VIEW POINT
Journal of Cellular Biochemistry 114:245–249 (2013)

Journal of Cellular Biochemistry

Vertical Inhibition of the PI3K/Akt/mTOR Pathway for the Treatment of Osteoarthritis

Jiezhong Chen,[1,2*] Ross Crawford,[1] and Yin Xiao[1**]

[1]*Institute of Health and Biomedical Innovation, Queensland University of Technology, Brisbane, Queensland 4059, Australia*

[2]*Illawarra Health and Medical Research Institute, University of Wollongong, Northfields Avenue, New South Wales 2522, Australia*

ABSTRACT

Osteoarthritis is characterized by degenerative alterations of articular cartilage including both the degradation of extracellular matrix and the death of chondrocytes. The PI3K/Akt pathway has been demonstrated to involve in both processes. Inhibition of its downstream target NF-kB reduces the degradation of extracellular matrix via decreased production of matrix metalloproteinases while inhibition of mTOR increased autophagy to reduce chondrocyte death. However, mTOR feedback inhibits the activity of the PI3K/Akt pathway and inhibition of mTOR could result in increased activity of the PI3K/Akt/NF-kB pathway. We proposed that the use of dual inhibitors of PI3K and mTOR could be a promising approach to more efficiently inhibit the PI3K/Akt pathway than rapamycin or PI3K inhibitor alone and produce better treatment outcome. J. Cell. Biochem. 114: 245–249, 2013.

KEY WORDS: OSTEOARTHRITIS; EXTRACELLULAR MATRIX METALLOPROTEINASES; AUTOPHAGY; PI3K/AKT PATHWAY

Osteoarthritis (OA) is a joint degenerative disease affecting mostly the aged population. OA is characterized by the degenerative alteration of articular cartilage although other joint tissues are involved [Pitsillides and Beier, 2011; Jaiprakash et al., 2012; Troeberg and Nagase, 2012]. This results in pain and dysfunction of the affected joint. Cartilage is an avascular structure consisting of chondrocytes and extracellular matrix (ECM). The degenerative alteration of OA includes both the death of chondrocytes and the degradation of ECM. In OA, ECM is degraded by increased matrix metalloproteinases (MMPs), and adamalysin with thrombospondin motif 5 (ADAMTS-5), which is a key destructive process [Prasadam et al., 2012a; Troeberg and Nagase, 2012]. The main components of ECM are aggrecan and type II collagen [Pitsillides and Beier, 2011]. Many MMPs have been found to be increased in OA. Among them MMP-13, MMP-3, and MT1-MMP are considered the most important. MMP-13 not only degrades aggrecan but also preferentially digests type II collagen rather than types I and III [Billinghurst et al., 1997]. Both MMP-3 and MT1-MMP activate proMMP-13 in OA [Knauper et al., 2002]. MMP-3 can also digest aggrecan and activate many other proMMPs including proMMP-2, -7, -8, and -9. In OA, Reactive oxygen species are increased as well as cytokines due to chronic inflammation and both processes result in increasing apoptosis of chondrocytes [Blanco et al., 1998; Facchini et al., 2011]. The macromolecules produced by apoptotic chondrocytes may further increase ROS and lead to more chondrocyte death [Lopez-Armada et al., 2006]. Cell signaling pathways have been reported in the regulation of chondrocyte hypertrophy, chondrocyte and osteoblast interactions, and degradation of ECM [Prasadam et al., 2010ab, 2012b]. The intracellular signaling pathway PI3K/Akt has also been demonstrated to be involved in both cellular and ECM alterations. Manipulation of this pathway could be a useful therapy for OA, which is currently difficult to cure.

PI3K/Akt PATHWAY IN OSTEOARTHRITIS

Activation of the PI3K/Akt pathway can increase MMP production by chondrocytes via its multiple downstream target proteins. The PI3K/Akt regulates a cascade of changes through its broad target

*Correspondence to: Dr. Jiezhong Chen, Institute of Health and Biomedical Innovation, Queensland University of Technology, 60 Musk Avenue, Kelvin Grove, Brisbane, QLD 4059, Australia. E-mail: chen.jiezhong@qut.edu.au

**Correspondence to: Yin Xiao, Professor, Institute of Health and Biomedical Innovation, Queensland University of Technology, 60 Musk Avenue, Kelvin Grove, Brisbane, QLD 4059, Australia. E-mail: yin.xiao@qut.edu.au

Manuscript Received: 31 May 2012; Manuscript Accepted: 15 August 2012

Accepted manuscript online in Wiley Online Library (wileyonlinelibrary.com): 28 August 2012

DOI 10.1002/jcb.24362 • © 2012 Wiley Periodicals, Inc.

proteins such as mTOR, NF-κB, GSK-3beta, and p53 [Hay, 2005; Chen and Huang, 2009; Kalaany and Sabatini, 2009; Chen, 2010, 2011, 2012]. NF-κB, a transcriptional factor, has been demonstrated to be a key regulator of MMP production [Lin et al., 2011]. NF-κB regulates MMP-13 production via two signaling pathways E74-like factor 3 (ELF3) and Hypoxia-induced factor 2alpha/unt-related transcription factor 2 (HIF2alpha/Runx2) (Fig. 1) [Goldring et al., 2011]. ELF3 can directly bind to the promoter of MMP-13 to regulate synthesis of MMP-13 [Otero et al., 2012]. SiRNA silence of ELF3 abolished IL-1beta induced MMP13 secretion. Runx-2 is correlated with the expression of MMP-13 and is necessary for IL-1beta induced MMP13 [Mengshol et al., 2001]. Runx-2 binds to the promoter of MMP13 and controls its transcription. Inhibition of the PI3K/Akt pathway and NF-κB has been considered as an option for the treatment of osteoarthritis. Indeed, inhibition of pAkt and NF-κB by curcumin decreased IL-1beta stimulating MMP secretion [Schulze-Tanzil et al., 2004; Shakibaei et al., 2007]. In addition, activator protein-1 (AP-1) is also involved in the regulation of MMP-13 with a binding site in the promoter of MMP-13 [Benderdour et al., 2002]. It has been demonstrated that JUN and its downstream target AP-1 are key mediators of IL-1 and TNF-alpha induced MMP-13 expression [Liacini et al., 2002, 2003]. Inhibition of JUN by SP600125 and AP-1 by norhihydroguaiaretic acid (NDGA) resulted in decreased MMP-13 mRNA stimulated by TNF-alpha [Liacini et al., 2003]. Inflammatory factors, which are increased in the progressive stage of OA can lead to ECM degradation. The most commonly increased cytokines are IL1beta, IL-6, IL-6 like cytokine oncostatin M, IL17, and TNF-alpha. Activated pAkt and NF-κB have been shown to play key roles in cytokine-induced secretion of MMPs [Mengshol et al., 2001; Shakibaei et al., 2007]. IL-6 like cytokine oncostatin M (OSM) has been shown to increase MMP-1 and MMP-13 via the PI3K/Akt pathway [Litherland et al., 2008]. PI3K specific inhibitor Ly294002 inhibited OSM-induced production of MMPs in chondrocytes [Litherland et al., 2008].

Another interesting sample is the role of the PI3K/Akt pathway in leptin-induced MMP secretion in chondrocytes [Koskinen et al., 2011; Hui et al., 2012]. Leptin is highly increased in obesity (30-fold) and thus this study is used to explain the mechanism for obesity-associated osteoarthritis which is demonstrated by epidemiological studies and animal models [Gill et al., 2011; Runhaar et al., 2011; Anandacoomarasamy et al., 2012; Killock, 2012]. Leptin, can increase the secretion of MMPs 1, 3, 9, and 13 by chondrocytes [Hui et al., 2012]. The PI3K/Akt pathway has been demonstrated to be important in the production of these MMPs. Ly294002, a PI3K specific inhibitor can abolish the effect of leptin on the production of MMPs in chondrocytes.

The downstream target protein of PI3K/Akt pathway-mTOR has also involved in the cell death of chondrocytes in OA. In OA, increased cell death is accompanied by autophagy, a cell survival mechanism. Under stress such as nutrient deprivation, the cells can degrade unnecessary cellular components to save energy for cell survival [Levine and Klionsky, 2004]. Therefore, autophagy can reduce macromolecules produced by dead cells from chondrocytes [Sasaki et al., 2012]. These molecules can produce ROS, resulting in more cell death. The decreased autophagy with age may be an explanation for age-related OA [Carames et al., 2010]. In the early stage of OA, there is increased apoptosis of chondrocytes in the superficial and middle zones of the cartilage as indicated by increased active caspase 3 and TUNEL signal [Almonte-Becerril et al., 2010]. Correspondingly autophagy is also increased as indicated by LC3II molecules. Inhibition of mTOR by rapamycin, an

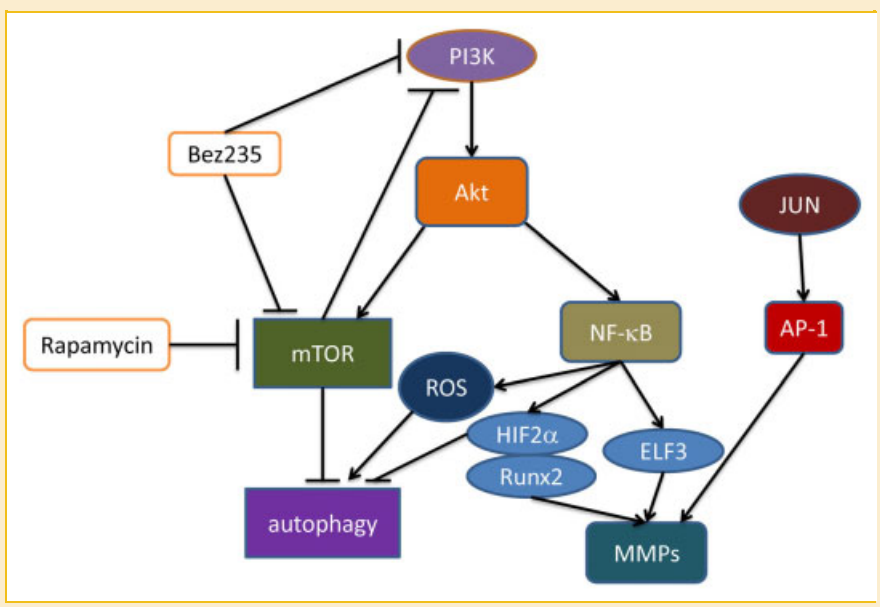

Fig. 1. PI3K/Akt pathway in cartilage degeneration. PI3K/Akt can activate mTOR to suppress autophagy. PI3K/Akt can also activate NF-κB, which in turn increases the synthesis of MMPs via HIF2α and ELF3. HIF2α inhibits autophagy while ROS increases autophagy. In addition, JUN and AP-1 also regulate MMPs. mTOR can feedback inhibit the activity of PI3K/Akt pathway. PI3K, phosphoinositide 3-kinase; Akt, protein kinase B; NF-κB, nuclear factor-kB; MMPs, matrix metalloproteinases; ELF3, E74-like factor 3; HIF-2α, hypoxia-induced factor 2α; Runx2, Runt-related transcription factor 2; mTOR, mammalian target of rapamycin; ROS, reactive oxygene species; AP-1, activator protein-1.

mTOR inhibitor has been shown to increase autophagy in several cell types [Sabers et al., 1995; Shigemitsu et al., 1999]. Recently, rapamycin has also been demonstrated to reduce autophagy of chondrocytes in an osteoarthritis animal model [Sasaki et al., 2012; Carames et al., 2012]. Rapamycin is more effective than PI3K specific inhibitor Ly294002 because mTOR is also activated by MAPK pathway, AMPK and serum amino acids. Carames et al. [2012] tested rapamycin in osteoarthritis in C57Bl/6J mice established by transaction of the medial meniscotibial ligament and the medial collateral ligament in the right knee. The effect of rapamycin on the pathway is detected by decreased phosphorylation of ribosomal protein S6. Increased autophagy is indicated by LC3II. Rapamycin was shown to decrease the severity of osteoarthritis-like changes such as cartilage degeneration, loss of surface lamina and fibrillations. The study also showed that ADAMTS-5, an aggrecan degradation proteinase, was decreased and Rapamycin preserved the cellularity and decreased synovial inflammation with decreased IL-1beta. However, the study did not examine MMPs.

DUAL INHIBITION OF PI3K AND mTOR

We think that a problem in using rapamycin is that it can increase the activity of the PI3K/Akt pathway. It has been demonstrated that mTOR negatively regulates PI3K/Akt activity [Harrington et al., 2005]. Silencing of mTOR with siRNA or inhibition by rapamycin of mTOR resulted in activation of the PI3K/Akt pathway [McDonald et al., 2008]. mTOR can increase p70S6K which in turn phosphorylates IRS1 and inhibits IRS1, a major upstream regulator of the PI3K/Akt pathway [Hay, 2005]. The feedback inhibition of mTOR has been considered as the reason for the limited efficacy of mTOR inhibition in cancer treatment [Cloughesy et al., 2008]. However, it is not well studied about the feedback inhibition of mTOR in the pathway when mTOR inhibitor is used for the treatment of OA. When mTOR is inhibited, the activity of PI3K/Akt pathway increases. Therefore, mTOR inhibition by rapamycin may result in the increase of the activity of the PI3K/Akt pathway. In the event of mTOR inhibition, Akt can activate NF-κB to affect MMP secretion. Therefore, rapamycin may increase autophagy to protect cartilage but it may increase MMP secretion. Evidence shows that rapamycin inhibits mTOR but increases NF-κB. In rat glomerular MC cells, rapamycin further amplified IL-beta-induced NF-κB activation [Osman et al., 2011]. This led to increased MMP-9 promoter activity although rapamycin inhibited MMP-9 mRNA stability via other mechanisms. No study has investigated the effect of rapamycin on the production of MMPs when it is used for increasing autophagy. Another adverse effect of the rapamycin is that NF-κB activated HIF2 is the suppressor of autophagy. It is not examined if this effect decreases the effect of rapamycin. Bohensky et al. [2009] showed that knockout of HIF2 by siRNA resulted in a robust increase of autophagy with decreased mTOR activity. Controversy, NF-κB can activate anti-oxidant enzymes such as superoxide dismutases and glutathione peroxidise to reduce ROS [Mathy-Hartert et al., 2008]. Inhibition of NF-κB can lead to increased ROS, which enhances autophagy [Scherz-Shouval et al., 2007].

We propose that the use of dual inhibitors of PI3K and mTOR could be a promising approach to more efficiently inhibit the PI3K/Akt pathway than rapamycin or PI3K inhibitor alone. The inhibitor of the PI3K may not be sufficient to decrease mTOR because mTOR is also regulated by the MAPK pathway and serum levels of amino acids independent of the PI3K/Akt pathway. However, inhibition of mTOR (by rapamycin) only may lead to increased activity of the PI3K/Akt/NF-κB/MMP pathway. Therefore, inhibitors with dual effect on both mTOR and PI3K/Akt are superior to PI3K or mTOR inhibitor alone. At present several dual inhibitors of PI3K and mTOR have been developed by various companies including XL765, SF1126, PI103, Bez235, BGT226 and GDC0980. Several of them have been used in clinical trials for cancer therapy and demonstrated to be well tolerated, indicating a potential application in the treatment of OA.

CONCLUSION

In conclusion, the PI3K/Akt/mTOR pathway is important in regulating the production of MMPs by chondrocytes. Therefore, selective inhibition of the pathway may prevent cartilage degeneration by reducing the production of MMPs. Here, we propose that dual inhibition of PI3K and mTOR may be a promising approach, as mTOR inhibitor alone (rapamycin) may increase PI3K/Akt activity due to the inhibitory role of mTOR on PI3K/Akt.

REFERENCES

Almonte-Becerril M, Navarro-Garcia F, Gonzalez-Robles A, Vega-Lopez MA, Lavalle C, Kouri JB. 2010. Cell death of chondrocytes is a combination between apoptosis and autophagy during the pathogenesis of osteoarthritis within an experimental model. Apoptosis 15(5):631–638.

Anandacoomarasamy A, Leibman S, Smith G, Caterson I, Giuffre B, Fransen M, Sambrook PN, March L. 2012. Weight loss in obese people has structure-modifying effects on medial but not on lateral knee articular cartilage. Ann Rheum Dis 71(1):26–32.

