Redox Biology 14 (2018) 72–81

Contents lists available at ScienceDirect

Redox Biology

journal homepage: www.elsevier.com/locate/redox

Research paper

PKR activation causes inflammation and MMP-13 secretion in human degenerated articular chondrocytes

Ching-Hou Ma[a], Chin-Hsien Wu[a], I.-Ming Jou[a], Yuan-Kun Tu[a], Ching-Hsia Hung[b,c], Pei-Ling Hsieh[d,*], Kun-Ling Tsai[b,*]

[a] Department of Orthopedics, E-Da Hospital/I-Shou University, Kaohsiung, Taiwan
[b] Department of Physical Therapy, College of Medicine, National Cheng Kung University, Tainan, Taiwan
[c] Institute of Allied Health Sciences, College of Medicine, National Cheng Kung University, Tainan, Taiwan
[d] Institute of Oral Sciences, Chung Shan Medical University, Taichung, Taiwan

ARTICLE INFO

Keywords:
Osteoarthritis
Human chondrocyte
PKR
Oxidative stress
MMP-13

ABSTRACT

Osteoarthritis (OA) is a degenerative joint disease affecting a large population of people. Although the elevated expression of PKR (double stranded RNA-dependent protein kinase) and MMP-13 (collagenase-3) have been indicated to play pivotal roles in the pathogenesis of OA, the exact mechanism underlying the regulation of MMP-13 by PKR following inflammatory stimulation was relatively unknown. The purpose of this study was to determine the signaling pathway involved in the PKR-mediated induction of MMP-13 after TNF-α-stimulation. In this study, cartilages of knee joint were obtained from OA subjects who underwent arthroplastic knee surgery. Cartilages were used for tissue analysis or for chondrocytes isolation. In results, the upregulated expression of PKR was observed in damaged OA cartilages as well as in TNF-α-stimulated chondrocytes. Phosphorylation of PKC (protein kinase C) was found after TNF-α administration or PKR activation using poly(I:C), indicating PKC was regulated by PKR. The subsequent increased activity of NADPH oxidase led to oxidative stress accumulation and antioxidant capacity downregulation followed by an exaggerated inflammatory response with elevated levels of COX-2 and IL-8 via ERK/NF-κB pathway. Activated ERK pathway also impeded the inhibition of MMP-13 by PPAR-γ. These findings demonstrated that TNF-α-induced PKR activation triggered oxidative stress-mediated inflammation and MMP-13 in human chondrocytes. Unraveling these deregulated signaling cascades will deepen our knowledge of OA pathophysiology and provide aid in the development of novel therapies.

1. Introduction

Osteoarthritis (OA) is one of the critical degenerative orthopedic diseases that affect a significant proportion of the population. Clinical symptoms included joint pain, stiffness and decrease of mobility, leading to physical disability and reduced quality of life [1]. To date, a number of factors are believed to trigger OA, such as abnormal mechanical stress, compressive forces, failure of nutrient intake or genetic issues [2]. However, current interventions are still restricted to pain control and total knee arthroplasty is often suggested for late-stage OA cases [3].

Chondrocytes, the only cell type present in the articular cartilage, express various genes to maintain homeostasis. And numerous genes involved in extracellular matrix (ECM) formation and oxidative damage defense have been found to be altered in late-stage OA cartilage [4]. In addition, it is well established that pathogenesis of OA is closely related with pro-inflammatory cytokines produced by chondrocytes, such as interleukin (IL)-1 and tumor necrosis factor (TNF)-α, which eventually results in activation of matrix metalloproteinases (MMPs) and deterioration of OA [5]. In particular, MMP-13 has been shown to be upregulated [6] and identified as a critical target during the progression of OA [7]. Also, it has been previously suggested structural cartilage damage in animal OA model is dependent on MMP-13 activity [8]. As such, elucidation of the detailed mechanism underlying the increased expression of MMP-13 may be beneficial to prevent the development of OA.

