CCL2 Inhibition Restores SSC-Mediated Fracture Repair in Aged Mice

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INTRODUCTION: Fragility fractures are frequent in older adults and carry substantial morbidity and mortality because bone repair is delayed and often incomplete. Skeletal stem cells (SSCs) coordinate endochondral regeneration, but aging distorts their lineage potential and dampens responsiveness to osteogenic cues. C-C motif chemokine ligand-2 (CCL2/MCP-1), produced largely by macrophages, is a key driver of age-related inflammation; single-cell profiling of aged marrow reveals macrophage subsets with elevated Ccl2 gene expression. We hypothesized that macrophage-derived CCL2 constrains SSC osteogenesis and that pharmacologic CCL2 blockade restores fracture repair in aged mice.

METHODS: Twenty-month-old mice (n=8) received standardized femoral fractures; callus tissue was harvested at 2 weeks. SSCs were isolated by FACS ($CD45^-Ter119^-TIE2^-ITGAV^+THY1^-6C3^-CD200^+CD105^-$) and cultured under osteogenic conditions with or without recombinant CCL2 (10 ng/mL); mineralization was quantified by Alizarin Red (A450). To test the effects of macrophages on SSC osteogenesis, neonatal (P3) SSCs were co-cultured in transwells with macrophages from 20-month-old marrow ± the CCL2 inhibitor Bindarit, and mineral deposition was again assessed by Alizarin Red. For *in vivo* validation, 20-month-old mice (n=3/group; Stanford APLAC-33042 approved) underwent femoral fracture and received control vehicle or Bindarit during healing; callus bone volume fraction (BV/TV) was quantified by micro-CT.

RESULTS SECTION: Exogenous CCL2 significantly reduced mineralization of aged fracture-callus SSCs versus vehicle (A450, $P<0.01$). Co-culture with aged macrophages suppressed osteogenesis of young SSCs, whereas Bindarit restored mineral deposition. Systemic Bindarit treatment in aged mice yielded higher BV/TV in the fracture callus at 2 weeks than vehicle ($P<0.05$), indicating improved bone formation.

DISCUSSION: Aging macrophages limit SSC-driven bone regeneration; this process involves Ccl2. Blocking this axis restores SSC osteogenesis ex vivo and enhances fracture healing in vivo in aged mice. These data position Ccl2 as a potential microenvironmental target to restore endogenous skeletal repair.
SIGNIFICANCE/CLINICAL RELEVANCE: Targeting the macrophage-Ccl2 pathway revitalizes SSC function and accelerates bone healing in aged individuals. Ccl2 inhibition may provide a translational strategy to improve outcomes after fragility fractures in older patients.

IMAGES AND TABLES:

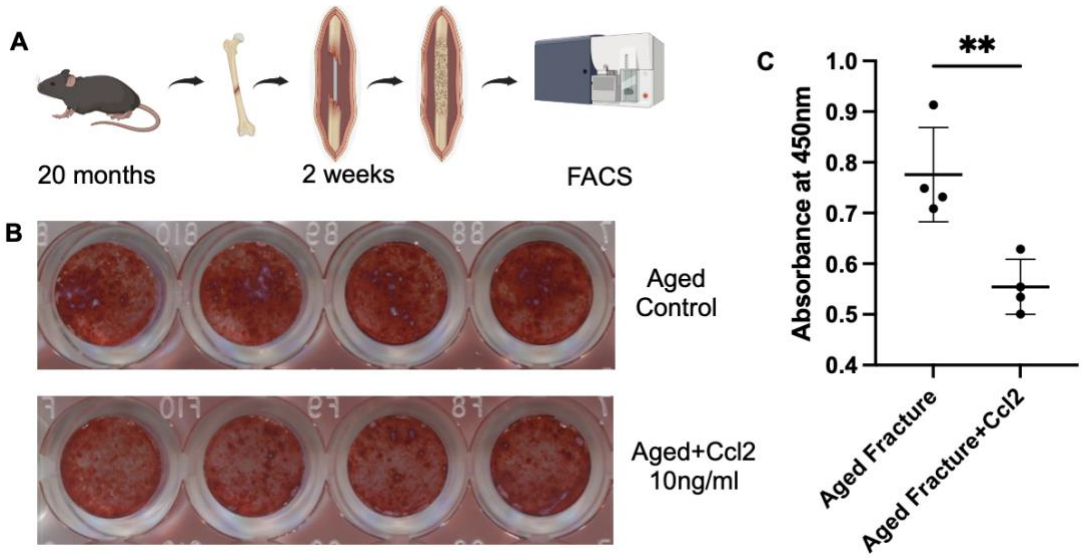

Figure1. Ccl2 inhibits osteogenic capacity of SSCs from aged mice

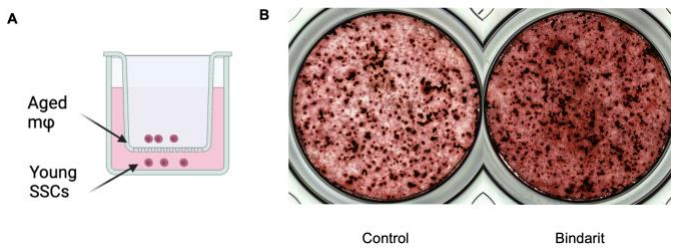

Figure2. Ccl2 blockade with Bindarit restores osteogenesis of young SSCs co-cultured with aged macrophages

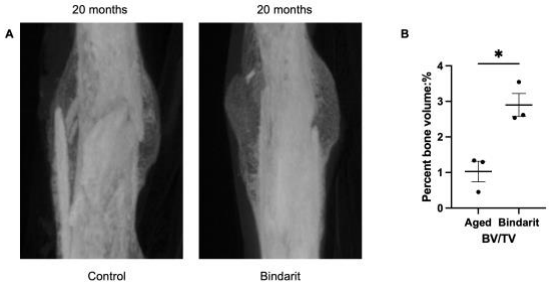

Figure3. Ccl2 blockade with Bindarit increases callus BV/TV and improves fracture healing in aged mice