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Norepinephrine inhibits mesenchymal stem cell chemotaxis migration by increasing stromal cell-derived factor-1 secretion by vascular endothelial cells *via* NE/abrd3/JNK pathway

Baolei Wu[b,c,1], Lei Wang[a,b,*,1], Xi Yang[a], Ming Mao[c], Chen Ye[a], Peng Liu[b], Zihui Yang[b], Xinjie Yang[b], Delin Lei[b], Chenping Zhang[a,b,*]

[a] *Department of Oral & Maxillofacial-Head & Neck Oncology, Ninth People's Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai Key Laboratory of Stomatology, No.639 Zhizaoju Road, Shanghai 200011, China*
[b] *State Key Laboratory of Military Stomatology, Department of Oral and Maxillofacial Surgery, School of Stomatology, the Fourth Military Medical University, No.145 West Changle Road, Xi'an 710032, China*
[c] *No. 3 Hospital of People's Liberation Army, Department of Oral and Maxillofacial Surgery, Baoji 721004, China*

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ABSTRACT

Mesenchymal stem cells (MSCs), which are physiologically maintained in vascular endothelial cell (VEC)-based niches, play a critical role in tissue regeneration. Our previous studies demonstrated that sympathetic denervation could promote MSC mobilization, thereby enhancing bone formation in distraction osteogenesis (DO), a self-tissue engineering for craniofacial and orthopeadic surgeries. However, the mechanisms on how sympathetic neurotransmitter norepinephrine (NE) regulates MSC migration are not well understood. Here we showed that deprivation of NE by transection of cervical sympathetic trunk (TCST) inhibited stromal cell-derived factor-1 (SDF-1) expression in the perivascular regions in rat mandibular DO. *In vitro* studies showed that NE treatment markedly upregulated p-JNK and therefore stimulated higher SDF-1 expression in VECs than control groups, and siRNA knockdown of the abrd3 gene abolished the NE-induced p-JNK activation. On the other hand, osteoblasts differentiated from MSCs showed an increase in SDF-1 secretion with lack of NE. Importantly, NE-treated VECs inhibited the MSC chemotaxis migration along the SDF-1 concentration gradient as demonstrated in a novel 3-chamber Transwell assay. Collectively, our study suggested that NE may increase the SDF-1 secretion by VECs *via* NE/abrd3/JNK pathway, thereby inhibiting the MSC chemotaxis migration from perivascular regions toward bone trabecular frontlines along the SDF-1 concentration gradient in bone regeneration.

1. Introduction

Distraction osteogenesis (DO) is widely used in orthopeadic and craniofacial surgeries for bone regeneration to reconstruct defects and deformities in long bones and the maxillofacial bone [1,14,22,39]. The new bone generated by DO is formed by the endogenous rather than exogenous stem cells [10,11]. Among the endogenous stem cells, a critical role is played by the mesenchymal stem cells (MSCs) that can self-renew and multi-differentiate into other cell types [6,12,29]. Under physiological conditions, MSCs are maintained in their stem cell niches, which are microenvironments located around blood vessels [8,28]. MSC niches particularly regulate proliferation, differentiation and self-renewal of MSCs [7,19,24,27,29]. During DO, MSCs detach from their niches, migrate to the osteogenic front to differentiate into osteoblasts and form new bones [11]. However, the exact mechanism that initiates MSC mobilization from their niches is not clearly understood.

Stromal cell-derived factor-1 (SDF-1 or CXCL12), which belongs to a CXC subfamily of chemokines, is known to activate MSC mobilization by binding to a G protein coupled receptor, CXCR4, on the surface of MSCs [38]. Furthermore, it has been demonstrated that SDF-1 is up-regulated to a greater extent during DO, when compared to normal

Abbreviations: MSC, mesenchymal stem cell; VEC, vascular endothelial cell; DO, distraction osteogenesis; NE, norepinephrine; TCST, transection of cervical sympathetic trunk; SDF-1, stromal cell-derived factor-1; JNK, c-Jun N-terminal kinase; BMD, bone mineral density; FACS, flow cytometry analysis

* Corresponding authors at: Department of Oral & Maxillofacial-Head & Neck Oncology, Ninth People's Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai Key Laboratory of Stomatology, No.639 Zhizaoju Road, Shanghai 200011, China.
E-mail addresses: wangleizyh@aliyun.com (L. Wang), zhang.chenping@hotmail.com (C. Zhang).
[1] These authors contributed equally to this work.

