THE IMPORTANCE OF THE MTOR REGULATORY NETWORK IN CHONDROCYTE BIOLOGY AND OSTEOARTHRITIS

***Elena V. Tchetina**

Senior Scientist, Clinical Immunology Laboratory, Nasonova Research Institute of Rheumatology, Russian Academy of Medical Sciences, Moscow, Russia
**Correspondence to etchetina@mail.ru*

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ABSTRACT

Osteoarthritis (OA) is a chronic disorder associated mainly with pain, limited range of motion, stiffness, joint inflammation, and articular cartilage (AC) destruction. Recent studies demonstrated the involvement of chondrocyte differentiation (hypertrophy) as one of the mechanisms of cartilage degradation in OA. This indicates the involvement of profound alterations in chondrocyte metabolism in the course of cartilage resorption orchestrated by principal changes in the regulation of cellular function. Mammalian target of rapamycin (mTOR) controls critical cellular processes such as growth, proliferation, and protein synthesis, and integrates extracellular signals from growth factors and hormones with amino acid availability and intracellular energy status. The importance of mTOR activity during AC destruction in OA is supported by considerable alterations in the mTOR regulatory network, involving multiple intracellular (availability of growth factors, adenosine triphosphate [ATP], and oxygen as well as autophagy) and extracellular (glucose, amino acid, lipid, and hexosamine) signals. Moreover, variable mTOR gene expression in the peripheral blood of OA patients is associated with increases in pain or synovitis, and indicates a profound metabolic dissimilarity among patients that might require differential approaches to treatment. These issues are discussed in the present review article.

Keywords: Mammalian target of rapamycin (mTOR), osteoarthritis, articular cartilage, peripheral blood, nutrient signalling pathways.

INTRODUCTION

Osteoarthritis (OA) is a systemic condition that can affect single or multiple joints, and involves degenerative changes in the articular cartilage (AC), remodelling of the subchondral bone, and limited synovial inflammation.[1-4] The disability in OA is related to pain and reduced mobility due to AC degeneration. Recent evidence has been presented that disease manifestation is preceded by phenotypic modification (hypertrophy) of articular chondrocytes similar to that observed in foetal chondrocytes during their maturation in the epiphyseal growth plate.[1,5-11] These phenotypic changes were associated with upregulation of genes involved in cartilage destruction, altered expression of apoptosis markers, regulatory growth, and transcription factors.[7-14] However, subsequent inhibition of cartilage degradation by genetic abrogation of the local proteolysis of aggrecan and collagen in animal studies reduced pain, experimental disease severity, and subchondral bone changes, whereas osteophyte development was not affected.[15,16] Moreover, clinical trials applying inhibitors of proteinases or inflammatory cytokines were also unsuccessful.[17-21] Therefore, identification of the upstream factors that regulate expression of catabolic molecules and/or chondrocyte hypertrophy in AC is important for a more profound understanding of the regulatory mechanisms that control articular chondrocyte function.[22]

Previous studies have demonstrated that the majority of the identified genes involved in OA encode signal transduction proteins,[23,24] and numerous signalling pathways have been shown to regulate chondrocyte activities.[24-27] These signal transduction pathways are flexible and, therefore, potentially liable to intervention and modification.[25] As AC destruction in OA is associated with chondrocyte hypertrophy, signalling molecules - which regulate chondrocyte activities both in the growth plate and adult AC during OA - could be of particular interest.[28] For example, it has been reported that ERK1/2 phosphorylation and suppression of p38 phosphorylation produce hypertrophic differentiation of AC chondrocytes.[29,30] At the same time, targeting specific signalling pathways in OA might not be easy due to the high variety and crosstalk among pathways.[31] For example, direct targeting of beta-catenin might be risky because of its importance both in the maintenance of articular chondrocyte phenotype stability and cancer development.[32]

With this information in mind, tracking nutrient signalling pathways, which are thought to be linked to seven of the top ten causes of sickness and death including heart disease, obesity, several cancers, diabetes, and others, is more promising.[33] Traditionally, nutrients such as amino acids, carbohydrates, and lipids were considered as substrates for the generation of high-energy molecules and biosynthetic precursors of macromolecules. However, at present, it is obvious that nutrients can function as signalling molecules in nutrient sensing signalling pathways, which regulate various aspects of energy metabolism and control cell growth, proliferation, and survival.[34]

