Expression of VEGF-A Signaling Pathway in Cartilage of ACLT-Induced Osteoarthritis Mouse Model

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Abstract

Background: The clear understanding of the underlying mechanism of Osteoarthritis(OA) remains elusive. Researches have shown that Vascular Endothelial Growth Factor(VEGF) induced angiogenesis and inflammation were important processes in the pathophysiology of OA. Now, Anterior Cruciate Ligament Transection surgery(ACLT) induced OA model was often used to investigate the molecular mechanism of OA, but till now the angiogenesis and inflammation reaction in different pathological stages of ACLT-induced OA model has never been revealed.

Methods: Moderate OA model was established by ACLT, and 1, 2, 4, 8 and 12 weeks after surgery, Hematoxylin–eosin(HE) and Safranin-O(S-O) staining were used to detect the pathological changes in mouse knee cartilage, and the matrix biomarkers A Disintegrin and Metalloproteinase with Thrombospondin Motifs 5(ADAMTS5), Collagen II(COL-II) were detected using Immunohistochemistry (IHC), CD31 was detected by Immunofluorescence(IF) to show the vascular invasion in cartilage, and proteins expression of VEGF-A pathway were detected by Western blot(WB). Meanwhile the inflammatory biomarkers Cyclooxygenase-2 (COX-2) and inducible Nitric Oxide Synthase(Inos) in cartilage were detected by WB.

Results: ACLT surgery can lead to degeneration of cartilage in mice, and the characteristics of the lesion were time dependent. The ADAMTS5 positive cells increased while COL-II decreased in OA cartilage with time, new blood vessel labeled by CD31 can be seen from 1 week in OA cartilage, and increased in 8 and 12 weeks. The expression of VEGF-A, VEGFR2, COX-2 and iNOS were higher than control groups, which were basically consistent with the degree of osteoarthritis.

Conclusions: VEGF-A related signaling pathway played an irreplaceable role in the occurrence and development of ACLT model, and the underlying mechanism may be related to the angiogenesis and inflammation in cartilage.

Full-text

Due to technical limitations, full-text HTML conversion of this manuscript could not be completed. However, the manuscript can be downloaded and accessed as a PDF.

Figures

Figure 1

Histopathological analysis of the cartilage tissues obtained from each group($\times 200$) to show the development in the ACLT model.A:After fixation,decalcification and embedding,4 mm frontal sections were cut from the knee joints,and were stained with HE staining.B:Safranin-O-fast green staining images of cartilage in each group.C:HE and Safranin-O-fast green staining of control group.D:Mankin score.The data in the figures represent mean values $\pm$ SD,* $p < 0.05$,** $p < 0.01$ compared with the control group,# $P < 0.05$,## $P < 0.01$ compared with the sham group at the same point.

Figure 2

The expression of cartilage matrix biomarkers ADAMTS5 and COL-II of knee joint. A: Immunohistochemistry staining of ADAMTS5 (scale bar: 20 μ m). B: Immunohistochemistry staining of COL-II (scale bar: 20 μ m). C: Immunohistochemistry staining of ADAMTS5 and COL-II in control group (scale bar: 20 μ m).

Figure 3

The protein level of inflammatory biomarkers COX-2 and iNOS in cartilage detected by Western blotting. The data in the figures represent mean values $\pm$ SD,*p<0.05,**p<0.01 compared with the control group,#P<0.05,##P<0.01 compared with the sham group at the same point.

Figure 4

Immunofluorescence staining for CD31 to identify angiogenesis in the cartilage of ACLT-induced mouse by a fluorescence microscope (OLYMPUS) (Scale bar:20 µm).

Figure 5

The level of the expression of VEGFA and VEGFR2 in cartilage. The data in the figures represent mean values $\pm$ SD, * $p < 0.05$, ** $p < 0.01$ compared with the control group, # $P < 0.05$, ## $P < 0.01$ compared with the sham group at the same point