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Liraglutide, a glucagon-like peptide-1 receptor agonist, ameliorates inflammation and apoptosis via inhibition of receptor for advanced glycation end products signaling in AGEs induced chondrocytes

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Abstract

Background This study aimed to investigate functions of GLP-1R agonist by liraglutide (LIRA) and revealing the mechanism related to AGEs/RAGE in chondrocytes.

Methods To illustrate potential effect of GLP-1R agonist on AGEs induced chondrocytes, chondrocytes were administrated by AGEs with LIRA and GLP-1R inhibitor exendin. Inflammatory factors were assessed using ELISA. Real-time PCR was used to evaluate the catabolic activity MMPs and ADAMTS mRNA level, as well as anabolic activity (aggrecan and collagen II). RAGE expression was investigated by Western blotting. TUNEL, caspase3 activity and immunofluorescence were performed to test the apoptotic activity.

Results Our results showed that treatment with LIRA at > 100 nM attenuated the AGE-induced chondrocyte viability. Western bolt demonstrated that GLP-1R activation by LIRA treatment reduced RAGE protein expression compared with the AGEs groups. ELISA showed that LIRA hindered the AGEs-induced production of inflammatory cytokines (IL-6, IL-12 and TNF-α) in primary chondrocytes. AGEs induced catabolism levels (MMP-1, -3, -13 and ADAMTS-4, 5) are also attenuated by LIRA, causing the retention of more extracellular matrix (Aggrecan and Collagen II). TUNEL, caspase3 activity and immunofluorescence results indicated that LIRA inhibited the AGEs-induced production of inflammatory cytokines in primary chondrocytes and attenuated the caspase 3 level, leading to the reduced apoptotic activity. All the protective effects are reversed by exendin (GLP-1R blockers).

Conclusions The present study demonstrates for the first time that LIRA, an agonist for GLP-1R which is commonly used in type 2 diabetes reverses AGEs induced chondrocyte inflammation and apoptosis through suppressing RAGE signaling, contributing to reduced catabolism and retention of more extracellular matrix. The above results indicate the possible effect of GLP-1R agonist on treating OA.

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Keywords GLP-1R, Liraglutide, Osteoarthritis, RAGE, AGEs

Background

Osteoarthritis (OA) is the frequently seen progressive and degenerative joint disorders causing limited physical activity, disability and tremendous economic burdens globally [1]. Different factors are associated with OA occurrence and development, like age, sex, body weight, mechanical injury and heredity [2]. Chondrocyte apoptosis and inflammation have found to be related to the occurrence and severity of articular cartilage been degradation, which also has been shown to increase with human OA cartilage [3–5]. Currently, anti-OA conservative treatments focus on inflammation and pain control with anti-inflammatory agents such as analgesics and nonsteroidal anti-inflammatory drugs, aiming to attenuate articular cartilage damage during early OA [6, 7]. However, most of the above treatments only achieve short-term effects, and can not avoid or mitigate OA development. Therefore, it is important to understand OA pathogenic mechanism to explore the new treatments.

Risk factors for knee and hip OA consist of age, sex, obesity and cardiometabolic factors, all of which are central characteristics of metabolic dysfunction. The overlap between OA and metabolic dysfunction enabled scientists to put forward that OA might become a component of the metabolic syndrome [8]. An obvious characteristic of OA refers to the modification of proteins by nonenzymatic glycation. Nonenzymatic glycation is the frequently seen protein modification at the posttranslational level resulting from the reducing sugars. Reducing sugars can spontaneously condense with free amino groups within arginine or lysine residues on proteins, which contributes to forming the reversible Schiff base that can be later stabilized via Amadori rearrangement. Afterwards, browning or Maillard reactions transform those intermediate products generated at first to the advanced glycation end-products (AGEs) [9]. The accumulation of AGEs in cartilage results in inferior mechanical properties [10] and a change in cartilage metabolism [11, 12]. To be specific, cartilage stiffness elevates significantly as AGE level increases, whereas the articular chondrocytes-synthesized matrix is damaged [10]. In a recent study, in vivo effects of AGEs have been detected in a canine model of OA induced experimentally by anterior cruciate ligament transection. Animals showing increased AGE concentrations are more susceptible to OA compared with those exhibiting normal AGE concentrations [13]. The mechanism of AGEs in affecting articular cartilage cellular function needs to be further investigated. Changes of matrix synthesis are regulated via receptors for AGEs. Some AGE receptors have been found, like scavenger receptors type I and type II, oligo saccharyl transferase 48 (AGE-R1), 80 K-H phosphoprotein (AGE-R2), galectin 3 (AGE-R3), and the receptor for advanced glycation end products (RAGE) [9]. RAGE belongs to the immunoglobulin superfamily of cell surface molecules, which can be detected on various cells [14].

During the past century, the discovery and characterization of the incretins, a family of gastrointestinal hormones stimulating insulin production, has stimulated the development of new therapies for the treatment of T2DM, a chronic disease featured by increased blood glucose level resulted from insulin resistance [15]. In addition to its insulinotropic effects, GLP-1 exerts valuable physiological activities, sustained obviously by strong anti-inflammatory activities [16]. GLP-1 can bind to GLP-1 receptor (GLP-1R), a G-coupled receptor readily found in pancreatic β cells, intestine and central nervous system and moderately expressed in blood vessels, pancreatic alpha cells, peripheral nervous system, lungs, heart, kidneys as well as joints [17]. The risk factors for OA overlap with those for metabolic syndrome, GLP-1 is found to exert anti-inflammatory effects on certain tissues and GLP-1R is expressed in joint tissues, so GLP-1 analogues are the promising candidates used to treat OA [18]. Glucagon-like peptide-1 mitigates the diabetes-related osteoporosis of Zucker diabetic fatty rat, probably via RAGE pathway [19]. Liraglutide hinders receptor for advanced glycation end products (RAGE)/reduced form of nicotinamide-adenine dinucleotide phosphate (NAPDH) signaling to ameliorate non-alcoholic fatty liver disease (NAFLD) in vivo and vitro [20]. Liraglutide, the glucagon-like peptide-1 analogue, can mitigate atherogenesis by suppressing AGE-mediated RAGE level in mice with apolipoprotein-E deficiency [21].

Therefore, this study was carried out to determine if GLP-1R activation by LIRA could inhibit AGEs induced chondrocyte inflammation and apoptosis by inhibiting RAGE signal, thus illustrating the mechanism of LIRA in resisting AGEs induced OA chondrocyte.

Materials and methods

AGEs preparation

AGEs preparation was carried out according to previous description after modifications [22]. In brief, bovine serum albumin (BSA, 10 mg/ml) and 0.1 mol/l D-glyceraldehydes contained in 0.2 mol/l phosphate buffer (PBS, pH 7.4) was incubated under 37 °C for 7 days. Thereafter, preparation dialysis was carried out (3 washes/18 h under 4 °C) in PBS (pH 7.4, 4 °C) to remove free D-glyceraldehyde, followed by separation in aliquots and storage under −20 °C before the analysis.

Chondrocyte culture

Chondrocyte was isolated from the knee joint of male Sprague–Dawley rats (3-month-old, weight, 250–300 g, Animal Center of the Chinese Academy of Sciences, Shanghai, China) [23]. The rats were sacrificed via CO_2 inhalation (40% vol/min for 5 min), the breathing and heartbeat of the rat will be reconfirmed as stopped, then their knee-joint cartilage was removed under aseptic conditions. The cartilage tissue was cut into pieces and incubated with collagenase II (2 mg/ml) in cell incubator for 6 h. Next, specimen was filtered via a filtration system (70 μm) and the solution was centrifuged and washed, followed by incubation with DMEM/F12 medium, containing 10% FBS. The medium was altered every two days, and chondrocytes were passaged once reaching 70–80% confluence. Chondrocytes at P2 were administrated with AGEs, LIRA or exendin. Afterwards, cells were harvested in subsequent tests. The animal experiments and procedures were ethically approved by the Animal Ethics Committee of ShangRao People's Hospital.

CCK-8 method

CCK-8 kit (Dojindo, Japan) was used to test cell viability in line with the specific instructions. In brief, cells were inoculated into the 96-well plates and exposed to or not to 24-h AGEs treatment at varying concentrations when they reached the 80–90% confluency, followed by treatment with/without different concentrations of LIRA. Thereafter, every well was introduced with 10 μL CCK-8 dye and incubated for 2 h. The 96-well plate reader was utilized to measure absorbance at 450 nm (Thermo, Rockford, IL, USA).

Table 1 The sequence of primers used for RT-PCR

Genes	Primer sequences
MMP-1	5′- GCTTAGCCTTCCTTTGCTGTTGC – 3′(forward);
	5′- GACGTCTTCACCCAAGTTGTAGTAG – 3′(reverse)
MMP-3	5′- CTGGGCTATCCGAGGTCATG – 3′(forward);
	5′- TGGACGGTTTCAGGGAGGC – 3′(reverse)
MMP-13	5′- AGCCACTTTATGCTTCCTGATG – 3′(forward);
	5′- GATGTTTAGGGTTGGGGTCTTC – 3′(reverse)
ADAMTS-4	5′-AAGCATCCGAAACCCTGTCAACG-3′ (forward);
	5′-AGCCATACCCAGAGCGTCAC-3′ (reverse)
ADAMTS-5	5′-AGAGTCCGAACGAGTTTACG-3′ (forward);
	5′-GTGCCAGTTCTGTGCGTC-3′ (reverse)
Aggrecan	5′- GTCAGATACCCCATCCACACTC-3′(forward);
	5′-CATAAAAGACCTCACCCTCCAT-3′ (reverse)
Collagen II	5′- CTCAAGTCGCTGAACAACCA – 3′(forward);
	5′- GTCTCCGCTCTTCCACTCTG – 3′ (reverse)
β-actin	5′-TCAGGTCATCACTATCGGCAAT-3′(forward);
	5′-AAAGAAAGGGTGTAAAACGCA-3′ (reverse)

Western blot analysis

Following specific treatments, we prepared cell extracts in PBS containing protease inhibitor cocktail. Protein concentration could be calculated with Bio-Rad Laboratories protein reagent (Bio-Rad, USA). Cellular lysates were separated through PAGE gel. Then, the isolated protein was transferred onto PVDF membranes for antibody blotting. The specific antibodies were used (anti-RAGE and anti-β-actin) over night following relative second antibodies. An ECL System (Amersham Biosciences, USA) was used to visualize the immunoreactive bands.

Quantitative RT-PCR

mRNA was isolated with TRIzol method. The concentration of specimen was measured spectrophotometrically at 260 nm, and cDNA was prepared using 1 μg RNA with cDNA Synthesis Kit (Thermo Scientific). PCR was conducted by SYBR Premix Ex Taq™ as follows, 10 min at 95 °C; 15s at 95 °C and 1 min at 60 °C. The targeted mRNA levels were compared with β-actin to acquire the Ct. The sequence of primers used for RT-PCR is showed in Table 1.

Immunofluorescent staining

The treated cells were rinsed by PBS twice and fixed using 3.7% formalin solution under room temperature for 10 min, followed by 10-min permeabilization using 0.5% Triton X-100 in PBS and 60 min blocking using 3%BSA in PBS. Slides were later incubated using specific primary antibody at 4 °C overnight and later rinsed twice by PBS Tween-20 (PBST) (10 min each), followed by 60 min incubation using secondary fuorescence antibodies in PBST under the room temperature. Later, DAPI was added for nuclear staining. Image capturing was completed with the laser confocal microscope (FV10-ASW, Olympus, Tokyo, Japan).

Apoptosis assay

Chondrocytes were harvested to detect cell apoptosis by TUNEL staining (Beyotime, Nanjing, China) in line with the specific instructions. After 30-min fixation using 4% paraformaldehyde under the room temperature, cells were subjected to resuspension using PBS that contained 0.3% Triton X-100. Cells were later incubated for another 5 min under the room temperature, TUNEL solution was added for the staining of apoptotic cells, whereas DAPI was introduced for nuclear staining. The fluorescence microscope was used for cell observation. In addition, the Caspase-3 Activity Assay Kit (Cell Signaling Technology, Beverly, USA) was adopted for measuring caspase-3 activity.

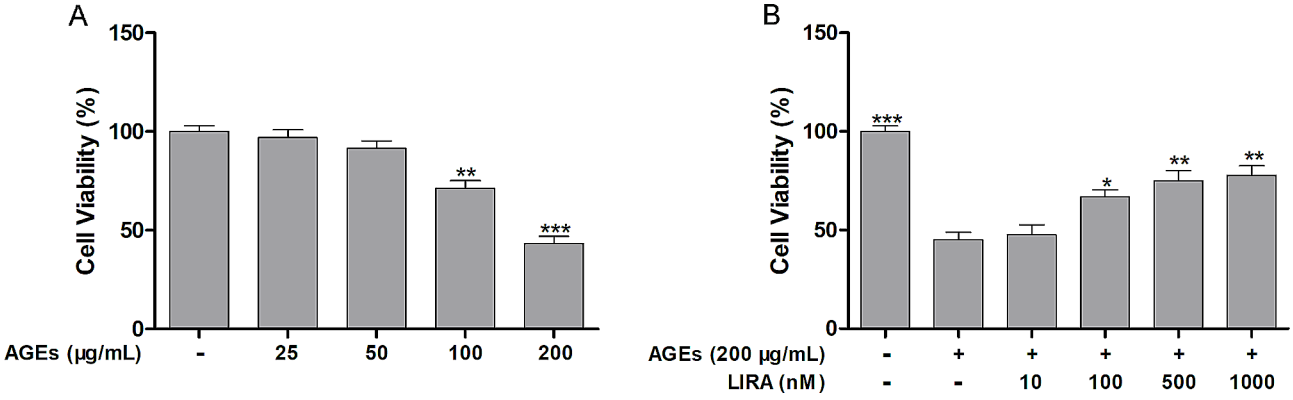

Fig. 1 Effects of GLP-1R on AGE-induced cell viability. **(A)** Cytotoxicity of AGEs varying doses for 24 h to chondrocytes measured by the CCK-8 method. **(B)** CCK-8 analysis on LIRA-exposed chondrocytes under the stimulation by AGEs. Data are presented as means ± SD. * $P < 0.05$, ** $P < 0.05$, *** $P < 0.05$

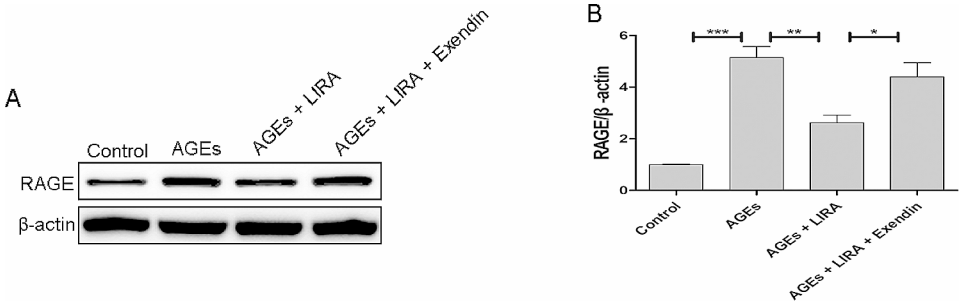

Fig. 2 GLP-1R activation attenuated AGEs-mediated Rage expression in chondrocytes. (**A**-**B**) RAGE protein expression within chondrocytes was measured through Western blotting. Data are presented as means ± SD. * $P < 0.05$, ** $P < 0.05$, *** $P < 0.05$

Enzyme-linked immunosorbent assay (ELISA)

Using the monoclonal antibody-based mouse IL ELISA kit, ELISA was performed to measure ILs (including IL-6, IL-12, and TNF-α) in line with specific protocols (R&D Systems, Minneapolis, MN, USA). Results were indicated in a form of mean±SEM from three or more individual assays and analyzed by ANOVA.

Statistical analysis

Data were represented by mean±standard deviation. One-way ANOVA (analysis of variance) was utilized to compare differences in means among diverse groups. Statistical analysis was carried out with SPSS 13.0 software (SPSS Inc, Chicago, IL, USA).

Results

Functions of GLP-1R in AGEs-mediated cell viability

The AGEs-induced cytotoxicity to chondrocytes was assessed by CCK-8 assay. According to the preliminary experimental results, 24-h treatment of chondrocytes with AGEs(25, and 50 μg/mL) did not induce obvious cytotoxicity, whereas AGEs 100 and 200 μg/mL treatment caused significant decrease of cell viability (Fig. 1A). Therefore, primary chondrocytes were later exposed to 24-h AGEs (200 μg/mL) treatment. Our results showed that treatment with LIRA at >100 nM attenuated the AGE-induced chondrocyte apoptosis (Fig. 1B). Therefore, LIRA at 100, 500 nM was selected in later analysis.

GLP-1R activation attenuated AGEs-mediated rage expression in chondrocytes

For analyzing mechanisms related to aggravation of OA chondrocytes, we determined RAGE level within AGEs induced chondrocytes. RAGE protein level was up-regulated in AGEs group relative to control group. GLP-1R activation by LIRA treatment reduced RAGE protein expression compared with the AGEs groups (Fig. 2). After adding exendin (GLP-1R blockers), RAGE protein expression was found to be lower than that of the AGEs+LIRA group.

GLP-1R activation via LIRA inhibits AGEs-mediated inflammatory cytokine production in primary chondrocytes

Inflammatory factor levels in the supernatant of chondrocyte culture were measured through ELISA. Relative to control group, AGEs significantly elevated IL-1β, IL-6, IL-12 and TNF-α levels (Fig. 3). Our results suggested that activation of GLP-1R by LIRA suppressed the AGEs-mediated increased inflammatory factor expression. After the addition of exendin, inflammatory factors were reversed and significantly increased relative to

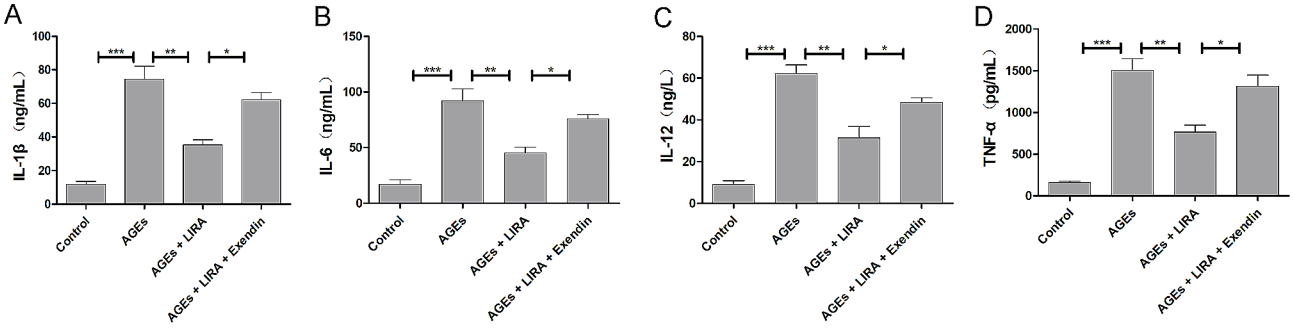

Fig. 3 Activation of GLP-1R by LIRA inhibits the AGEs-induced production of inflammatory cytokines in primary chondrocytes. Levels of IL-1β **(A)**, IL-6 **(B)**, IL-12 **(C)** and TNF-α **(D)** proteins were examined through ELISA. Data are presented as means ± SD. * $P < 0.05$, ** $P < 0.05$, *** $P < 0.05$