THE MTOR SIGNALLING PATHWAY

In humans, gene expression is regulated by nutrients interacting with signalling pathways primarily involving mammalian target of rapamycin (mTOR), which integrates contributions from amino acids, growth factors, and molecules involved in the energy status of the cell.[34-37] mTOR is a catalytic subunit of two different complexes including mTOR complex 1 (mTORC1) and mTOR complex 2 (mTORC2). These complexes are distinguished through the binding of mTOR to accessory proteins. Raptor is a rapamycin (RAP)-sensitive regulatory protein associated with mTORC1. mTORC1 is regulated through actions on the tuberous sclerosis (TSC) 1/2 tumour suppressor protein complex. TSC1 has no catalytic activity, whereas TSC2 functions as GTPase-activating protein that inhibits Ras homolog enriched in brain (Rheb). Inactivation of the TSC complex results in activation of mTOR[38] (Figure 1). It has been shown recently that mTORC1 could also be activated by RAS-like GTPase RALB.[39] mTORC1 is in charge of the growth factor and nutrient responses, and therefore, critically regulates proliferation, metabolism, and cell survival. Rictor is a RAP-insensitive companion of mTORC2.[40] The activity of mTORC2 is associated with cell migration, glycogen metabolism, and possible regulation of gluconeogenesis.[41]

MTOR REGULATION

Chondrocyte Function in Foetal Development

The mTOR signalling pathway is responsible for positive regulation of chondrocyte maturation, proliferation, cartilage matrix production, and cell growth during skeletal development.[42-46] (Table 1). RAP administered to young rats significantly reduced endochondral bone growth as evidenced by enlargement of the hypertrophic zone (due to decreased parathyroid hormone/parathyroid hormone-related peptide [PTH/PTHrP] expression and increased Indian hedgehog [Ihh] expression) and a decrease in chondrocyte proliferation associated with downregulation of mTOR. This was accompanied by a reduced number of tartrate-resistant acid phosphatase (TRAP)-positive multinucleated chondro/osteoclasts and decreased expression of receptor activator of nuclear factor kappa-B ligand (RANKL), and vascular endothelial growth factor (VEGF).[44] RAP also reduced insulin-induced growth of foetal rat metatarsal explants due to a selective effect on the hypertrophic zone but not cell proliferation. In the ATDC5 chondrogenic cell line, RAP inhibited proteoglycan (PG) accumulation, Type 10 collagen (COL10A1), and Ihh expression.[47] In the case of nutrient starvation, stress, or reduced availability of growth factors, cellular metabolic adjustments involve inhibition of mTOR activity and induction of autophagy, which serves to promote cell survival. Autophagy was shown to affect foetal chondrocyte differentiation,[48,49] as it developed in terminally differentiated chondrocytes, and permitted these cells to survive in the local microenvironment.[50,51]

Autophagy

During an autophagic state, the cell cannibalises itself to generate energy and/or to remove

