SMGroup

Role of Chondrocyte Energy Sensors in Articular Cartilage Homeostasis and Their Potential as Therapeutic Targets in OA

Ru Liu-Bryan[1,2]*

[1]VA San Diego Healthcare System. 2. Department of Medicine, University of California San Diego, USA.

***Corresponding author:** Ru Liu-Bryan, VASDHS, 3350 La Jolla Village Drive, 111K, San Diego, CA 92161, USA, Tel: Ph: 858 552 8585; Fax: 858 552 7425; Email: ruliu@ucsd.edu

Published Date: May 24, 2016

ABSTRACT

Osteoarthritis **(OA)** is the most common form of arthritis. Articular cartilage degeneration is the hallmark of OA. Low-grade chronic inflammation either resulted from systemic metabolic disturbance or induced by endogenous molecular products derived from cellular stress and extracellular matrix disruption in local joint can promote OA progression. Emerging evidence indicates that bioenergy sensors couple metabolism with inflammation to switch physiological and clinical phenotypes. AMP-activated protein Kinase **(AMPK)** and sirtuins (e.g. SIRT1) are critical cellular energy sensors. Dysregulation of AMPK and sirtuins has been implicated in diverse human diseases and aging, and effective regulation of cellular energy metabolism is important for tissue homeostasis. Recent studies reveal that dysfunction of AMPK and SIRT1 in articular chondrocytes alters energy metabolism, which can lead to disturbance of cartilage matrix homeostatic balance. Because a sustained activity of AMPK and SIRT1 in articular chondrocytes appears to be critical for cartilage homeostasis, targeted activation of AMPK and SIRT1 could be an attractive and novel therapeutic strategy for OA.

Keywords: AMPK (AMP-activated protein kinase); Sirtuins; Energy metabolism; Cartilage homeostasis

INTRODUCTION

Osteoarthritis **(OA)**, the most common form of arthritis, is a leading cause of disability [1]. Aging and prior joint injury are the major risk factors for development of OA [1]. OA is considered a progressive degenerative process which involves whole joint [1-3]. It is characterized by degeneration of articular cartilage, a hallmark of the diesase, low-grade synovial inflammation and subchondral bone remodeling [1-3]. Chondrocytes, the only type of cells embedded in articular cartilage, play an important role in cartilage matrix homeostasis by maintaining a balance between anabolic and catabolic activities. Dysfunction of chondrocytes favors catabolism by increasing activities of matrix degrading enzymes such as Metalloproteinases (MMP-1,-3 and -13) and decreasing production of type II collagen and aggrecan which are the major components of matrix in articular cartilage, leading to cartilage degradation [1]. Articular cartilage is avascular and hypoxic connective tissue. Glucose transport and glycolysis, and less so mitochondrial oxidative phosphorylation, provide the primary sources of metabolic energy in articular chondrocytes [12,13]. Biomechanical demands, inflammatory mediators, aging and other factors [12] could alter articular chondrocyte energy balance and metabolism. Disturbances in the maintenance of cellular energy balance trigger cell stress and induce inflammation [8-10]. Effective regulation of cellular energy metabolism is critical for tissue homeostasis [11]. Recent studies have implicated that AMP-activated protein Kinase **(AMPK)** and sirtuins (e.g. SIRT1) which are cellular energy sensors, play important roles in cartilage homeostasis by regulating energy balance and coordinates several housekeeping mechanisms to increase cell stress resistance and maintain quality control.

AMPK IN ARTICULAR CHONDROCYTES

AMPK, an evolutionary conserved serine/threonine protein kinase existing in essentially all eukaryotic cells, functions as a master regulator of cellular energy balance [14,15]. AMPK is a heterotrimeric complex consisting of a catalytic α-subunit and two regulatory β- and α-subunits. In mammals all three subunits have multiple isoforms (α1, α2; β1, β2; γ1, γ2, and γ3) encoded by distinct genes [10,11]. Articular chondrocytes express α1, α2, β1, β2 and γ1 isoforms of AMPK subunits, and α1 appears to be the predominantly expressed AMPK α isoform [12]. Phosphorylation of a conserved threonine residue within the activation loop of the kinase domain (Thr172) is required for the kinase activity of AMPK [10,11]. AMPK is activated in response to an increase in the cellular AMP to ATP ratio by metabolic stress either increasing ATP consumption (e.g., exercise/muscle contraction) or decreasing ATP production (e.g., ischemia or hypoxia) [10,11]. In this manner, AMPK allows cells to adjust to changes in energy demand [10,11]. AMPK can be activated by pharmacological compounds such as the nucleotide mimetic AICAR (5-aminoimidazole-4- caboxamide 1-b-D-ribofuranoside), and A-769662 which is a selective and direct AMPK activatorthrough binding to β1 subunit [13]. Some drugs already in the clinic for arthritis and other conditions (e.g., sodium salicylate, high dose aspirin, methotrexate, metformin),

and a variety of natural plant products (e.g. berberine, resveratrol, curcumin) are also able to activate AMPK [13,14]. However, most of them activate AMPK through indirect mechanisms.

Phosphorylation of AMPKα Thr172 is found to be constitutively present in normal articular chondrocytes/cartilage, but is decreased in human knee OA chondrocytes/cartilage [12] and in mouse knee OA cartilage [15]. In addition, mouse knee cartilage exhibits aging-associated reduction of phosphorylation of AMPKα [15]. Moreover, *in vitro* studies demonstrated that inflammatory cytokines IL-1β and TNFα, as well as biomechanical injury, can cause de-phosphorylation of AMPKα in normal articular chondrocytes, which is correlated with increased catabolic responses (e.g. increased MMP-3 and MMP-13 release), and these effects are inhibited by pre-treatment of chondrocytes with AMPK pharmacological activators [12,15]. Furthermore, chondrocytes deficient in both AMPKα1 and AMPKα2 (achieved via siRNA transfection) appear to have significant increase in catabolic responses to IL-1β and TNFα [12]. These data indicate that cartilage with decreased chondrocyte AMPK activities is susceptible to degradation. This conclusion is supported by our recent *in vivo* finding that activation of AMPK by berberine limits both surgical knee instability-induced and aging-related OA in mice [16], reflecting by significantly less proteoglycan loss and cartilage degradation [16]. Collectively, sustained AMPK activity in articular chondrocytes appears to be chondroprotective.

SIRTUINS IN ARTICULAR CHONDROCYTES

Sirtuins are a conserved family composed of seven members **(SIRT1-7)**, which are ubiquitously expressed and possess Nicotinamide Adenine Dinucleotide **(NAD+)**- dependent protein deacetylase, deacylase, and ADP-ribosyl transferase activities [17,18]. They regulate cellular stress, inflammation, genomic stability, and energy metabolism [17,18]. Sirtuins exhibit different subcellular locations. SIRT1 and SIRT6 are predominately found in the nucleus (SIRT1 is also found in the cytosol), whereas SIRT7 is located within the nucleolus [17,18]. SIRT2 is predominantly located in the cytoplasm, whereas SIRT3, SIRT4 and SIRT5 are localized to the mitochondria [17,18].

