Autophagy modulates osteoarthritis-related gene expressions in human chondrocytes

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Introduction: Osteoarthritis (OA) is characterized by degeneration of articular cartilage. During development and progression of OA, abnormal qualitative and quantitative changes in chondrocyte gene expression and increased apoptosis have been observed. Understanding the molecular mechanisms that regulate these alterations in chondrocytes during the development of OA may lead to strategies for preventing or arresting progression of this disease.

Autophagy, an evolutionarily conserved process for the bulk degradation of cytoplasmic components, has been reported to be a cell survival mechanism under starved condition 1, 2. The main functions of autophagy are housekeeping and quality control of proteins and organelles, therefore dysfunction of autophagy has been indicated as a cause of some degenerative diseases 3, 4. The purpose of this study is to elucidate the role of autophagy in human chondrocytes and pathophysiology of OA.

Materials and Methods: Articular cartilage tissues were obtained from femoral condyles of patients with OA or femoral heads of patients with femoral neck fracture and used as OA cartilage and normal cartilage respectively. Primary chondrocytes were also isolated from the articular cartilage samples of patients with OA and used as OA chondrocytes. Normal Human Articular Chondrocytes-knee was purchased and used as normal human chondrocytes. Autophagy in the articular cartilage samples and the chondrocytes was assessed by immunohistochemistry and immunoblotting using antibodies for autophagy markers, LC3II and beclin1. We examined the effects of inhibition or induction of autophagy under the stimulation with IL-1β. Autophagy was inhibited by small interfering RNA (siRNA) targeting Atg5, an autophagy essential gene and autophagy was induced by rapamycin under IL-1β stimulation. The effects of inhibition or induction of autophagy were examined by real-time PCR for MMP13, ADAMTS5, aggrecan and COL2A1 mRNA. We also examined the effects of inhibition or induction of autophagy on apoptosis by western blotting for cleaved caspase9 and cleaved PARP.

Results: Autophagy in articular cartilage tissues and primary chondrocytes

LC3II and beclin1 were strongly expressed in the osteoarthritic cartilage samples compared with the non-osteoarthritic cartilage of the femoral heads. In addition, OA chondrocytes strongly expressed LC3II and beclin1 compared with non-osteoarthritic normal chondrocytes (Fig.1).

The effects of inhibition or induction of autophagy under the stimulation with IL-1β

The inhibition of autophagy by Atg5 siRNA under the stimulation of IL-1β caused significantly increased MMP13 and ADAMTS5 expressions while the induction of autophagy by rapamycin reduced these gene expressions. Additionally, the inhibition of autophagy downregulated the expressions of aggrecan and COL2A1 while the induction of autophagy upregulated the expressions of aggrecan and COL2A1 (Fig.2).

The effect of inhibition or induction of autophagy on apoptosis of human chondrocytes

The transfection of siRNA for Atg5 alone caused a slight increase in the level of cleaved caspase9 and cleaved PARP protein, and treatment with IL-1β alone strongly increased cleaved caspase9 and cleaved PARP. In addition, IL-1β and siRNA for Atg5 further increased cleaved caspase9 and cleaved PARP, while rapamycin substantially inhibited the increase in cleaved caspase9 and cleaved PARP induced by treatment with IL-1β (Fig.3), suggesting that enhanced apoptosis by inhibition of autophagy and reduced autophagy response to protect cells and autophagy play protective roles in chondrocytes. Further studies about autophagy in chondrocytes will provide novel insights into the pathophysiology of OA.

Discussion: In this study, we found that autophagy was increased in human osteoarthritic cartilage. In addition, the inhibition of autophagy enhanced OA-like gene expression changes induced by IL-1β while the induction of autophagy prevented. Furthermore, the inhibition of autophagy enhanced apoptotic changes induced by IL-1β, whereas the activation of autophagy suppressed the apoptotic changes. These observations suggested the increased autophagy might be an adaptive response to protect cells and autophagy play protective roles in chondrocytes. Further studies about autophagy in chondrocytes will provide novel insights into the pathophysiology of OA.