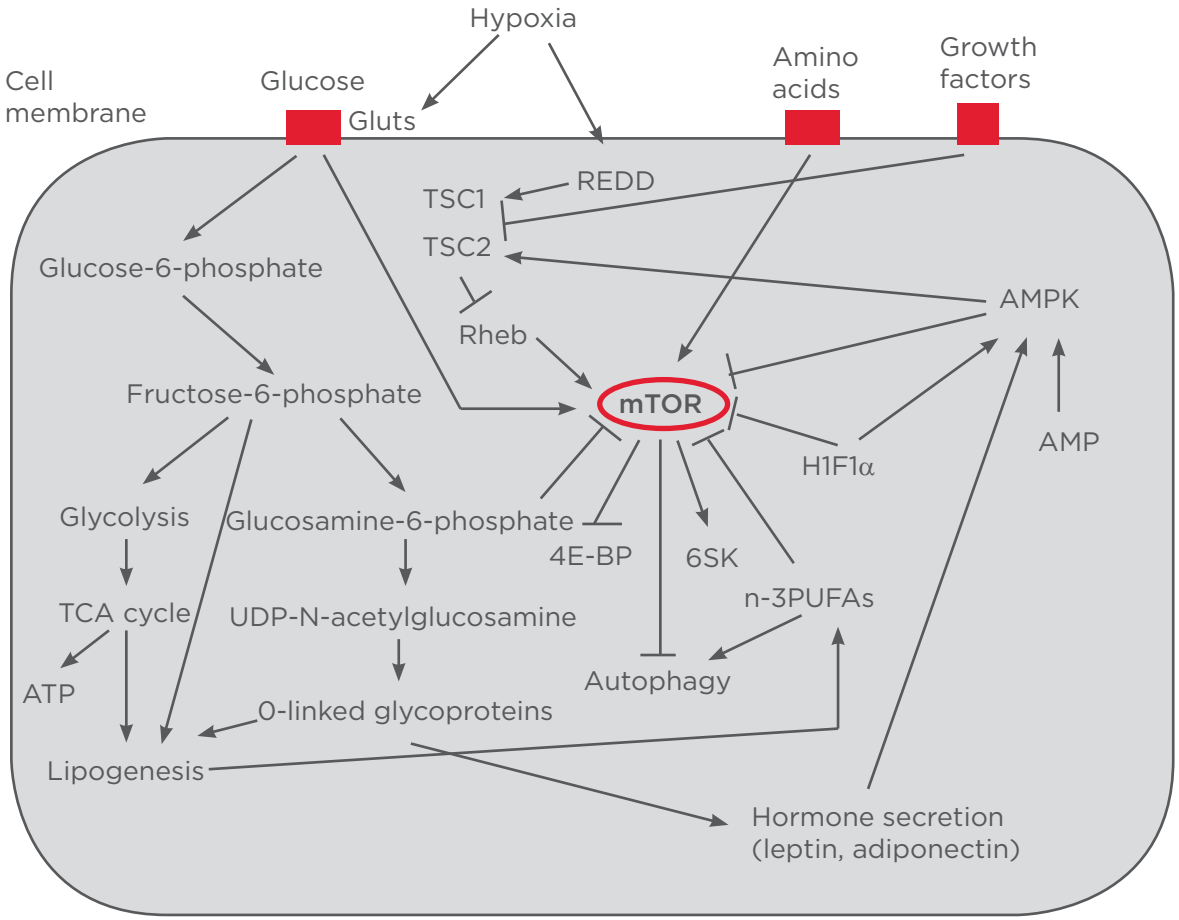

Figure 1: mTOR regulatory network in chondrocyte.
mTOR: mammalian target of rapamycin; Gluts: glucose transporters; REDD: regulated in development and DNA damage responses; TSC1/2: tuberous sclerosis 1/2 tumour suppressor protein complex; Rheb: Ras homolog enriched in brain; AMPK: AMP-activated protein kinase; AMP: adenosine monophosphate; ATP: adenosine triphosphate; TCA: tricarbonic acid cycle; HIF1α: hypoxia inducible factor 1α; 4E-BP: eukaryotic translation initiation factor 4E binding protein; 6SK: ribosomal protein S6 kinase; n-3PUFAs: omega 3 polyunsaturated fatty acids.

defective organelles. When autophagy is extended, Type 2 apoptosis can be activated.[52,53] In chondrocytes, autophagy is regulated by adenosine monophosphate (AMP)-activated protein kinase (AMPK) and mTOR activities in a hypoxia-inducible factor (HIF)-dependent manner.[42,54,55] Increased autophagy was associated with mTOR inhibition upon cell growth cessation.[56,57] Autophagy might be protective during cell stress conditions, as it is increased in normal chondrocytes under nutritional (1% foetal bovine serum [FBS]) or catabolic (interleukin-1 beta [IL-1β] or nitric oxide [NO]-generating agent, sodium nitroprusside) stresses.[58]

Autophagy was observed in the superficial and mid-zones of AC in early animal OA,[59] and in human normal and OA articular chondrocytes.[42,60] Increased autophagy was also noted in mild human OA AC versus normal and severely damaged specimens, and in cultured human OA chondrocytes when compared to normal.[58] However, some studies have also described a decreased autophagic response in mild and severe OA cartilage compared to normal cartilage in humans.[60-62]