Among all sirtuins, SIRT1 is best characterized, and mostly studied in articular chondrocytes. Similar to AMPK, SIRT1activity is generally increased in response to energy/nutrient stress [17,18]. As for phosphorylation of AMPKα, decreased expression of SIRT1 is observed in both human and mouse knee OA cartilage, and in aged mouse knee cartilages [19-21]. *In vitro* studies show that increased apoptosis and enhanced pro-catabolic responses to IL-1β and TNFα in chondrocytes with loss of SIRT1 [22-24]. Additionally, SIRT1 is shown to promote cartilage-specific gene expression [25], protect chondrocytes from radiation-induced senescence [26], and inhibit apoptosis in chondrocytes [22,27,28]. Specifically, SIRT1 enhances human OA chondrocyte survival by repressing Protein Tyrosine Phosphatase 1B **(PTP1B)**, a potent pro-apoptotic protein [22]. *In vivo* studies demonstrated that adult heterozygous Sirt1 knockout mice and mice with a Sirt1 point mutation lacking SIRT1 enzyme activity exhibit increased OA progression [28,29],

and cartilage-specific Sirt1 Knockout **(KO)** mice develop accelerated OA progression [21]. Taking together, a sustained SIRT1 expression and activity in articular chondrocytes is important for cartilage homeostasis.

Nicotinomide Phosphotransferase **(NAMPT)** activity is known to stimulate the synthesis of NAD^+ through salvage pathway [30]. Recent studies showed that hypoxia-inducible factor-2α (HIF-2α) activates NAMPT-NAD^+ -SIRT axis by up-regulating NAMPT in articular chondrocytes, which stimulates NAD+ synthesis, leading to activation of sirtuin family members [30]. The activated NAMPT-NAD^+ -SIRT pathway, in turn, promotes HIF-2α protein stability and transcriptional activity by negatively regulating its hydroxylation and 26S proteasome-mediated degradation [30]. Interestingly, sirtuin family members display isoform-specific regulationof HIF-2α stability and transcriptional activity. Overexpression of SIRT2 and SIRT4 increases, whereas overexpression of SIRT3 inhibits HIF-2α protein stability and transcriptionalactivity [30]. Overexpression of SIRT1, SIRT5, SIRT6, or SIRT7 do not have any effect [30]. Expression of HIF-2α is increased in both human and mouse knee OA cartilages [31]. Overexpression of HIF-2α by intra-articular (IA) injection of Ad-Epas1, the gene encodes HIF-2α, results in spontaneous mouse knee OA development [31]. Remarkably, overexpression SIRT2 in joint tissue through IA injection of Ad-Sirt2 in mice does not cause cartilage degradation [30]. However, IA co-injection of Ad-shSirt2 (SIRT2 knockdown) together with either Ad-Epas1 or Ad-Nampt significantly inhibits HIF-2α - and NAMPT-induced expression of catabolic MMPs and cartilage destruction [30]. It should note that SIRT2 stabilizes HIF-2α protein without affecting its acetylation status in chondrocytes [30], suggesting that SIRT2 deacetylase activity is not necessary for regulation of HIF-2α protein stability. Since SIRT2 can inhibit expression of NF-KB-dependent genes by deacetylating p65 subunit of NF-KB at lysine 310 [17,18], SIRT2 deficiency caused increased expression NF-KB-dependent MMPs could result from increased acetylation of NF-KB. It remains to be determined if SIRT2 expression is decreased in OA cartilage, and if SIRT2 deficiency accelerates OA development and progression.

Expression of SIRT6 is significantly decreased in human OA chondrocytes [32]. Depletion of SIRT6 in human chondrocytes causes increased DNA damage and telomere dysfunction, and subsequent premature senescence, which are processes implicated in cartilage degeneration in OA [33]. Overexpression of Sirt6 is shown to prevent OA development by reducing both NF-KB-dependent inflammatory response and chondrocytes senescence [32]. The endocrine function of Infrapatellar Fat Pad **(IPFP)**, a unique fat depot that is located intra-capsularly and extra-synovially in the joint and is in close contact with articular cartilage, is thought to contribute to initiation and progression of OA [34]. SIRT6 haploinsufficency (SIRT6+/-) in mice promotes expression of inflammatory cytokines in the IPFP [34]. The aged SIRT6+/-mice fed on High Fat Diet **(HFD)** exhibit accelerated OA progression, evidenced by chondrocyte hypertrophy, advanced degeneration of articular cartilage, and osteophyte formation [34]. This associated with enhanced inflammation of the IPFP and impaired glucose tolerance [34]. These results indicate a pivotal role of SIRT6 in the cross talk among aging, metabolic syndrome and OA.

The functions of SIRT4, SIRT5 and SIRT7 in articular chondrocytes are not yet known. Changes in SIRT3 activity have been shown tobe an important determinant in the acetylation state of mitochondria in response to nutrient availability [35]. The acetylation of many mitochondrial proteins can alter their catalytic/biological function [35]. As such, loss of SIRT3 activity results in profound aberrations in mitochondrial function. We observed decreased SIRT3 expression in human knee OA chondrocytes, and SIRT3 knock-down chondrocytes have increased catabolic responses to IL-1β [36], suggesting that SIRT3 is also involved in cartilage matrix metabolism.

SIGNALING OF AMPK, SIRT1 AND SIRT3 IN ARTICULAR CHONDROCYTES

AMPK activity is regulated positively through phosphorylation by upstream kinases, or negatively via de-phosphorylation by protein phosphatases [10,11]. Liver Protein Kinase B1 **(LKB1)** is shown to be the primary upstream kinase that phosphorylates AMPKα Thr172 in articular chondrocytes, because phosphorylation of AMPK α is nearly completely inhibited in LKB1 knockdown chondrocytes [15]. Concomitant reduction of phosphorylation of both LKB1 and AMPKα is observed in primary human knee OA chondrocytes, in mouse knee OA cartilage, in aged mouse knee cartilage, and in chondrocytes challenged with mechanical injury [15]. This suggests that dysregulation of LKB1 in aged and OA cartilage may contribute to suppression of AMPK activation. Expression of protein phosphatases 2Cα (PP2Cα) is enhanced by IL-1β and TNFα in chondrocytes. Notably, phosphorylation of AMPK TNFα (Thr172) is increased in PP2C TNFα knock-down chondrocytes, indicating that PP2Cα is, at least in part, responsible for decreased phosphorylation of AMPKα induced by IL-1β and TNFα (unpublished observation). Activation of AMPK is shown to stimulate the expression and activity of SIRT1 by increasing the intracellular concentrations of NAD^+ via induction of expression of NAMPT in articular chondrocytes [37]. Interestingly, SIRT1 can deacetylate LKB1, which subsequently increase LKB1 activity, leading to AMPK activation [9,38]. This positive feedback loop could potentiate the function of AMPK, and effectively control cellular energy balance [9,38]. Activation of AMPK also can promote expression of mitochondrial SIRT3 (unpublished observation) in articular chondrocytes. Normal cellular function is dependent on a number of highly regulated homeostatic mechanisms. Signaling of AMPK and sirtuins particularly SIRT1 can coordinate several housekeeping mechanisms to increase cell stress resistance [9,38].

