Senescent cells impair fracture repair through elevating ubiquitin-proteasome system activity in aged mice

Wu, T; Liu, J; Wang, J; Boyce, BF; Xing, L; Zhang, HW
Department of Pathology and Laboratory Medicine; Center for Musculoskeletal Research
University of Rochester Medical Center, Rochester, NY, USA
Disclosures: The authors have nothing to disclose. Email: hengwei_zhang@urmc.rochester.edu

Introduction:

Senescent cells (SCs) inhibit the growth of fracture callus-derived mesenchymal progenitor cells (MPCs), and senolytic drugs enhance fracture repair in aged mice by removing SCs. However, intrinsic molecular mechanisms underlying the regulatory influence of SCs on MPCs, particularly via senescence-associated secretory phenotype (SASP), remain inadequately understood. Numerous studies indicate that aged individuals have increased Ubiquitin-Proteasome System (UPS) activity, wherein inflammatory cytokines contribute to increased protein ubiquitination (Ub) and degradation. We reported previously that the proteasome inhibitor, bortezomib enhanced MPC growth and fracture repair in aged mice. Based on these findings, we hypothesize that SCs impair fracture repair by increasing UPS activity via SASPs.

Methods:

1. 4-m- (young) and 20-m-old (aged) C57BL/6J male mice received open tibial fracture surgery and were treated with the following agents or combinations at day 1, 3, and 5 post-fracture: 1) Senolytic drugs, dasatinib (D, 5 mg/kg) and quercetin (Q, 50 mg/kg); 2) Proteasome inhibitor, bortezomib (Btz, 0.6mg/kg, i.p.); 3) PDGF (1.5μg/callus injection); 4) PDGFRβ selective inhibitor, su16f (10mg/kg by gavage).
2. Callus-derived MPCs (CaMPCs) were treated with SC conditioned medium (CM) to examine the inhibition of cell growth.
3. Total protein from the callus of young and aged mice was used for the expression of PDGFRβ and Ub-PDGFRβ.
4. CaMPCs were 3D cultured using Decellularized Wharton jelly matrix (DWJM) discs from the umbilical cord.

Results:

1. Aged CM increases levels of Ub-protein and reduces growth of CaMPCs, which is prevented by D+Q or Btz (Fig. 1). We previously reported that SCs impaired fracture repair in aged mice by inhibiting CaMPCs growth. In the present study, we found that CM from aged mice (aged CM) increased total Ub-protein levels, which was prevented by D+Q treatment in aged mice (A). The increased protein degradation caused by aged CM was prevented by Btz, but not by the lysosome inhibitor, chloroquine (CQ) (B). Aged CM inhibited CaMPCs growth and fracture repair in aged mice, and enhanced Ub-protein and fracture repair were all prevented by D+Q. Btz did not have an addictive effect on D+Q (D-F).

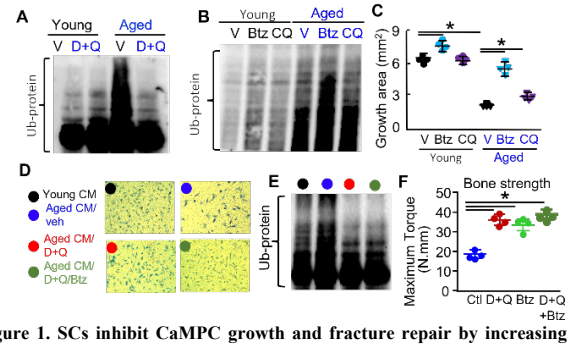

Figure 1. SCs inhibit CaMPC growth and fracture repair by increasing UPS activity. CaMPCs treated with CM±D+Q or Btz/CQ. Expression of Ub-proteins by WB in (A-B). (C) CaMPCs growth examined in Btz/CQ-treated cells. (D) CaMPCs were 3D cultured and treated with young or aged CM± D+Q±Btz. (E) Expression of total Ub-protein in cells from (D) by WB. (F) Fractured aged (20-m) mice treated with D+Q±Btz. Bone strength was examined at d35 by biomechanical testing.