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conditions without DO and bone fracture in the internal callus [3,4]. Recent studies have shown that MSCs can migrate from a region with low SDF-1 levels to a region with high SDF-1 levels [18,24]. It has been proved that c-Jun N-terminal kinase (JNK) pathway involves SDF-1 release from osteoblasts and vascular endothelial cells (VECs) [9]. However, it is not known whether VECs, which are key components of the stem cell niche, are involved in the regulation of SDF-1 expression during DO.

Bone regeneration and MSCs regulation are complex processes and are controlled by a number of systems including the immune system and the nervous system [23,33,35,36,41]. In previous studies, we found that loss of norepinephrine (NE), a major sympathetic neurotransmitter, could facilitate the mobilization of MSCs during mandibular DO both *in vivo* and *in vitro* [4,11,37]. However, the mechanism by which NE inhibits MSCs' escape from their niches and migration to bone-forming sites is unknown. In this study, we tested the hypothesis that NE affects the secretion of SDF-1 by VECs and thereby regulates the MSC migration in the distraction gap under a tension stress. We demonstrate that NE increases the SDF-1 secretion by VECs *via* NE/abrd3/JNK pathway, thereby inhibiting MSC chemotaxis migration from perivascular regions toward bone trabecular frontlines along the SDF-1 concentration gradient in bone regeneration.

2. Material and methods

2.1. Mandibular distraction osteogenesis in rats

Twenty male Sprague-Dawley (SD) rats (10–20 weeks, 280–320 g) were used in this study. All animal protocols were approved by the Animal Care and Use Committee at the Fourth Military Medical University, China. All rats were maintained in the Animal Care Center of School of Stomatology, Fourth Military Medical University, China. Animals were randomly divided into two groups (Fig. 1A–C): the Experimental group (n=10) received the transaction of cervical sympathetic trunk (TCST) and right mandibular DO; the Control group (n=10) received only right mandibular DO. Animal anesthesia was performed by administering 1% sodium pentobarbital solution (30 mg/kg) through the abdomen. In the control group, submandibular incision was used to expose the right mandibular body and ramus. Then, a titanium distractor (Zhongbang Titanium Biomaterials Corporation, Xi'an, China) was fixed following the buccal surface of the right mandible. A vertical corticotomy was made at the mid-mandible level to avoid injuries to the teeth. The distractor was operated several times to confirm that corticotomy was complete. The incision was carefully sutured with the force section out of the skin (Fig. 1D). In the Experimental group, TCST was performed as described in our previous study [37]. After a latency period of 5 days, the distraction was performed at a rate of 0.2 mm twice a day for 10 days. The regenerated bone was allowed to consolidate for 15 days. Animals were sacrificed with an overdose of sodium pentobarbital solution and the callus at the distraction site was harvested..

2.2. NE and SDF-1 expression in callus in vivo

On the harvest day, the mandibles were obtained from the rats with the surrounding soft tissue removed, and immersed in 4% paraformaldehyde for 36 h to immobilize the proteins and subsequently used for bone mineral density (BMD) analysis with micro-computed tomography (micro-CT) as previously described [37]. Then, half of the callus was harvested and homogenized in 0.01 N HCl in the presence of 0.15 mM EDTA and 4 mM sodium metabisulfite. NE ELISA E-5200 kit (Rocky Mountain Diagnostics, Colorado Springs, CO, USA) was used for detection of NE in the callus. The remaining callus and residual mandibles were decalcified in 10% ethylene diamine tetraacetic acid (EDTA) for 4 weeks. Then, the distraction section was paraffin-embedded and serially sectioned to produce 5 μm thick paraffin

