Pdgfrβ Signaling inhibits BMP2-Mediated Osteogenesis in Bone Healing

S. Novak, L. Shum, J. Madunic, M. Vucetic, X. Wang and I. Kalajzic
UConn Health, Department of Reconstructive Sciences, Farmington, CT, USA

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INTRODUCTION. Bone regeneration depends on a pool of bone/cartilage progenitor cells and mechanisms regulating their differentiation. During regeneration, mesenchymal stem/progenitor cells (MSCs) have to be recruited, proliferate and under appropriate stimuli differentiate into osteogenic cells. Understanding the mechanisms controlling cell fate is crucial for developing efficient treatment to enhance bone healing process. BMP2 is known as an important growth factor involved in bone healing and has been used in clinical settings. However, the supraphysiological doses used can cause serious side-effects. Platelet-derived growth factor (PDGF), is a mitogenic factor highly expressed during bone healing, has also been used to induce bone formation. Our previous results showed that in periosteal cell cultures PDGF BB inhibits BMP2-induced osteogenesis (Wang et al. 2018). The aim of this study was to evaluate role of PDGF signaling in two different injury models; 1) using targeted deletion of PDGFRβ during femoral fracture healing, and 2) determine role of PDGF BB on BMP2-induced osteogenesis in critical size long bone defect model.

METHODS. To determine expression level of PDGFRβ in periosteal progenitor cells during the fracture healing, tibia fractures were created in 8-10-week-old αSMA9 mice (αSMACreERT2 crossed with Ai9 reporter mice; TdTomato). TdTomato expression was induced injecting tamoxifen on -1- and day of fracture. Periosteal callus cells were extracted for flow cytometric analysis on 0 (unfractured periosteum), 4- and 10-days post fracture (DPF). Furthermore, fracture healing process was evaluated in male animals with induced deletion of PDGFRβ in αSMACre+ osteoprogenitor cells and compared to Cre- littermates. For induction of PDGFRβ deletion, tamoxifen was injected on a day of a fracture and 2 DPF. Histological analysis of fractured femurs was performed on 7, 12 and 21 DPF to evaluate callus size, cartilage and mineralized area and osteoclast (TRAP stain). μCT and torsion testing was performed 21 and 42 DPF. Effect of PDGF BB (2 μg) and BMP2 (0.5 or 5 μg) or their combination on bone healing was determined using critical size femoral defects healing model with external stabilization (mouse ExFix, Switzerland). Growth factors were applied to infuse scaffold (Medtronic, MN, USA) at the time of surgery. For tracing osteoprogenitors and osteoblasts, male 6-8 months old SMA9/Col2.3GFP mice were used. Two and nine weeks after the surgery, femora were fixed and healing process evaluated by μCT (bone volume) and histology (SMA9+ or Col2.3GFP+ cells within the defect area ± 30% of the defect where bone response was observed). Statistical differences were determined by Student's t test, or one-way ANOVA when appropriate and data presented as mean value ± SEM. Animal procedures were approved by an institutional animal care and use committee.

RESULTS. During early healing process αSMA+ progenitor cells are quickly expanding and expression of PDGFRβ is significantly increased compared to CD45-/Ter119-/CD31- cells in unfractured ($p<0.01$) and four-day old callus tissue ($p<0.05$). We evaluated in vivo effect of PDGFRβ deletion in αSMA osteoprogenitors in stabilized fracture healing model αSMACreER/PDGFRβ$^{fl/fl}$ mice. Callus area ($p<0.01$) and cartilage area ($p<0.001$) were significantly increased 7 DPF in male mice with PDGFRβ deletion compared to their Cre- controls. 21 DPF increased number of osteoclast and osteoclast surface per bone surface was present in remodeling callus of Cre+ animals compared to Cre-. PDGFRβ deletion led to increased callus density, callus bone mass, and increased bone stiffness ($p<0.5$) 21 DPF. Critical size femoral defects healing was evaluated using BMP2 and PDGF BB. Treatment with 5 μg BMP2 significantly increased progenitor cell number (SMA9+) within the defect compared to all other treatment groups ($p<0.001$). When scaffold was treated with 5 μg BMP2 and 2 μg of PDGF BB combined, lower number of SMA9+ cells were present within the defect area compared to BMP2 treatment alone. Histological analysis of defects 9 weeks post defect showed increased number of Col2.3GFP cells when treated with 5 μg BMP, and PDGF BB decreased osteoblast number compared to BMP2 treatment. PDGF inhibition of BMP2-induced bone healing was confirmed by μCT where significantly decreased bone volume was observed within the bone defect in combined PDGF+BMP2 treatment compared to BMP2 treatment only (5 μg) ($p<0.01$).