2. Btz enhances fracture repair in aged mice through PDGFRβ (Fig. 2). We found that PDGFRβ is the major targeted protein of Btz in MPCs by Ub-proteomics (not shown). PDGFRβ plays important roles in MPC expansion, but its relationship with SCs has been not studied. We found that PDGFRβ expression was significantly decreased in aged bone samples (A). CaMPCs from aged mice did not proliferate with PDGF treatment, which was rescued by co-treatment with Btz and abrogated by the PDGFRβ inhibitor, su16f (B). PDGF failed to enhance fracture repair in aged mice. In contrast, Btz+PDGF synergistically increased callus volume

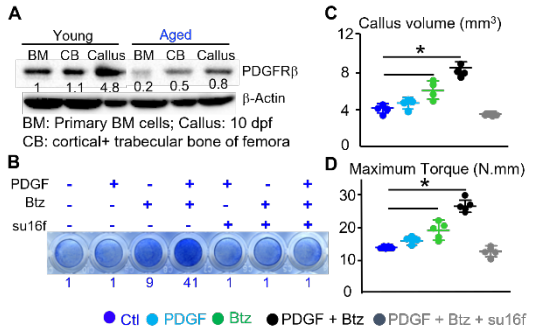

Figure 2. Btz enhances fracture repair in aged mice through PDGFRβ. Fractured young (4-m) and aged (20-m) mice were sacrificed at d10. (A) PDGFRβ protein levels in various bone tissue by WB. (B) CaMPCs from aged mice were treated with PDGF-BB±Btz±su16f. (C-D) Fractured aged mice were treated with PDGF-BB±Btz±su16f. Callus was examined by microCT at d10 or biomechanical testing at

and bone strength in aged mice, which was blocked by su16f, suggesting that Btz acts by affecting PDGFR signaling.

3. TGFβ1 expressed by SCs induces Ub and proteasomal degradation of PDGFRβ (Fig. 3). We previously identified TGFβ1 as the most highly expressed SASP in callus of aged mice. D+Q and TGFβ neutralization enhanced fracture repair in aged mice. Here, we found that TGFβ neutralizing Ab blocked the effect of aged CM on CaMPC growth, Ub-protein and PDGFRβ expression, which were inhibited by the PDGFRβ inhibitor, su16f (A-B). Data suggest that SCs in aged mice impaired fracture repair through regulating PDGFRβ via TGFβ1.

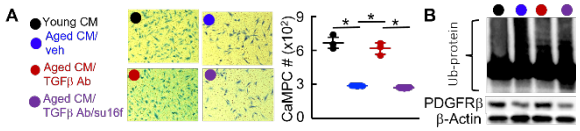

Figure 3. SC-derived TGFβ1 induces ubiquitination and proteasomal degradation of PDGFRβ in CaMPCs. (A) CaMPCs were 3D cultured and treated with young or aged CM±TGFβ Ab±su16f. (B) Expression of total Ub-protein and PDGFRβ in cells from (A) were examined.

Discussion: SCs accumulate in multiple organs during aging. Removal of SCs represents a promising therapeutic approach for age-related disorders. Although SCs removal was reported to alleviate the aging phenotype in bone by regulating osteoblasts and osteoclasts through Sost and RANKL respectively, the precise molecular mechanism has yet to be thoroughly examined. In the current study, we demonstrated that SCs increased protein ubiquitination and degradation in CaMPCs. Both senolytic drugs or Btz alone enhanced CaMPC growth and fracture repair in aged mice, but no addictive effect was observed when senolytic drugs and Btz were administered together. We also found that Btz has no direct effect on cell senescence. Collectively, our data imply that SCs impair fracture repair in aged mice by increasing UPS activity in CaMPCs. Furthermore, our findings show that PDGFRβ is an important target protein of the UPS pathway in senescent CaMPCs. Although SCs produce high levels of SASPs and the UPS has multiple protein targets in CaMPCs, our investigation has revealed that UPS-PDGFRβ is a pivotal signaling pathway in CaMPCs trigged by SCs that may regulate the interaction between SCs and CaMPCs during fracture repair in aged mice.

Significane: Our study has revealed a new molecular mechanism whereby SCs regulate the function of CaMPCs during fracture repair in aged mice and opens up avenues for optimizing treatment strategies with senolytic drugs on aging fracture, such as their combination with PDGF.

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