

A combined treatment of BMP2 and VEGFR1 promotes tendon-bone healing by regulating injury-activated skeletal stem cell lineage

Linfeng Wang,^{a,b,c,d}, Tao Zhang,^{a,b,c,d}, Changbiao Guan,^{a,b,c,d}, Chengjun Li^{a,b,c,e}, Jianzhong Hu,^{a,b,c,e}, Daqi Xu,^{a,b,c,d,#}, Hongbin Lu,^{a,b,c,d,#}

a Department of Sports Medicine, Xiangya Hospital, Central South University, Changsha, China;

b Key Laboratory of Organ Injury, Aging and Regenerative Medicine of Hunan Province, Changsha, China;

c Hunan Engineering Research Center of Sports and Health, Changsha, China;

d National Clinical Research Center for Geriatric Disorders, Xiangya Hospital, Central South University, Changsha, China;

e Department of Orthopedics, Xiangya Hospital, Central South University, Changsha, China.

Abstract

INTRODUCTION: Bone morphogenetic protein 2 (BMP2) is an appealing osteogenic and chondrogenic growth factor for the promotion of tendon-bone (T-B) healing. Recently, it has been well reported that VEGFR1 (a VEGF receptor antagonist) could enhance BMP2-induced bone repair and cartilage regeneration, so their combined application may represent a promising treatment to improve T-B healing. Moreover, BMP2 could stimulate skeletal stem cells (SSCs) expansion and formation, which are responsible for wounded tendon-bone interface (TBI) repair. However, whether co-delivery BMP2 and VEGFR1 increase tendon enthesis injury-activated SSCs better than sole BMP2 need further research. Here, we intended to study the effect of BMP2 combined with VEGFR1 on T-B healing and injury-activated skeletal stem cell lineage.

METHODS: A total of 128 C57BL/6 mice that underwent unilateral supraspinatus tendon (ST) detachment and repair were randomly assigned to 4 groups: untreated control group, hydrogel group (received a local injection of the blank hydrogel at the injured site), BMP2 group (received an injection of hydrogel with BMP2), and BMP2 with VEGFR1 group (received an injection of hydrogel with BMP2 and VEGFR1). Histology, Micro-computed tomography (Micro-CT), and biomechanical tests were conducted to evaluate T-B healing at 4 and 8 weeks after surgery. In addition, flow cytometry was performed to detect the proportion of SSCs and their downstream differentiated subtypes including BCSPs (bone, cartilage, and stromal progenitors), OPs (osteoprogenitors), and PCPs (pro-chondrogenic progenitors) within supraspinatus tendon enthesis at 1 week postoperatively.

RESULTS: The repaired interface in BMP2 with VEGFR1 group showed an increased amount of fibrocartilage, greater newborn bone, and elevated mechanical properties when compared to the other three groups. And there were more SSCs, BCSPs, OPs, and PCPs in BMP2 with VEGFR1 group than that in the other groups.

DISCUSSION: To our knowledge, this is the first study to investigate the effects of BMP2 combined with VEGFR1 on T-B healing and SSCs lineage within enthesis. In the current study, using a mice RC reconstruction model, the combination of BMP2 with VEGFR1 was shown to have a higher therapeutic potential for T-B healing when compared with BMP2 alone, as confirmed by histology, micro-CT, and biomechanical testing. In addition, our results indicated that BMP2 with VEGFR1 could stimulate the expansion of SSCs and its downstream differentiated cells including BCSPs, OPs, and PCPs at repaired TBI. Our study suggests that the combined delivery of BMP2 and VEGFR1 could promote T-B healing and stimulate the expansion of SSCs and their downstream progeny within injured TBI.

CLINICAL RELEVANCE: The combination of BMP2 with VEGFR1 may be a good clinical treatment for wounded tendon/ligament enthesis healing.

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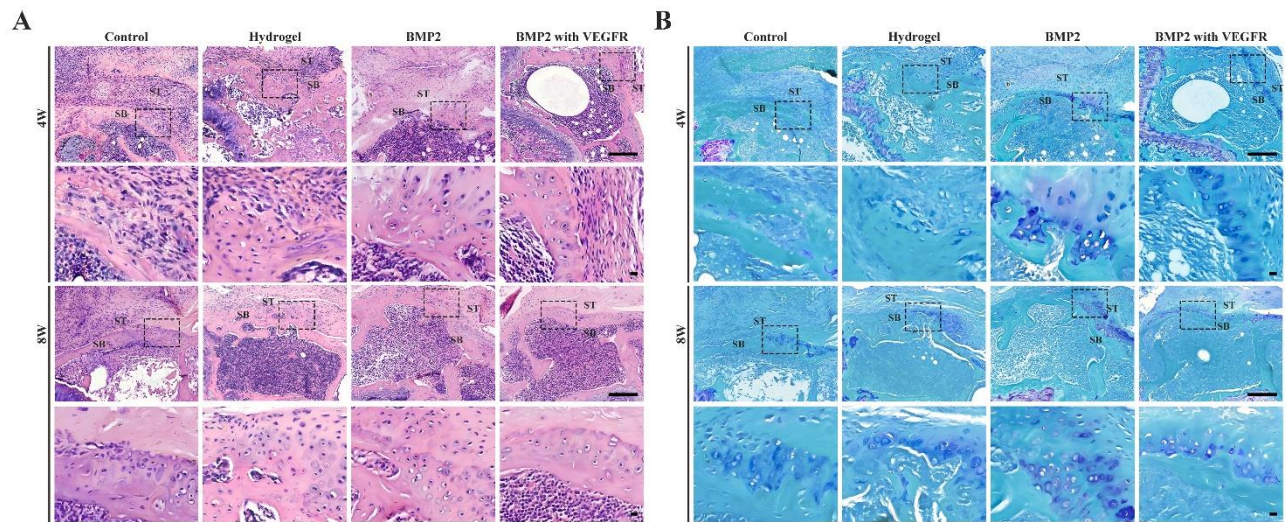