DISCUSSION. PDGF is a mitogenic growth factor highly expressed during bone healing. PDGF and BMP2 individually have abilities to induce bone. However, our previous in vitro work showed that PDGF BB inhibits BMP2-induced osteogenesis in periosteal progenitor cells. Here we show expansion of PDGFRβ expressing progenitor cells compared to CD45-/Ter119-/CD31- cell population during healing. Induced deletion of PDGFRβ in osteoprogenitor cells resulted with improved healing. BMP2 treatment induced expansion of αSMA+ progenitor cells within the defect. This effect was decreased when BMP2 was combined with PDGF BB. μCT data showed that PDGF BB inhibited BMP2-induced bone formation, demonstrated by the decrease in bone volume in the defect area. PDGF inhibition of BMP2-induced osteogenesis in critical size femoral defect confirmed our in vitro data. Our study defines mechanisms controlling BMP2-induced osteogenesis leading to better treatment options.

SIGNIFICANCE/CLINICAL RELEVANCE: Data presented indicate that modulating PDGF signaling could potentially led to decreasing BMP2 dose to more physiological concentrations, decrease its side effects with preserving healing ability in clinical settings.

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REFERENCE: Wang X, Matthews BG, Yu J, Novak S, Grcevic D, Sanjay A, Kalajzic I. PDGF Modulates BMP2-Induced Osteogenesis in Periosteal Progenitor Cells. JBMR Plus. 2019 Jan 15;3(5):e10127. doi: 10.1002/jbm4.10127.

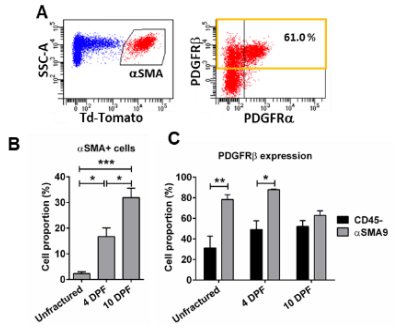

Figure 1. A. Gating strategy for αSMA cells and PDGFRα/β cells in periosteal callus. B. αSMA9 and C. PDGFRs expression analyzed within non-hematopoietic cells. Values are expressed as mean + SEM. n = 3.

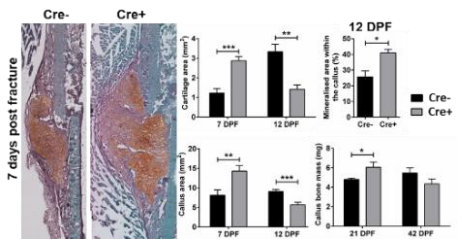

Figure 2. Callus and cartilage area were determined 7 DPF and callus, cartilage and mineralized area on day 12. Cre- (n=7), Cre+ (n=10) on day 7, and Cre- (n=9), Cre+ (n=11) 12 DPF. Callus bone mass determined 21 DPF (Cre- n=6, Cre+ n=10) and 42 DPF (n=5).

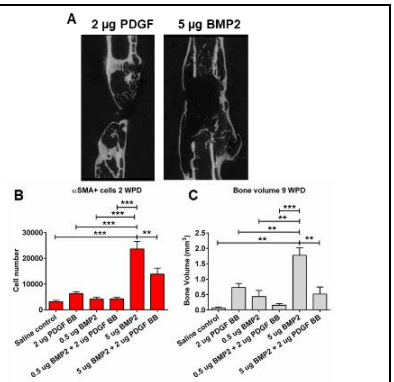

Figure 3. A. Representative 2D images of μCT morphometry of defect femurs 9 WPD. B. αSMA+ progenitor cells 2 WPD surgery. n=4-6 C. Bone volume within the defect area. n=2-9.