Discussion: Inhibitory mechanism of PGE2 on MMPs and NGF involves the up-regulation of DUSP-1 resulting in the dephosphorylation of MAP kinases. Selective COX-2 inhibitors are useful for inflammatory pain, but they may have a limited efficacy for joint destruction and NGF-related pain because these drugs would attenuate the inhibitory action of PGE2 on MMPs and NGF expressions. DUSP-1 would be a novel target molecule for OA by regulating MAP kinases and following MMPs and NGF expressions.

PI-11.
Two cases in which nerve preservation was carried out by in situ preparation method for femoral liposarcoma and wrist squamous cell carcinoma

P1-12.
Role of DUSP1 on the regulation of NGF and MMPs in human intervertebral disc cells

[Introduction] For tumors existing close to important tissues such as neurovascular structures, by using in situ preparation method (ISP method), safe margins can be secured without unnecessary sacrifice of neurovascular structures, if there is no risk of seeding of tumor cells during surgery. Furthermore, it is possible to prevent residual tumor cells by subjecting the remaining neurovascular structures to alcohol treatment.

[Methods] ISP method is performed according to 5 steps. Step 1 is that the tumor is excised en bloc, including the tumor and nerve/vessels, maintaining an adequate wide margin. However, the continuity of nerve/vessels is preserved. Step 2 is that the tissue mass is then isolated from the surgical bed using a sheet. Step 3 is that it is separated to release the nerve/vessels from the tumor. Step 4 is that If the invasion of the tumor reaches the perineurium or the adventitia of the blood vessel, soaking the nerve/vessels in pure alcohol. Step 5 is that If the nerve/vessels completely adhere to the tumor, they are sacrificed.

[Results] ISP method was used for 2 cases (myxofibrosarcoma, squamous cell carcinoma), local recurrence was not observed for 1 year and half a year, and the affected limb function could be preserved.

[Discussion] ISP method can be compensation for deficits in preoperative diagnosis and surgical technique is easy and special equipment is unnecessary. But there is restriction for a high malignant tissue type and extravascular invasion types. Therefore, adaptation of ISP method should be used with caution.
Results: DUSP1 gene was significantly suppressed about 70% (p<0.01) by DUSP1 siRNA. While phosphorylation of MAPKs by IL-1 stimulation was transient in untransfected cells, enhanced and sustained phosphorylation was observed in DUSP1 knocked-down cells. In addition, NGF and MMPs expressions were exaggerated in the DUSP1 knocked-down cells.

Conclusions: DUSP1 was involved in dephosphorylation of MAPKs in intracellular signaling of IL-1 stimulation in IVD cells, and it was found to be an important molecule involved in NGF and MMPs regulation. DUSP1 can be a new target molecule in conservative therapy for discogenic low back pain.

PI-13.
Regulation of endogenous interleukin (IL)-1 by exogenous IL-1 in human intervertebral disc

(社会人大学院博士課程 2年整形外科学)
○小西 隆允
(整形外科)
澤地 恭昇、遠藤 健司、日下部拓哉
前川 麻人、山本 謙吾

Objective: Intervertebral disc (IVD) degeneration by matrix metalloproteinase (MMP) and nerve invasion by nerve growth factor (NGF) are involved in the development of discogenic low back pain (LBP). Interleukin (IL)-1 is a cytokine that induces various inflammatory proteins including NGF and MMPs in IVD cells, and is considered as a key molecule for the pathogenesis of discogenic LBP. However, the regulation of endogenous IL-1 in IVD cells are largely unknown. We investigated the regulation of endogenous IL-1 expression by exogenous IL-1 by focusing on mitogen-activated protein (MAP) kinases (ERK, p38, JNK) and their endogenous phosphatase, dual-specificity phosphatase (DUSP) 1.

Methods: Human IVD cells were isolated from IVD obtained during lumbar surgery. IVD cells were stimulated with IL-1 in the presence of MAP kinases inhibitors. DUSP1 was knocked-down by transfecting siRNA. Phosphorylation of MAP kinases was evaluated by Western blotting. The expression of endogenous IL-1 and DUSP1 was analyzed by real time-PCR.

Results: Endogenous IL-1 expression was induced by exogenous IL-1. This expression was suppressed by JNK and ERK inhibitor, while it was enhanced by p38 inhibitor. Knocking down of DUSP1 resulted in exaggeration of the phosphorylation of the three MAP kinases compared to untransfected cells. The expression of endogenous IL-1 after IL-1 stimulation was significantly higher in the DUSP1 knock-down cells.

Conclusion: Because the IVD tissue is avascular and composed solely of IVD cells, inflammatory cytokines are to be produced by the IVD cells. Our results demonstrated that exogenous IL-1 stimulates the induction of endogenous IL-1 which may stimulate the same or neighboring cells in an autocrine manner. This positive feedback may cause prolonged local inflammation in IVD tissue. DUSP1 would be a novel target molecule that could attenuate the positive feedback loop of IL-1.

PI-14.
Analysis of testicular specific genes for autoimmune orchitis in mice

(社会人大学院博士課程 3年人体構造学)
○永岡 健太
(人体構造学)
河田 晋一、表原 拓也、宮宗 秀伸
李 忠連、小川 夕暦、伊藤 正裕

Spermatozoa don’t come into existence in the seminiferous epithelium until puberty, when immune tolerance has already been established. Therefore, there are various autoimmunogenic antigens in testicular germ cells (TGC) recognized as foreign by the self-immune system. However, the blood-testis barrier formed by Sertoli cells and the blood-epididymal barrier formed by epididymal epithelial cells protect autoimmunogenic spermatozoa from attack by the self-immune system. We previously showed that immunization of susceptible mice with TGC alone is sufficient to induce experimental autoimmune orchitis (EAO) without the use of any adjuvant. Various testicular autoantigens have been reported by many studies, but antigenic genes for EAO