PRESERVATION OF MITOCHONDRIAL BIOGENESIS CAPACITY AND FUNCTION IN ARTICULAR CHONDROCYTES

The classic role of mitochondria is to produce ATP mainly through the process of Oxidative Phosphorylation **(OXPHOS)**, transduced by the respiratory complexes (I to IV) andthe ATP synthase (complex V) [39,40]. Mitochondria also coordinate numerous metabolic reactions through the Krebs cycle and fatty acid metabolism [41]. Mitochondrial function is known to decline with aging [41]. As cells age, the efficacy of the mitochondrial respiratory chain tends

to diminish, thus increasing electron leakage that leads to increases in Reactive Oxygen Species **(ROS)** production and oxidative damage, and reduced ATP generation [41]. Mitochondrial function is impaired in OA chondrocytes, reflected by decreased numbers of mitochondria and activity of respiratory complexes I, II and III [39-42]. Although the majority of the ATP in chondrocytes is made by glycolysis rather than by OXPHOS, ATP levels per chondrocyte are reduced despite glycolysis is increased in OA chondrocytes [43], which not only contributes to decreased mitochondrial bioenergetic reserve [44-46], but also adversely affects cellular redox balance [47-49], and chondrocyte homeostatic functions dependent on physiological generation of low levels of ROS [48,49]. The cell's mitochondrial mass is closely regulated by the complex cellular signaling pathways that respond to energy demand and is adjusted through mitochondrial biogenesis, which is important for maintenance of mitochondrial function [50]. Mitochondrial biogenesis is a complex process that involves close cooperation between nuclear and mitochondrial genome [50]. Deregulation of AMPK, SIRT1 and SIRT3 signaling can induce mitochondrial dysfunction [50].

AMPK phosphorylates PGC-1α (peroxisome proliferator-activated receptor γ co-activator 1α) protein that subsequently allows SIRT1 to deacetylate and activate PGC-1α [38,50]. PGC-1α, a transcriptional co-activator, is a master regulator of mitochondrial biogenesis and function [38,50]. Expression of PGC-1α is found to decrease in both mouse knee OA cartilage and in aged mouse knee cartilage [37]. In addition, mitochondrial biogenesis capacity and function are significantly reduced in advanced human knee OA chondrocytes, indicated by deceased mitochondrial DNA content and mitochondrial mass, and reduced oxygen consumption rate and intracellular ATP level, all of which werecorrelated with concomitant reduction of phosphorylation of AMPKα, expression of SIRT1 and PGC-1α, increased acetylation of PGC-1α, and reduced expression of transcription factors involved in mitochondrial biogenesis such as nuclear respiratory factor 1 (NRF1), NRF2, and mitochondrial transcription factor A (TFAM), as well as reduced expression of respiratory complexes [37]. Moreover, the established impairments in mitochondrial biogenesis and function in advanced human knee OA chondrocytes can be reversed by either AMPK pharmacologic activation through SIRT1-PGC-1α signaling [37]. Decreased SIRT6 expression in human knee OA chondrocytes may also contribute to reduced capacity of mitochondrial biogenesis, as SIRT6 is recently shown to act as a transactivator for NRF2 [51], suggesting a novel role for SIRT6 in the control of oxidative homeostasis.

Human OA chondrocytes exhibit mitochondrial DNA **(mtDNA)** damage, evidenced by the presence of the 4977 bp mtDNA deletion, the most frequent and common mtDNA mutation associated with oxidative damage [52]. Mitochondrial DNA **(mtDNA)** damage can cause mitochondrial respiratory chain dysfunction and augment production of ROS [53]. We recently observed reduced SIRT3 expression in human knee OA chondrocytes and aged mouse knee cartilages. This is correlated with reduced phosphorylation of AMPKα and expression of human 8-Oxoguanine-DNA Glycosylase 1**(OGG1**), a DNA repair enzyme executing the excision of

7,8-dihydro-8-oxoguanine (8-oxoG), an oxidative form of guanine and a mutagenic base generated as a result of exposure to ROS [36]. Sirt3 is shown to interact with OGG1 that contributes to repair of mitochondrial DNA and protects from apoptotic cell death under oxidative stress [54]. We found that acetylation of OGG1 was increased in SIRT3 knockdown chondrocytes [36]. In addition, AMPK pharmacological activator A-769662 increased expression of SIRT3 and OGG1, and limited ROS-induced the common 4977 bp mtDNA deletion in human knee chondrocytes [36], suggesting that importance of AMPK-SIRT3-OGG1 signaling in maintaining mtDNA integrity.

INHIBITION OF OXIDATIVE STRESS AND INFLAMMATORY RESPONSES

FOXO3a, a transcription factor that belongs to the Forkhead Box O **(FOXO)** family, and PGC-1α are closely related [55]. FOXO3 is a direct transcriptional regulator of PGC-1α. PGC-1α itself can augment the transcriptional activity of FOXO3a [55]. AMPK directly phosphorylates FOXO3a, and SIRT1 deacetylates and activates FOXO3a [9,38]. Both PGC-1α and FOXO3a have been shown to limit cellular oxidative stress by up-regulating antioxidant enzymes, including SOD2 and catalase [55,56]. As for PGC-1α, expression of FOXO3a is reduced in both mouse knee OA cartilage andin aged mouse knee cartilage [46], correlated with decreased phosphorylation of AMPKα. AMPK pharmacological activator A-769662 inhibits excessive oxidative stress in articular chondrocytes via PGC-1α and FOXO3a through increased expression of SOD2 and catalase [57] Overexpression of SIRT3 in chondrocytes also exhibits inhibition of excessive oxidative stress [36]. Since acetylation of SOD2 is increased in SIRT3 knock-down chondrocytes, AMPK pharmacologic activation may also exert its anti-oxidant effect via SIRT3.

Elevated levels of ROS resulted from mitochondrial dysfunction promotes cartilage degradation directly by cleaving collagen and aggrecan and indirectly by activating MMPs [58,59]. ROS also indirectly modulate redox-sensitive NF-KB and other signaling pathways that increase chondrocyte catabolic activity [60-62]. Both AMPK and SIRT1 have anti-inflammatory effects in diverse types of cells and tissues [9,38]. Activation of AMPK inhibits NF-KB activation via SIRT1, which deacetylates p65 NF-KB subunit, ultimately primes p65 for proteasome degradation [63,64]. Activation of AMPK or SIRT1 inhibits catabolicresponses to IL-1β and TNFα via attenuation of NF-KB activation in articular chondrocytes [12,23,24,65-67]. In addition, PGC-1α and FOXO3a, at least in part, mediate AMPK to inhibit NF-KB activation and inflammatory cytokine-induced catabolic responses in chondrocytes [57]. Both IL-1β and TNFα can decrease phosphorylation of AMPKα and expression of SIRT1 [12,23,24] in articular chondrocytes. TNFα reduces SIRT1 activity in chondrocytes by inducing cathepsin B-mediated cleavage of SIRT1 [68]. In addition, chondrocytes with reduced activity of AMPK and SIRT1 exhibit increased responsiveness to inflammatory cytokines [12,24]. These data suggest that inflammatory cytokines cause dysregulation of AMPK and SIRT1 signaling in chondrocytes, which reduces capacity of chondrocyte to resist inflammatory stress and further provoke inflammatory responses.

