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

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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 disease, 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-aminimidazole-4- carboxamide 1-b-D-ribofuranoside), and A-769662 which is a selective and direct AMPK activator through 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, SIRT1 activity 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 regulation of HIF-2α stability and transcriptional activity. Overexpression of SIRT2 and SIRT4 increases, whereas overexpression of SIRT3 inhibits HIF-2α protein stability and transcriptional activity [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-ƙB-dependent genes by deacetylating p65 subunit of NF-ƙB at lysine 310 [17,18], SIRT2 deficiency caused increased expression NF-ƙB-dependent MMPs could result from increased acetylation of NF-ƙB. 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-ƙB-dependent inflammatory response and chondrocytes senescence [32]. The endocrine function of Infraapatellar 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 haploinsufficiency (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 to be 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) and the 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 and 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-ƙB 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-ƙB activation via SIRT1, which deacetylates p65 NF-ƙB subunit, ultimately primes p65 for proteasome degradation [63,64]. Activation of AMPK or SIRT1 inhibits catabolic responses to IL-1β and TNFα via attenuation of NF-ƙB activation in articular chondrocytes [12,23,24,65-67]. In addition, PGC-1α and FOXO3a, at least in part, mediate AMPK to inhibit NF-ƙB 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 (eIF2α) 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 generation and 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], indicating a 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 autophagy or 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 1101BX002234, National Institutes of Health grant AR1067966 and an Innovative Science Grant from the Arthritis Foundation.

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