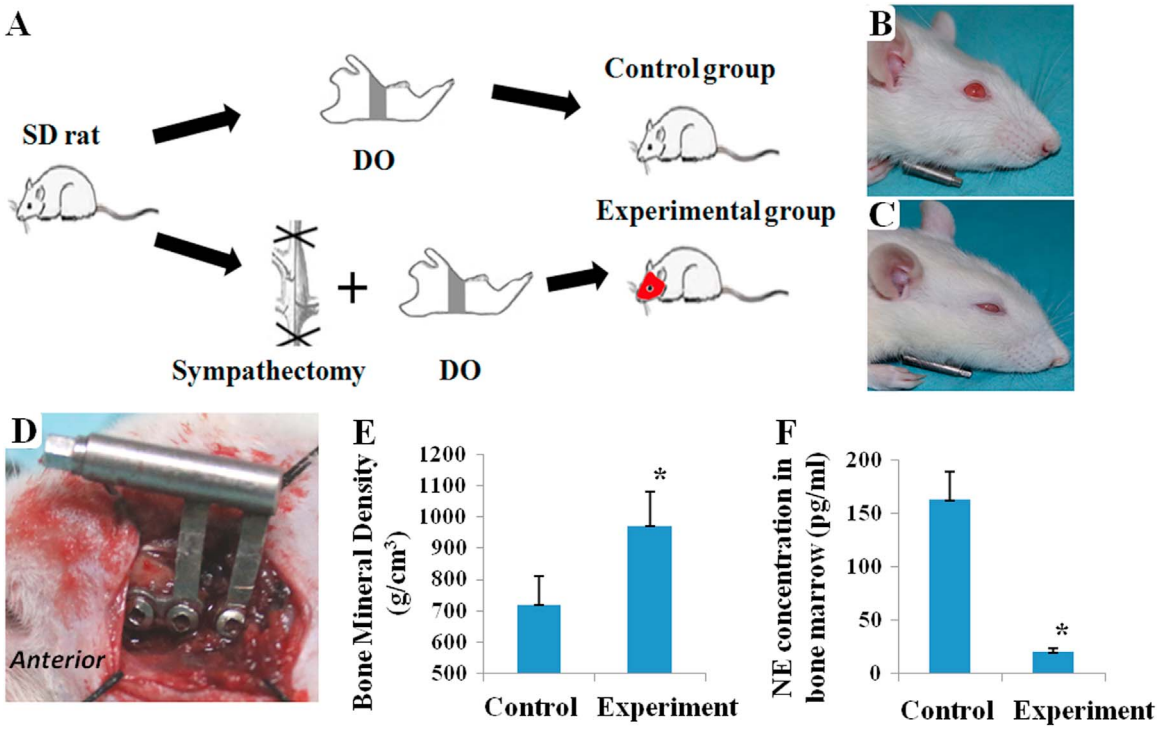

Fig. 1. Sympathetic denervation induced deprivation of norepinephrine (NE) and improved bone mineral density *in vivo*. **(A)** Scheme for groupings for Sprague-Dawley rat mandibular distraction osteogenesis (DO), in which Control group received DO treatment alone whereas Experiment group received both transection of cervical sympathetic trunk (TCST) and DO (n=10 each). **(B-C)** Ptosis symptom was shown in all the rats of Experiment group but not Control group postoperatively. **(D)** Insertion of DO devices during surgeries in the rats of both groups. **(E)** Sympathetic denervation significantly improved the bone mineral density of mandibles in Experimental group. **(F)** NE was decreased in the bone callus of Experimental group when compared with Control group as analyzed using a NE ELISA kit. Scale bar =25 μm. Data represent the mean ± SEM of 3 independent experiments. $^{*}P < 0.05$ by Student's *t* test.