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CKIP-1 silencing promotes new bone formation in rat mandibular distraction osteogenesis

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Objective. This study investigated the effects and possible molecular mechanism of casein kinase-2 interacting protein-1 (CKIP-1) silencing on bone regeneration during rat mandibular distraction osteogenesis (DO).

Study Design. CKIP-1 silencing by chitosan/si-CKIP-1 was employed and analyzed both in rat mandibular DO models in vivo and in cultured rat mandible bone marrow stromal cells (BMSCs) in vitro.

Results. Gross observation, micro–computed tomography analysis, and hematoxylin and eosin (H&E) staining revealed that new bone formation in the distraction gap of the chitosan/si-CKIP–treated group was better compared with the chitosan/si-NC and phosphate buffered saline–treated groups in both quantity and quality. Proliferation assay, flow cytometry, and alizarin red staining indicated that CKIP-1 silencing significantly inhibited apoptosis, but promoted osteogenic differentiation of cultured BMSCs. Additionally, CKIP-1 silencing significantly promoted the expression of Wnt3 a, β-catenin, and osteocalcin both in new bone formation of DO models in vivo and in the osteogenic differentiation process of BMSCs in vitro.

Conclusions. Promotion of bone formation after CKIP-1 silencing in rat mandibular distraction osteogenesis appears to be mediated through the Wnt3 a/β-catenin signaling pathway. (Oral Surg Oral Med Oral Pathol Oral Radiol 2016;■:e1-e9)

Distraction osteogenesis (DO) has become a widely accepted method for endogenous bone regeneration in oral and maxillofacial surgery.[1] DO has been applied in the reconstruction of bone deformities and defects caused by congenital malformation, injury, or tumorectomy.[1] However, the prolonged treatment period and the uncertain quality of bone formation are considered major disadvantages of DO,[2,3] which increase the risk of complications and limit further clinical application of this surgical procedure.[3-5]

To improve the clinical effectiveness and quality of bone formation in DO, many strategies have been tried, including stem cell therapy,[6,7] gene therapy,[8,9] sympathetic inhibition,[10] physical stimulation,[11] and adjuvant therapy with various adjuvant substances and delivery methods.[12,13] Gene therapy using small interfering RNA (siRNA therapy) is a highly target-specific technique to silence a negative-regulator gene of bone formation and regulate its corresponding protein expression in DO.[8,14] Considering the high specificity, intrinsic biological response, and silencing efficiency of siRNA, it is safe to say that siRNA therapy has great potential in DO.[8,14] Casein kinase-2 interacting protein-1 (CKIP-1) is a recently identified negative regulator of bone formation.[15,16] A number of studies have found that therapeutic siRNA targeting CKIP-1 promotes bone formation in healthy and osteoporotic mice.[15-17] Additionally, cross-species CKIP-1 siRNA sequences were identified and no detectable immunostimulatory activity was induced by such sequences.[16] Our previous study demonstrated that siRNA targeting CKIP-1 delivered by chitosan significantly increased the osteogenic differentiation of bone marrow stromal cells (BMSCs).[18] However, the role of CKIP-1 in DO remained inexplicit. In the present study, to investigate the role and possible molecular mechanism of CKIP-1 in DO, CKIP-1 silencing by si-CKIP-1 was employed and analyzed both in rat mandibular DO models in vivo and in cultured rat mandible BMSCs in vitro.

Statement of Clinical Relevance

This study demonstrates that CKIP-1 silencing promotes new bone formation in distraction osteogenesis, suggesting that CKIP-1 may represent a potential small interfering RNA target in the development of novel bone regeneration therapy.