Chondrocyte Function in Experimental OA

In mouse experimental OA upregulation of mTOR expression in the knee AC was associated with downregulation of autophagy[61,63] (Table 1). Autophagy has been shown to be capable of ameliorating OA as its activation on mTOR inhibition by RAP or by mTOR deletion reduced disease severity in animal studies.[61,63] This was accompanied by reduced cartilage degradation,

decreased A Disintegrin, and Metalloproteinase with Thrombospondin Motifs (ADAMTS)-5, matrix metalloproteinase (MMP)-13, IL-1β expression, and synovitis.[63] Moreover, activation of autophagy on mTOR gene deletion was associated with a reduction of PG loss, synovial fibrosis, transforming growth factor beta/Mothers against decapentaplegic homolog 3 (TGF-β/SMAD3) signalling, MMP-13, MMP-induced Type 2 collagen degradation, and apoptosis.[61]

Chondrocyte Function in Human AC

At present, some studies also indicate the importance of mTOR signalling in articular chondrocyte metabolism, extracellular matrix (ECM) maintenance, and OA development, as mTOR expression has been reported in human normal and OA articular cartilage.[61,62,64] mTOR upregulation in end-stage OA articular cartilage was associated with downregulation of autophagy, cyclin-dependent kinase inhibitors, and upregulation of regulators of cell death and apoptosis, increased expression of chondrocyte hypertrophy-related COL10A1, and ECM degrading MMP-9 and MMP-13[61,62] (Table 1). The value of mTOR signalling in chondrocyte biology is further supported by studies on the role of mTOR regulators in AC function, as being a major regulator of various cellular processes it is itself a target of regulation.

Functions of Positive mTOR Regulators in Chondrocytes

Nutrients such as amino acids and glucose act through mTOR and directly affect chondrocyte differentiation and long bone growth.[43,47]

Amino acids

Essential amino acids are considered a limiting factor as they are required as substrates for protein synthesis and also act as signalling molecules in several regulatory pathways. Leucine is the most potent regulator of mTOR signalling.[65] The chondroprotective and anti-inflammatory effects of a herbal leucine mix have been demonstrated by a strong inhibition of inducible nitric oxide synthase (iNOS), MMP-9 and MMP-13, NO-production, glycosaminoglycan (GAG) release, and upregulation of COL2A1 expression in human OA chondrocytes and cartilage explants stimulated by IL-1β.[66] In contrast, leucine restriction produced a dose-dependent inhibition of foetal rat metatarsal explant growth. This was accompanied by reduced cell proliferation and hypertrophy, and partial inhibition of mTOR activity. In chondrogenic ATDC5 cells, leucine restriction inhibited cell numbers and PG accumulation as well as COL10A1 and Ihh expression.[43]

Glucose

Glucose regulates mTOR by several mechanisms including inhibition of mTOR during glucose limitation due to a decreased ATP/AMP ratio and a concomitant AMPK activation,[67] and by a glyceraldehyde-3-phosphate dehydrogenase (GAPDH)-dependent inhibition of Rheb.[68] mTOR might be an important player in chondrocyte glucose metabolism as it is subject to regulation by glucose.

As AC is an avascular non-insulin-sensitive tissue, it utilises glucose as a main energy source, a precursor for GAG synthesis, and a regulator of gene expression.[69] In a hypoxic milieu of AC, anaerobic glycolysis is considered a central element in generating ATP to support ECM synthesis and chondrocyte viability, while mitochondrial oxidative phosphorylation (OXPHOS) serves as a physiologic reserve for ATP production[70] and a source of oxidants generated in mitochondrial electron transport chain (ETC) to maintain cellular redox balance in favour of glycolysis.[71] The importance of glycolysis in AC PG synthesis has been confirmed by enhanced inhibition of this process by a glycolysis inhibitor compared to an OXPHOS uncoupler. Moreover, oxidation of GAPDH by hydrogen peroxide resulted in inhibition of PG core protein synthesis *in vitro* and in an animal model of acute arthritis.[72]