The double stranded (ds) RNA-dependent protein kinase, PKR, is a ubiquitously expressed serine/threonine kinase. Once PKR is activated by dimerization and autophosphorylation, it subsequently phosphorylates the α-subunit of the eukaryotic translation initiation factor 2 (eIF2α) [9]. Various studies have implicated PKR signaling pathways in the cartilage degradation that occurs in arthritic diseases [10–12]. It has been proven that PKR inhibitor could antagonize the IL-1α-activated eIF2α phosphorylation, thereby suppressing proteoglycan

* Corresponding authors.
E-mail addresses: akinosha@hotmail.com (P.-L. Hsieh), kunlingtsai@mail.ncku.edu.tw (K.-L. Tsai).

http://dx.doi.org/10.1016/j.redox.2017.08.011
Received 11 July 2017; Received in revised form 31 July 2017; Accepted 15 August 2017
Available online 24 August 2017

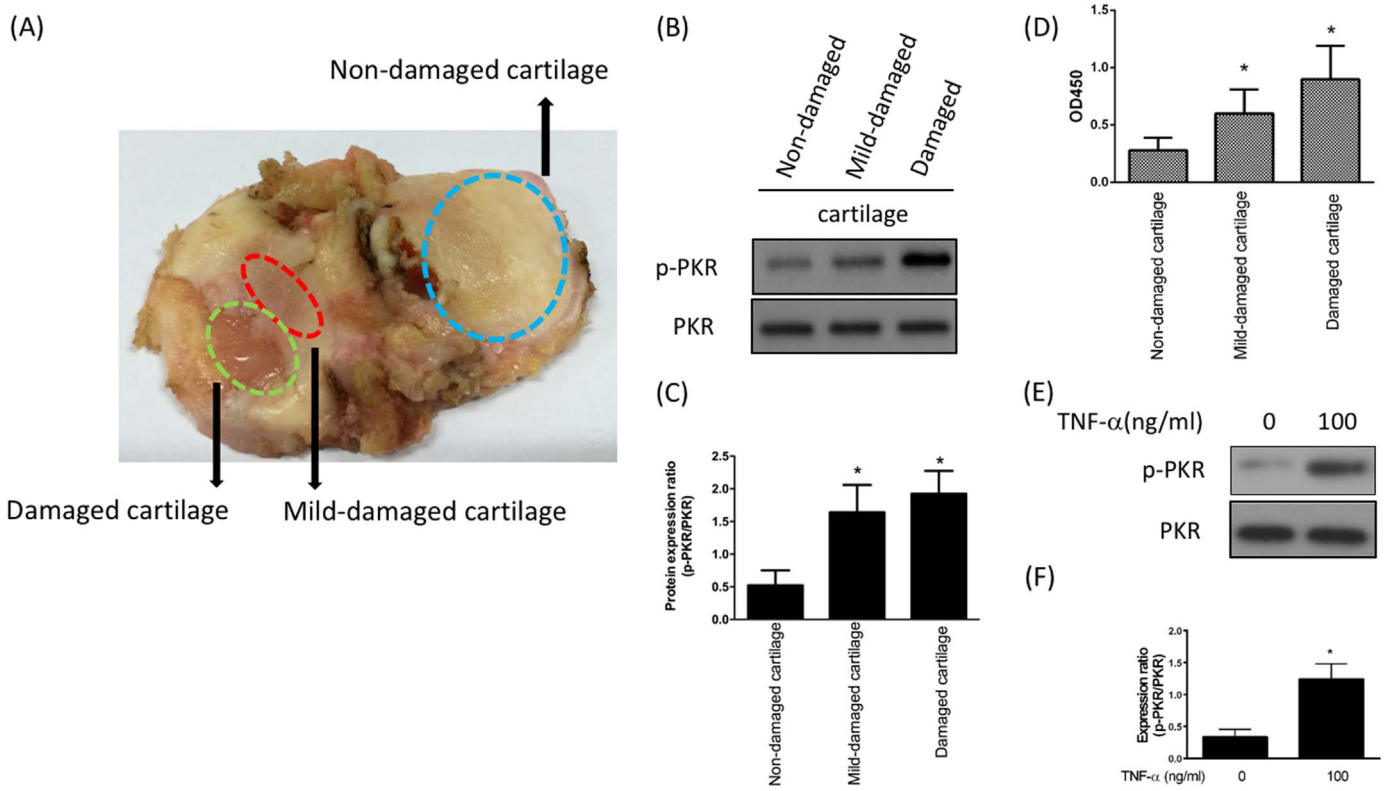

Fig. 1. Upregulation of PKR following cartilage inflammation. A. Representative image of cartilage from patient with total knee replacement showing non-damaged, mid-damaged and damaged regions; Protein expression (B) and ratio (C) of p-PKR to total PKR; (D) Kinase activity of p-PKR from three different regions; protein expression (E) and ratio (F) of p-PKR to total PKR after exogenous administration of TNF-α for 12 h in human chondrocytes. (n = 3; * $p < .05$ compared to non-damaged cartilage or control group).

degradation and cyclooxygenase (COX)−2 accumulation [11]. PKR also regulates the TNF-α-induced proteoglycan degradation and chondrocyte cell death [10]. Moreover, PKR has been indicated to mediate the TNF-α-induced activation of MMP-2 and −9 [10]. Hence, it is tempting to speculate that PKR regulates MMP-13 expression in OA cartilages, which leads to ECM degradation.

The aim of the present study was to decipher the mechanism of PKR-mediated MMP-13 upregulation in chondrocytes following TNF-α stimulation. To this end, we employed the chondrocytes obtained from OA patients and examined the associated signaling pathways in order to understand the role of PKR in the onset of OA. Our data also demonstrated the relationship between accumulated oxidative stress and increased inflammation and MMP-13.

2. Materials and methods

2.1. Reagents

