THE INVOLVEMENT OF SDF-1/CXCR4 AXIS IN ENDOCHONDRAL BONE REPAIR

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INTRODUCTION
A chemokine, SDF-1, and its receptor, CXCR4, have been well known to play a crucial role in homing of hematopoietic stem cells (HSCs) to bone marrow niche. Additionally accumulating data have shown that SDF-1 is highly expressed in some damaged organs, such as myocardial infarction (1) and brain injury (2), and recruits circulating mesenchymal stem cells (MSCs) to the damaged lesion, allowing them to start normal organ repair. But it is still unknown whether SDF-1 is involved in bone repair. Using murine autograft and allograft models, we tested the hypothesis that SDF-1 is essential in endochondral bone repair.

METHODS
Murine autograft and allograft models. All animal studies were conducted in accordance with principles and procedures approved by Kyoto University Committee of Animal Resources. We used a segmental femoral autograft and allograft models as previously described (3). Briefly, a 4-mm diaphyseal segment was osteotomized and removed from the femoral shaft of C57Bl/6 mice. For autograft model, the freshly removed bone graft was transplanted back to the same mouse, and secured by a metal pin. For allograft model, bone grafts, which had been harvested from ICR mice and kept frozen at –70°C, were used. For primary MSCs harvested from bone marrow of C57Bl/6 mice were applied. The chemotactic effect of rmSDF-1 on these cells was analyzed as previously described (4).

RNA extraction and RT-PCR. Harvested tissue was snap frozen, homogenized using POLYTRON, and extracted total RNA using TRIsol. For four grafts were used for each RNA sample. To detect SDF-1 mRNA the primer sequences were: forward, 5'-CGCCAGAGCCAACGTCAAGC-3'; reverse, 5'-TTCGGGTCAATGCACACTTG-3'. The primer sequences for actin were: forward, 5'-AGATGTGGATCAGCAAGCAG-3'; reverse, 5'-GCGGCAAGTTAGGTITTTCTA-3'.

In vitro and chemotaxis assay. In vitro cell migration was assayed using inserts with an 8-µm pore membrane. Primary MSCs harvested from bone marrow of C57Bl/6 mice were applied. The chemotactic effect of rmSDF-1 on these cells was analyzed as previously described (4).

RESULTS
Radiographical analysis showed sufficient callus formation around the autograft, but few callus formed around allograft (Fig. 1A). The mRNA expression of SDF-1 was increased in autograft at days 2 after transplantation, while no increase was detected in allograft (n=4 each), indicating the involvement of SDF-1 for the acute phase of endochondral bone repair (Fig. 1B). The new bone formation around the autograft was significantly inhibited by the peritoneal injection of anti-SDF-1 antibody and CXCR4 antagonist, suggesting that SDF-1/CXCR4 axis has an essential and functional effect on normal bone repair (Fig. 2).

DISCUSSION
The present results provided an evidence that SDF-1/CXCR4 axis plays an essential role in endochondral bone repair. The increased mRNA of SDF-1 was detected in autograft at days 2 by RT-PCR, while no promoted expression was observed in allograft. Moreover, the new bone formation around autograft was significantly reduced by the treatment with anti-SDF-1 antibody and CXCR4 antagonist. In vitro chemotaxis assay showed the migratory effect of SDF-1 on MSCs in a dose-dependent manner, which was inhibited by TF14016 (data not shown).

REFERENCES
(2) Ji JF et al. Stem cells. 2004;22(3):415-27

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