Figure 1. (A) Representative images of supraspinatus tendon enthesis stained with hematoxylin and eosin at weeks 4 and 8 after surgery.; (B) Representative images of supraspinatus tendon enthesis stained with toluidine blue and fast green at weeks 4 and 8 after surgery. The area selected by the rectangle dashed line is the local magnified area. scale bar = 200 μ m. SB, subchondral bone; ST, supraspinatus tendon

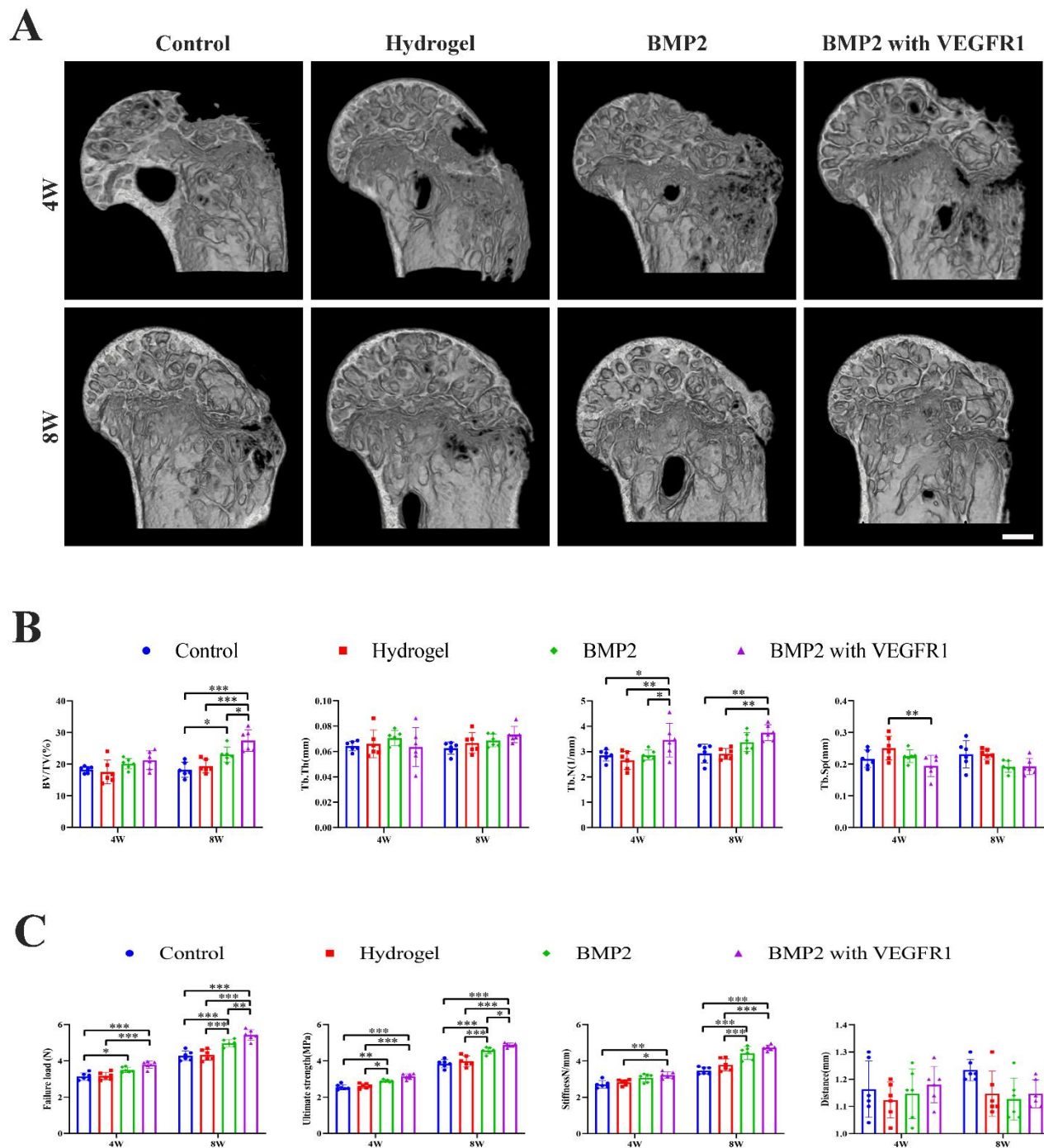


Figure 2. (A) three-dimensional reconstruction image of micro-CT for the humeral head, Scale bar =500 μ m. (B) The morphological measurement result of newly formed bone at the healing site. n = 6 per group. (C) Biomechanical properties at the tendon insertion site of ST-humerus complexes at postoperative weeks 4 and 8. BV/TV, bone volume / total volume; Tb.Th, trabecular thickness; Tb. N, trabecular number; Tb.Sp, trabecular separation; ST, supraspinatus tendon. Data are shown as mean \pm SD, *p < 0.05, **p < 0.01, ***p < 0.001.

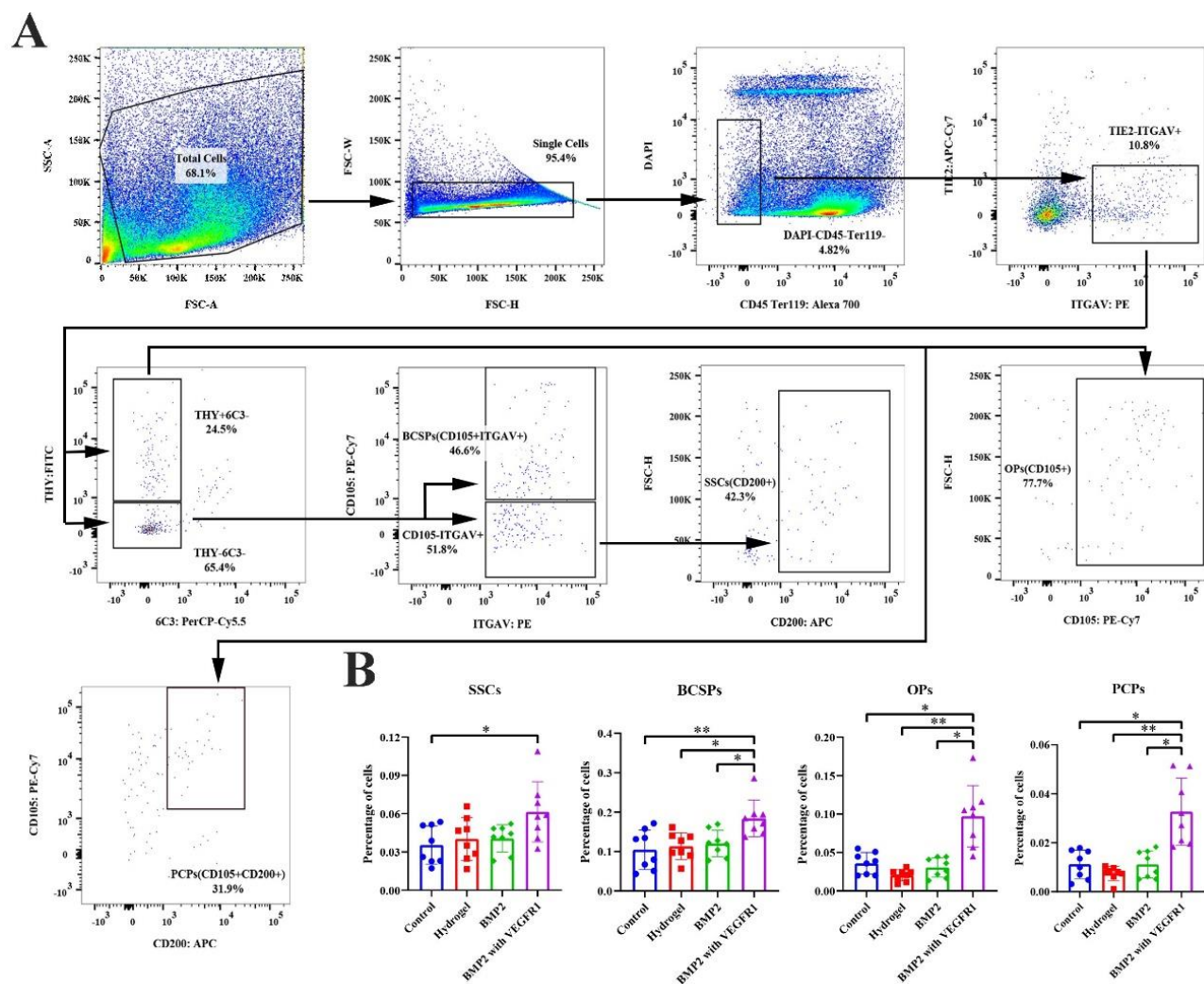


Figure 3. (A) Representative flow cytometry plots gating strategy used to determine SSCs lineage. (B) Quantification of SSCs, BCSPs, OPs, and PCPs

within ST enthesis at different groups. n = 8 per group. Data are shown as mean \pm SD, *p < 0.05, **p < 0.01.