Rapamycin Rejuvenates Skeletal Stem Cells and Restores Youthful Bone Formation

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INTRODUCTION: Age-related bone loss and fragility fractures remain a major and growing clinical challenge, with limited therapeutic options. A central driver of skeletal decline is dysfunction of skeletal stem and progenitor cells (SSPCs), which are essential for maintaining bone homeostasis and regeneration. Current osteoporosis therapies can slow bone loss but fail to restore regenerative stem cell function, leaving the underlying deficit unaddressed.
Rapamycin, an FDA-approved inhibitor of mTOR signaling, has attracted wide attention for its ability to rejuvenate stem cells across multiple tissues and to extend lifespan in diverse species. Despite this promise, its effects on skeletal aging remain largely unknown, and its impact on SSPCs in particular has scarcely been investigated. Here, we set out to test whether rapamycin could restore regenerative function in aged SSPCs and, in doing so, improve bone structure and integrity *in vivo*.

METHODS: Male and female aged (18–22 months) C57BL/6J mice were randomized to receive either control chow or rapamycin-supplemented chow (42 ppm encapsulated rapamycin) for 12 weeks (n = 6 control, 7 rapamycin-treated). All studies were approved by the NYU Langone Health IACUC in accordance with USDA, PHS, and NIH guidelines. SSPC frequency and clonogenic potential were assessed by flow cytometry and colony-forming unit-fibroblast (CFU-F) assays. Bone-forming capacity was evaluated through *in vitro* using osteogenic differentiation assays. Skeletal structure was analyzed *in vivo* by micro-computed tomography (µCT). To interrogate molecular mechanisms, single-cell RNA sequencing (scRNA-seq) was performed on FACS-purified SSPCs from young (3–4 months, n = 4), aged (n = 4), and aged rapamycin-treated mice (n = 3). Statistical comparisons were made using unpaired two-tailed t-tests or one-way ANOVA with Bonferroni correction (GraphPad Prism); $p < 0.05$ was considered significant.

RESULTS: Rapamycin significantly expanded the frequency of SSPCs in aged bone marrow (0.4% vs 0.2% in controls, p = 0.0004; n = 7 and 5/group). Functional assays confirmed this expansion was accompanied by enhanced clonogenicity, with CFU-F assays showing a 2.7-fold increase in colony formation (p = 0.0004). Differentiation assays revealed that rapamycin rescued the age-related decline in osteogenesis: aged control SSPCs formed 1.5 ± 0.5 mineralized nodules (n = 6), whereas rapamycin-treated aged SSPCs formed 2.0 ± 0.3 (n = 7, p = 0.04). µCT analysis demonstrated corresponding improvements in skeletal architecture, with trabecular bone volume fraction (BV/TV) nearly tripling in treated aged mice compared to controls (4.5 ± 2.6 vs 1.7 ± 0.5, p = 0.04). At the molecular level, scRNA-seq revealed that SSPC subpopulations undergo distinct transcriptional shifts with age, including loss of osteogenic programs and enrichment of adipogenic ones. Rapamycin broadly reversed these changes, restoring youthful gene expression networks. RNA velocity analysis further predicted that rapamycin biased SSPC lineage trajectories toward an *Itga6*$^{+}$ subpopulation, which we identified as an osteo-chondroprogenitor with high bone-forming potential and reduced adipogenic fate.

DISCUSSION: mTOR inhibition with rapamycin rejuvenated SSPCs in aged mice at multiple levels: increasing their abundance, enhancing self-renewal, restoring osteogenic capacity, and reversing transcriptomic hallmarks of skeletal aging. These cellular and molecular improvements translated into measurable gains in trabecular bone mass and architecture *in vivo*.

SIGNIFICANCE / CLINICAL RELEVANCE: Our findings demonstrate that rapamycin, an FDA-approved drug with an established safety profile, rejuvenates aged skeletal stem cells and restores regenerative bone formation. By targeting the root cellular and molecular drivers of skeletal aging, this approach moves beyond current symptom-focused osteoporosis therapies and provides a translationally feasible strategy to restore bone health and fracture resistance in older adults.

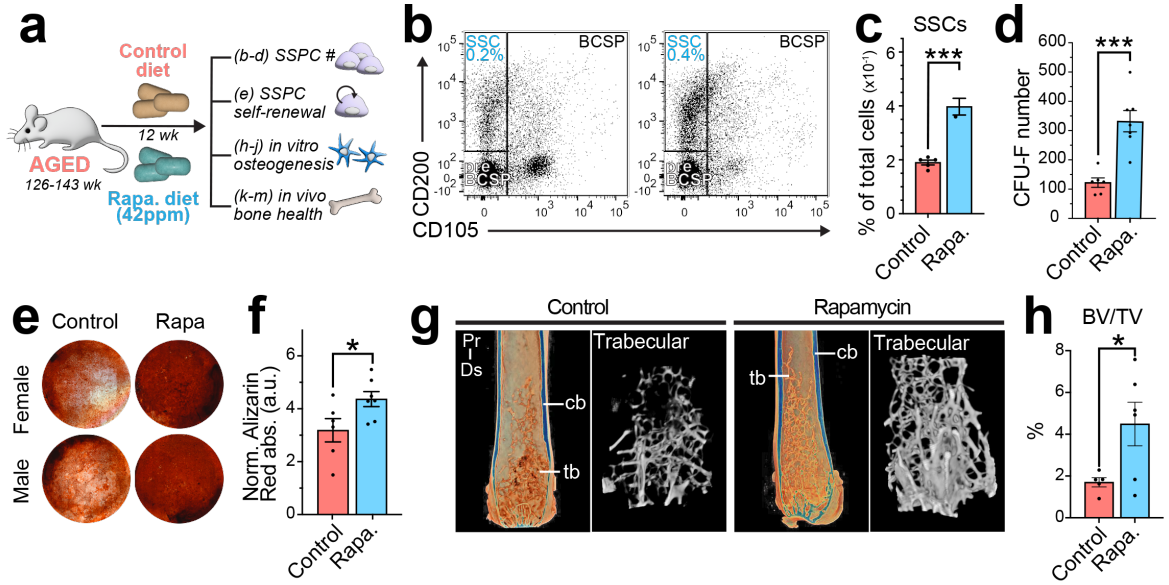

Figure 1. Rapamycin restores skeletal stem cell abundance and enhances osteogenic potential. a Experimental design. Aged (126–143-week-old) wild-type mice were administered either vehicle control (Eudragit encapsulation material) or a rapamycin-enriched diet (42 ppm) *ad libitum* for 12 weeks. Cells were then isolated from hindlimb bones for quantification of skeletal stem/progenitor cell (SSPC) number, self-renewal capacity, and osteogenic differentiation potential, or whole bones were harvested for microCT and histomorphometric analysis. **b** Representative flow cytometry plots and **c.** corresponding quantification of Skeletal Stem Cell (SSC) abundance. **d** Absolute colony-forming unit–fibroblast (CFU-F) number. **e** Representative Alizarin Red staining of bone marrow cells from control and rapamycin-treated mice after 14 days of osteogenic differentiation. **f** Quantification of Alizarin Red staining. **g** Representative 3D-rendered coronal microCT cross-sections of femurs from control and rapamycin-treated aged mice. Lower panels show isolated metaphyseal trabecular bone. **h** Quantification of trabecular BV/TV, bone volume fraction. Data are presented as mean ± s.e.m., with each point representing a single biological replicate. *$p \leq 0.05$; ***$p \leq 0.001$.