REGULATION OF ER STRESS RESPONSES AND AUTOPHAGY

It is known that all secretory and integral membrane proteins are folded and post-translationally modified in the Endoplasmic Reticulum **(ER)**, which is also a site of calcium storage and lipid biosynthesis [69,70]. Stresses that compromise the ER homeostasis such as perturbations in calcium homeostasis, energy stores, redox state, and metabolic and inflammatory challenges result in the accumulation of misfolded proteins and activation of a stress response termed the Unfolded Protein Response **(UPR)** [69,70], which helps to re-establish cellular homeostasis. Several adaptive signaling pathways have evolved to restore an efficient protein-folding environment through the induction of chaperones, degradation of misfolded proteins and attenuation of protein translation [69,70]. Inositol-Requiring Kinase 1 **(IRE1)**, ER eukaryotic translation Initiation Factor 2 (eIF2a) kinase (PERK), and Activating Transcription Factor 6 **(ATF6)** are the three branches of UPR signaling cascade, which are triggered by disassociation of the chaperon GRP78 upon ER stress [69,70]. However, when ER stress is too severe or chronic, or the UPR is unable to resolve the protein-folding defects, cells undergo apoptosis through induction of C/EBP Homologous Protein **(CHOP)** [69,70]. Induction of expression of GRP78 and CHOP and generation of alternatively spliced and transcriptionally activated X-Box Protein 1 (XBP1) are observed in OA cartilage, suggesting activation of UPR pathways [71,72]. ATF6 upregulates XBP1 expression in OA chondrocytes by promoting direct binding to XBP1 promoter [73], and increased XBP1s expression accelerates chondrocyte hypertrophy [73]. XBP1 expression is also increased by IL-1β in chondrocytes [74]. Inhibition of XBP1 expression in chondrocytes via siRNA attenuates nitric oxide and MMP-3 release induced by IL-1β [74]. Several factors implicated in OA pathogenesis including biomechanical injury, IL-1β, nitric oxide and Advanced Glycation End Products **(AGEs)** upregulate expression of GRP78 and CHOP in cultured articular chondrocytes [74-79]. CHOP potentiates the capacity of IL-1β to induce catabolic responses, superoxide generationand apoptosis in chondrocytes, and does so by inhibiting AMPK activity [71]. CHOP-mediated apoptosis is shown to contribute to the progression of cartilage degeneration in mice [79]. However, pharmacologic AMPK activation blunts CHOP expression and catabolic responses induced by IL-1β and biomechanical injury [15,71], indicatinga role of AMPK in alleviating ER stress in chondrocytes.

Autophagy is a cellular housekeeping and protein quality control mechanism, which can remove damaged or defective proteins and organelles, e.g. damaged mitochondria [9,80]. It is also critical to provide energy and molecular building blocks by recycling macromolecules in response to nutrient and environmental stress [80]. AMPK controls autophagy through Mammalian Target of Rapamycin **(mTOR)** and Unc-51-Like Kinase 1 **(ULK1)** signaling [80]. mTOR is a highly conserved serine/threonine kinase and a master regulator of cell growth and metabolism. It is activated in response to nutrients, growth factors and cellular energy. mTOR signaling contributes to chondrocyte differentiation, cartilage growth and development [81]. ULK1 is a critical kinase that governs the cascade of events triggering autophagy. AMPK can inhibit activity

of mTOR Complex **(mTORC1)** either by directly phosphorylating Raptor, a regulatory component of mTORC1, or by phosphorylating Tuberous Sclerosis Protein 2 [TSC2], which subsequently suppresses mTOR activity [80]. AMPK stimulates autophagy by dissociating mTORC1 from the ULK1 complex via the phosphorylation of the Raptor component, as well as by directly binding to the ULK1 complex and phosphorylating ULK1 [80]. In addition, AMPK can enhance the later steps in autophagosome formation through SIRT1 by deacetylating several autophagy-related proteins (e.g. Atg5, Atg7 and Atg8) [9]. SIRT3 can also initiate mitochondrial autophagyor mitophagy, an organelle-specific form of autophagy that homeostatically controls excessive ROS production by eliminating dysfunctional mitochondria, via deacetylation of mitochondrial proteins including FOXO3a under oxidative stress conditions [82,83]. Chondrocyte autophagy is known to be a constitutive homeostatic mechanism in articular cartilage [84], which can be promoted by AMPK signaling [85,86] through mTOR suppression. Expression of mTOR is up-regulated, but autophagy is reduced with a linked increase in apoptosis in human knee OA, mouse knee OA and aged mouse knee cartilages [84]. Suppressed autophagy also is observed in cartilage ex *in vivo* response to mechanical injury [87]. Inhibition of autophagy in chondrocyte exacerbated IL-1β-induced OA-like gene expression changes and apoptotic signals, while activation of autophagy inhibited them, possibly through modulation of ROS in chondrocytes *in vitro* [88].Cartilage-specific mTOR KO mice showed significant protection from surgery-induced OA, associated with increased autophagy and decreased articular chondrocyte cell death [89], suggesting a potential role for mTOR inhibition to restore homeostasis during OA. Indeed, inhibition of mTOR signaling by rapamycin upregulates autophagy and reduces the severity of experimental OA *in vivo* [90].

CLINICAL RELEVANCE AND IMPLICATION

Dysregulation of AMPK and sirtuins has been linked to a variety of age-related diseases such as diabetes, atherosclerosis, cardiovascular disease, cancer, and neurodegenerative diseases [9,38,91]. Studies have revealed that responsiveness of AMPK activation declines during the aging process [9], and low-grade inflammation present in aging tissues may be at least in part responsible for suppressing AMPK signaling [9]. Loss of mitochondrial function is a hallmark of aging and age-related diseases, which is linked to decreased concentrations of NAD+ and reduced activity of sirtuins [92]. The nuclear sirtuins such as SIRT1 and SIRT6 regulate the activity of key transcription factors and cofactors of numerous metabolic pathways in almost all tissues by linking nutrient signals with the cellular responses to energy demands [17,18]. The mitochondrial sirtuin SIRT3 regulate the activity of important mitochondrial enzymes and drive metabolic cycles in response to fasting and calorie restriction [17,18]. Nutritional factors are known to affect AMPK signaling. Caloric restriction stimulates, but nutritional overload impairs activities of AMPK, which can induce insulin resistance in many tissues [9]. The metabolic disturbance can cause low-grade inflammation leading to development of metabolic syndrome such as obesity and diabetes [9], which are often associated with OA [93]. The prevalence of OA increases with aging and metabolic syndrome supports the concept that a dysfunction of AMPK is involved in the disease process.