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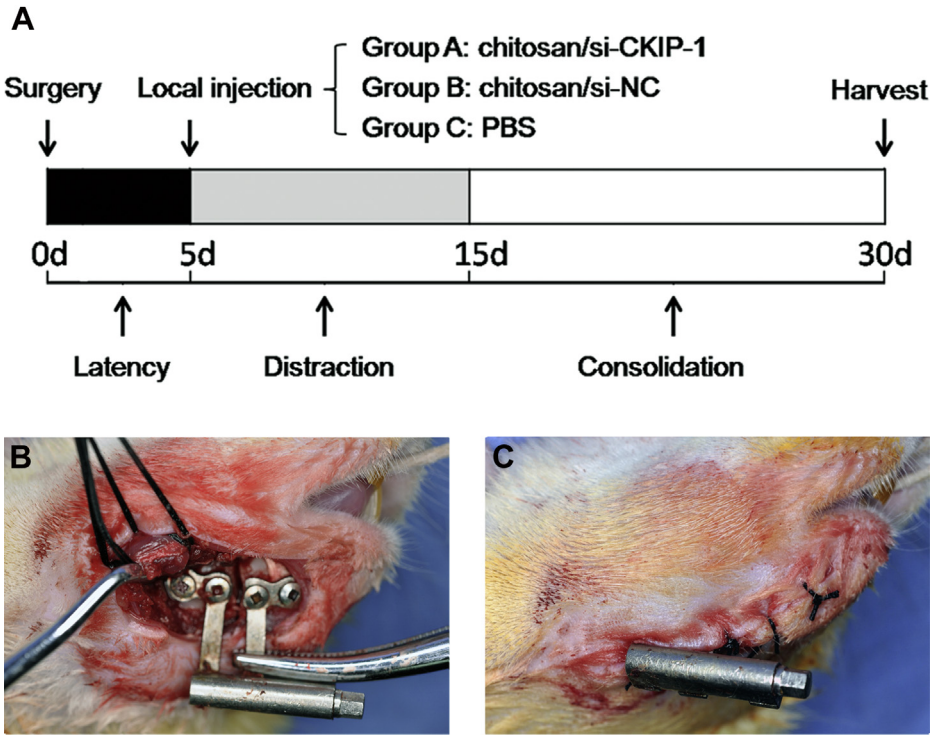

Fig. 1. Distraction plan and groupings for the rat mandibular distraction osteogenesis (DO) experiment. **A,** The distraction plan and groupings. After a 5-day latency period, 24 rats were randomly divided into 3 groups: group A (n = 8), given a local injection of 0.1 mL chitosan/si− casein kinase-2 interacting protein-1 (CKIP-1) (20 μM); group B (n = 8), given a local injection of 0.1 mL chitosan/si-NC (20 μM); group C (n = 8), given a local injection of 0.1 mL phosphate buffered saline (PBS). The distraction was initiated and allowed to take place for 10 days. After a consolidation period of 15 days, samples were harvested for study. **B,** The right mandibular body and ramus were exposed and a vertical osteotomy was performed between the molars and the mandibular ramus. Then a titanium distraction device was used for fixation and distraction of the rat mandible. **C,** Establishment of the rat mandibular DO models.

MATERIALS AND METHODS

Animal modeling and grouping

This study was approved by the Animal Care and Use Committee at Fourth Military Medical University. All the animals were housed in the university's Animal Care Center at the School of Stomatology.

A total of 24 male Sprague-Dawley rats (12 weeks old, weight 200 ± 10 g) were used in this study, whose plan is shown in Figure 1A. All the animals were anesthetized with 1% pentobarbital sodium (30 mg/kg). The right mandibular body and ramus were exposed through a submandibular incision and then a vertical osteotomy was performed between the molars and mandibular ramus. A titanium distraction device (Zhongbang Titanium Biomaterials Corporation, Xi'an, China) was used for fixation and distraction (Figures 1B and C). After a latency period of 5 days, the 24 rats were randomly divided into 3 groups. Group A (n = 8) underwent DO and received local injection of 0.1 mL chitosan/si-CKIP-1 silencing solution containing 20 μL si-CKIP-1 (20 μM); group B (n = 8) underwent DO and received local injection of 0.1 mL chitosan/si-NC silencing solution containing 20 μL si-NC (20 μM); group C (n = 8) underwent DO and received local injection of 0.1 mL phosphate buffered saline (PBS). Here, chitosan was used as a nontoxic and sustained release system[19] to load the si-CKIP-1 (sense 5′-GGACUUGGUAGCAAGGAA AdT*dT-3′, antisense 5′-UUCCUUGCUACCAAGUC CdT*dT-3′; Shanghai GenePharma, Shanghai, China) or si-NC (sense 5′-UUCUCCGAACGUGUCACG UTT-3′, antisense 5′-ACGUGACACGUUCGGAGA ATT-3′; Shanghai GenePharma). The silencing solution consisted of a mixture of chitosan solution and siRNA silencing complexes. The methods of preparing the chitosan solution and siRNA silencing complexes were described in our previous study.[18]

The distraction protocols were as follows: The rods were distracted at a rate of 0.2 mm twice a day for 10 days, followed by a consolidation period of 15 days. At the end of the consolidation period, all the animals were sacrificed with an overdose of pentobarbital sodium. Then the mandibles were harvested and used for photographic, micro−computed tomography (MicroCT),