主　論　文

The distribution of vascular endothelial growth factor in human meniscus and meniscal injury model
（半月板における VEGF の局在と役割）

[Introduction]

In adult human meniscus, only the outer 10-25% is vascularized, whereas the inner 70-80% is composed of avascular tissue. Cells in the vascularized area (red-red region) are more fibroblast-like in appearance, while cells in the middle (white-red region) and inner (white-white region) zones show more chondrocyte-like shapes. Based on these findings, healing potential of the outer meniscus was higher than that of the inner meniscus.

Vascular endothelial growth factor (VEGF) is a major angiogenic factor. VEGF promotes both physiological and pathological vascularization. VEGF can aid the healing of tissues by promoting vascularization. In rabbits, VEGF expression in the avascular meniscus zone shows a significant increase 2 weeks after injury. This increased expression of VEGF may lead to healing of the meniscal tear and may be associated with an increase in the expression of anti-angiogenic factors, notably endostatin. However, the role and distribution of VEGF in human meniscus are unclear. In the current study, we investigated the localization of VEGF in human native meniscus and in an ex vivo model of meniscal injury.

[Materials and Methods]

Tissue, cells, and cell culture

Macroscopically intact lateral menisci were obtained at total knee arthroplasty in 6 patients (4 females and 2 males, average age 73-year-old) suffering from osteoarthritis of the knee. Meniscal tissues were divided into two groups: (1) native and (2) injured. In the native group, inner and outer meniscus cells were prepared from the meniscal samples. Inner and outer meniscal tissues were prepared by careful cutting. Inner and outer meniscus cells were prepared by organ culture.

In the injured meniscus group, full-thickness, radial, 5-mm-long lesions in both the inner and outer meniscal area were created with a microsurgical blade. Subsequently, the injured tissue was divided into two groups: one was used for immunohistochemistry (IHC) and the other was separated into inner 2/3 and outer 1/3 menisci for the polymerase chain reaction (PCR) and cell culture.

RT-PCR and quantitative RT-PCR

RNA samples were obtained from superficial zone-excluded meniscal tissues and cultured meniscus cells. The following specific primer sets were used: $VEGF_{exon3\text{-}4}$, hypoxia-inducible factor-1α (HIF-1α) and glyceraldehyde-3-phosphate dehydrogenase (G3PDH).

Immunohistochemical assay

Rabbit anti-VEGF (sc-152) antibody was used. Staining density of VEGF was quantified by Image J. Relative staining densities of VEGF, and VEGF-positive cell counts per 2500 μm^2.

[Results]

Expression of VEGF and HIF-1α mRNA

PCR was unable to detect VEGF or HIF-1α mRNA in freshly isolated superficial zone-excluded inner or outer meniscal tissues. However, in cultured meniscus cells, VEGF and HIF-1α mRNA could be detected in both the inner and outer meniscal regions. In outer meniscal cells, more VEGF mRNA expression was observed than in inner cells. Real time PCR results for VEGF and HIF-1α gene expression in meniscal tissue and cells paralleled these data.

VEGF and HIF-1α mRNAs were detected in injured, but not native or cultured, inner and outer meniscal tissue. VEGF mRNA levels were greater in the outer injured tissue while HIF-1α mRNA levels were similar between inner and outer injured meniscal tissues. Quantitative real time PCR results were similar to the PCR data. Specifically, native and cultured meniscal tissues showed similar and low levels of VEGF and HIF-1α mRNAs in both the inner and outer regions. HIF-1α mRNA levels were increased by injury, with the greatest increase evident in the outer region.

VEGF deposition in the meniscus

In native meniscal tissue, VEGF protein was detected primarily in the outer and superficial zones by immunohistochemical analyses. The deposition of VEGF was observed mainly in intracellular, pericellular, and extracellular regions of outer meniscus cells. VEGF was weakly detected in inner meniscus cells. VEGF staining density and the VEGF-positive cell counts were higher in the outer than inner meniscus region.

In cultured injured meniscal tissue, VEGF was detected mainly around the tear. In particular, the deposition of VEGF was higher in the outer tear region than in cultured outer meniscal tissue. Moreover, around the inner tear area, VEGF deposition increased. However, in cultured inner meniscus, VEGF deposition remained at a low level. VEGF staining density and VEGF-positive cell counts were higher in the injured region than in the cultured meniscus. These results were consistent with the PCR data.

[Discussion]

Hypoxia is an established feature in injured tissues and a key stimulus that induces transcription of VEGF. And HIF-1α is a key mediator of hypoxic responses. In the absence of HIF-1α, VEGF mRNA levels are not induced by hypoxia. In our study, we demonstrated that changes in the expression of VEGF and HIF-1a paralleled each other. This suggests that VEGF is stimulated by HIF-1α.

In the current study, we detected the expression of VEGF and HIF-1α in both the native and cultured tissues. However, mRNA expressions of these genes were different between organ and cell cultures. We consider that expressions of VEGF and HIF-1α may be maintained at a low level in the normal meniscal tissue because the vascularization status may be low at the inner region and several anti-angiogenic factors may prevent these gene expressions. On the other hand, cultured cells were free from cell-surrounding extracellular matrices. In injured meniscus tissue, the expression of VEGF and HIF-1α mRNAs increased compared to native meniscus tissue. We consider that this phenomenon is related to the vascularization of injured meniscus that then promotes the healing of meniscal injury.

VEGF exists in both the inner and outer meniscus regions. However, only outer meniscus is vascular area. Anti-angiogenic factors which mainly exist in the human inner meniscus and may

contribute to avascularization of the inner meniscus.

In our posttraumatic model, the number of cells immunostained for VEGF increased approximately 4-fold around the injured area compared to the uninjured area. Meniscal injury caused by the cut and the cannula punctures necessary for meniscal suturing during surgery may have upregulated VEGF mRNA expression

There are some limitations in this study. Firstly, we did not directly prove the VEGF-mediated vascularization during meniscal tissue healing. In addition, the dose-dependent effects of VFGF on the neovascularization and endothelial cell behaviors in meniscal tissues were not investigated. Secondly, our samples were obtained from OA patients. However, we solved these limitations through some paper or technology.

[Conclusion]

Our study demonstrates that the meniscus contains VEGF in both the inner and outer regions. HIF-1α expression was elevated and VEGF deposition increased in injured meniscus. These results suggest that VEGF may be a key angiogenic factor during the posttraumatic situation after meniscal injury, especially in the outer meniscus.