Depletion of CCR7 prevents bony growth imbalance after physeal injury in mice
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INTRODUCTION: Growth plate (GP) is responsible for longitudinal bone growth. However, it is weakest structure in the skeleton of a child and a frequent site of an injury or fracture, leading to progressive skeletal growth imbalance and deformity, and significant physical problems. Current treatment involves surgical resection of the bar and replacement with an interpositional material to preserve normal growth in the remaining physis. However, these procedures are invasive and cause substantial burden to the child and their family. Chemokine is low Molecular Weight protein of 8-10kD and known to play a crucial role in recruiting stem cells or precursor cells. Besides, we have observed that depletion of chemokine receptor CCR7 in mice deteriorate articular cartilage regeneration (1). We therefore hypothesized that CCR7 would play crucial roles in physeal cartilage regeneration. To test this hypothesis, we employed CCR7 knockout mice (KO) to analyze the role of CCR7 in the GP cartilage repair process.

METHODS: All experimental procedures fully complied with the related laboratory animal regulations. GP injury was made in the left proximal tibial GP in 3-week-old C57BL/6 mice (WT) and CCR7 knockout mice (2). Briefly, under general anesthesia, the skin over the proximal tibia was incised with a scalpel and opened with forceps to render the tibiae visible. The proximal tibia GP was entirely pierced in a lateral-medial direction with a 25-G needle. Right tibial GPs were sham operated with skin incision only. The tibiae were harvested and analyzed at 3 and 5 weeks postoperatively. The length of tibiae was measured macroscopically using Image J software and the height of proximal tibial growth plate were measured by micro-CT. The tibiae were harvested from costal cartilage of 3-week-old mice and subjected to qPCR analysis. All results were statistically compared using unpaired t-tests to evaluate differences between pairs of groups. Significance was accepted with a p value < 0.05. All statistical analyses were done using statistical software JMP Pro 16.0 (SAS Institute, Cary, NC, USA).

RESULTS: Immunhistological analysis revealed that CCR7 was expressed at injury site 1 day postoperatively, therefore we employed CCR7 knockout mice (Figure 1A). 3 and 5 weeks postoperatively, the drop ratio in KO mice significantly lower than those in WT mice (mean ± standard deviation, 2.76 ± 0.86 % in WT vs 0.83 ± 0.86 % in KO at 3 weeks, p < 0.01; 2.51 ± 0.84 % in WT vs 1.01 ± 0.74 % in KO at 5 weeks, p < 0.01, Figure 1B). Histological examination revealed that WT showed a denser and more continuous bony bar starting to form at 1 week, while bony bar in KO showed rather sparse, unstable and discontinuous shape, partly empty, especially at 5 weeks (Figure 2). Bone volume of the physeal bridge in KO mice was significantly lower than those in WT mice at both 3 and 5 weeks (143.16 ± 9.21 HU in WT vs 130.37 ± 7.62 HU in KO at 3 weeks, p < 0.01; 150.29 ± 4.59 HU in WT vs 142.84 ± 5.07 HU in KO at 5 weeks, p < 0.01). The osteogenic markers such as VEGF, MMP13 in physeal cartilage were significantly downregulated, in contrast the expression of Runx2, Sox9 and ERK were almost equal between WT and KO (Figure 3).

DISCUSSION: The current study demonstrated that depletion of CCR7 in mice inhibited physeal bridge formation and ameliorated growth imbalance after physeal injury. These results suggested that CCR7 might have important roles in physeal bridge formation. Since surgical resection of physeal bar may affect the viability of remaining cartilage, we must pay much attention to decision of additional non-surgical intervention. The treatment strategy to inhibit physeal bridge formation by manipulation of CCR7 may be future directions after the growth plate injury.

Significations: Depletion of CCR7 ameliorated growth imbalance after physeal injury in mice.


![Figure 1. (A) CCR7 expression after 1 day GP injury. (B) Drop ratio of tibiae after GP injury.](image1)

![Figure 2. Histological analysis after GP injury.](image2)

![Figure 3. QPCR analysis from physeal cartilage harvested from costal cartilage of 3-week-old mice.](image3)