SDF-1/CXCR4 Axis on Endothelial Progenitor Cells Regulate Both Osteogenesis and Vasculogenesis for Bone Fracture Healing

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Introduction
CXCR4 is an alpha-chemokine receptor specific for stromal derived-factor 1 (SDF-1) endowed with potent chemotactic activity for various lymphocytes and endothelial progenitor cells (EPCs). 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 enhanced vasculogenesis/angiogenesis and osteogenesis in damaged skeletal tissue in mice. 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. Based on these scientific backgrounds, we speculated a pivotal mechanism of SDF-1/CXCR4 signaling on EPCs in bone fracture healing. To study the strengthening function of CXCR4 on EPCs for bone fracture healing, we used a bone fracture model of the Tie2-Cre CXCR4 knockout mouse, in which bone fracture healing was delayed in the CXCR4 KO group. We believed that our findings contribute to future therapeutic strategy of bone healing and repair.

Materials and Methods

Animal model: The institutional animal care and committees of RIKEN Center for Developmental Biology approved all animal procedures. Tie2-CreER transgenic mice were crossed with CXCR4floxed mice. To disrupt CXCR4 in endothelial lineage cells postnatally, tamoxifen was injected 4-weeks-old mice intraperitoneally. To confirm the tamoxifen-induced disruption of CXCR4, mononuclear cells were collected from bone marrow and peripheral blood and flow cytometry were performed using antibodies for Tie2 and CXCR4. To simulate clinical situation of fracture, we applied a reproducible model of closed femoral fracture in 10-week-old mice. We set two groups of CXCR4 knockout (CXCR4 KO) and wild type mice (control group).

Morphological fracture healing: We examined the radiographical and histological assessment after fracture to confirm morphological healing process of bone fracture.

Histological vascular staining and blood flow: To examine angiogenesis and functional blood flow recovery during fracture healing process, quantitative capillary density by isolectin B4 staining and Laser Doppler Perfusion Imaging (LDPI) were performed.

Molecular analysis of the fractured tissue: We performed real time RT-PCR of angiogenic markers (CD31, VE cadherin, vascular endothelial growth factor) at one week after fracture and osteogenic markers (osteocalcin, collagen1A1, bone morphogenetic protein 2) two weeks after fracture.

Incorporation of EPCs in vivo: To evaluate the incorporation of EPC, Dil labeled EPCs from wild type mice and CXCR4 knockout mice in each were intravenously injected to fracture model. One week after fracture creation, we performed double immunostaining for Dil and isletin B4 as an endothelial cell marker.

Stimulation of SDF-1: To evaluate gain function of the SDF-1/CXCR4 pathway, three groups of the SDF-1 intraperitoneally injected group, wild type group, and SDF-1 injected CXCR4 KO group were evaluated by radiography and histology after fracture.

Results

Confirmation of conditional knockout of CXCR4 on Tie2 expressing cells: Analysis of flow cytometry and immunostaining demonstrated that there exists rare Tie2+/CXCR4+ cells in peripheral blood and bone marrow cells, indicating successful generation of the conditional CXCR4 knockout.

Morphological fracture healing: Radiological and histological examinations demonstrated that relative callus area at week 2 was significantly greater in the control group than in the CXCR4 KO group (p<0.05). These results indicated that the fracture healing was delayed in the CXCR4 KO group.

Immunohistochemical vascular staining and blood flow: Quantitative analysis of capillary density at week 1 showed significant decrease in the CXCR4 KO group, which was higher in the wild type group than the CXCR4 KO group (p<0.05). LDPI analysis demonstrated that severe reduction of blood flow at fracture sites were found compared to contralateral sites immediately after fracture creation, and that the control group represented a significantly higher perfusion value at fracture sites than the CXCR4 KO group (1.28±0.04 mm2) at week 1 (p<0.05).

Molecular analysis of fractured tissue: Real time RT-PCR analysis showed that the gene expressions of angiogenic and osteogenic markers were higher in the wild type group than the CXCR4 KO group (p<0.05).

Incorporation of EPCs: In the EPC incorporation assay, double immunofluorescent staining for isletin B4 and Dil demonstrated that double stained cells were found in the mice injected EPCs from wild type mice, but not in the mice injected EPCs from CXCR4 KO mice (p<0.05).

Stimulation of SDF-1: In the SDF-1 injected study, radiographical and histological assessment demonstrated that the fracture in the SDF-1 injected group was significantly faster healed with bridging callus formation than the wild type group (p<0.05).

Discussion

The present findings showed that bone fracture healings in Tie2-Cre CXCR4 knockout mice were delayed compared to the wild type mice via down-regulation of angiogenesis and osteogenesis. Our results also indicated that mobilization and incorporation of EPCs in bone fracture healing process was through SDF-1/CXCR4 pathway. The current series of study suggested that the promotion of CXCR4/SDF-1 signal on EPCs lead to the acceleration of bone fracture healing for new therapeutic strategies to enhance bone repair.

Conclusion

In the present study, using Tie2-Cre CXCR4 knockout mouse, we showed the important role of SDF-1/CXCR4 signal on EPCs for bone fracture healing. We believe that our findings contribute to future therapeutic strategy of bone healing and repair.

References

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