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Endoplasmic reticulum stress participates in the progress of senescence and apoptosis of osteoarthritis chondrocytes

Yake Liu [a, 1], Hai Zhu [a, b, 1], Xin Yan [a, b], Haoye Gu [a, b], Zhifeng Gu [b, c, *], Fan Liu [a, **]

[a] Department of Orthopaedics, Affiliated Hospital of Nantong University, Nantong, Jiangsu Province, China
[b] Research Center of Clinical Medicine, Affiliated Hospital of Nantong University, Nantong, Jiangsu Province, China
[c] Department of Rheumatology, Affiliated Hospital of Nantong University, Nantong, Jiangsu Province, China

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ABSTRACT

Endoplasmic reticulum stress (ERS) has been shown to participate in many disease pathologies. Recent reports have reported that ERS exists in human osteoarthritis (OA) chondrocytes. During OA, chondrocytes exhibit increased level of some senescence marker, such as senescence-associated β-galactosidase (SA β-gal) activity. However, the persistence and accumulation of senescent cells in various tissues can also impair function and have been involved in the pathogenesis of many age-related diseases, including OA. In this present study, we used IL-1β (10 ng/ml) to mimic OA chondrocytes and we found that IL-1β stimulated chondrocytes caused the increasing expression of ADAMTS5 and MMP13, decreasing COL2A1 expression, which were in accord with OA chondrocytes changes. Our data also showed that ERS is involved in the OA chondrocytes, SA β-gal activity significantly increases and inhibition of ERS can decrease the SA β-gal activity, apoptosis of OA chondrocytes and increase cell viability. These results help us to open new perspectives for the development of molecular-targeted treatment approaches and thus present an effective novel therapeutic approach for OA.

1. Introduction

Osteoarthritis (OA), one of the representative age-related diseases, is the most common joint disease, which affects the majority of individuals over the age of 65 [1], leading to the degradation of articular cartilage, synovial inflammation and subchondral bone remodelling. Chondrocytes, the only cell type in articular cartilage, are prerequisite for the maintenance of the cartilage homeostasis [2]. However, it is still unclear as to what mechanisms control the fate of chondrocytes within the articular cartilage during normal versus OA conditions.

Interleukin-1β (IL-1β), a key pro-inflammatory factor, is one of the most mediators of OA [3,4]. However, inflammation usually accompanies progression of OA and deteriorates cartilage degradation [5]. OA patients display increasing IL-1β levels in the cartilage, synovial fluid and synovial membrane [6], and inflammatory cytokines, such as IL-1β and tumor necrosis factor-α(TNF-α), can interdict generation of cartilage-structural proteins, including type II collagen and aggrecan [7,8]. Finally, IL-1β induces degradation of cartilage matrix through activation of extracellular matrix proteases, as well as via inhibition of synthesis of extracellular matrix proteins, such as matrix metalloproteinases (MMPs), especially MMP13, the major MMP responsible for remodeling type II collagen(Col II) in cartilage tissue, and a disintegrin and metalloproteinase with thrombospondin motifs (ADAMTSs), which plays an important role in cartilage degradation [9]. MMPs and ADAMTSs are probably the primary enzymes involved in digestion of the ECM, which is a critical step in the loss of articular cartilage in OA.

Cellular senescence is a state of stable proliferation arrest of cells. The senescence pathway has many beneficial impacts and is to be activated in damaged or stressed cells, as well as during wound healing and embryonic development. Nevertheless, senescent cells can also damage function when the senescent cells are continuity and accumulation in various tissues and have been implicated in the pathogenesis of many age-related diseases. Senescence finally results in the loss of cellular replicative capacity because of the inability of these cells to express proliferation

* Corresponding author. Department of Rheumatology, Research center of clinical medicine, Affiliated Hospital of Nantong University, 20 Xisi Road, Nantong, Jiangsu, 226001, China.
** Corresponding author. Institute of Orthopaedic Surgery, Affiliated Hospital of Nantong University, 20 Xisi Road, Nantong, Jiangsu 226001, China.
E-mail addresses: guzhifeng@126.com (Z. Gu), liufannt57@163.com (F. Liu).
[1] Yake Liu and Hai Zhu contributed equally to this work.

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[10,11]. It was reported that senescent cells were observed near the osteoarthritic lesions, but not in intact cartilage from the same patients and normal donor [12]. Accumulation of senescent cells may predispose articular joints to the development and/or progression of OA [13]. Senescence can cause loss of cell proliferation, which contributes to OA progression [14]. In accordance with above results, transplanted senescent cells induce an OA-like state in mice [15]. In addition, senescent cells can secrete damage associated molecular patterns released from dying cells communicating cellular damage [16]. Furthermore, chondrocytes undergoing senescence actively produce cartilage degrading enzymes such as MMP13 and aggrecanases, which promote the degradation of aggrecan and Col II [17]. This would suggest that targeting senescent cells might be an attractive therapeutic modality for treatment of OA.

