Original Paper

Cyclic Mechanical Stretching Induces Autophagic Cell Death in Tenofibroblasts Through Activation of Prostaglandin E2 Production

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Key Words

Prostaglandin E2 • Autophagy • Cell death • Tendinopathy

Abstract

Background/Aims: Autophagic cell death has recently been implicated in the pathophysiology of tendinopathy. Prostaglandin E2 (PGE2), a known inflammatory mediator of tendinitis, inhibits tenofibroblast proliferation *in vitro*; however, the underlying mechanism is unclear. The present study investigated the relationship between PGE2 production and autophagic cell death in mechanically loaded human patellar tendon fibroblasts (HPTFs) *in vitro*. **Methods:** Cultured HPTFs were subjected to exogenous PGE2 treatment or repetitive cyclic mechanical stretching. Cell death was determined by flow cytometry with acridine orange/ethidium bromide staining. Induction of autophagy was assessed by autophagy markers including the formation of autophagosomes and autolysosomes (by electron microscopy, AO staining, and formation of GFP-LC3-labeled vacuoles) and the expression of LC3-II and BECN1 (by western blot). Stretching-induced PGE2 release was determined by ELISA. **Results:** Exogenous PGE2 significantly induced cell death and autophagy in HPTFs in a dose-dependent manner. Blocking autophagy using inhibitors 3-methyladenine and chloroquine, or small interfering RNAs against autophagy genes *Becn-1* and *Atg-5* prevented PGE2-induced cell death. Cyclic mechanical stretching at 8% and 12% magnitudes for 24 h significantly stimulated PGE2 release by HPTFs in a magnitude-dependent manner. In addition, mechanical stretching induced autophagy and cell death. Blocking PGE2 production using COX inhibitors indomethacin and celecoxib significantly reduced stretching-induced autophagy and cell death. **Conclusion:** Taken together, cyclic mechanical stretching induces autophagic cell death in tenofibroblasts through activation of PGE2 production.

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Introduction

Tendinopathy is a common clinical problem in sports and occupational settings in modern society [1]. Chronic or acute tensile overload can lead to tendon inflammation and degeneration. Prostaglandin E₂ (PGE₂) is a key inflammatory mediator in tendinopathy. When the rotator cuff is torn, interleukin-1 β (IL-1 β) is produced in the torn tendon, stimulating cyclooxygenase 2 (COX-2) expression and subsequent prostaglandin E₂ (PGE₂) production [2]. In *in vitro* model systems, mechanical loading often don fibroblasts or isolated tendon increases PGE₂ production and release [3-5]. Exogenous PGE₂ treatment inhibits tendon fibroblast proliferation and collagen synthesis *in vitro* [6] and causes localized tendon disorganization and degeneration *in vivo* [7, 8]. However, the mechanism by which PGE₂ inhibits tendon fibroblast proliferation is unclear.

Excessive cell death in degenerative tendon can impair collagen synthesis and repair, contributing to the pathophysiology of tendinopathy [9]. Apoptosis and autophagy are both forms of programmed cell death. Apoptosis is a recognized causative factor in tendinopathy. Increased apoptosis has been described in human tendinopathic tissues [10, 11] as well as in animal tendon after high-strain mechanical loading [12]. In *in vitro* systems, mechanical stretching can induce apoptosis in human tendon fibroblasts via oxidative stress-associated pathways [13]. Recently, autophagy has been implicated as another contributing factor in tendinopathy. Autophagy, a basic catabolic mechanism to remove unnecessary or dysfunctional cellular components through the actions of lysosomes, promotes cellular survival during starvation by maintaining cellular energy levels [14]. However, autophagy can cause degradation of proteins and organelles essential for cell survival, which eventually leads to cell death [15]. Both apoptosis and autophagic cell death have been observed in human tendinopathic tissues [16, 17], with up to 51.9% autophagic cell death being detected in tendon samples collected from patients undergoing surgery for rotator cuff tears [17]. However, the factors causing autophagic cell death in tendinopathic tissues are not clear. In the present study, we investigated the effects of exogenous PGE₂ and repetitive mechanical loading on the induction of autophagy and cell death in human patellar tendon fibroblasts *in vitro*. The relationship between mechanical loading, PGE₂ production, and autophagic cell death were investigated.