Glycolysis is regulated by glucose transporter (GLUT) expression via cytokines. Both anabolic (TGF-β1) and catabolic (IL-1β) factors have been shown to be equally capable of accelerating glucose transport in normal human cultured chondrocytes. However, TGF-β1-stimulated glucose transport was not associated with increased expression of GLUTs (1, 3, 6, 8, 10), and involved protein kinase C (PKC) and extracellular signal-regulated kinase (ERK) activation. However, in a study of IL-1β-stimulation, glucose transport was accompanied by increased expression of GLUT1 and 6, dependent on PKC and p38 mitogen-activated protein (MAP) kinase, and produced higher levels of lactate indicating glycolysis activation.[69]

Table 1: Effects of mTOR signalling on chondrocyte function.

Condition or treatment	mTOR response	Tissue/cell response	Tissue, animal or cell type	Ref
Human OA disease	Upregulation	Decreased expression of autophagy marker ULK1, CDKN1A (p21), increased expression of Type X collagen, MMP-13, and MMP-9	Human end-stage knee articular cartilage	62
Human OA disease	Upregulation	Downregulation of 20 autophagy related genes including ULK1, LC3B, Beclin 1, ATG-3, -5, -13, GABARAPL1, and also BNIP3, CDKN1B (p27), FAS, HSP90AA1, and HSPA8. Upregulation of 5 autophagy related genes and cell death/apoptosis regulators APP, CTSB, BCL2, and BAD	Human end-stage knee articular cartilage	61
Surgical OA in animals	Upregulation	Downregulation of autophagy markers LC3 and ATG5	Dog and mouse knee articular cartilage	61
Inducible cartilage-specific mTOR knock-out mice	Downregulation	Increased expression of ULK1, AMPK1, ATG5, BNIP3, and LC3, protection from cartilage degradation, reduction of proteoglycan loss and articular chondrocyte cellularity, reduction of synovial fibrosis, TGF-β/SMAD3 signalling, MMP-13, and MMP-induced Type 2 collagen degradation, and apoptosis	Knee articular cartilage	61
RAP treated animals	Downregulation	Reduced severity of cartilage degradation, decrease in synovitis, expression of ADAMTS5 and IL-1β, and activation of autophagy marker LC3	Mouse experimental OA articular cartilage	63
Increase in the endogenous n-3 PUFAs	Downregulation	Decreased cartilage destruction and osteophytosis, downregulated MMP-13 and ADAMTS5 expression, reduced chondrocyte loss and ECM degradation, and increased autophagy	Fat-1 transgenic mice articular cartilage	116
Artificially induced temporomandibular condylar cartilage degeneration	Downregulation	Increased expression of autophagy markers Beclin 1 and LC3, and reduced MAPK4K3 activity	Cartilage of rat temporomandibular joint	57
RAP treated mechanically injured cartilage	Downregulation	Enhanced expression of autophagy markers ULK1, Beclin 1, and LC3, cell viability, and decreased sulfated glycosaminoglycan loss	Human and bovine articular cartilage explants	125
RAP treated cells	Downregulation	Reduction of proteoglycan accumulation, Type 10 collagen and Ihh expression	ATDC5 chondrogenic cell line	47
mTOR silenced by siRNA in cells	Downregulation	Increased autophagy marker LC3 expression, less association of BCL2 with Beclin 1	Mouse chondrocytes	42
RAP treated cells	Downregulation	Increased autophagy marker LC3 expression	Mouse chondrocytes	42
RAP treated cells	Downregulation	Decrease of IGF-1-stimulated proteoglycan synthesis	Normal human articular chondrocytes	77
Glucosamine treated cells	Downregulation	Increased autophagy marker LC3 expression	Normal human articular chondrocytes	93
Leucine restriction	Partial downregulation	Inhibition of metatarsal bone growth, reduction of proliferation and hypertrophy	Foetal rat metatarsal explants	43
Leucine restriction	Partial downregulation	Inhibition of cell numbers, proteoglycan accumulation, Type 10 collagen and Ihh expression	ATDC5 chondrogenic cell line	43

Table 1 continued.

