SDF-1α/CXCR4 Axis Regulate Both Osteogenesis and Vasculogenesis for Bone Fracture Healing

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Introduction: CXC chemokine receptor 4 (CXCR4) is an alphachemokine receptor specific for stromal -derived-factor 1 (SDF-1) endowed with potent chemotactic activity for various lymphocytes. SDF-1/CXCR4 interaction contributes to the regulation of endothelial progenitor cell (EPC) recruitment in ischemic tissues. Lately, we reported that EPCs may play an essential role in fracture healing by promoting a favorable environment through neovascularization and osteogenesis in damaged skeletal tissue. On the other hand, the other group reported that circulating bone marrow-derived osteoblast progenitor cells are recruited in the bone formation through the SDF-1/CXCR4 pathway.

According to these backgrounds, there may be several ways of the SDF-1/CXCR4 pathway for the fracture healing. Thus, in this study, we used a bone fracture model made a Tie2-Cre CXCR4 knockout mouse by the dosage of the tamoxifen on five days, and to confirm the knockout of CXCR4 in Tie2 expression which is known as a marker of EPCs. Using this knockout mouse, we could perform the examination in a near state to a physiologic condition. The purpose of this study is to investigate the influence of the SDF-1/CXCR4 pathway on EPCs in the bone formation and the contribution to the development of future bone therapy.

Materials and Methods: The institutional animal care and use committees of RIKEN Center for Developmental Biology approved all animal procedures. We made a Tie2-Cre CXCR4 knockout mouse (C57BL6) by the dosage of the tamoxifen on five days, and to confirm the knockout of CXCR4, we performed flow cytometry of bone marrow and peripheral blood. To simulate clinical situation of fracture, we applied a reproducible model of closed femoral fracture (10-week-old). We set two groups of CXCR4 knockout (CXCR4 KO) and the wild type mice (control group). And we examined the radiographical and histological assessment after fracture to confirm a morphological bone fracture healing process. To examine angiogenesis and functional blood flow recovery during fracture healing process, quantitative capillary density by isoelectin B4 staining and Laser Doppler Perfusion Imaging (LDPI) were performed. In addition, we performed real time RT-PCR of angiogenic the markers (CD31, VE cadherin, vascular endothelial growth factor) at one week after fracture and osteogenenic markers (osteocalcin, collagen1A1, bone morphogenetic protein 2) at two weeks after fracture. To evaluate gain function of the SDF-1/CXCR4 pathway, we also set two groups of the SDF-1 intraperitoneally injected group and the wild type group, and studied the radiographical and histological assessment after fracture.

Results: Morphological fracture healing in each group was evaluated by radiological and histological examinations. Relative callus area at week 2 was significantly greater in control group than in CXCR4 KO group (p<0.05)(IMAGE 1). These results indicated that the fracture healing was delayed in CXCR4 KO group. Quantitative analysis of capillary density at week 1 showed significant decrease in the CXCR4 KO group compared to the control group (p<0.05). LDPI analysis demonstrated a severe reduction of blood flow at the fracture sites compared to contralateral sites immediately after fracture creation, and at week 1 the control groups had a significantly higher perfusion value at fracture site than the CXCR4 KO group (p<0.05)(IMAGE 2). Real time RT-PCR analysis showed that the gene expressions of angiogenic and osteogenic markers were higher in wild type group than the CXCR4 KO group (p<0.05). In the SDF-1 injected study, we found that the fracture in the SDF-1 injected group is significantly faster healed with bridging callus formation than the wild type group (p<0.05).

Discussion: Our present study showed that the bone fracture healings in CXCR4 knockout mic were delayed compared to the wild type mice via down-regulation of angiogenesis and osteogenesis. These results may indicate that the SDF-1/CXCR4 pathway including mobilization and incorporation of EPCs is an important pathway in the fracture healing. It is thought that the promotion of this pathway lead to the acceleration of the fracture healing. Our study may have essential implications for the new therapeutic strategies to enhance bone repair.