Materials and Methods

Reagents

A Acridine orange (AO), ethidium bromide (EB), 3-MA, chloroquine, were purchased from Sigma-Aldrich Canada (Oakville, ON, Canada). Hoechst 33342 staining kit was purchased from Molecular Probes (Eugene, OR). All the antibodies used in this study were bought from Cell Signaling Technology, Inc. (Beverly, MA, USA).

Isolation and culture of human patellar tendon fibroblasts

Human patellar tendon fibroblasts (HPTFs) were isolated from tendon pieces trimmed from patellar tendon autografts of two healthy male donors (16 and 31 years old) and one healthy female donors (25 years old) who underwent reconstruction of the anterior cruciate ligament. The protocol for obtaining the tendon samples was approved by the Research Ethics Committee of Shanghai Sixth People's Hospital (Shanghai, China). HPTFs were isolated and expanded as described previously [4]. Briefly, the tendon samples were thoroughly washed with phosphate buffered saline (PBS), minced, and incubated in a 100-mm Petri dish in DMEM containing 10% heat-inactivated fetal bovine serum (FBS, Invitrogen, Carlsbad, CA, USA) and 1% penicillin/streptomycin (P/S; Invitrogen) at 37°C, 5% CO₂ in a humidified incubator. When the cells grew to 100% confluence, they were harvested and subcultured. Cells after 5 to 7 passages were used in stretching experiments.

Cell death determination

Cell death was determined by flow cytometry (BD FACS Calibur) using acridine orange/ethidium bromide (AO/EB) staining. Live cells are permeable to AO but not EB and stained green. Dead cells are permeable to both AO and EB and stained orange. Data were acquired and analyzed using CellQuest software (Becton Dickinson, USA). In addition, cell membrane permeability was assessed microscopically using both AO/EB and Hoechst staining.

Quantification of Acidic Vesicular Organelles (AVO) with AO Staining

Autophagy is the process of sorting cytoplasmic constituents into vesicles known as AVOs (autophagosomes and autolysosomes) for lysosomal degradation. To assess the induction of autophagy, we measured the formation of AVOs using flow cytometry with AO staining as described previously [18, 19]. AO is a fluorescent weak base that accumulates in acidic spaces. AVOs fluoresce bright red in AO-stained cells [19]. Briefly, cells were collected by centrifugation, resuspended in 1 ml PBS, and stained with AO (100 µg/ml) for 15 - 20 min. After washing twice with PBS, cells were resuspended in 0.3 ml PBS and analyzed on a flow cytometer (BD FACS Calibur) using CellQuest software.

Detection of autophagosomes with GFP-LC3 labeling

GFP-LC3-expressing constructs were obtained from Addgene (Cambridge, MA, USA). Cells were transfected with 1 µg of GFP-LC3 cDNA in a mammalian expression vector using Lipofectamine 2000 reagent (Invitrogen) for 4 h. The transfected cells were seeded in six-well plates (2 × 10⁵ cells/well) and cultured overnight. On the next day, cells were treated with PGE₂ at 37°C for 24 h, and GFP-LC3-labeled vacuoles (autophagosomes) were counted as described previously [18] under an Olympus microscope equipped with a coolsnap camera. When 3-MA (2 mM) or chloroquine (30 µM) was included in the treatment, cells were pre-incubated with the compound at 37°C for 1 h prior to PGE₂ treatment.

Silencing of Becn-1 and ATG5 by siRNAs

The small interfering RNAs (siRNA) specific for human Becn-1 and ATG5 were purchased from Dharmacon (Lafayette, CO, USA). A scrambled RNA from was used as the control. The same amount of cells were seeded in a Petri plate (100×20 mm) and incubated at 37°C overnight. On the next day, cells were transfected with siRNA (scrambled, Becn-1, or ATG5) for 6 h using Lipofectamine RNAiMax (Invitrogen) following manufacturer's instructions. The transfected cells were collected, seeded in six-well plates (2 × 10⁵ cells/well), and cultured overnight. On the next day, cells were treated with PGE₂ at 37°C for 24 h. Cell death and the formation of AVOs were analyzed as above. To assess the formation of autophagosomes, cells were transfected with GFP-LC3 prior to siRNA transfection.

Ultrastructural analysis by electron microscopy

Cells were fixed in 2% paraformaldehyde for 2 h and then post-fixed in 1% OsO₄ for 1.5 h. After washing with PBS, the cells were stained with 3% aqueous uranyl acetate for 1 h, washed with PBS, dehydrated with graded alcohol, and embedded in Epon-Araldite resin (Canemco). Ultrathin sections were cut on a Reichert ultramicrotome, counterstained with 0.3% lead citrate, and examined on a Philips EM420 electron microscope.

Western blot analysis

Cells were lysed in NP-40 lysis buffer (50 mM HEPES, 150 mM NaCl, 50 µM ZnCl₂, 50 µM NaF, 2 mM EDTA, 1 mM sodium vanadate, 1.0% NP-40, 2 mM phenylmethylsulfonyl fluoride; pH 7.25). The protein concentration was determined using the Bradford assay. Proteins were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to nitrocellulose membranes (Millipore, USA). After blocking with 5% non-fat dry milk for 1 h, the membranes were incubated with anti-LC3-I, anti-LC3-II, anti-BECN1, anti-Atg5, and anti-β-actin antibodies, respectively, at 4°C overnight. After washing, the membranes were incubated with secondary antibodies conjugated to horseradish peroxidase. Protein bands were visualized using enhanced chemiluminescence reagents (Pierce (Thermo Scientific), Waltham, USA). Tris-Tricine SDS-PAGE was used for the detection of LC3-I and LC3-II. Tris-glycine SDS-PAGE was used for the detection of all other proteins.

In vitro cell stretching experiments

Cyclic mechanical stretching was applied to cultured HPTFs in an *in vitro* model system as described previously [4, 20, 21]. Briefly, A total of 2×10^5 HPTFs were grown on the microgrooved surface of a custom-made silicone dish in DMEM containing 10% FBS and 1% P/S. The silicon dish was pre-coated with 10 µg/ml of ProNectin-F (Sigma, USA) to promote cell attachment. After 24 h, the medium was replaced with fresh DMEM containing 1% FBS and 1% P/S. After another 36–48 h incubation, the cells were cyclically stretched with 4%, 8%, or 12% magnitude at a constant stretching frequency of 0.5, 1, or 1.5 Hz for a duration of 24 h. Non-stretched HPTFs were used as the control. After a 4 h rest, the concentration of PGE₂ in the culture media was determined by ELISA. Cell death and the formation of AVOs were analyzed as described above. To assess the formation of autophagosomes, cells transfected with GFP-LC3 were used in the stretching experiments. When indomethacin (25 µM) or celecoxib (40 µM) was included in the treatment, cells were pre-incubated with the compound for 1 h prior to mechanical stretching.

PGE₂ release by ELISA

The PGE₂ concentration in the culture media was determined using a PGE₂ ELISA kit from R&D Systems Inc. (USA) following manufacturer's instructions. The absorbance at 405 nm was measured on a Spectra MAX190 microplate reader (Molecular Devices, USA). The PGE₂ concentration was calculated using a standard curve created with PGE₂ solutions of known concentrations.

Statistical analysis

All data are expressed as means ± s.e. (standard error). All experiments were performed at least three times. Each experiment was performed in two or more replicates. Data were analyzed using Excel and SigmaPlot. Results from different groups were compared using Student's *t*-test. Differences with a P value less than 0.05 were considered statistically significant.

Results

PGE₂ induces cell death in tenofibroblasts

We isolated and expanded HPTFs as described previously [4]. Sufficient amounts of cells were obtained after 5 passages for experimental studies. To test the effects of PGE₂ on tenofibroblast viability, HPTFs were treated with exogenous PGE₂ at increasing concentrations (range: 1 - 15 ng/ml) for a duration of 12, 24, or 48 h. Immediately after treatment, cells were stained with either AO/EB or Hoechst 33342 and examined under a fluorescence microscope. PGE₂ exposure increased the number of cells stained positive for EB (Fig. 1A) and Hoechst (Fig. 1B) dyes, both indicating increased plasma membrane permeability. Flow cytometric analysis of AO/EB-stained cells showed that exogenous PGE₂ induced HPTF cell death in a dose and time-dependent manner (Fig. 1C). Significant induction of cell death was observed at all PGE₂ concentrations except 1 ng/ml, the lowest concentration used in the study.

PGE₂ induces autophagy in tenofibroblasts

Autophagy is characterized by the formation of AVOs (autophagosomes and autolysosomes). To find out whether exogenous PGE₂ induces autophagy in tenofibroblasts, we treated HPTFs with 10 ng/ml PGE₂ for 24 h, and examined the morphological changes in subcellular organelles by electron microscopy. AVOs were detected in PGE₂-treated but not control cells (Fig. 2A). To quantify the effects of PGE₂ on AVO formation, cells were treated with 0 - 15 ng/ml PGE₂ for 24 h, immediately stained with the AVO marker AO, and subjected to flow cytometric analysis. AVOs fluoresce bright red in AO-stained cells. As shown in Fig. 2B, the percentage of cells stained positive for AVOs increased with the PGE₂ concentration. Compared with untreated cells, cells treated with 5, 10 or 15 ng/ml PGE₂ had significantly greater AVO contents. LC3 is a widely used protein marker for autophagosomes. To directly visualize the formation of autophagosomes, cells were transfected with GFP-LC3 and subjected to PGE₂ treatment. Using fluorescence imaging, we detected GFP-LC3-labeled

Fig. 1. PGE₂ induces cell death in tenofibroblasts. HPTFs were treated with 1, 5, 10 or 15 ng/ml PGE₂ for a duration of 12, 24 or 48 h. Untreated cells were used as the control. Plasma membrane permeability was assessed by AO/EB and nuclear fragmentation or condensation was measured via Hoechst 33342 staining. (A) Fluorescent microscopic images of AO/EB staining. (B) Fluorescent microscopic images of Hoechst 33342 staining. The white arrows show fragmented or condensed nuclei. (C) Cell death determined by flow cytometric analysis with AO/EB staining. n = 3, *P < 0.05 vs. control.

double-membrane vacuoles (autophagosomes) in PGE₂-treated but not control cells (Fig. 2C). Moreover, the percentage of cells with GFP-LC3-labeled vacuoles increased with the PGE₂ concentration (Fig. 2C, bar graph). These data were consistent with results from flow cytometric detection of AVOs using AO staining. To further assess the induction of autophagy, we determined the conversion of LC3-I to LC3-II and the protein level of BECN1 using western blot analysis. The conversion of soluble LC3-I to lipid bound LC3-II is associated with the formation of autophagosomes in autophagy. BECN1 plays an essential role in autophagy initiation. Both are well-recognized protein markers for autophagy. After 24 h PGE₂ treatment, LC3-I stayed essentially unchanged while LC3-II showed a dose-dependent increase (Fig. 2D). Similar to LC3-II, BECN1 was induced by PGE₂ in a dose-dependent manner (Fig. 2D). These changes in autophagy protein markers provide further evidence that PGE₂ induces autophagy in tenofibroblasts.

Blocking autophagy prevents PGE₂-induced cell death in tenofibroblasts

Having demonstrated that PGE₂ induces both cell death and autophagy in tenofibroblasts, we investigated whether autophagy contributes to PGE₂-induced cell death. Autophagy protein 5 (Atg-5) is an E3 ubiquitin ligase required for autophagosome elongation. Silencing of Becn-1 or Atg-5 by siRNA significantly decreased the percentage of cells with AVOs or GFP-LC3-labeled vacuoles after PGE₂ treatment (Fig. 3A). Intriguingly, Becn-1 or Atg-5 silencing also significantly attenuated PGE₂-induced cell death (Fig. 3A). Similarly, blocking autophagy with autophagy inhibitors 3-methyladenine (3-MA) or chloroquine significantly reduced PGE₂-induced cell death (Fig. 3B). Collectively, these results demonstrate that PGE₂-induced cell death in tenofibroblasts is mediated by autophagy.

Fig. 2. PGE₂ induces autophagy in tenofibroblasts. HPTFs were treated with 1, 5, 10 or 15 ng/ml PGE₂ for 24h. Untreated cells were used as the control. (A) Electron microscopic images. Arrows represent double-membrane vacuoles in PGE₂-treated cells (enlarged image). N represents the nucleus. (B) The percentage of cells with AVOs determined by flow cytometry. n = 3, *P < 0.05 vs. control. (C) Representative fluorescent microscopic images showing GFP-LC3-labeled vacuoles (puncta) (left) and the percentage of cells with GFP-LC3 puncta (right). Cells were transfected with GFP-LC3 prior to PGE₂ treatment. n = 3, *P < 0.05 vs. control. (D) The conversion of LC3-I to LC3-II and the expression of BECN1 were determined by western blot. β-Actin was used as loading control.

Fig. 3. Blocking autophagy prevents PGE₂-induced cell death in tenofibroblasts. HPTFs were subjected to 24 h treatment with 0 - 15 ng/ml PGE₂ for 24h. Autophagy (the formation of AVOs and GFP-LC3-labeled vacuoles) and cell death were assessed. (A) Cells were transfected with scrambled siRNA, Becn-1 siRNA, or Atg-5 siRNA prior to PGE₂ treatment. Gene silencing was confirmed by western blot. n = 3, *P < 0.05 vs. scramble. (B) Cells were pre-incubated with 3-MA (2 mM), chloroquine (30 μM), or vehicle only (control) prior to PGE₂ treatment. n = 3, *P < 0.05 vs. control.

Cyclic mechanical stretching induces autophagic cell death in tenofibroblasts through activation of PGE₂ production

Previous studies have shown that repetitive mechanical loading stimulates PGE₂ production in tenofibroblasts [3, 4]. Since we had found that exogenous PGE₂ induces

Fig. 4. Cyclic mechanical stretching induces autophagic cell death in tenofibroblasts through activation of PGE₂ production. HPTFs grown in a custom-made silicone dish were cyclically stretched for 24 h followed by a 4 h rest. Non-stretched cells were used as the control. (A) The PGE₂ concentration in the culture media after 4%, 8%, or 12% stretching at 1 Hz. $5 \leq n \leq 10$, * $P < 0.05$ vs. control. (B) The PGE₂ concentration in the culture media after 12% stretching at 0.5, 1.0, or 1 Hz. $5 \leq n \leq 10$, * $P < 0.05$ vs. control. (C - F) The PGE₂ concentration in the culture media (C), the formation of AVOs and GFP-LC3-labeled vacuoles (D), the LC3-I, LC3-II, and BECN1 protein expression (E), and cell death (F) after 12% stretching at 1.5 Hz. When indomethacin (25 μ M) or celecoxib (40 μ M) was included in the treatment, cells were co-incubated with the compound during stretching. $5 \leq n \leq 10$, * $P < 0.05$.

autophagic cell death in tenofibroblasts, we speculated that repetitive mechanical loading could cause autophagic cell death in tenofibroblasts by stimulating endogenous PGE₂ production. To test this hypothesis, we utilized a previously reported *in vitro* stretching model [4, 20, 21]. HPTFs grown on a custom-made silicon dish were cyclically stretched for 24 h with 4%, 8% or 12% magnitude at a fixed frequency of 1 Hz. After 4 h rest, the PGE₂ concentration in the culture media was determined by ELISA. Although 4% stretching did not show significant effect, 8% and 12% stretching significantly increased PGE₂ release in a magnitude-dependent manner (Fig. 4A). When cells were stretched at a fixed frequency of 0.5, 1, or 1.5 Hz, PGE₂ release significantly increased with the frequency of stretching (Fig. 4B). These results were consistent with previous reports [4]. The PGE₂ concentration in the media reached about 7 ng/ml after 24 h stretching with 12% magnitude at a frequency

of 1.5 Hz (Fig. 4B). According to our studies with exogenous PGE₂, this concentration (7 ng/ml) of PGE₂ was sufficient to induce autophagic cell death in HPTFs. Indeed, we detected significantly higher percentage of cells with AVOs and GFP-LC3-labeled vacuoles (Fig. 4D), elevated LC3-II and BECN1 levels (Fig. 4E), and increased cell death (Fig. 4F) in HPTFs mechanically stretched under these conditions. Blocking prostaglandin synthesis by COX inhibitor indomethacin or celecoxib significantly reduced stretching-induced PGE₂ release (Fig. 4C), as well as autophagy and cell death (Fig. 4D – 4F). Taken together, these data demonstrate that cyclic mechanical stretching induces autophagic cell death in tenofibroblasts through activation of PGE₂ production.

Discussion

In addition to its function as a metabolic recycling system to promote cellular homeostasis and survival, autophagy regulates vital processes such as programmed cell death, inflammation, and adaptive immune mechanisms [22]. Autophagy has been implicated in the pathogenesis of many diseases including cancer, neuro degenerative, cardiovascular, pulmonary, and autoimmune diseases [23, 24]. Although pharmacologic modulation of autophagy has shown some early promises, current therapeutic targeting of autophagy is limited by an incomplete understanding of how autophagy contributes to pathogenesis [23]. Targeting disease-specific autophagy pathways rather than general autophagy may be a more effective strategy for development of therapeutic agents.