Condition or treatment	mTOR response	Tissue/cell response	Tissue, animal or cell type	Ref
RAP treated cells stimulated by IL-1β	Downregulation	Enhanced lysosomal activity, increased expression of autophagy markers Beclin 1 and LC3, COL2A1, aggrecan, reduced expression of MMP-13 and ADAMTS5	Normal human articular chondrocytes	58
RAP treated cells	Downregulation	Increased autophagy markers LC3 and ULK1; AMPK1, Type 2 collagen and aggrecan expression, decreased MMP-13, CCL5/RANTES, and CCL2/MCP-1 expression	Human OA chondrocytes	61
IL-1β-treated cells	Upregulation	Increased expression of MMP-13, CCL2, and CCL5; decreased expression of Type 2 collagen	Human OA chondrocytes	61
Pten-deficient mice	Upregulation	Accelerated hypertrophic differentiation, increased expression of Type 10 collagen, alkaline phosphatase, PDK1, and PI3K signalling	Mouse long bone growth plate	126
RAP treated animals	Downregulation	Reduction of body and tibia growth, decrease in chondrocyte proliferation, enlargement of growth plate hypertrophic zone, increase in Ihh and reduction in PTH/PTHrP, RANKL, VEGF expression, and decline in TRAP-positive multinucleated cells	Weanling rat growth plate	44
RAP treated explants	Downregulation	Decreased insulin-induced bone growth stimulation	Foetal rat metatarsal explants	47
Endochondral ossification: proliferative zone	Upregulation	Inhibition of autophagy	Proliferative growth plate chondrocytes	48, 50
Endochondral ossification: hypertrophic zone	Downregulation	Increase in autophagy and AMPK activity	Terminally differentiated growth plate chondrocytes	48, 50

mTOR: mammalian target of rapamycin; OA: osteoarthritis; MMP: matrix metalloproteinase; TGF-β: transforming growth factor beta; ADAMTS-5: A Disintegrin and Metalloproteinase with Thrombospondin Motifts-5; IL-1β: interleukin-1 beta; PUFAs: n-3 polyunsaturated fatty acids; ECM: extracellular matrix; RAP: rapamycin; IGF-1: insulin-like growth factor 1; PTH/PTHrP: parathyroid hormone/parathyroid hormone related peptide; RANKL: receptor activator of nuclear factor kappa-B ligand; VEGF: vascular endothelial growth factor; TRAP: tartrate resistant acid phosphatase; AMPK: AMP-activated protein kinase; siRNA: small interfering RNA; PDK1: pyruvate dehydrogenase kinase-1; PI3K: phosphoinositide 3 kinase; MAP4K3: mitogen-activated kinase kinase kinase kinase 3.

Glycolysis inhibition by sodium fluoride (NaF) induced a dose-dependent decrease in ATP production, inhibition of chondrocyte proliferation and differentiation, and cell death promotion in a human chondrocytic cell line. Moreover, chondrocyte treatment by a combination of NaF and lactate upregulated the expression of several genes associated with chondrocyte hypertrophy, including alkaline phosphatase (ALP), VEGF, COL10A1, and MMP-13 and MMP-9.[73] Altered glycolysis function has also been shown to be involved in OA. For example, development of spontaneous OA in guinea pigs was associated with depletion of knee chondrocyte intracellular ATP by 50% despite a lack of mitochondrial ultrastructure abnormalities and the presence of an adaptive augmentation of glycolysis indicated by an increased ratio of lactate to pyruvate.[70]

However, proteomic studies in human OA chondrocytes revealed decreased concentrations of proteins involved in glycolysis (enolase, GAPDH, and fructose bisphosphate aldolase).[74] Moreover, a GAPDH inhibitor, monosodium acetate, caused chondrocyte apoptosis evidenced by upregulation of cytochrome-oxidase C and caspase-3 protein levels and reactive oxygen species (ROS) production.[75] In addition, a significant reduction in GLUT1 mRNA observed in clinical OA cartilage samples resulted in failure of OA cartilage repair.[76]

