INTRODUCTION:
Ligament healing is composed of several phases, including acute inflammation and reaction phase, repair and regeneration phase, and remodeling phase. At the molecular level, these phases are regulated by a complex interplay among inflammatory cells, fibroblasts, and the molecules, such as inflammatory cytokines, proteinases, extracellular matrices, and etc, which are produced by these cells. Macrophage migration inhibitory factor (MIF), which was discovered in 1966 as the first lymphokine, has been recognized as a pivotal mediator of inflammation in various pathological conditions including sepsis, arthritis, ARDS, and etc. Recent studies have shown that MIF plays an important role in proliferation and differentiation of cells, generation of organs, and wound healing. However, a role of MIF on the ligament healing process has not been elucidated as of yet. Based on the above-described knowledge, we have hypothesized that MIF may enhance healing of the injured medial collateral ligament (MCL). Recently, a murine MCL injury model has attracted much notice because it is useful to clarify the effect of specific factor ablation on MCL healing by using genetically altered mice. A specific hypothesis to be tested in this study using MIF gene-deficient (MIF KO) mice is that the genetic ablation of MIF may inhibit natural improvement of the structural and mechanical properties of the injured MCL.

MATERIALS AND METHODS:
Study Design: Animal experiments were carried out in Hokkaido University School of Medicine under the Rules and Regulations of the Animal Care and Use Committee. Six Balb/C wild-type (WT) mice and 6 Balb/C background-MIF KO mice (10 weeks, male) were used. In each animal, a 1-cm skin incision was made in the mid-medial aspect of the knee. Surgery, and the femur-MCL-tibia complex was harvested from both sides. All mice were allowed unrestricted cage activity after surgery. Each animal was sacrificed on 28 days after surgery, and the femur-MCL-tibia complex was harvested from both knees.

Biomechanical evaluation: The specimens frozen at -80°C were thawed at 5°C. Tensile tests were performed using a previously reported Micro-tensile tester. The femur and the tibia were potted in 6.0-mm diameter aluminum tubes with polymethyl methacrylate, and mounted on the tensile tester so that the knee was flexed at 45 degrees of flexion. Then, all soft tissues except for the MCL were removed from the mounted knee specimen with a dissecting microscope. The cross-sectional area was measured using video dimension analyzer. After preconditioning (elongation rate of 1.7% for 2 minutes) in saline solution, each specimen was stretched to failure at a rate of 10 mm/min. The strain was measured using a video dimension analyzer. Statistical analyses were performed between the MIF KO and WT mice using the Student’s t-test. The significance level was set at p<0.05.

RESULTS:
Failure modes showed midsubstance tear in all tested specimens. Concerning the uninjured MCL, there were no significant differences in the structural or mechanical properties between the WT and MIF KO mice. The maximum load, the tensile strength, and the tangent modulus were significantly lower (p=0.006, p=0.0013, and p=0.0167, respectively) in MIF KO mice (2.8N, 11.1MPa, and 117.0MPa) than in the WT mice (4.7N, 33.2MPa, and 235.0MPa). In addition, the injured/uninjured ratio, which was defined as a ratio (%) of a value in the injured knee to a value in the uninjured knee in each animal, was significantly lower in the maximum load, the tensile strength, and the tangent modulus (p=0.0079, p=0.0027, p=0.0385, respectively) in MIF KO mice (46.8%, 22.8%, 44.5%) than in the WT mice (76.1%, 62.5%, 76.9%).

DISCUSSION:
This study clearly demonstrated that the genetic ablation of MIF inhibited natural improvement of the structural and mechanical properties of the injured MCL. This result suggested a possibility that MIF may enhance healing of the injured MCL. We speculated the possible mechanisms of this phenomenon. First, MIF prevents migration of macrophages from an inflammation site. Wright et al. reported that local administration of blood into an injured site in the MCL significantly induces the number of macrophages and gene expression of type I collagen. Therefore, in the present study, the MIF gene deficiency might allow migration of macrophages from the injured site at the acute inflammation phase, resulting in the inhibition of MCL healing on the 28 days after injury. Secondly, MIF is a potent inducer of MMP expression. It is reported that MMP-12 deficiency resulted in impaired healing of the injured MCL. Therefore, in the present study, the MIF gene deficiency might reduce MMP expression, resulting in the inhibition of MCL healing on the 28 days after injury. As to clinical relevance from the present study, further molecular biological studies are needed to verify a possible effect of localized enhancement of MIF expression on ligament healing, because MIF has a potential to enhance ligament healing.

REFERENCES: