Experimental Cell Research 348 (2016) 201–208

Contents lists available at ScienceDirect

Experimental Cell Research

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

PEP-1-SIRT2-induced matrix metalloproteinase-1 and -13 modulates type II collagen expression via ERK signaling in rabbit articular chondrocytes

Seong-Hui Eo [a], Soo Young Choi [b], Song Ja Kim [a,*]

[a] Department of Biological Sciences, College of Natural Sciences, Kongju National University, 56 Gongjudaehak-ro, Gongju, Chungnam 32588, Republic of Korea
[b] Department of Biomedical Science and Research Institute of Bioscience and Biotechnology, Hallym University, Chunchon, Republic of Korea

ARTICLE INFO

Article history:
Received 1 September 2016
Received in revised form
27 September 2016
Accepted 30 September 2016
Available online 30 September 2016

Keywords:
Matrix Metalloproteinases
Type II collagen
PEP-1-SIRT2
Chondrocytes
ERK signaling

ABSTRACT

Matrix metalloproteinases (MMPs) are critical for the degradation of the extracellular matrix (ECM), which includes cartilage-specific collagen types I, II and XI. We previously found that PEP-1-sirtuin (SIRT) 2 could induce dedifferentiation of articular chondrocytes; however, the underlying mechanisms remains unclear. We addressed this in the present study by examining the association between PEP-1-SIRT2 and the expression of MMP-1 and MMP-13 and type II collagen in rabbit articular chondrocytes. We found that PEP-1-SIRT2 increased MMP-1 and -13 expression in a dose- and time-dependent manner, as determined by western blotting. A similar trend in MMP-1 and -13 levels was observed in cultures during expansion to four passages. Pharmacological inhibition of MMP-1 and -13 blocked the PEP-1–SIRT2-induced decrease in type II collagen level. Phosphorylation of extracellular regulated kinase (ERK) was increased by PEP-1–SIRT2; however, treatment with the mitogen-activated protein kinase inhibitor PD98059 suppressed PEP-1–SIRT2-induced MMP-1 and -13 expression and dedifferentiation while restoring type II collagen expression in passage 2 cells. These results suggest that PEP-1–SIRT2 promotes MMP-induced dedifferentiation via ERK signaling in articular chondrocytes.

1. Introduction

Osteoarthritis (OA) is a degenerative disease characterized by loss of chondrocyte function in mammals and affecting the functioning of articular cartilage tissue in joints, resulting in the destruction of surrounding extracellular matrix (ECM) [1,2]. The main components of cartilage ECM are collagens and sulfate proteoglycans; the degradation of aggrecan and type II collagen in the ECM can lead to severe joint pain and loss of movement. These proteins can be directly cleaved by matrix metalloproteinases (MMPs) and aggrecanases [3–5]. For example, in pathological conditions such as OA and rheumatoid arthritis (RA), chondrocytes upregulate the expression of these enzymes, resulting in aberrant cartilage destruction [6].

MMPs are a large family of zinc-dependent proteolytic enzymes that degrade target proteins in the ECM [6]. At least 28 members have been identified thus far, including the collagenases MMP-1, −8, −13, and −18 that cleave interstitial collagen types I, II, and III. MMP-2 and −9 are gelatinases that degrade both collagens and gelatins, while MMP-3, −10, and −11 are stromelysins. These enzymes are expressed in various cell types and play a key role in diverse cellular processes [7,8]; their expression is regulated by the mitogen-activated protein kinase (MAPK) pathway in fibroblast-like chondrocytes and osteoblasts [9].

Seven sirtuins (SIRT1–7) have been identified in mammals that exhibit NAD+-dependent deacetylase (SIRT1–3, 5, and 7) or ADP-ribosyl-transferase (SIRT4 and 6) activity [10,11]. SIRTs are involved in many biological process such as DNA repair, cell cycle regulation, cellular metabolism, apoptosis, and aging [12–14]. SIRT6 overexpression protected against OA by inhibiting the inflammatory response and chondrocyte senescence [15]. Recent studies have reported that SIRT1 proteins levels are reduced in chondrocytes derived from OA as compared to normal cartilage [16]. Mammalian SIRT2 is predominantly cytoplasmic [14], and deacetylates various cytoskeletal proteins including α-tubulin, histones, and forkhead proteins [17]. Some studies have indicated that SIRT2 expression levels increase during mitosis, and that its

Abbreviation: PB, phosphate-buffered saline; MMPs, Matrix Metalloproteinases; ECM, extracellular matrix; ERK, extracellular regulated kinase; OA, Osteoarthritis; RA, rheumatoid arthritis; SIRT, Sirtuin; MAPK, mitogen-activated protein kinase; JNK, c-Jun N-terminal kinase; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; MMPI, MMP inhibitor; PD, PD98059

* Corresponding author.
E-mail addresses: eoseonghee@kongju.ac.kr (S.-H. Eo), sychoi@hallym.ac.kr (S.Y. Choi), ksj85@kongju.ac.kr (S.J. Kim).

http://dx.doi.org/10.1016/j.yexcr.2016.09.024
0014-4827/

activity is required for cell survival [18]. SIRT2 inhibition has shown beneficial effects in Parkinson's and Huntington's disease models; on the other hand, its overexpression was found to protect against neurodegeneration in mouse models of Alzheimer's disease [17,19].

Protein transduction domains such as Tat and PEP-1 can be used to deliver hydrophilic macromolecules such as proteins into cells or tissues [20]. A recent study reported the use of PEP-1 to transport SIRT2 into epithelial and RAW264.7 cells. Transducing cells with PEP-1–SIRT2 reduced inflammation by attenuating cytokine expression and nuclear factor (NF)-κB and MAPK activation [21,22]. We previously showed that PEP-1–SIRT2 is efficiently transduced into rabbit chondrocytes and induces dedifferentiation of rabbit articular chondrocytes by stimulating cyclooxygenase-2 expression via MAPK signaling [23].

Components of the MAPK pathway include extracellular signal-regulated kinase (ERK)-1/2, p38, and c-Jun N-terminal kinase (JNK); these proteins convert extracellular stimuli into specific cellular responses such as chondrocyte proliferation and differentiation [24]. For example, gallotanin induces differentiation and inflammation via ERK-1/-2 and p38 pathways [25], while salinomycin causes dedifferentiation via ERK activation in rabbit articular chondrocytes [26]. We also found that resveratrol stimulates differentiation and inflammation in these cells via ERK, p38, and Akt pathways [27]. However, it is unclear how PEP-1–SIRT2 induces MMP-mediated differentiation of articular chondrocytes.

We addressed this in the present study by investigating the effects of PEP-1–SIRT2 on MMP-1 and -13 and collagen type II expression and ERK activation in rabbit articular chondrocytes. We found that PEP-1–SIRT2 attenuates MMP-1 and -13 and type II collagen expression via upregulation of ERK signaling.

2. Materials and methods

2.1. Antibodies and proteins

PET–SIRT2 [control (C-)SIRT2] and PEP-1–SIRT2 proteins were a gift from Prof. Choi (Department of Biomedical Science and Research Institute of Bioscience and Biotechnology, Hallym University, Korea). Primary antibodies specific for MMP-13, type II collagen, glyceraldehyde 3-phosphate dehydrogenase (GAPDH), and SIRT2 were obtained from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Antibodies against MMP-1 and phosphorylated (p) ERK were from Calbiochem (San Diego, CA, USA) and Cell Signaling Technology (Beverly, MA, USA), respectively.

2.2. Rabbit articular chondrocyte culture

Rabbit articular chondrocytes were harvested from 2-week-old New Zealand white rabbits (Koatech, Pyeougtaek, Korea). Cartilage slices were digested for 8 h in 0.2% collagenase type II (381 U/ml of solid) in Dulbecco's modified Eagle's medium (DMEM; Invitrogen, Carlsbad, CA, USA), and cells were collected by brief centrifugation at 1000 rpm and 25 °C for 10 min and resuspended in DMEM supplemented with heat-inactivated 10% (v/v) bovine calf serum (Invitrogen), 50 μg/ml streptomycin (Sigma-Aldrich, St. Louis, MO, USA), and 50 U/ml penicillin (Sigma-Aldrich). Cells were seeded at 5×10^4 cells/cm^2 in 35-mm culture dishes and incubated for 3 days at 37 °C in a humidified incubator of 5% CO_2/95% air. After about 3 days, the medium was replaced with fresh medium and cells were treated with PEP-1–SIRT2. To induce dedifferentiation, passage (P)0 cells were expanded every 3 days (P1–4) by seeding at density of 5×10^4 cells/cm^2 per subculture. The study protocol was approved by the ethics committee of Kongju National University, Gongju, Korea (IRB no. 2011-2).

2.3. Cell viability assay

Cells were seeded in 96-well plates (1×10^4 cells/well) and incubated overnight to allow attachment. The next day, cells were treated with various concentrations of PEP-1–SIRT2 or C–SIRT2 or were left untreated in the absence or presence of MMP inhibitor (MMPI) or MAPK kinase inhibitor PD98059 (PD) for 24 h. The medium was replaced with fresh medium, and 10 μl of 10 mg/ml 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium (MTT) reagent solution (Sigma-Aldrich) was added to each well, followed by incubation at 37 °C for 4 h; 100 μl of MTT reagent II [solubilization buffer containing 10% sodium dodecyl sulfate (SDS) with 0.01 N HCl and dimethylsulfoxide] were then added to each well, and cells were incubated overnight at 37 °C. The optical density of each well was measured at 595 nm using a microplate reader.

2.4. Western blot analysis

For western blotting analysis, cells were lysed in cold radioimmunoprecipitation assay buffer composed of 50 mM Tris-HCl (pH 7.4), 150 mM NaCl, 1% Nonidet P-40%, and 0.1% SDS and supplemented with protease and phosphatase inhibitors. Proteins in cell lysates were resolved by 8% SDS-polyacrylamide gel electrophoresis and transferred to a nitrocellulose membrane (Whatman Schleicher and Schuell; Dachen, Germany), which was blocked with 5% non-fat dry milk for 1 h at room temperature and incubated overnight at 4 °C with primary antibodies. The membrane was then washed three times with Tris-buffered saline with 0.1% Tween-20 and incubated with horseradish peroxidase-conjugated secondary antibody (Sigma-Aldrich) for 2 h at room temperature. Proteins were detected by chemiluminescence and bands were visualized using the LAS-4000 imaging system (GE Healthcare, Little Chalfont, UK).

2.5. Immunocytochemistry

Chondrocytes were fixed with 3.5% paraformaldehyde in phosphate-buffered saline (PBS) for 20 min at room temperature, and permeabilized with 0.1% Triton X-100 in PBS for 15 min. Cells were washed three times with PBS and incubated for 1 h with antibodies against MMP-1 and -13, type II collagen, and His. After washing with PBS, cells were incubated with secondary antibodies for 1 h, washed again with PBS, and observed under a fluorescence microscope (Olympus, Tokyo, Japan).

2.6. Alcian Blue staining

Rabbit chondrocytes were fixed with 3.5% paraformaldehyde in PBS at room temperature for 20 min. The cells were stained overnight with 0.1% Alcian Blue in 0.1 M HCl, washed three times with PBS, and then incubated with 6 M guanidine HCl for 6 h. The production of sulfated proteoglycan was measured at 620 nm using a microplate reader.

2.7. Statistical analysis

At least three independent experiments were carried out in triplicate for each analysis. Data are presented as the mean ± standard error of the mean. Results were analyzed by one-way analysis of variance, and all pairwise comparisons between groups were carried out using the Turkey post-hoc test. P values ≤ 0.05 were considered statistically significant.