INTRODUCTION
The therapeutic potential of hematopoietic stem/endothelial progenitor cells (HSCs/EPCs) for fracture healing has been demonstrated with mechanistic insight of vasculogenesis/angiogenesis and osteogenesis enhancement in sites of fracture. (1) Lnk is expressed in hematopoietic cell lineages, and bone marrow (BM) cells of Lnk-deficient mice are superior in hematopoietic population of those of wild-type mice. Lnk has recently been proved an essential inhibitory signaling molecule in stem cell factor (SCE)-c-Kit signaling pathway for stem cell self-renewal demonstrating enhanced hematopoietic and osteogenic reconstitution in Lnk-deficient mice. (2) In terms of expanding clinical application, we therefore investigated the hypothesis that down regulation of Lnk signaling enhances regenerative response via vasculogenesis and osteogenesis in fracture healing.

MATERIALS AND METHODS
siRNA preparation: Synthetic 21-nt RNAs (Lnk siRNA and negative control siRNA) were purchased from Dharmaco in deprotected, desalted and annealed form.

Cell Preparation for In-vitro Study: To investigate the effect of Lnk siRNA for mice ostoblasts (OBs) and Endothelial cells (ECs) in vitro, we isolated mouse calvarial OBs from 3-5-day old mice, and mouse endothelial commitment of c-kit+8c-12-Lineage (KSL) subpopulations of bone marrow cells from 10-12wk old mice BM. Lnk and control siRNAs were delivered into these cells by lipofection.

Animal Model: A reproducible model of femoral fracture was created in mice (C57BL/6, CLEA Japan, Inc. male 10-12wk old). All animal procedures were performed in accordance with the Japanese Physiological Society Guidelines for the care and Use of Laboratory Animals.

Experimental Groups: Immediately after fracture creation, mice received local administration of the following materials with in each group (n=20 in each group): (1)10μM Lnk siRNA (LnkiRNA group), (2)10μM control scramble siRNA (control siRNA group) .

RESULTS AND DISCUSSION
Lnk siRNA transfected cells show high capacity of colony formation and osteogenic differentiation in vitro. After 15 days in culture with methylcellulose-based medium, number of small and large colonies was significantly higher in the Lnk siRNA transfected cell group compared with control siRNA transfected cell group. When Lnk siRNA transfected OBs were cultured in osteogenic differentiation medium, calcium deposit assessed by Alizarin red staining was striking and mRNA expressions of osteocalcin and collagen I were significantly high. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and Western blotting: The results of Western blotting using tissue samples collected from perfurcture site demonstrated that Lnk expression was decreased significantly in animals of Lnk siRNA transfected group until 2week post-fracture.

Radiographic and histological evidence of fracture healing: In 60% (6 out of 10) of Lnk siRNA group mice at week 2 and 100% (10 out of 10) at week 3, the fracture healed with bridging callus formation radiographically, while WT mice showed only 30% (3 out of 10) fracture healing at week 2 and 80% (8 out of 10) at week 3 (p<0.05 in week 2). Micro CT analysis presented such results more clearly. (Fig.1)

FUNCTIONAL BONE HEALING BY THREE POINT BENDING TEST: Biomechanical evaluation by 3-point bending test was performed at week4 in both groups. The percentage ratios of ultimate stress and extrinsic stiffness in the fractured femur versus contralateral intact femur in Lnk siRNA group were superior to control group.

DISCUSSION AND CONCLUSION
Down regulation of Lnk system may have a clinical potential for faster fracture healing, which contributes to reduce delayed unions or nonunions.

SIGNIFICANCE
In our series of experiment, we clarified that negatively controlled Lnk system contributed to a favorable environment for fracture healing by enhancing vasculogenesis/angiogenesis and osteogenesis, which leads to prompt recovery from fracture.

REFERENCES