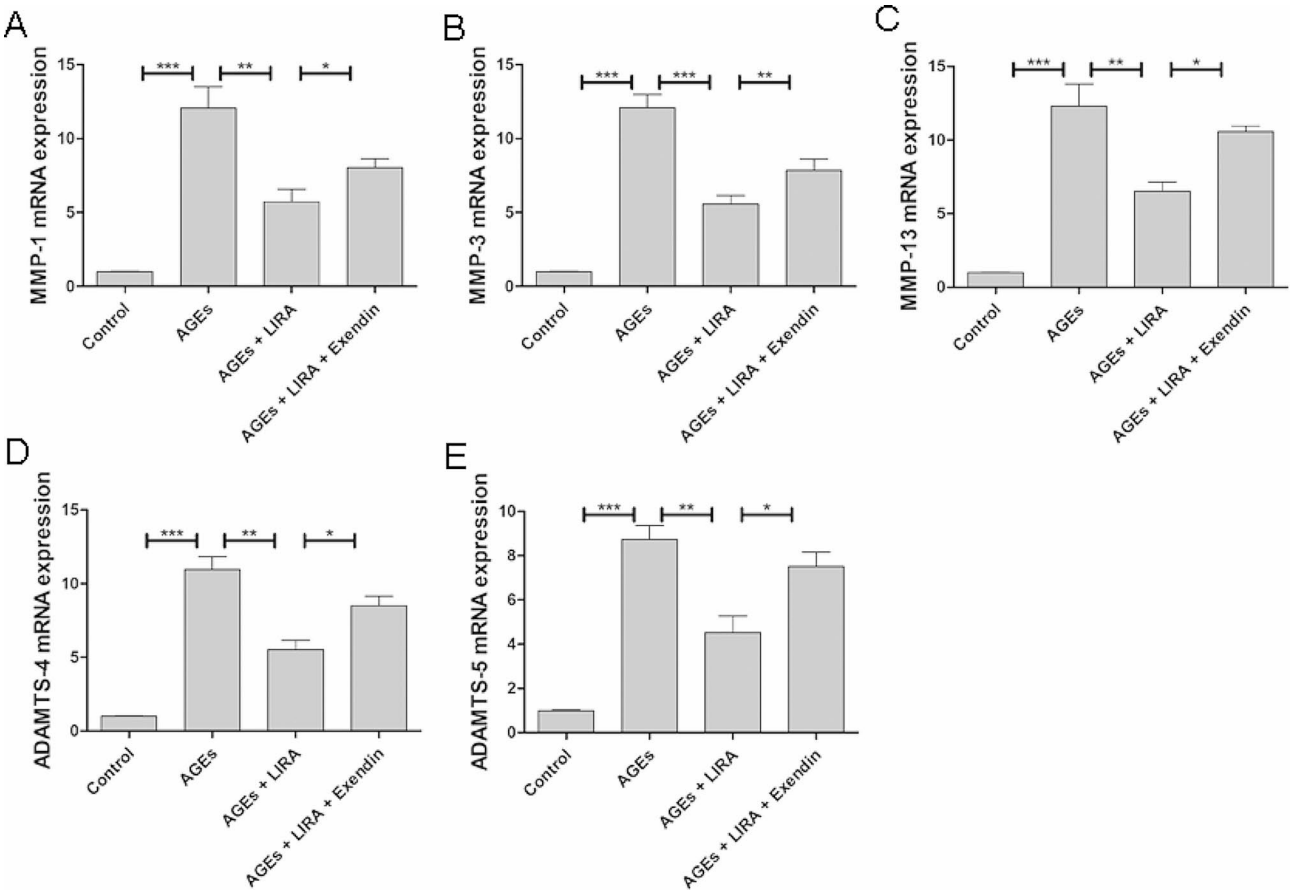

Fig. 4 GLP-1R activation decreases AGEs induced catabolic metabolism in chondrocytes. MMP-1 **(A)**, MMP-3 **(B)**, MMP-13 **(C)**, ADAMTS-4 **(D)** and ADAMTS-5 **(E)** mRNA expression was measured through qPCR. Results are performed as the means ± SD. * $P < 0.05$, ** $P < 0.05$, *** $P < 0.05$

AGEs + LIRA group, indicating that GLP-1R exerted anti-inflammatory effects on AGEs-induced chondrocytes.

GLP-1R activation decreases AGEs induced catabolic metabolism in chondrocytes

Since catabolic enzymes, including MMPs and ADAMTS, have vital functions during OA progression, we explored the production of catabolic factors MMP1, MMP3, MMP13, ADAMTS4 and ADAMTS5, which exert essential roles in cartilage degeneration. These results showed that AGEs enhanced the release of MMP1, MMP3, MMP13, ADAMTS4 and ADAMTS5, during which GLP-1R activation by LIRA decreased the release of the catabolic factors in AGEs-stimulated group. Exendin, again, could reverse the effects of LIRA on the expression of catabolic factors, indicating that GLP-1R activation decreased catabolic activities in AGEs induced chondrocytes (Fig. 4A-E).

Activation of GLP-1R by LIRA promotes anabolic metabolism in AGEs-induced chondrocytes

We detected the major matrix proteins Aggrecan and Type II collagen in chondrocytes by PCR. In our work, GLP-1R activation by LIRA attenuated AGEs-mediated reduction in ECM components (Aggrecan and Type II collagen) (Fig. 5A-B), while this effect was partially reversed by the GLP-1R inhibitor exendin. Immunofluorescence evaluation of Type II collagen protein expression is consistent with the mRNA results (Fig. 5C). Together, all the above results demonstrate that GLP-1R activation via LIRA enhances anabolic metabolism in AGEs-induced chondrocytes.

GLP-1R activation via LIRA suppressed AGEs-induced apoptotic activity in chondrocytes

To explore the effects of GLP-1R activation on resisting apoptosis in AGEs treated chondrocytes, TUNEL method was performed to measure the apoptotic activity of chondrocytes. TUNEL results demonstrated that GLP-1R activation via LIRA significantly reduced apoptotic cell number among AGEs-induced chondrocytes (Fig. 6A-B), which was attenuated by the GLP-1R inhibitor exendin. Based on the Caspase-3 activity assay, LIRA suppressed AGEs-mediated caspase-3 activation, and such effect was abolished via exendin (Fig. 6C). This indicates that activation of GLP-1R by LIRA suppressed AGEs-induced apoptotic activity in chondrocytes.

