#955 - Experimental Study / Posters

TNIIIA2 As A Candidate For Preventing Articular Cartilage Degeneration

Orthopaedics / Knee & Lower Leg / Miscellaneous

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Background
Tenascin-C (TNC) is an extracellular matrix glycoprotein. TNC is almost completely absent in adult articular cartilage, but TNC reappear in osteoarthritis (OA) cartilage. It was reported intra-articular injection of TNC could prevent cartilage degeneration in OA model mice. But intra-articular injection of the domain of TNC induce synovitis. TNIIIA2 is the peptide of TNC. It was reported TNIIIA2 induce activation of β1-integrins, and activation of β1-integrin promote chondrocyte proliferation and inhibit chondrocyte apoptosis. We hypothesized that TNIIIA2 have the effect of preventing cartilage degeneration without inflammation of synovium.
Objectives
We evaluated the expression of TNIIIA2 in human OA cartilage. And we evaluated intra-articular injection of TNIIIA2 would induce synovitis in mice or not, and evaluated the effect of intra-articular injection of TNIIIA2 in OA model mice.
Study Design & Methods
The peptide, termed TNIIIA2, was synthesized using the Boc and Fmoc solid phase strategy. Rabbits were immunized with a synthetic peptide containing the active sequence coupled with thyroglobulin. The IgG fraction of rabbit serum was applied to Sepharose beads coupled with the synthetic peptide immunogen. Eluted IgG was used as the anti-TNIIIA2 antibody.
In the study of human cartilage, human cartilage specimens were obtained from patients who underwent total knee joint arthroplasty for the treatment of OA. Immunolabeling of TNC and TNIIIA2 was performed in tissue specimens of OA cartilage (n=3). We used tibia cartilage of who was underwent TKA for femur neoplasm for control (n=1). In the experiment on animals, Male 8-week-old mice were used. 10μg/ml of TNIIIA2 was injected into the knee joint of mice (group II n=12). The control group had an injection of phosphate buffered saline (PBS) (group I n=12). We evaluated at 2 and 4 weeks after injection. OA model: Both knee joints were exposed following a medial capsular incision and the anterior cruciate ligament and medial collateral ligament were transected. After the articular capsule was closed, 10μg/ml of TNIIIA2 was injected into the knee joint (group IV n=35). The control group had an injection of PBS (group III n=35). We evaluated 2,4,8 and 12 weeks postoperatively. Histological examinations were made using hematoxylin & eosin and

safranin-O staining. Immunohistochemical evaluation was performed using anti-TNC antibody and anti-TNIIIA2 antibody. Synovitis was evaluated using synovitis score. Cartilage degeneration was evaluated using Mankin score. Statistical significance was determined using the Mann-Whitney U-test.

Results

In the study of immunofluorescence for OA cartilage specimens, both anti-TNC and anti-TNIIIA2 staining was observed in the OA cartilage, but not in normal cartilage.

In the study of intra-articular injection of mice, low grade synovitis was occurred at 2weeks in both groups, but it was improved at 4 weeks in both groups. There was no difference between both groups. In the study of OA model mice, no development of OA was found in both groups at 2 weeks. Mankin score were significantly higher in group III than in group IV at 4 weeks and 8 weeks. There was no significant difference in both group at 12 weeks.

Conclusions

TNIIIA2 was expressed in OA cartilage. We demonstrated that 10μg/ml of TNIIIA2 could prevent articular cartilage degeneration without synovitis for 8 weeks. TNIIIA2 could be an important candidate for preventing articular cartilage degeneration.