A systemic review and meta-analysis showed that knee extensor muscle weakness was associated with an increased risk of developing knee OA in both men and women [94]. Muscle strengthening to and improve muscle quality in knee OA patients is recommended. Evidence supports the benefits of various types of exercise for improving pain and function in knee OA [95]. In fact, exercise is recommended for the management of OA in all clinical guidelines irrespective of disease severity, pain levels, and functional status [95]. Studies in both animals and humans demonstrate that skeletal muscle contraction and exercise activate AMPK in an intensity and time-dependent manner [96,97], and increased AMPK activation promotes adaptation to muscle endurance exercise though PGC-1α [96,97]. Diet rich in n-3 long chain Polyunsaturated Fatty Acids **(PUFAs)** is considered as a nutritional tool to prevent insulin resistance associated to type 2 diabetes and obesity [98]. This is probably at least in part owing to the ability of n-3 PUFAs to stimulate activation of AMPK-SIRT1 signaling [99-102]. Recent studies in mice demonstrated that OA was significantly associated with dietary fatty acid content, and small amounts of ω-3 PUFAs (8% by kcal) in a high-fat diet were sufficient to mitigate injury-induced OA [103]. Interestingly, in a randomized, double-blind, multicenter trial enrolled patients with knee OA and regular knee pain, the low-dose fish oil group exhibit greater improvement in pain and function scores at 2 years compared with the high-dose group [104].

Methotrexate and metformin, drugs already in the clinic for rheumatoid arthritis and type II diabetes respectively, are AMPK activators [13]. A recent randomized placebo-controlled small trial of methotrexate in symptomatic knee OA showed significant improvement in physical function associated with reduced pain and synovitis [105]. Patients with metabolic syndrome (e.g. type II diabetes patients) have increased risk of OA [93]. Given that metformin activates AMPK, metformin treatments to these patients may provide additional beneficial effect on limiting OA development. Some natural plant products either present in traditional medicine or derived from food (e.g. berberine, resveratrol, curcumin, quercetin) appealed to have ''nutraceutical'' properties exhibit their ability to activate AMPK [14]. Arandomized double-blind placebo-controlled small trial of curcuminoid (closely-related to curcumin) in treatment of knee OA also showed significant improvements in pain and physical function [106]. Whether the beneficial effects of the agents mentioned abovein the OA clinical trails are in part resulted from AMPK activation remains to be investigated.

CONCLUSION

Reduced activities of AMPK and sirtuins (e.g. SIRT1) in articular cartilage, likely in other joint tissues as well, could limit energy availability for cellular maintenance, trigger significant cell stress by inducing mitochondrial dysfunction, oxidative stress and inflammation that compromise cell survival and tissue integrity and function, ultimately leading to OA development and progression. Because sustained activities of AMPK and SIRT1 are important to cartilage homeostasis, targeted activation of AMPK and SIRT1 through diet, exercise, nutraceuticals, pharmacologics or combination of some of these approaches could be an attractive and novel therapeutic strategy for OA.

ACKNOWLEDGMENT

Dr. Ru Liu-Bryan's research is supported by the Department of Veterans Affairs grant 1I01BX002234, National Institutes of Health grant AR1067966 and an Innovative Science Grant from the Arthritis Foundation.

References

1. Loeser RF, Goldring SR, Scanzello CR, Goldring MB. Osteoarthritis: a disease of the joint as an organ. Arthritis Rheum. 2012; 64: 1697-1707.
2. Scanzello CR, Goldring SR. The role of synovitis in osteoarthritis pathogenesis. Bone. 2012; 51: 249-257.
3. Goldring MB, Goldring SR. Articular cartilage and subchondral bone in the pathogenesis of osteoarthritis. Ann N Y Acad Sci. 2010; 1192: 230-237.
4. Mobasheri A, Vannucci SJ, Bondy CA, Carter SD, Innes JF, et al. Development and cartilage degradation in osteoarthritis. Histol Histopathol. 2002; 17: 1239-1267.
5. Blanco FJ, López-Armada MJ, Maneiro E. Mitochondrial dysfunction in osteoarthritis. Mitochondrion. 2004; 4: 715-728.
6. O'Neill LA, Hardie DG. Metabolism of inflammation limited by AMPK and pseudo-starvation. Nature. 2013; 493: 346-355.
7. Liu TF, Brown CM, El Gazzar M, McPhail L, Millet P, et al. Fueling the flame: bioenergy couples metabolism and inflammation. J Leukoc Biol. 2012; 92: 499-507.
8. Fullerton MD, Steinberg GR, Schertzer JD. Immunometabolism of AMPK in insulin resistance and atherosclerosis. Mol Cell Endocrinol. 2013; 366: 224-234.
9. Salminen A, Kaarniranta K. AMP-Activated Protein Kinase (AMPK) controls the aging process via an integrated signaling network. Ageing Res Rev. 2012; 11: 230-241.
10. Steinberg, GR, Kemp BE. AMPK in Health and Disease. Physiol Rev. 2009; 89: 1025-1078.
11. Witczak, CA, Sharoff CG, Goodyear LJ. AMP-activated protein kinase in skeletal muscle: from structure and localization to its role as a master regulator of cellular metabolism. Cell Mol Life Sci. 2008; 65: 3737-3755.
12. Terkeltaub R, Yang B, Lotz M, Liu-Bryan R. Chondrocyte AMP-activated protein kinase activity suppresses matrix degradation responses to inflammatory cytokines IL-1β and TNFα. Arthritis Rheum. 2011; 63: 1928-1937.
13. Hardie DG, Ross FA, Hawley SA. AMP-activated protein kinase: a target for drugs both ancient and modern. Chem Biol. 2012; 19: 1222-1236.
14. Hwang JT, Kwon DY, Yoon SH. AMP-activated protein kinase: a potential target for the diseases prevention by natural occurring polyphenols. N Biotechnol. 2009; 26: 17-22.
15. Petursson F, Husa M, June R, Lotz M, Terkeltaub R, et al. Linked decreases in Liver Kinase B1 and AMP-activated protein kinase activity modulate matrix catabolic responses to biomechanical injury in chondrocytes. Arthritis Res Ther. 2013; 15: R77.
16. Zhao X, Wang Y, Lee HS, Kim HJ, Alifah Akasdi A, et al. Activation of AMP-activated Protein Kinase (AMPK) by Berberine Limits Both Surgical Knee Instability-induced And Aging-related Osteoarthritis in Mice. 2014 American College of Rheumatology Meeting. Arthritis & Rheumatology. 2014; S2949.
17. Giblin W, Skinner ME, Lombard DB. Sirtuins: guardians of mammalian health span. Trends Genet. 2014; 30: 271-286.
18. Covington JD, Bajpeyi S. The sirtuins: Markers of metabolic health. Mol NutrFood Res. 2016; 60: 79-91.
19. Dvir-Ginzberg M, Steinmeyer J. Towards elucidating the role of SirT1 in osteoarthritis. Front Biosci. 2013; 18: 343-355.
20. Fujita N, Matsushita T, Ishida K, Kubo S, Matsumoto T, et al. Potential involvement of SIRT1 in the pathogenesis of osteoarthritis through the modulation of chondrocyte gene expressions. J Orthop Res. 2011; 29: 511-515.
21. Matsuzaki T, Matsushita T, Takayama K, Matsumoto T, Nishida K, et al. Disruption of Sirt1 in chondrocytes causes accelerated progression of osteoarthritis under mechanical stress and during ageing in mice. Ann Rheum Dis. 2014; 73: 1397-1404.
22. Gagarina V, Gabay O, Dvir-Ginzberg M, Lee EJ, Brady JK, et al. SirT1 enhances survival of human osteoarthritic chondrocytes by repressing protein tyrosine phosphatase 1B and activating the insulin-like growth factor receptor pathway. Arthritis Rheum. 2010; 62: 1383-1392.
23. Moon MH, Jeong JK, Lee YJ, Seol JW, Jackson CJ, et al. SIRT1, a class III histone deacetylase, regulates TNF-α-induced inflammation in human chondrocytes. Osteoarthritis Cartilage. 2013; 21: 470-480.

24. Matsushita T, Sasaki H, Takayama K, Ishida K, Matsumoto T, et al. The overexpression of SIRT1 inhibited osteoarthritic gene expression changes induced by interleukin-1*β* in human chondrocytes. J Orthop Res. 2013; 31: 531-537.

25. Dvir-Ginzberg M, Gagarina V, Lee EJ, Hall DJ. Regulation of cartilage-specific gene expression in human chondrocytes by SirT1 and nicotinamid phosphoribosyltransferase. J Biol Chem. 2008; 283: 36300-36310.

26. Hong EH, Lee SJ, Kim JS, Lee KH, Um HD, et al. Ionizing radiation induces cellular senescence of articular chondrocytes via negative regulation of SIRT1 by p38 kinase. J Biol Chem. 2010; 285: 1283-1295.

27. Takayama K, Ishida K, Matsushita T, Fujita N, Hayashi S, et al. SIRT1 regulation of apoptosis of human chondrocytes. Arthritis Rheum. 2009; 60: 2731-2740.

28. Gabay O, Oppenhiemer H, Meir H, Zaal K, Sanchez C, et al. Increased apoptotic chondrocytes in articular cartilage from adult heterozygous SirT1 mice. Ann Rheum Dis. 2012; 71: 613-616.

29. Gabay O, Sanchez C, Dvir-Ginzberg M, Gagarina V, Zaal KJ, et al. Sirtuin 1 enzymatic activity is required for cartilage homeostasis in vivo in a mouse model. Arthritis Rheum. 2013; 65: 159-166.

30. Oh H, Kwak JS, Yang S, Gong MK, Kim JH, et al. Reciprocal regulation by hypoxia-inducible factor-2*α* and the NAMPT-NAD(+)-SIRT axis in articular chondrocytes is involved in osteoarthritis. Osteoarthritis Cartilage. 2015; 23: 2288-2296.

31. Yang S, Kim J, Ryu JH, Oh H, Chun CH, et al. Hypoxia-inducible factor-2alpha is a catabolic regulator of osteoarthritic cartilage destruction. Nat Med. 2010; 16: 687-693.

32. Wu Y, Chen L, Wang Y, Li W, Lin Y, et al. Overexpression of Sirtuin 6 suppresses cellular senescence and NF-κB mediated inflammatory responses in osteoarthritis development. Sci Rep. 2015; 5: 17602.

33. Nagai K, Matsushita T, Matsuzaki T, Takayama K, Matsumoto T, et al. Depletion of SIRT6 causes cellular senescence, DNA damage, and telomere dysfunction in human chondrocytes. Osteoarthritis Cartilage. 2015; 23: 1412-1420.

34. Ailixiding M, Aibibula Z, Iwata M, Piao J, Hara Y, et al. Pivotal role of Sirt6 in the crosstalk among ageing, metabolic syndrome and osteoarthritis. Biochem Biophys Res Commun. 2015; 466: 319-326.

35. Parihar P, Solanki I, Mansuri ML, Parihar MS Mitochondrial sirtuins: emerging roles in metabolic regulations, energy homeostasis and diseases. Exp Gerontol. 2015 61: 130-141.

36. Liu-Bryan R, Wang Y, Terkeltaub R. Activation of AMP-Activate Protein Kinase (AMPK) limits mitochondrial DNA damage in human knee OA chondrocytes by upregulation of SIRT3 and OGG1. Osteoarthritis and Cartilage. 2015; 23: A157-A158.

37. Wang Y, Zhao X, Lotz M, Terkeltaub R, Liu-Bryan R. Mitochondrial biogenesis is impaired in osteoarthritis chondrocytes but reversible via peroxisomeproliferator-activated receptor *γ* coactivator 1*α*. Arthritis Rheumatol. 2015; 67: 2141-2153.

38. Ruderman NB, Xu XJ, Nelson L, Cacicedo JM, Saha AK, et al. AMPK and SIRT1: a long-standing partnership? Am J Physiol Endocrinol Metab. 2010; 298: E751-60.

39. Blanco FJ, López-Armada MJ, Maneiro E. Mitochondrial dysfunction in osteoarthritis. Mitochondrion. 2004; 4: 715-728.

40. Blanco FJ, Rego I, Ruiz-Romero C. The role of mitochondria in osteoarthritis. Nat Rev Rheumatol. 2011; 7: 161-169.

41. López-Otín C, Blasco MA, Partridge L, Serrano M, Kroemer G. The hallmarks of aging. Cell. 2013; 153: 1194-1217.

42. Maneiro E, Martín MA, de Andres MC, López-Armada MJ, Fernández-Sueiro JL, et al. Mitochondrial respiratory activity is altered in osteoarthritic human articular chondrocytes. Arthritis Rheum. 2003; 48: 700-708.

43. Johnson K, Svensson CI, Etten DV, Ghosh SS, Murphy AN, et al. Mediation of spontaneous knee osteoarthritis by progressive chondrocyte ATP depletion in Hartley guinea pigs. Arthritis Rheum. 2004; 50: 1216-1225.

44. Lotz M, Loeser RF. Effects of aging on articular cartilage homeostasis. Bone. 2012; 51: 241-248.

45. Lee RB, Urban JP. Evidence for a negative Pasteur effect in articular cartilage. Biochem J. 1997; 321: 95-102.

46. Lee RB, Urban JP. Functional replacement of oxygen by other oxidants in articular cartilage. Arthritis Rheum. 2002; 46: 3190-3200.

47. Martin JA, Martini A, Molinari A, Morgan W, Ramalingam W, et al. Mitochondrial electron transport and glycolysis are coupled in articular cartilage. Osteoarthritis Cartilage. 2012; 20: 323-329.

48. Henrotin Y, Kurz B, Aigner T. Oxygen and reactive oxygen species in cartilage degradation: friends or foes? Osteoarthritis Cartilage. 2005; 13: 643-654.

49. Henrotin YE, Bruckner P, Pujol JP. The role of reactive oxygen species in homeostasis and degradation of cartilage. Osteoarthritis Cartilage. 2003; 11: 747-755.

50. Brenmoehl J, Hoeflich A. Dual control of mitochondrial biogenesis by sirtuin 1 and sirtuin 3. Mitochondrion. 2013; 13: 755-761.

51. Pan H, Guan D, Liu X, Li J, Wang L, et al. SIRT6 safeguards human mesenchymal stem cells from oxidative stress by coactivating NRF2. Cell Res. 2016; 26: 190-205.

52. Chang MC, Hung SC, Chen WY, Chen TL, Lee CF, et al. Accumulation of mitochondrial DNA with 4977-bp deletion in knee cartilage--an association with idiopathic osteoarthritis. Osteoarthritis Cartilage. 2005; 13: 1004-1011.

53. McInnes J. Mitochondrial-associated metabolic disorders: foundations, pathologies and recent progress. Nutr Metab (Lond). 2013; 10: 63.

54. Cheng Y, Ren X, Gowda AS, Shan Y, Zhang L, et al. Interaction of Sirt3 with OGG1 contributes to repair of mitochondrial DNA and protects from apoptotic cell death under oxidative stress. Cell Death Dis. 2013; 4: e731.

55. Olmos Y, Valle I, Borniquel S, Tierrez A, Soria E, et al. Mutual dependence of Foxo3a and PGC-1alpha in the induction of oxidative stress genes. J Biol Chem. 2009; 284: 14476-1484.

56. Kang C, Li Li Ji. Role of PGC-1alpha signaling in skeletal muscle health and disease. Ann N Y Acad Sci. 2012; 1271: 110-117.

57. Zhao X, Petursson F, Viollet B, Lotz M, Terkeltaub R, Liu-Bryan R. Peroxisome Proliferator-Activated Receptor *γ* Coactivator 1*α* and FoxO3A mediate Chondroprotection by AMP-Activated Protein Kinase. Arthritis Rheumatol. 2014; 66: 3073-3082.

58. Rajagopalan S, Meng XP, Ramasamy S, Harrison DG, Galis ZS. Reactive oxygen species produced by macrophage-derived foam cells regulate the activity of vascular matrix metalloproteinases in vitro. Implications for atherosclerotic plaque stability. J Clin Invest. 1998; 98: 2572-2579.

59. Petersen SV, Oury TD, Ostergaard L, Valnickova Z, Wegrzyn J, et al. Extracellular superoxide dismutase (EC-SOD) binds to type I collagen and protects against oxidative fragmentation. J Biol Chem. 2004; 279: 13705-13710.

60. Loeser RF. Molecular mechanisms of cartilage destruction in osteoarthritis. J Musculoskelet Neuronal Interact. 2008; 8: 303-306.

61. Henrotin YE, Bruckner P, Pujol JP. The role of reactive oxygen species in homeostasis and degradation of cartilage. Osteoarthritis Cartilage. 2003; 11: 747-755.

62. Henrotin Y, Kurz B, Aigner T. Oxygen and reactive oxygen species in cartilage degradation: friends or foes? Osteoarthritis Cartilage. 2005; 13: 643-654.

63. Salminen A, Hyttinen JM, Kaarniranta K, AMP-activated protein kinase inhibits NF-*κ*B signaling and inflammation: impact on health span and lifespan. J Mol Med. 2011; 89: 667-676.

64. Kauppinen A, Suuronen T, Ojala J, Kaarniranta K, Salminen A. Antagonistic crosstalk between NF-*κ*B and SIRT1 in the regulation of inflammation and metabolic disorders. Cell Signal. 2013; 25: 1939-1948.

65. Lei M, Wang JG, Xiao DM, Fan M, Wang DP, et al. Resveratrol inhibits interleukin 1*β*-mediated inducible nitric oxide synthase expression in articular chondrocytes by activating SIRT1 and thereby suppressing nuclear factor-κB activity. Eur J Pharmacol. 2012; 674: 73-79.

66. Csaki C, Mobasheri A, Shakibaei M. Synergistic chondroprotective effects of curcumin and resveratrol in human articular chondrocytes: inhibition of IL-1beta-induced NF-kappaB-mediated inflammation and apoptosis. Arthritis Res Ther. 2009; 11: R165.

67. Shakibaei M, Csaki C, Nebrich S, Mobasheri A. Resveratrol suppresses interleukin-1beta-induced inflammatory signaling and apoptosis in human articular chondrocytes: potential for use as a novel nutraceutical for the treatment of osteoarthritis. Biochem Pharmacol. 2008; 76: 1426-1439.

68. Dvir-Ginzberg M, Gagarina V, Lee EJ, Booth R, Gabay O, et al. Tumor necrosis actor *α*-mediated cleavage and inactivation of SirT1 in human osteoarthritic chondrocytes. Arthritis Rheum. 2011; 63: 2363-2373.

69. Zhang K, Kaufman RJ. The unfolded protein response: a stress signaling pathway critical for health and disease. Neurology. 2006; 66: S102-109.

70. Dufey E, Sepúlveda D, Rojas-Rivera D, Hetz C. Cellular Mechanisms of Endoplasmic Reticulum Stress Signaling in Health and Disease. 1. An overview. Am J Physiol Cell Physiol. 2014; 307: C582-C594.

71. Husa M, Petursson F, Lotz M, Terkeltaub R, Liu-Bryan R. C/EBP homologous protein drives pro-catabolic responses in chondrocytes. Arthritis Res Ther. 2013; 15: R218.

72. Takada K, Hirose J, Senba K, Yamabe S, Oike Y, et al. Enhanced apoptotic and reduced protective response in chondrocytes following endoplasmic reticulum stress in osteoarthritic cartilage. Int J Exp Pathol. 2011; 92: 232-242.

73. Guo FJ, Xiong Z, Lu X, Ye M, Han X, Jiang R. ATF6 upregulates XBP1S and inhibits ER stress-mediated apoptosis in osteoarthritis cartilage. Cell Signal. 2014; 26: 332-342.

74. Liu Y, Zhou J, Zhao W, Li X, Jiang R, et al. XBP1S associates with RUNX2 and regulates chondrocyte hypertrophy. J Biol Chem. 2012; 287: 34500-34513.

75. Oliver BL, Cronin CG, Zhang-Benoit Y, Goldring MB, Tanzer ML. Divergent stress responses to IL-1beta, nitric oxide, and tunicamycin by chondrocytes. J Cell Physiol. 2005; 204: 45-50.

76. Takada K, Hirose J, Yamabe S, Uehara Y, Mizuta H. Endoplasmic reticulum stress mediates nitric oxide-induced chondrocyte apoptosis. Biomed Rep. 2013; 1: 315-319.