Discussion

OA is a frequent arthritic disorder with the feature of degraded extracellular matrix and progressively lost articular cartilage. Chondrocyte apoptosis and inflammation have been found to be related to articular cartilage degradation occurrence and severity, which also has been shown to increase within human OA cartilage [3, 4]. The apoptosis of chondrocytes is a main factor associated with cartilage degeneration; besides, it is also the potential anti-OA therapeutic target [24–26]. Caspase-3, which belongs to the caspase family, is capable of cleaving the DNA repair proteins, causing DNA fragmentation and the final cell apoptosis [27]. More and more studies have demonstrated that the occurrence of inflammatory reaction is one of the main causes of the pain, swelling and other symptoms of OA. Proinflammatory factors, like TNF-α, IL-1, IL-6, and IL-12, are up-regulated in bone, synovium, and cartilage, thus facilitating OA [28]. The pro-inflammatory factors, exert an essential effect on accelerating OA development by promoting cartilage degrading enzymes production, such as A disintegrin-like and metalloproteinase with thrombospondin motifs (ADAMTSs) matrix metalloproteinases (MMPs) [29–31]. Matrix-degrading enzymes belonging to MMP family have critical effects on cartilage degradation in OA, where MMP-3 and MMP-13 are the key components during OA catabolism [32, 33]. They are responsible for hydrolyzing protein structures within articular cartilage ECM, including aggrecan and type II collagen [34, 35]. In addition, aggrecanases like ADAMT-4/5 are found to promote ECM degradation through inducing aggrecan and proteoglycan cleavage in the matrix [36]. Currently, anti-OA conservative treatments focus on inflammation and pain control with anti-inflammatory agents such as analgesics and nonsteroidal anti-inflammatory agents, aiming to attenuate articular cartilage damage during early OA [6]. Nonetheless, most of the above treatments only achieve short-term effects, and can not avoid or mitigate OA development.

AGEs production and accumulation are the typical age-associated alterations. AGEs are known to promote tissue stiffness, reduce collagen and proteolytic production and have a certain influence on numerous cellular processes [37, 38]. AGEs have been increasingly suggested to exist at an increased level within the cartilages in patients with OA [39]. The promoted AGEs occurrence and accumulation exert an essential effect on OA occurrence and development [40]. Nonetheless, it remains largely unclear the relation of AGEs with OA genesis and progression. Reducing sugars are spontaneously condensed with free amino groups within arginine or lysine residues on proteins, as a result, the reversible Schiff base is formed, and it can be later stabilized through Amadori rearrangement. Afterwards, browning or Maillard reactions transform those intermediate products generated initially to AGEs [9]. AGEs will thus accumulate within cartilage, causing the less favorable mechanical properties [10] and the changed cartilage metabolism [11, 12]. Apart from the typical AGE generation pathway, AGE formation is discovered to be initiated through lipid peroxidation and metal-catalyzed glucose autooxidation (thus providing the relation of lipid metabolism with OA occurrence). Such diverse reaction pathways can lead to various AGEs chemical structures. Certain AGEs can be adducts to proteins, whereas additional other AGEs show the inter-protein crosslinks. Articular cartilage collagen shows an extremely long half-life, its low turnover helps to accumulate AGEs within articular cartilage [8], because the AGE accumulation rate can be substantially determined by protein turnover rate [11]. RAGE, together with the ligand accumulation (including amyloid fibrils, AGEs, S100/calgranulins, together with high mobility group box chromosomal protein 1 (referred to as amphoterin before), is related to some pathological conditions, like tumors, diabetes, dialysis-related amyloidosis, and immune/inflammatory disorders [14, 41]. RAGE activation via its ligands will enhance inflammatory response in some diseases, while in others, it is related to cell migration and/or tumor metastasis [14].

