Role of Parasubthalamic Nucleus in Distraction Osteogenesis in a Mouse Model

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INTRODUCTION: Distraction osteogenesis (DO) is surgical technique used in the treatment of bone defects, limb deformities or limb length discrepancy to promote bone regeneration via controlled mechanical stimulation. Despite its clinical application for various bone diseases, the underlying mechanisms of DO remain understudied. This study aims to address two key issues: the lack of a well-established mouse model for DO research, which limits molecular investigations using transgenic mice, and the potential involvement of the central nervous system (CNS) in DO, beyond the current focus on angiogenesis and stem cell mobilization.

METHODS: A novel and reusable distraction device was custom-made with titanium alloy. This device is a unilateral frame with four horizontally aligned holes. This frame can be fixed to the mouse femur by four thread screws with a drill guide forcep. Four thread screws were installed to the drilled holes via the guide forcep. For establishment of the mouse DO model, 12-week-old male C57/BL6J mice were used. Transverse osteotomy was conducted in the midline of femur after fixation with the distraction device. Animals were divided into 3 groups: fracture (F), acute distraction (AD), and gradual distraction (GD). In F group, the mice were treated with osteotomies only. In AD group, a 3-mm distraction was performed on the sixth day after the osteotomy. The GD protocol consisted of 5 days of latency, 10 days of distraction at a rate of 0.3 mm daily and 28 days of consolidation. Samples were harvested 43 days after the surgery (POD 43) for micro-CT and histological analysis. To investigate the involvement of the CNS in DO, brain mapping was performed to explore brain regions relevant to the DO callus, pseudorabies virus - red fluorescence protein (PRV-RFP) was injected into the distraction gap or the femoral growth plate. To explore the regulatory role of interested brain region in DO, adeno-associated virus (AAV)-DIO-mCherry or AAV-DIO-hM4Di-mCherry was injected to the targeted brain region of $Tac1^{+}$ cre mice for inhibition of the neuronal excitatory status of the PSTN. Fluorescence at the injection sites were determined by microscopy. Bone parameters of DO callus were determined by micro-CT at 2 weeks after the distraction phase.

RESULTS: Remarkable bone regeneration in calluses were observed in the F and GD groups, while bone defect still could be found in the AD group (Figure 1A-B). At the end point (POD 43), the GD group showed significantly higher bone volume (BV) compared to the AD group (Figure1B, $P < 0.05$, n = 3-7). A number of brain regions were labeled by PRV-RFP injected in the growth plate or the distraction gap, including BLA, PVN, NTS, etc. The parasubthalamic nucleus (PSTN) was only labeled by PRV-RFP injected in distraction gap, but not in the growth plate (Figure 2), showing a DO-dependent manner. Micro-CT analysis showed lower BV in the chemoinhibition (mCherry-hM4Di) group compared to control (mCherry) group, indicating a potential regulatory role of PSTN in bone regeneration (Figure 3, $P < 0.05$, n = 4-5).

DISCUSSION: This DO model successfully replicates the bone regeneration process observed in clinical practice. This finding provides evidence supporting a potential regulatory role of the PSTN in DO, which opens up an opportunity for future research aimed at exploring the underlying neuronal mechanisms and identifying potential therapeutic strategies to enhance bone regeneration.

SIGNIFICANCE/CLINICAL RELEVANCE: Understanding the regulatory role of the Parasubthalamic nucleus in distraction osteogenesis could lead to the development of targeted therapeutic strategies to enhance bone regeneration.

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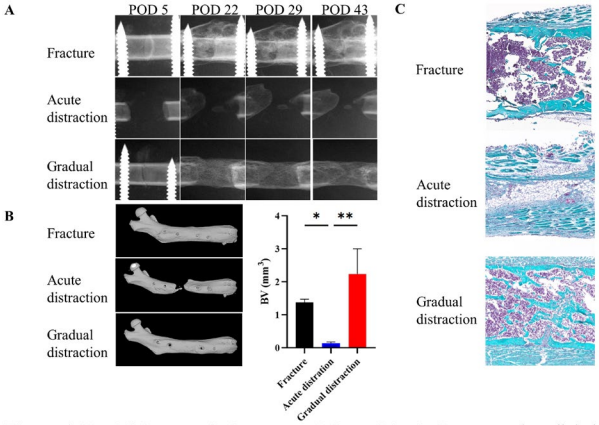

Figure 1 Establishment of the mouse DO model. A. Representative digital radiography images of operated femurs at POD 5, 22, 28 and 43. B. Representative micro-CT images and quantitative analysis of operated femurs in F, AD and GD group collected at POD 43. C. Fast green staining of operated femurs of F, AD and GD group collected at POD 43.

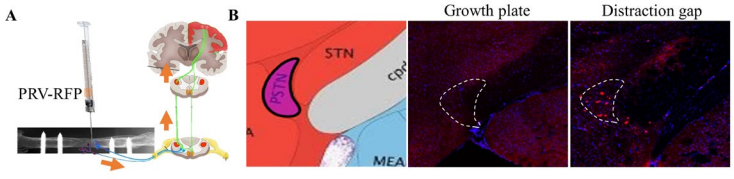

Figure 2 Retrograde tracing from the operated femurs of the mouse DO model. A. Schematic of retrograde tracing using PRV-RPF. B. Schematic of anatomical characterization of the mouse PSTN (Images modified from the Allen Brain Atlas) and representative images of PRV-RFP labeled cells in brain sections of mice with PRV-RFP injected at the growth plate or the distraction gap. White dashed lines indicate the outline of PSTN.

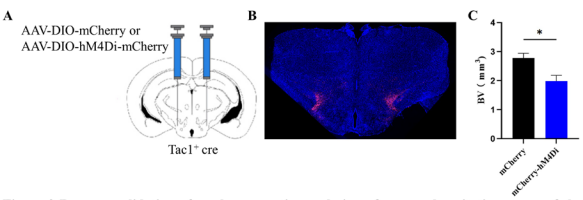

Figure 3 Bone consolidation after chemogenetic regulation of neuronal excitation status of the PSTN. A. Schematic of bilateral PSTN injection of $Tac1^{+}$ cre mice with AAV-DIO-mCherry or AAV-DIO-hM4Di-mCherry. B. Representative images of fluorescence at the injection sites. C. Representative 2D micro-CT images and quantification of bone volume (BV) of distraction gap from mice injected with AAV-DIO-mCherry or AAV-DIO-hM4Di-mCherry.