SDF-1/CXCR4 is Essential for In Vivo 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 mesenchymal stem cells (MSCs) to the damaged lesion, allowing them to start normal organ repair. But it is not fully understood whether and how SDF-1 functions in bone repair. At this annual meeting last year we reported the involvement of SDF-1/CXCR4 axis in in vivo endochondral bone repair. In mouse autograft and allograft models, the expression of SDF-1 mRNA was increased in autograft at days 2 after transplantation, while no increase was detected in allograft (Fig. 1). Moreover the administration of anti-SDF-1 antibody inhibited the new bone formation around the autograft. In the present study we further investigated the localization and functional role of SDF-1 using mouse autograft model.

Materials and Methods: Mouse autograft model. All animal studies were conducted in accordance with principles and procedures approved by Kyoto University Committee of Animal Resources. Segmental femoral autograft model was created as previously described (3). Briefly, a 4-mm diaphyseal segment was osteotomized and removed from the femoral shaft of C57BL/6 mice. Then the freshly removed bone graft was transplanted immediately back to the same mouse and secured by a metal pin.

Immunohistochemistry. Bone grafts and surrounding tissue from autograft model were harvested at days 2 and processed to paraffin sections. The expression of SDF-1 protein was detected by immunostaining using anti-SDF-1 antibody.

Primary cell culture and cell labeling. Rat/mouse bone marrow stromal cells (rBMSCs/mBMSCs) were harvested by flushing the bone marrow from the femoral shafts of Wister rats/C57BL6 mice, respectively. The plastic adhesive cells were cultured as BMSCs (p.p. 0). To label mBMSCs, cells were incubated in the culture media supplemented with bromodeoxyuridine (BrdU) for 24 h before cell harvest.

In-vitro migration assay. Migration was assayed using inserts with an 8-μm pore membrane. 1 x 105 rBMSCs were applied to each upper well. For chemotaxis assay, the lower wells were filled with medium supplemented with various doses of rSDF-1 (0, 10, 100 ng/mL), and cultured for 24 h. For chemokinesis assay, the medium in the lower well was supplemented with 100ng/mL of rSDF-1 and 50 or 100 μM of TN14003, an antagonist for CXCR4. The number of the cells that had migrated to the undersurface of the membrane was counted.

In-vivo migration assay. We investigated whether SDF-1 can recruit MSCs towards the fractured ends of the autograft in vivo. BrdU-labeled mBMSCs (1.5 x 105 cells) were injected through the tail vein immediately after the surgery. To assess the inhibitory effect of the antagonist on in vivo migration, these mice received continuous administration of TN14003 dissolved in PBS (10 mM) or vehicle using Alzet micro-osmotic pumps. The mice were sacrificed at days 7 and applied for anti-BrdU immunostaining assay.

Results: The mRNA expression of SDF-1 was increased in autograft at days 2 after transplantation, while no increase was detected in allograft (Fig. 1). SDF-1 protein expression was observed at the periosteum of the autograft bone at day 2 (Fig. 2a,b). In contrast, the periosteum of the host bone didn’t appear to express SDF-1 protein (Fig. 2c).

In-vitro migration assay showed that rSDF-1 induced the migration of rBMSCs in a dose-dependent manner. Moreover, the treatment with TN14003 reduced this effect, suggesting the functional effect of SDF-1/CXCR4 axis on the in-vitro migration of MSCs. We also investigated whether SDF-1 can recruit MSCs towards the fractured ends of the autograft in vivo. In the specimen treated with the vehicle, positive BrdU staining was detected in the developing callus around the host-graft junction, indicating that the i.v. injected mBMSCs migrated towards the autograft lesion. On the contrary, the treatment with TN14003 significantly reduced the number of migrated cells. Adjacent sections immunostained with PBS resulted in no positive staining. These results strongly indicated that SDF-1 has a crucial role on the in-vivo recruitment of MSCs towards the graft lesion at the early phase of the structural autograft repair.

Discussion: We previously reported that SDF-1 mRNA was increased in autograft at days 2 by RT-PCR and also that anti-SDF-1 antibody significantly inhibited the bone formation. In the present study we showed that SDF-1 protein was expressed at the periosteum of autograft bone at days 2, indicating that the periosteum is a main source of SDF-1 protein in endochondral bone repair.

In this study we also demonstrated in-vitro and in-vivo migratory effect of SDF-1 on MSCs. In in-vivo migration assay, the positive BrdU staining was detected in the developing callus around the host-graft junction, indicating that pre-labeled mBMSCs not only migrated towards the autograft lesion but also actually participated in the endochondral bone repair. These results again corroborated that SDF-1 has a crucial role on the recruitment of MSCs towards the graft lesion at the early phase of the autograft repair.

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