77. Yamabe S, Hirose J, Uehara Y, Okada T, Okamoto N, et al. Intracellular accumulation of advanced glycation end products induces apoptosis via endoplasmic reticulum stress in chondrocytes. FEBS J. 2013; 280: 1617-1629.

78. Rasheed Z, Haqqi TM. Endoplasmic reticulum stress induces the expression of COX-2 through activation of eIF2*α*, p38-MAPK and NF-*κ*B in advanced glycation end products stimulated human chondrocytes. Biochim Biophys Acta. 2012; 1823: 2179-2189.

79. Uehara Y, Hirose J, Yamabe S, Okamoto N, Okada T, et al. Endoplasmic reticulum stress-induced apoptosis contributes to articular cartilage degeneration via C/EBP homologous protein. Osteoarthritis Cartilage. 2014; 22: 1007-1017.

80. Alers S, Löffler AS, Wesselborg S, Stork B. Role of AMPK-mTOR-Ulk1/2 in the regulation of autophagy: cross talk, shortcuts, and feedbacks. Mol Cell Biol. 2012; 32: 2-11.

81. Phornphutkul C, Wu KY, Auyeung V, Chen Q, Gruppuso PA. mTOR signaling contributes to chondrocyte differentiation. Dev Dyn. 2008; 237: 702-712.

82. Tseng AH, Shieh SS, Wang DL. SIRT3 deacetylates FOXO3 to protect mitochondria against oxidative damage. Free Radic Biol Med. 2013; 63: 222-234.

83. Webster BR, Scott I, Han K, Li JH, Lu Z, et al. Restricted mitochondrial protein acetylation initiates mitochondrial autophagy. J Cell Sci. 2013; 126: 4843-4849.

84. Caramés B, Taniguchi N, Otsuki S, Blanco FJ, Lotz M. Autophagy is a protective mechanism in normal cartilage, and its aging-related loss is linked with cell death and osteoarthritis. Arthritis Rheum. 2010; 62: 791-801.

85. Srinivas V, Bohensky J, Shapiro IM. Autophagy: a new phase in the maturation of growth plate chondrocytes is regulated by HIF, mTOR and AMP kinase. Cells Tissues Organs. 2009; 189: 88-92.

86. Bohensky J, Leshinsky S, Srinivas V, Shapiro IM. Chondrocyte autophagy is stimulated by HIF-1 dependent AMPK activation and mTOR suppression. Pediatr Nephrol. 2010; 633-642.

87. Caramés B, Taniguchi N, Seino D, Blanco FJ, D'Lima D, et al. Mechanical injury suppresses autophagy regulators and pharmacologic activation of autophagy results in chondroprotection. Arthritis Rheum. 2012; 64: 1182-1192.

88. Sasaki H, Takayama K, Matsushita T, Ishida K, Kubo S, et al. Autophagy modulates osteoarthritis-related gene expression in human chondrocytes. Arthritis Rheum. 2012; 64: 1920-1928.

89. Zhang Y, Vasheghani F, Li YH, Blati M, Simeone K, et al. Cartilage-specific deletion of mTOR upregulates autophagy and protects mice from osteoarthritis. Ann Rheum Dis. 2015; 74: 1432-1440.

90. Caramés B, Hasegawa A, Taniguchi N, Miyaki S, Blanco FJ, et al. Autophagy activation by rapamycin reduces severity of experimental osteoarthritis. Ann Rheum Dis. 2012; 71: 575-581.

91. Poulose N, Raju R. Sirtuin regulation in aging and injury. Biochim Biophys Acta. 2015; 1852: 2442-2455.

92. Verdin E. NAD□ in aging, metabolism, and neurodegeneration. Science. 2015; 350: 1208-1213.

93. Sellam J, Berenbaum. Is osteoarthritis a metabolic disease? Joint Bone Spine. 2015; 80: 568-573.

94. Øiestad BE, Juhl CB, Eitzen I, Thorlund JB. Knee extensor muscle weakness is a risk factor for development of knee osteoarthritis. A systematic review and meta-analysis. Osteoarthritis Cartilage. 2015; 23: 171-177.

95. Bennell KL, Dobson F, Hinman RS. Exercise in osteoarthritis: moving from prescription to adherence. Best Pract Res Clin Rheumatol. 2014; 28: 93-117.

96. Richter EA, Ruderman NB. AMPK and the biochemistry of exercise: implications for human health and disease. Biochem J. 2009; 418: 261-275.

97. Friedrichsen M, Mortensen B, Pehmøller C, Birk JB, Wojtaszewski JF. Exercise-induced AMPK activity in skeletal muscle: role in glucose uptake and insulin sensitivity. Mol Cell Endocrinol. 2013; 366: 204-214.

98. Fedor D, Kelley DS. Prevention of insulin resistance by n-3 polyunsaturated fatty acids. Curr Opin Clin Nutr Metab Care. 2009; 12: 138-146.

99. Jelenik T, Rossmeisl M, Kuda O, Jilkova ZM, Medrikova D, et al. AMP-activated protein kinase *α*2 subunit is required for the preservation of hepatic insulin sensitivity by n-3 polyunsaturated fatty acids. Diabetes. 2010; 59: 2737-2746.

100. Suchankova G, Tekle M, Saha AK, Ruderman NB, Clarke SD, et al. Dietary polyunsaturated fatty acids enhance hepatic AMP-activated protein kinase activity in rats. Biochem Biophys Res Commun. 2005; 326: 851-858.

101. Rossmeisl M, Flachs P, Brauner P, Sponarova J, Matejkova O, et al. Role of energy charge and AMP-activated protein kinase in adipocytes in the control of body fat stores. Int J Obes Relat Metab Disord. 2004; 28: S38-44.

102. Gabler NK, Radcliffe JS, Spencer JD, Webel DM, Spurlock ME. Feeding long-chain n-3 polyunsaturated fatty acids during gestation increases intestinal glucose absorption potentially via the acute activation of AMPK. J Nutr Biochem. 2009; 20: 17-25.

103. Hill CL, March LM, Aitken D, Lester SE, Batterysby R, et al. Fish oil in knee osteoarthritis: a randomised clinical trial of low dose versus high dose. Ann Rheum Dis. 2016; 75: 23-29.

104. Hershman DL, Unger JM, Crew KD, Awad D, Dakhil SR, et al. Randomized Multicenter Placebo-Controlled Trial of Omega-3 Fatty Acids for the Control of Aromatase Inhibitor-Induced Musculoskeletal Pain: SWOG S0927. J Clin Oncol. 2015; 33: 1910-1917.

105. Abou-Raya A, Abou-Raya S, Khadrawe T. Methotrexate in the treatment of symptomatic knee osteoarthritis: randomised placebo-controlled trial. Ann Rheum Dis. 2014.

106. Panahi Y, Rahimnia AR, Sharafi M, Alishiri G, Saburi A, et al. Curcuminoid Treatment for Knee Osteoarthritis: A Randomized Double-Blind Placebo-Controlled Trial. Phytother Res. 2014; 28: 1625-1631.