Growth factors

Growth factors, primarily insulin-like growth factor 1 (IGF-1), are known to be mTOR positive regulators in many tissues. In AC, growth factor-related ECM maintenance has also been shown to be mediated by mTOR. Accordingly, a decrease in IGF-1-stimulated PG synthesis in cultured normal human articular chondrocytes was observed upon inhibition of mTOR.[77]

Functions of Negative mTOR Regulators in Chondrocytes

AMPK

AMPK is a heterotrimeric serine-threonine kinase, which is activated when intracellular energy is limiting. It stimulates ATP catabolism and inhibits its synthetic activity.[78] In mammals, AMPK activates the TSC2-TSC1 complex, thus inhibiting mTOR.[79] AMPK regulates energy homeostasis and cellular metabolism, and also exerts anti-inflammatory effects in multiple tissues.

AMPK activity also supports AC homeostasis, as it is constitutively present in normal articular chondrocytes and cartilage but decreased in OA articular chondrocytes and cartilage as well as in normal chondrocytes treated with IL-1β or tumour necrosis factor alpha (TNFα). Attenuation of AMPK resulted in enhanced catabolic responses to IL-1β and TNFα in human and mouse chondrocytes, and was associated with increased MMP-3 and MMP-13 release. Moreover, AMPK activators suppressed cartilage/chondrocyte procatabolic responses to IL-1β and TNFα, and the capacity of TNFα and IL-8 to induce COL10A1 expression.[80-82]

Hypoxia

Hypoxia regulates mTOR via REDD (regulated in development and DNA damage response) 1/2 proteins. REDD1 inhibition of mTOR is mediated by the TSC1/2 complex.[83] A hypoxic environment is optimal and protective for AC as chondrocyte exposure to hypoxia inhibited caspase-8 and the generation of ROS, which were induced in primary articular chondrocytes co-treated with the proteasome inhibitor and apoptosis stimulator, TNF-related apoptosis inducing ligand (TRAIL), under normoxic conditions.[84] In the presence of an optimal (5%) oxygen concentration for porcine articular chondrocytes, maximum ATP generation and the highest protection against IL-1β and NO stimulation were observed. However, in the presence of 20% or 1% oxygen, reduced ATP levels and increased AMPK expression were demonstrated.[85,86] Moreover, hypoxia stimulation induced by cobalt chloride (a hypoxia mimetic) increased glucose uptake and lactate production, and upregulated GLUT1 mRNA expression in primary articular chondrocytes.[76,87]

HIF transcription factors represent a central control mechanism of oxygen sensing.[88] HIF activity is important in both foetal and adult AC. For example, HIF-1α is expressed in the central part of the growth plate,[89] and its inactivation in foetal chondrocytes dramatically inhibits anaerobic energy generation and ECM synthesis.[90] In adult AC, HIF-1α was detected both in normal and OA chondrocytes while an increase in HIF-1α expression was associated with disease severity in OA cartilage.[90] Moreover, HIF-1α was suggested to be involved in cartilage repair, as hyaline-like matrix synthesis was increased upon HIF-1α overexpression in the presence of IGF-1 or BMP-2 in the periosteal cells from animal chondral knee lesions.[91]

Hexosamine pathway

The hexosamine signalling pathway is an additional glucose sensor and is responsible for glucose redistribution either for ATP production or conservation in lipids and/or glycogen. This pathway may be involved in leptin and adiponectin synthesis, which are capable of activating AMPK and inhibiting mTOR function.[34] Another mechanism for the involvement of the hexosamine pathway in mTOR inhibition was observed in normal articular chondrocytes, where glucosamine activated autophagy and inhibited glucose uptake in a manner consistent with the actions of a competitive inhibitor.[92,93]

Glucosamine is an amino sugar widely used to relieve symptoms associated with OA likely because chondrocytes utilise this sugar as a structural component for ECM (glycosaminoglycan) synthesis.[94] Glucosamine has been shown to decrease both foetal and articular chondrocyte proliferation, differentiation, and mineralisation[95,96] due to downregulation of catabolic MMPs, aggrecanases, pro-inflammatory mediators, and the induction of pro-anabolic hyaluronic acid *in vitro*.[97-99]

