**THE BASIC STUDY FOR THE NON-SURGICAL SYNOVECTOMY WITH ASK1 GENE.**

+*Terauchi, R; +Takahashi, K; +Arai, Y; +Ikeda, T; +Ohashi, S; +Tokunaga, D; **Mazda, O; **Imanisni, J; +Kubo, T.
+Kyoto Prefectural University of Medicine, Kyoto, Japan

**Introduction:**

Rheumatoid Arthritis (RA) is a chronic autoimmune inflammatory disease resulting in systemic cartilage damages and bone destructions. The symptoms of RA are closely related to the degree of inflammation and hyperplasia of synovial tissue. Proliferation of the synovial tissue leads to pannus tissue that overgrows and invades the cartilage, resulting in destruction of cartilage and bone, and produces the inflammatory cytokines and macrophages. The control of synovial tissue would be one of the useful methods for RA therapy.

Apoptosis signal-regulating kinase (ASK1) was identified as a mitogen-activated protein kinase kinase kinase (MAPKKK) which is involved in the stress-induced apoptosis-signaling cascade. The expression of ASK1 in cells would lead the apoptosis in them.

If the transduction of ASK1 gene into the synoviocytes could leads the apoptosis of the synovial tissue, it would be one of the newer therapies for RA. In this study, we transduced ASK1 gene to synoviocytes with adenovirus vectors, and investigated the efficacy of apoptosis in vitro.

**Methods:**

Synovial tissue samples were obtained during total knee arthroplasty from 6 RA patients. The diagnosis of RA was based on the 1987 ACR criteria for RA. The synovial tissue was minced into small pieces and digested with 1.0mg/ml collagenase. The cells were placed into culture dishes and incubated. Three types of adenovirus; AxCALNL-ASK1?N, AxCAN-Cre, AxCA-LacZ were used. ASK1 was transduced with Cre/loxP recombination using adenovirus vectors with cassette for ASK1(AxCALNL-ASK1?N) and Cre recombinase (AxCAN-Cre). LacZ was transduced with adenovirus vector (AxCA-LacZ).

After transfection with adenovirus (500 MOI AxCALNL-ASK1?N + 50 MOI AxCAN-Cre), cells were observed by microscopy at 48, 60, 72, and 96 hours. The effects of ASK1 on apoptosis were investigated by using the Hoechst 33342 staining, Terminal transferase dUTP Nick-End Labeling (TUNEL) staining, and MTT assay at 60 hours after transfection with adenovirus (500 MOI AxCALNL-ASK1?N + 50 MOI AxCAN-Cre, 500 MOI AxCALNL-ASK1?N, or 500 MOI AxCA-LacZ + 50 MOI AxCAN-Cre). The cells without transfection were used as control.

**Results:**

The number of cells began to decrease at 60 hrs. Tunnel positive cells (Fig. 1) and the apoptotic cells in Hoechst staining were only found in the ASK1 and Cre cotransfected groups. In the MTT assay, OD was 131.4±5.0 in the control cells, 110.2±20.7 in AxCALNL-ASK1?N transduced cells, 116.8±5.8 in AxCA-LacZ + AxCAN-Cre transduced cells, and 26.4±1.9 in AxCALNL-ASK1?N + AxCAN-Cre transduced cells (n = 5) (Fig.2).

**Discussion:**

In this study, ASK1 gene was transduced with Cre/loxP recombination system using adenovirus vectors. It is useful to control of harmful gene expression, such as ASK1 inducing apoptosis.

Sixty hours after cotransduction of AxCALNL-ASK1?N and AxCAN-Cre, the apoptosis has started. The Tunnel staining and Hoechst staining show the expression of apoptosis in AxCALNL-ASK1?N and AxCAN-Cre transduced cells. But it was not easy to find the cell death in other groups. It shows that the ASK1 gene expression was successfully controlled by Cre/loxP recombination system.(2) Also in MTT assay, OD in AxCALNL-ASK1?N transduced cells was significantly different from the other groups, it shows the ASK1 gene expression.

Removal of the synovium is useful for RA patients by decreasing inflammation and preventing destruction of adjacent structures. In clinical practice, the synovial tissue can be removed by surgical synovectomy. But if synovial tissue could be removed by gene transduction with simple intra articular injection, it would be an attractive alternative treatment strategy. Synovial tissue is well known to be transduced by adenovirus vectors with intra articular injection. This study shows the possibility of ASK1 gene therapy for RA with Cre/LoxP recombination system with adenovirus.

**References:**

1. Ichijo, H.; Oncogene, 1999; 18: 6084-93.

*Department of Orthopaedic Surgery, Kyoto Prefectural University of Medicine, Kyoto, Japan.
**Department of Microbiology, Kyoto Prefectural University of Medicine, Kyoto, Japan.

---

**Fig.1. Tunnel staining**

A is control, B is 500 MOI AxCALNL-ASK1?N, C is 500 MOI AxCA-LacZ + 50 MOI AxCAN-Cre, D is 500 MOI AxCALNL-ASK1?N + 50 MOI AxCAN-Cre.

**Fig.2. MTT assay**

ASK means the cell was transduced by 500 MOI AxCALNL-ASK1?N, LacZ + Cre means by 500 MOI AxCA-LacZ + 50 MOI AxCAN-Cre, ASK + Cre means by 500 MOI AxCALNL-ASK1?N + 50 MOI AxCAN-Cre. Data are expressed as mean ± S.E. * p < 0.01

---

49th Annual Meeting of the Orthopaedic Research Society
Poster #0527