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Proteomic analysis of osteoarthritic chondrocyte reveals the hyaluronic acid-regulated proteins involved in chondroprotective effect under oxidative stress☆

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ABSTRACT

Osteoarthritis (OA), the most common type of arthritis, is a degenerative joint disease. Oxidative stress is well known to play important roles in cartilage degradation and pathogenesis of OA. The intra-articular injection of hyaluronic acid (IAHA) is accepted as an effective clinical therapy for OA, but we do not yet fully understand the mechanisms underlying the effects of HA on OA chondrocytes under oxidative stress. Here, we show for the first time that IAHA significantly reduces the synovial fluid levels of hydrogen peroxide (H_2O_2) and superoxide (O_2^-) in patients with knee OA. We also demonstrate that HA suppresses H_2O_2-induced cell death in human OA chondrocytes. Proteomic approaches (2-DE combined with mass spectrometry) allowed us to identify 13 protein spots corresponding to 12 non-redundant proteins as HA-regulated proteins in OA chondrocytes under oxidative stress. The expression levels of three putative HA-regulated proteins (TALDO, ANXA1 and EF2) in control, H_2O_2-, HA- and HA/H_2O_2-treated OA chondrocytes were verified by Western blotting and the results indeed support the notion that HA acts in

Abbreviations: OA, osteoarthritis; HA, hyaluronic acid; IAHA, intra-articular injection of hyaluronic acid; H_2O_2, hydrogen peroxide; O_2^-, superoxide; SF, synovial fluid; ROS, reactive oxygen species; 2-DE, two-dimensional electrophoresis; GAG, glycosaminoglycan; LC–MS/MS, liquid chromatography tandem mass spectrometry; pI, isoelectric point; Mr, molecular weight; TALDO, transaldolase; ANXA1, annexin A1; EF2, elongation factor 2; PBS, phosphate buffered saline; RT-PCR, reverse transcription-polymerase chain reaction; PCR, polymerase chain reaction; DTT, dithiothreitol; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenytetrazolium bromide.

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anti-oxidation, anti-apoptosis, and the promotion of cell survival. Our results collectively demonstrate the utility of proteomic approaches and provide new insights into the chondroprotective effects of HA on OA.

Biological significance

In the present study, we show for the first time that IAHA reduces the levels of H_2O_2 and O_2^- in synovial fluids from OA patients. We used primary cultured human OA chondrocytes as a model, treated cells with H_2O_2 to partly mimic their physiological conditions under oxidative stress, and examined the protection effects of HA. The proteomic approach allowed us to identify candidate proteins regulated by H_2O_2 and/or HA in OA chondrocytes. We found that proteins functioning in stress responses, apoptosis and protein synthesis were consistently regulated by HA in chondrocytes under oxidative stress. These novel results contribute to our understanding of the molecular mechanisms underlying HA-mediated chondroprotection.

1. Introduction

Osteoarthritis (OA), which is also known as osteoarthroses or degenerative joint disease, is the most common type of arthritis worldwide [1–3]. OA is characterized by the erosion of articular cartilage, which leads to joint-space narrowing, subchondral sclerosis, subchondral cysts, synovial inflammation, and marginal osteophyte formation [2,4]. Individuals with OA can suffer from pain, muscle weakness, decreasing ranges of motion, and increasing disability. Documented contributors to this pathophysiology include genetic predisposition, trauma, inflammation, and metabolic changes [2,5,6].

Hyaluronate or hyaluronic acid (HA) is a high molecular weight glycosaminoglycan (GAG) composed of repeating disaccharide units of N-acetylglucosamine and glucuronic acid [7]. HA is the most abundant GAG in mammalian tissues, and is present at high concentrations in connective tissues, such as skin, cartilage, vitreous humor and umbilical cord. The largest single reservoir of HA is the synovial fluid (SF) of the diarthrodial joints, where concentrations of 0.5–4 mg/ml are achieved [1,8] and HA has been shown to mediate the elastic properties and viscosity of SF. Notably, however, the concentration and molecular weight of HA are dramatically reduced in OA joints [9,10]. Intra-articular injection of HA (IAHA) has been accepted as an effective therapy for OA and several trials have attempted to evaluate the benefits of IAHA for knee OA (e.g., improvements in painful knee movements and general pain relief) [9,11–20]. HA has a very short half-life (12 to 17 h) once injected into the joint, but its clinical effects can last six to 12 months [7,15]. Thus, HA appears to provide its clinical benefits by providing viscous fluid replacement and triggering multifunctional biological responses.

Reactive oxygen species (ROS), such as hydrogen peroxide (H_2O_2), hypochlorite ion (OCl^-), hydroxyl radical (.OH) and superoxide anion (O_2^-), are derived from oxygen and involve in both normal intracellular signal transduction and degenerative cellular processes [21,22]. Elevated production of ROS and/or depletion of antioxidants has been observed in a variety of pathological conditions, including inflammatory joint diseases [22–24]. Specifically, studies have shown that chondrocyte apoptosis and articular cartilage degradation can be caused by elevated ROS production, contributing to the aging of cartilage and the pathogenesis of OA [21–23,25–27]. Recently, Miki et al. demonstrated that HA treatment could attenuate mechanical stress-enhanced ROS synthesis and reverse proteoglycan synthesis inhibited by mechanical stress in bovine cartilage, supporting the antioxidant activity of HA [28]. However, we do not yet fully understand the molecular mechanisms through which HA offers its chondroprotective effects under oxidative stress. Here, we report that the levels of H_2O_2 and O_2^- in the SF of OA patients were significantly reduced following IAHA therapy, and that external HA treatment of human OA chondrocytes suppressed H_2O_2-induced cell death in vitro. To gain new insights into the HA-regulated proteins that contribute to its chondroprotective effects under oxidative stress, we used 2-DE combined with mass spectrometry to identify proteins that were differentially expressed in OA chondrocytes treated with H_2O_2 and/or HA. The obtained candidate proteins included subsets involved in stress, apoptosis/anti-apoptosis and protein synthesis, indicating that HA plays multiple biological roles in OA chondrocytes under oxidative stress.

2. Materials and methods

2.1. Patient population and clinical specimens

This study was approved by the Institutional Review Board for Research Ethics at the National Taiwan University Hospital Taipei, Taiwan. Written informed consent was obtained from each patient prior to enrollment. Cartilage specimens were collected from 14 OA patients (1 man and 13 women; mean age, 68 years; range, 28–81 years) who received total knee replacements. The clinical characteristics of these OA patients, including gender, age, diagnosis, OA grade, and OA sites were summarized in Supplementary Table S1. The cartilage samples were subjected to chondrocyte isolation immediately after surgery. SF samples were collected from 19 OA patients (7 men and 12 women; mean age, 56 years; range, 40–71 years) who received IAHA therapy (ARTZ® purified sodium hyaluronate, MW 620–1170 kDa; Seikagaku Corp, Tokyo, Japan). The SF samples (~2 ml) were aspirated from patients before and after IAHA treatments. The SF samples were then stored at −80 °C for further analysis.