Some clinical studies reported a decrease in pain and reduction of knee joint space loss after glucosamine treatment.[100] These could be associated with the induction of tissue TGF-β1 and connective tissue growth factor (CTGF) expression, as well as reduction of cartilage oligomeric matrix protein, an AC degradation marker.[101,102] However, the majority of clinical trials have reported numerous non-responders or the absence of this effect when compared with non-pharmacological treatment methods, such as exercise or weight loss.[103-105]

Lipids

Obesity is one of the main risk factors for OA. Activated white adipose tissue increases synthesis of proinflammatory cytokines while adipokines are capable of promoting synovial inflammation, upregulation of cartilage degrading enzymes, and bone matrix remodelling.[106,107] For example, adiponectin induced an increase in MMPs and collagen degradation activity in OA cartilage or cultured human chondrocytes, which was mediated by an mTOR inhibitor, AMPK.[108-110]

Altered lipid metabolism associated with OA involved increased cellular phospholipid and lipid deposition in the joint[111-113] and distorted cholesterol and fatty acid metabolism in OA chondrocytes.[113-115] It has been shown recently that n-3 polyunsaturated fatty acids (PUFAs) supplement significantly alleviated AC destruction and decreased MMP-13 and ADAMTS5 expression in an animal model of OA. Both exogenous and endogenous n-3 PUFAs downregulated mTORC1 activity and promoted autophagy in articular chondrocytes. Moreover, enhancement in synthesis of endogenous n-3 PUFAs from n-6 PUFAs was shown to be capable of delaying the incidence of OA.[116]

MTOR AS A MARKER OF SYSTEMIC OA MANIFESTATIONS

The data described above demonstrate the importance of mTOR signalling in chondrocytes both in normal and OA cartilage. However, systemic OA manifestations require additional studies focusing on tissues outside of the AC which play a role in OA.[117] Alterations in non-tissue-specific regulatory protein expression associated with disease manifestation may suggest differential gene expression in tissues other than cartilage. This is supported by the observation of modified expression of genes associated with foetal chondrocyte differentiation, such as bone morphogenetic proteins 2, 4, and 6, as well as runt-related transcription factor 2 (RUNX2), in the peripheral blood of OA patients.[118]

Assessment of gene expression changes measured in the whole blood is an emerging approach in OA research. Blood-based transcriptome and microarray gene expression analyses appeared capable of distinguishing OA patients from control subjects.[119,120] Moreover, upregulation of IL-1β gene expression in the blood was accompanied by increased pain and predicted a higher risk of radiographic progression of the disease,[121] while high expression of TNFα was associated with high mTOR expression and a higher incidence of synovitis.[62]

Upregulation of mTOR gene expression in the PBMCs might occur concomitantly with increased AC destruction as a positive correlation between mTOR gene expression in the blood and AC was noted in end-stage OA patients.[62] In addition, elevated mTOR gene expression was observed in both peripheral blood and AC of end-stage OA patients.[62] At the same time, excessive inhibition of mTOR expression is also deleterious as it might result in significantly more pain upon joint function,[62] which might be associated with the ERK pathway activation in sensory neurons.[122] Considering this information, although treatment of mice by mTOR inhibitors has been shown to be capable of reducing the severity of experimental OA[60,63] and inflammatory arthritis,[64,123] and is suggested for treatment of human OA,[124] mTOR inhibition in OA patients should be considered with caution.

CONCLUSION

The importance of mTOR regulation in chondrocyte biology and altered activity of positive and negative regulators of mTOR signalling pathway associated with OA suggest its involvement in the disease onset, progression, and outcome. However, the majority of studies on mTOR signalling associated with OA were performed using animal models and cultured chondrocytes. The results gained in these conditions do not necessary imply that exactly the same processes are involved in human OA. Therefore, clinical studies are warranted in order to truly identify the role of mTOR signalling in OA. As mTOR regulation involves both environmental nutrient signalling and is capable of modulating chondrocyte energy turnover, cell growth, proliferation, and survival, further detailed studies of mTOR signalling in OA patients might provide opportunities for the identification of new targets for therapeutic intervention, which could lead to secure and efficient therapies that reduce the symptoms and slow the progression of OA.

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