Many studies suggested that sustained autophagy could lead to autophagic type II programmed cell death [25, 26]. Autophagic cell death is morphologically defined as a type of cell death that occurs in the absence of chromatin condensation but is accompanied by large-scale autophagic vacuolization of the cytoplasm [27]. In mammals, there is no clear evidence indicating the presence of such autophagic cell death under physiological settings. However, numerous reports showed the pro-death function of autophagy and situations where cell died by the form of “autophagic cell death,” mostly from in vitro cell culture models [28]. In human cancer cells, there is a report showing a novel anti-cancer function of autophagic cell death in response to chemotherapy [29]. Such a finding is important as it provides evidence for possible exploration of inducing autophagic cell death for a therapeutic purpose in cancer.

Recently, excessive autophagic cell death has been detected in human tendinopathic tissues [16, 17], suggesting that autophagy plays an important role in the pathogenesis of tendinopathy. However, the factors causing autophagic cell death in tendinopathy are unclear. PGE₂, a well-known inflammatory mediator of tendinopathy, has been reported to inhibit tenofibroblasts proliferation [6]. In the present study, we found that exposure to exogenous PGE₂ induces cell death in cultured human tenofibroblasts via induction of autophagy. Incubation of HPTFs with exogenous PGE₂ significantly increased cell death (Fig. 1), as well as the formation of autophagosomes and autolysosomes (assessed by electron microscopy, AO staining, and GFP-LC3 puncta) (Fig. 2A - 2C), a widely used marker for autophagy. PGE₂-treated cells also showed stimulated the conversion of LC3-I to LC3-II, and upregulated the expression of BECN1 (Fig. 2D). Blocking autophagy using inhibitors 3-methyladenine and chloroquine, or small interfering RNAs against autophagy genes *Becn-1* and *Atg-5* prevented PGE₂-induced cell death (Fig. 3). These findings indicate that exogenous PGE₂ causes cell death in HPTFs through activation of autophagy. We subsequently demonstrated that repetitive mechanical loading stimulates PGE₂ production in HPTFs, which in turn induces autophagic cell death. Similar to previous findings [4], repetitive mechanical stretching at 8% and 12% magnitude for 24 h significantly increased PGE₂ release by HPTFs in a magnitude-dependent manner. In addition, we observed increased cell death as well as activation of autophagy with 12% stretching. Blocking PGE₂ production using COX inhibitors indomethacin and celecoxib significantly reduced stretching-induced autophagy and cell death, indicating that PGE₂ is the responsible factor for autophagic cell death. To our knowledge, this is the

first evidence linking PGE₂ to autophagic cell death in tendinopathy. Although apoptotic cell death was not directly assessed in the present study, our data suggested that it also contributed to cell death induced by PGE₂. Treatment of HPTFs with 15 ng/ml PGE₂ for 24 h caused approximately 46% cell death; however, AVOs were detected in only about 16% of cells (Fig. 3B). Therefore, about 30% cell death was possibly attributed to apoptosis, which is a well-recognized causative factor in tendinopathy. Further efforts are needed to elaborate the detailed death mechanisms involved in PGE₂ activation.

The signaling pathways leading to autophagy are not fully understood; however, oxidative stress is among the main causative factors [30]. Repetitive mechanical stretching can activate oxidative stress-associated pathways in human tenofibroblasts [13]. The oxidative stressor H₂O₂ induces autophagic cell death in rotator cuff tenofibroblasts, and this is prevented by cyanidin, a strong antioxidant [31]. These findings suggest that oxidative stress-associated pathways are involved in the induction of autophagic cell death in tendinopathy, likely through cross talking with the IL-1β/COX-2/prostaglandin E₂ pathway. Further studies are required to find out how these pathways crosstalk with each other to induce autophagic cell death in tendinopathy.

Currently, there are few good treatments for tendinopathy because of the lack of understanding of its pathogenesis. The new management strategies might target tendon stem cell proliferation and repair and restoration of matrix integrity. Advances in the research on autophagic cell death may help identify check points suitable for pharmaceutical intervention, and eventually help the development of effective therapy for tendinopathy.

Disclosure Statement

We declare that we have no conflicts of interest.

Acknowledgments

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