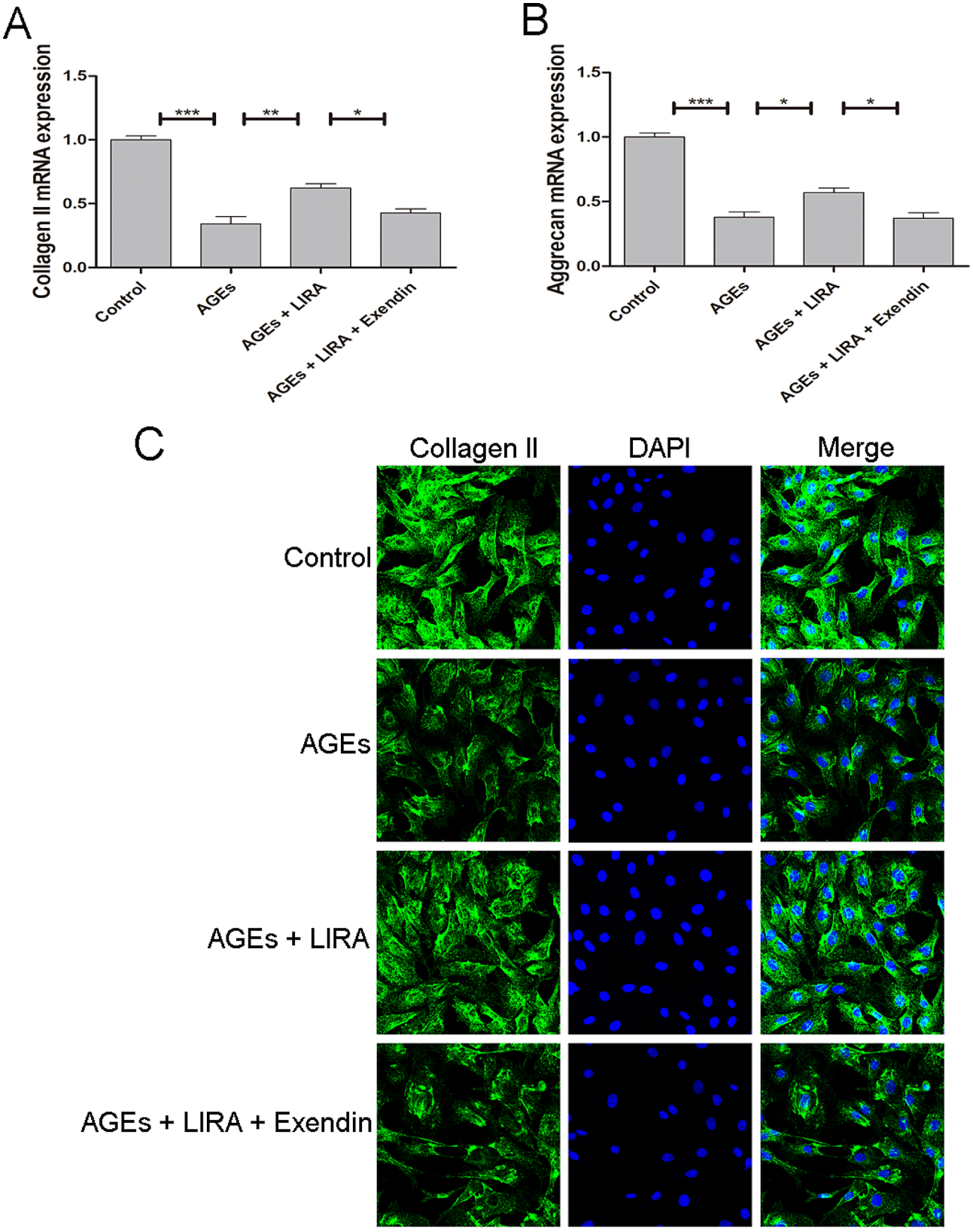

Fig. 5 Activation of GLP-1R by LIRA promotes anabolic metabolism in AGEs-induced chondrocytes. (**A**-**B**) Relative collagen-II and Aggrecan mRNA levels of different groups. **(C)** Immunofluorescence evaluation of collagen-II protein in chondrocytes. The results are indicated as the means ± SD. * $P < 0.05$, ** $P < 0.05$, *** $P < 0.05$

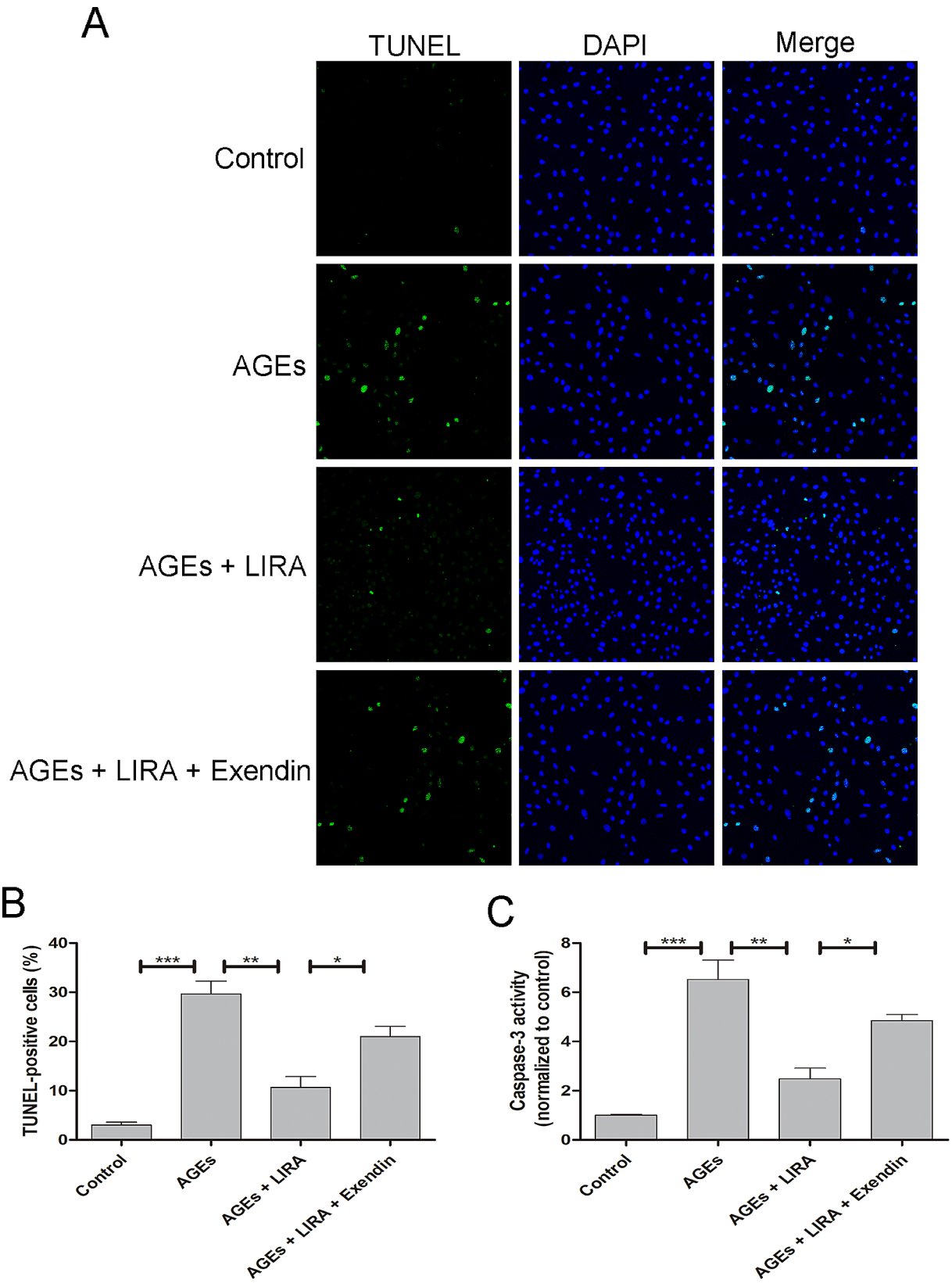

Fig. 6 GLP-1R activation by LIRA suppressed AGEs-induced apoptotic activity of chondrocytes. (**A**-**B**) TUNEL assay used to detect apoptosis. Blue symbolizes nuclear DAPI staining. **(C)** Caspase3 activity in chondrocyte of each group. The results are indicated as the means ± SD. * $P < 0.05$, ** $P < 0.05$, *** $P < 0.05$

