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

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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 Ub-proteomics (not shown), PDGFβR plays important roles in MPC expansion, but its relationship with SCs has been not studied. We found that PDGFβR 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 PDGFβR inhibitor, su16f (B). PDGF failed to enhance fracture repair in aged mice. In contrast, Btz/PDGF synergistically increased callus volume

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 (1.5 ug/callus injection); 4) PDGFβR 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 PDGFβR 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 CaMPC 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 CaMPC 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 additive 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 CMs: 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 d15 by biomechanical testing.

2. Btz enhances fracture repair in aged mice through PDGFβR (Fig. 2). We found that PDGFβR is the major targeted protein of Btz in MPCs by PDGFβR assays (not shown). PDGFβR plays important roles in MPC expansion, but its relationship with SCs has been not studied. We found that PDGFβR 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 PDGFβR 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 PDGFβR. Fractured young (4-m) and aged (20-m) mice were sacrificed at d10. (A) PDGFβR protein levels in various bone tissue by WB. (B) CaMPCs from aged mice were treated with PDGF-BB/Bztsu16f. (C-D) Fractured aged mice were treated with PDGF-BB/Bztsu16f. Callus was examined by microCT at d10 or biomechanical testing at bone strength in aged mice, which was blocked by su16f, suggesting that Btz acts by affecting PDGF signaling.

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

Figure 3. SC-derived TGFβ1 induces ubiquitination and proteasomal degradation of PDGFβR in CaMPCs. (A) CaMPCs were 3D cultured and treated with young or aged CMs/TGFβ AbSU16f. (B) Expression of total Ub-protein and PDGFβR 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 additive 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 PDGFβR 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-PDGFβR is a pivotal signaling pathway in CaMPCs triggered by SCs that may regulate the interaction between SCs and CaMPCs during fracture repair in aged mice.

Significance: 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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