Trypsin-EDTA and Dulbecco's modified Eagle's medium (DMEM) were bought from Gibco (Grand Island, NY, USA). PD98059, dihydroethidium (DHE), Apocynin, Diphenyleneiodonium (DPI), Polyinosinic-polycytidylic acid (poly(I:C)), collagenase B, Pyrrolidine dithiocarbamate (PDTC), penicillin and streptomycin were all purchased from Sigma (St. Louis, MO, USA). Anti-PKR, anti-p-PKR,anti-PKC, anti-p-PKC, anti-NOX-1, anti-p47, anti-Rac-1, were all obtained from Abcam (Cambridge, UK). Anti-β-actin, anti-ERK, anti-p-ERK, anti-COX-2, anti-PPAR-γ were all obtained from Santa Cruz Biotechnology (Santa Cruz, CA, USA). HRP-conjugated anti-rabbit secondary antibodies were purchased from Transduction Laboratories (CA, USA). Antioxidant enzymes kits were obtained from EMD Millipore (Calbiochem, Gibbstown, NJ). IL-8 ELISA kit was purchased from R & D Systems (Minneapolis, MN, USA).

2.2. Human chondrocytes isolation

The study group included 30 patients diagnosed with knee OA over 5 years. The study protocol was approved by the Ethics Committee of E-Da Hospital (EMRP-105-077), and each participant provided written informed consent. Cartilages of knee joint were obtained from OA subjects who underwent arthroplastic knee surgery. Articular cartilage tissues were gathered from the resected bone and cartilages. The damaged cartilage tissues were gathered for tissue analysis. The non-damaged cartilage tissues were gathered for tissue analysis and digested for in vitro investigations. Cartilage samples were cut into small pieces and washed with PBS for three times. Cartilage fragments were digested with collagenase B in DMEM at 37 °C overnight on a shaker. The isolated chondrocytes were centrifuged and washed two times with PBS. Chondrocytes were cultured in MDEM with 10% FBS, 2 mM L-glutamine, 25 mM HEPES, 100 U/ml penicillin, and 100 mg/ml streptomycin at 37 °C in a humidified atmosphere of 95% air and 5% CO2 [13].

2.3. Western blot analysis

RIPA, which was purchased from Millipore, was used to extract lysate. The proteins were transferred on to a PVDF membrane after the proteins were separated by SDS/PAGE. The membranes were blocked by the buffer for 1 h at 37 °C. Then, the membranes were incubated with primary antibodies overnight at 4 °C, followed by hybridization with HRP (horseradish peroxidase)-conjugated secondary antibody for