The increased AGE expression can activate catabolic pathways via RAGE, because chondrocytes stimulated by AGEs leads to increased inflammatory factors (TNF-α, IL-1β, IL-6, IL-12) and increased apoptotic level, contributing to increased catabolic metabolism (MMP-1, -3, -13 and ADAMTS-4, 5). Therefore, chondrocytes stimulated by AGEs exhibited lower levels of matrix synthesis (Aggrecan and Collagen II). Meanwhile, AGEs can significantly increase the level of RAGE in chondrocytes to enhance the pathway effect of AGEs/RAGE.

OA and type 2 diabetes mellitus (T2DM), the frequently seen disorders, are related to each other. OA is found to be linked with diabetes partially via hyperglycemia, and it can induce tissue and cellular toxicities in joint tissues [42]. Glucagon-like peptide-1 (GLP-1) stands for a major incretin hormone that can promote glucose-dependent insulin production via pancreatic β cells. Nonetheless, native human GLP-1 can be rapidly degraded via dipeptidyl peptidase 4 (DPP-4), which can restrict its clinical application while promoting GLP-1 analogue development (such as, liraglutide, exenatide, semaglutide, lixisenatide) with resistance to DPP-4 cleavage, and they are currently used to treat T2DM [43]. Apart from the insulinotropic activity, GLP-1 displays the favorable physiological effects, which is markedly maintained via the potent anti-inflammatory effects [16, 44]. GLP-1 can bind to GLP-1 receptor (GLP-1R), the G-coupled receptor discovered within pancreatic β cells, intestine together with central nervous system, with moderate expression within pancreatic alpha cells, blood vessels, peripheral nervous system, hearts, lungs, kidneys and joints [17]. As OA shares certain risk factors with metabolic syndrome, GLP-1 is suggested with anti-inflammatory effect on multiple tissues, GLP-1R is expressed within joint tissues, and GLP-1 analogues are the potential candidates used to treat OA [18]. Previous studies find that GLP-1R receptor agonists can protect against various diseases by regulating the AGEs/RAGE signaling. Glucagon-like peptide-1 mitigates diabetic osteoporosis of Zucker diabetic fatty rat, probably via RAGE pathway [19]. Liraglutide suppresses the RAGE/NAPDH pathway for mitigating NAFLD in vivo and in vitro [20]. Liraglutide, the glucagon-like peptide-1 analogue, can alleviate atherogenesis by suppressing AGEs-induced RAGE expression in mice with apolipoprotein-E deficiency [21].

Then, we investigated the function of GLP-1R Agonist by LIRA in AGEs induced chondrocytes. Our results showed that treatment with LIRA at >100 nM attenuated the AGE-induced chondrocyte apoptosis. LIRA reduced RAGE protein expression compared with the AGEs groups. LIRA inhibits the AGEs-induced production of inflammatory cytokines in primary chondrocytes and attenuated the caspase 3 level, causing the reduced apoptotic activity. As a result, AGEs induced catabolism levels (MMP-1, -3, -13 and ADAMTS-4, 5) are also attenuated by LIRA, leading to the retention of more extracellular matrix (Aggrecan and Collagen II). All the protective effects are reversed by exendin (GLP-1R blockers), indicating that the GLP-1R agonist can directly protect chondrocytes stimulated by AGEs by regulating the RAGE signaling pathway.

The study exploring (LIRA's impact on chondrocytes in the context of AGEs-induced damage presents several limitations, including its reliance on in vitro models that may not fully mimic in vivo conditions, and limited evaluation of potential species-specific differences, and lack of long-term efficacy and safety data. Addressing these limitations through expanded research is crucial for assessing LIRA's therapeutic potential for osteoarthritis.

To sum up, this study is the first to suggest that LIRA, an agonist for GLP-1R frequently utilized to treat type 2 diabetes, abolishes the AGEs-mediated chondrocyte inflammation and apoptosis via suppressing RAGE signaling, contributing to reduced catabolism and retention of more extracellular matrix. Our findings suggest the potential therapeutic value of GLP-1R agonist for the treatment of OA, especially OA with diabetes.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12891-024-07640-6.

Supplementary Material 1

Supplementary Material 2

Acknowledgements

Not applicable.

Author contributions

XZ, JC, GW and YG designed the study. XZ, JJ and JC performed the experiments and analyzed the data. XZ, JX and JC contributed the essential reagents or tools and wrote the manuscript. All authors contributed to revising the manuscript and approved the final version to be submitted.

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Data availability

The datasets used or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

All methods are reported in accordance with ARRIVE guidelines. All procedures were conducted in strict accordance with the institutional guidelines for laboratory animal treatment. The animal experiments and procedures were ethically approved by the Animal Ethics Committee of ShangRao People's Hospital (2022-85).

Competing interests

The authors declare no competing interests.

Consent for publication
Not applicable.

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