healthy primary osteocytes and bone marrow stromal cells (BMSCs), focusing on changes in cytoskeletal mechanics and membrane viscoelasticity. To our knowledge, this is the first study to explore these interactions from the mechanobiology angle.

METHODS: Primary osteocytes were isolated from the vertebrae of C57Bl/6 WT mice using a protocol established by Linda Bonewald's group (Fig. 1A). Senescence was induced in these osteocytes through low-dose irradiation (10 Gy). Senescence status was confirmed by assessing SA-β-Gal activity (Fig. 1B), as well as the expression of senescence markers, including p16^{Ink4a} and p21^{Cip1}, using RT-qPCR. Key genomic markers specific to primary osteocytes were also evaluated. The endpoint of this primary culture was set at 21 days post-irradiation (Fig. 1C). On day 21, a conditioned medium (CM) containing SASP factors was collected from the senescent osteocyte cultures. To assess the systemic effects of senescent osteocyte accumulation on the bone marrow microenvironment, BMSC spheroids were generated and cultured in CM from senescent osteocytes. The BMSC spheroids were treated with CM from day 21 post-irradiated senescent osteocytes every 3-5 days. Optical fiber-based interferometry nanoindentation (Pavone, Optics11Life) was used with two indenter tips (R = 3 µm, stiffness = 0.019 N/m; R = 26.5 µm, stiffness = 0.0019 N/m) to measure senescence-associated changes in cytoskeletal modulus and membrane viscoelasticity in both live cells (i.e., control, Sn-D7, Sn-D14, and Sn-D21) and spheroids (i.e., control, SnCM-D9, and SnCM-D14). The effective Young's modulus was obtained using the Hertzian contact model with a Poisson's ratio (ν) of 0.5. Statistical analyses were performed using one-way ANOVA with Tukey's multiple comparisons test at a 95% confidence interval. Statistical significance was set at $p < 0.05$.

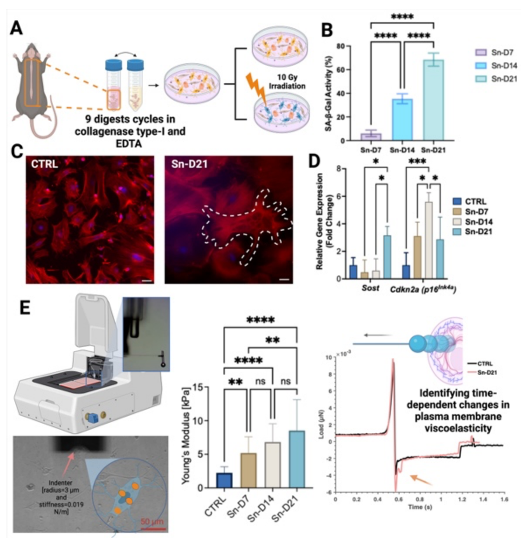

Fig. 1: (A) Isolation of primary osteocytes, **(B)** SA-β-Gal assay showing senescent cell population at D21, **(C)** morphological changes (F-Actin/nuclei), **(D)** increased Sost and p16 expression, and **(E)** nanoindentation analysis showing increased modulus and altered membrane viscoelasticity (CTRL vs. Sn-D21).

RESULTS: Our study revealed a significant increase in *p16^{Ink4a}* expression, a potent cyclin-dependent kinase inhibitor, along with a notable upregulation of *Sost*, an inhibitor of the Wnt/β-catenin signaling pathway, at day 21 post-irradiation (Sn-D21) (Fig. 1D). Young's modulus of cells significantly increased at D7, D14, and D21 post-irradiation compared to the CTRL group, indicating cytoskeletal stiffening (Fig. 1E). Analysis of the unloading portion of the indentation curves showed a smoother recovery phase with low adhesion force (range: 0.002–0.005 µN) in the CTRL group, suggesting predominantly elastic behavior in healthy primary cells. In contrast, senescent cells exhibited higher minimum adhesion forces (range: 0.006–0.01 µN) and a secondary peak or 'bump' before returning to baseline load (Fig. 1E), indicating prolonged recovery times and complex cell-membrane interactions. These findings imply that senescent osteocytes experience significant alterations in cytoskeletal architecture and membrane function, with delayed mechanical recovery and increased adhesion forces. In BMSC spheroids treated with SASP-CM, we observed a decreasing trend in Young's modulus and an increasing trend in membrane adhesion, with statistically significant differences at SnCM-D14 compared to CTRL (Fig. 2). This indicates enhanced cellular 'stickiness' in senescent cultures. Interestingly, senescent osteocytes and SASP-exposed BMSCs exhibited opposite trends in mechanical properties, potentially due to differential cellular responses to SASP factors.

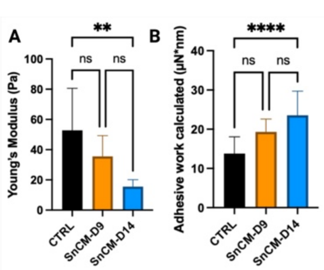

Fig. 2: Reduction in modulus and increased adhesion work in BMSC spheroids treated with SASP-CM from D21 senescent osteocytes compared to control.

DISCUSSION: Our study identifies critical structural changes in irradiated osteocytes and BMSC spheroids treated with SASP-CM, including changes in cytoskeletal stiffening and increased membrane adhesion. These findings suggest cellular senescence results in distinct biophysical changes, providing unique mechanobiological fingerprints. Previous studies have shown that senescent mesenchymal stem cells undergo cytoskeletal reorganization, characterized by stress fiber formation, increased crosslinking of actin filaments, and a denser actin network, which contributes to increased stiffness and reduced deformability. Our observations align with these findings, suggesting that cytoskeletal stiffening in senescent osteocytes may be driven by similar mechanisms, ultimately impairing mechanical function and stress relaxation. Additionally, the increased Sost expression observed in our study suggests a potential link between senescence and Wnt/β-catenin signaling, indicating that Sost may play a role in cytoskeletal reorganization and the resultant increased stiffness. **SIGNIFICANCE:** This study provides critical insights into how cellular senescence alters osteocyte structure and mechanics, specifically through cytoskeletal stiffening and increased membrane adhesion, likely contributing to age-related bone degeneration and impaired tissue repair. Our findings emphasize the role of senescent osteocytes in disrupting bone homeostasis and mechanotransduction, highlighting the importance of the Wnt/β-catenin pathway and SASP factors in these processes. By examining the effects of SASP on the bone marrow microenvironment, we establish a foundation for future research aimed at understanding the systemic impacts of senescent cells in the bone microenvironment and developing targeted therapies to counteract senescence-induced bone fragility. These insights could ultimately lead to novel therapeutic approaches for mitigating age-related bone diseases and improving skeletal health in the elderly.