WNT-modulating gene silencers as a gene therapy for osteoporosis, bone fracture, and critical-sized bone defects

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INTRODUCTION: Treating osteoporosis and associated bone fractures remains challenging for drug development in part due to potential off-target side effects and the requirement for long-term treatment. Here, we identify recombinant adeno-associated virus (rAAV)-mediated gene therapy as a treatment not only for only counteracted bone loss in both postmenopausal and senile osteoporosis but also promoted the healing of bone fractures or critical-sized bone defects.

METHODS: rAAVs were used to deliver long-lasting targeting of WNT signaling components to osteoblast-lineage cells. rAAV-mediated silencing of Schnurri-3 (SHN3) or Sclerostin (SOST) in osteoblast-lineage cells enhanced WNT signaling, osteoblast function, and bone formation in mice. Combination therapy targeting both factors further increased anabolic responses along with reduced bone resorption, compensating for the limitations of single gene silencers, as the expression of SOST and SHN3 is connected via a negative feedback mechanism. First, we confirmed that bone-targeted AAV gene silencers increase bone formation in mice. Two month-old WT mice were i.v. injected with rAAV9.DSS carrying amiR-ctrl, amiR-shn3, amiR-sost, or amiR-sost/shn3, and, 4 weeks later, mRNA levels of Shn3 and Sost and Axin2 and Lef1 in the tibia were measured by RT-PCR. Femoral bone mass was assessed by microCT, and dynamic histomorphometry was performed in the metaphysis of AAV-treated femurs. Next, we tested that bone-targeted AAV gene silencers reverse bone loss in osteoporosis. Sham or ovariectomy surgery was performed on 3-month-old female mice, and 6 weeks later, mice were i.v. injected with rAAV9-mediated gene silencers. Eight weeks later, mRNA levels of Shn3 and Sost in the tibia were assessed by RT-PCR. Twenty-month-old male mice were i.v. injected with rAAV9-mediated gene silencers, and, 2 months later, mRNA levels of Shn3 and Sost in the tibia were assessed by RT-PCR. MicroCT assessed femoral bone mass, and Histomorphometric quantification of BFR/BS and MAR was performed 8 weeks post-injection. Then, three-month-old mice were i.v. injected with rAAV9-mediated WNT modulators, and 2 weeks later, femoral osteotomy and intramedullary fixation were performed. Six weeks after the surgery, mRNA levels of Shn3, Sost and Axin2 in the tibia were assessed by RT-PCR. Union rate at the fracture sites was quantitated by microCT. At last, the decellularized isograft was incubated with rAAV9-mediated gene silencers for one hour and then rAAV9.DSS isograft was implanted into the osteotomy sites of the left femur. Twelve weeks later, the total bridging between the implanted isograft and the host bone was assessed by radiography and microCT. An autograft bone was implanted into the osteotomy sites as a positive control. All animals in this study were used following the NIH Guide for the Care and Use of Laboratory Animals and were handled according to protocols approved by our School Institutional Animal Care and Use Committee.

RESULTS: A single systemic administration of rAAVs effectively reversed bone loss in both postmenopausal and senile osteoporosis (Fig 1). Moreover, the healing of bone fractures and critical-sized bone defects was also markedly improved by systemic injection or transplantation of AAV-bound allograft bone to the osteotomy sites (Fig 2).

Discussion: Collectively, our data demonstrate the clinical potential of bone-specific gene silencers to treat skeletal disorders of low bone mass and impaired fracture repair.

IMAGES AND TABLES:

Fig 1.

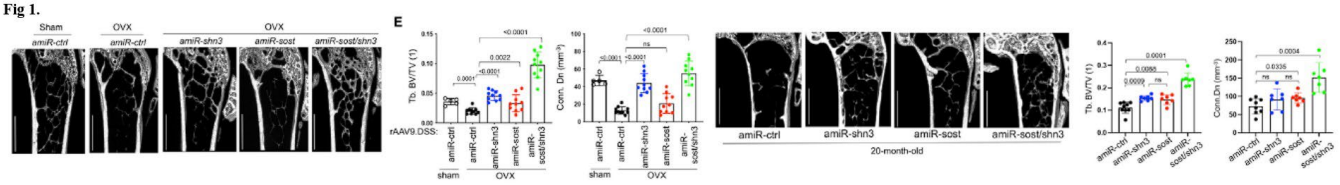

Fig 2.