Recent report has demonstrated that endoplasmic reticulum stress (ERS) exists in human OA cartilage and that chondrocytes are sensitive to ERS [18]. ERS, which is provoked by an imbalance between the load of unfolded proteins in the ER and the capacity of the ER, causes the accumulation of unfolded or misfolded proteins in the ER. The ERS response has attracted attention as a new area of research in OA cartilage biology, which plays a key role in cell survival and homeostatic regulation. Disruption of ER homeostasis results in the accumulation of unfolded proteins and apoptosis [19,20]. In cultured chondrocytes, the ERS inducer tunicamycin mediates Chop expression and apoptosis, decreases aggrecan and Col II mRNA expression [21], and increases the expression of MMP13 mRNA [22]. Excessive ERS results in apoptosis [23]. 78-KDa glucose-regulated protein (GRP78) is a central regulator of ER function due to its roles in protein folding and assembly, targeting misfold protein for degradation, ER Ca^{2+}-binding and controlling the activation of *trans*-membrane ERS sensors [24,25].

Our previous study reported ERS participated in the progress of senescence of bone-marrow-derived mesenchymal stem cells and led to cell apoptosis [26]. In this current study, we attempt to clarity the impact of ERS on senescence and ERS mediated apoptosis in chondrocytes.

2. Materials and methods

2.1. Materials

Dulbecco's Modified Eagle Medium was obtained from Corning. Penicillin, streptomycin and fetal bovine serum (FBS) were obtained from Gibco BRL (Grand Island, NY, USA). Recombinant human IL-1β was obtained from R&D Systems (Minneapolis, MN). Annexin V-FITC/propidium iodide(Annexin V-FIFC/PI) apoptosis kit was from BD Biosciences. A CCK8 kit was purchased from Dojindo (Kumamoto, Japan). 4-PBA was purchased from Sigma (Sigma-Aldrich, Shanghai, China). SA-β-gal staining kit was obtained from Beyotime (China). The antibodies used in this study are as follow: GRP78 from Cell Signaling Technology (Danvers, MA); β-actin from Abcam (Cambridge, UK). Predesigned primers for MMP13, ADAMTS5, COL2A1, and GAPDH were obtained from Biomics Biotechnologies (Nantong, China).

2.2. Isolation and culture of primary chondrocytes

The normal human articular cartilages from 6 trauma patients with mean 46.8 years (age mean, 28–59 years) were incubated with trypsin (Hyclone) at 37 °C for 10 min. After the trypsin solution was removed, the tissue slices were treated for 16 h with type IIcollagenase (Sigma-Aldrich, Shanghai, China) in DMEM with 10% FBS. The isolated chondrocytes were recovered in DMEM supplemented with 10% FBS, 50U/ml penicillin and 50 mg/ml streptomycin. The cells were incubated at 37 °C in the presence of 5% CO_2.

2.3. Cell viability assays

Cell Counting Kit-8 (CCK8) was employed to assess the number of viable cells according to the manufacturer's protocol. Chondrocytes were plated in 96-well plates at a density of 5000 cells/well and then cultured overnight in complete medium in the presence or absence of IL-1β (10 ng/ml). 10 μl CCK8 solution was added to each well and incubated for 1 h at 37 °C. Finally, the absorbance was read by the microplate reader (Japan Interned, Japan) at 450 nm, which represented the cell viability.

2.4. Apoptosis analysis by flow cytometry

Cell apoptosis were measured using an Annexin V-FITC/PI apoptosis kit. Briefly, chondrocytes were pretreated with 4-PBA, then cells were cultured with IL-1β (10 ng/ml) for 24 h. Cells were harvested, and washed twice with ice-cold PBS. Apoptotic cells were evaluated by double staining with Annexin V–FITC and PI in binding buffer using by flow cytometry (Becton Dickinson).

2.5. Senescence-associated β-galactosidase staining

The cells were plated in 6-well plates and then cultured overnight in complete medium in the presence or absence of IL-1β (10 ng/ml) for 24 h. Senescence-associated β-galactosidase (SA-β-gal) activity was detected with an SA-β-gal staining kit following the manufacturer's protocol.

2.6. Real-time PCR analysis

Cells were cultured in 6-well plates, and total RNA was extracted using Trizol (beyotime) according to the manufacturer's protocol. 1 μg of total RNA was reverse-transcribed to first-strand complementary DNA (cDNA). The converted cDNA samples were amplified in triplicate by real-time PCR (Roche cobas z 480) in a final volume of 20 μl using SYBR Green. When evaluating the effect of a treatment, the expression level of each control was assigned an arbitrary value of 1, and the treated cells were evaluated as fold change over control and calculated as $2^{-\Delta\Delta Ct}$. GAPDH was used as invariant housekeeping gene internal control.

2.7. Western blot

Cells were lysed in RIPA with 1 mM phenylmethanesulfony fluoride (PMSF, Beyotime) and the protein content of the lysates was determined using a bicinchoninic acid (BCA) protein assay reagent (Pierce, Rockford, USA). Cell lysates were adjusted to equal equivalents of protein and then were applied to SDS–polyacrylamide gels for electrophoresis as described before [27].

2.8. Statistical analysis

The statistical analysis was performed with SPSS 20.0 software. All data were displayed as the mean ± SD. The experiments were performed in triplicate. Differences were made using one-way analysis of variance. P values less than 0.05 were considered significant.

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