Benderdour M, Tardif G. Pelletier JP, Di Battista JA, Reboul P, Ranger P, Martel-Pelletier J. 2002. Interleukin 17 (IL-17) induces collagenase-3 production in human osteoarthritic chondrocytes via AP-1 dependent activation: Differential activation of AP-1 members by IL-17 and IL-1beta. J Rheumatol 29(6):1262–1272.

Billinghurst RC, Dahlberg L, Ionescu M, Reiner A, Bourne R, Rorabeck C, Mitchell P, Hambor J, Diekmann O, Tschesche H, Chen J, Van Wart H, Poole AR. 1997. Enhanced cleavage of type II collagen by collagenases in osteoarthritic articular cartilage. J Clin Invest 99(7):1534–1545.

Blanco FJ, Guitian R, Vazquez-Martul E, de Toro FJ, Galdo F. 1998. Osteoarthritis chondrocytes die by apoptosis. A possible pathway for osteoarthritis pathology. Arthritis Rheum 41(2):284–289.

Bohensky J, Terkhorn SP, Freeman TA, Adams CS, Garcia JA, Shapiro IM, Srinivas V. 2009. Regulation of autophagy in human and murine cartilage: Hypoxia-inducible factor 2 suppresses chondrocyte autophagy. Arthritis Rheum 60(5):1406–1415.

Carames B, Taniguchi N, Taniguchi N, Miyaki S, Blanco FJ, Lotz M. 2010. Autophagy is a protective mechanism in normal cartilage, and its aging-related loss is linked with cell death and osteoarthritis. Arthritis Rheum 62(3):791–801.

Carames B, Hasegawa A, Otsuki S, Blanco FJ, Lotz M. 2012. Autophagy activation by rapamycin reduces severity of experimental osteoarthritis. Ann Rheum Dis 71(4):575–581.

Chen J. 2010. The Src/PI3K/Akt signal pathway may play a key role in decreased drug efficacy in obesity-associated cancer. J Cell Biochem 110(2):279–280.

Chen J. 2011. Multiple signal pathways in obesity-associated cancer. Obes Rev 12(12):1063–1070.

Chen J. 2012. Targeted therapy of obesity-associated colon cancer. Transl Gastrointest Cancer 1(1):44–57.

Chen J, Huang X. 2009. Activation of p53 for the treatment of cancer. J Cell Biochem 107(4):567–568.

Cloughesy TF, Yoshimoto K, Nghiemphu P, Brown K, Dang J, Zhu S, Hsueh T, Chen Y, Wang W, Youngkin D, Liau L, Martin N, Becker D, Bergsneider M, Lai A, Green R, Oglesby T, Koleto M, Trent J, Horvath S, Mischel PS, Mellinghoff IK, Sawyers CL. 2008. Antitumor activity of rapamycin in a Phase I trial for patients with recurrent PTEN-deficient glioblastoma. PLoS Med 5(1):e8.

Facchini A, Stanic I, Cetrullo S, Borzi RM, Filardo G, Flamigni F. 2011. Sulforaphane protects human chondrocytes against cell death induced by various stimuli. J Cell Physiol 226(7):1771–1779.

Gill RS, Al-Adra DP, Shi X, Sharma AM, Birch DW, Karmali S. 2011. The benefits of bariatric surgery in obese patients with hip and knee osteoarthritis: A systematic review. Obes Rev 12(12):1083–1089.

Goldring MB, Otero M, Plumb DA, Dragomir C, Favero M, El Hachem K, Hashimoto K, Roach HI, Olivotto E, Borzi RM, Marcu KB. 2011. Roles of inflammatory and anabolic cytokines in cartilage metabolism: Signals and multiple effectors converge upon MMP-13 regulation in osteoarthritis. Eur Cell Mater 21:202–220.

Harrington LS, Findlay GM, Lamb RF. 2005. Restraining PI3K: mTOR signalling goes back to the membrane. Trends Biochem Sci 30(1):35–42.

Hay N. 2005. The Akt-mTOR tango and its relevance to cancer. Cancer Cell 8(3):179–183.

Hui W, Litherland GJ, Elias MS, Kitson GI, Cawston TE, Rowan AD, Young DA. 2012. Leptin produced by joint white adipose tissue induces cartilage degradation via upregulation and activation of matrix metalloproteinases. Ann Rheum Dis 71(3):455–462.

Jaiprakash A, Prasadam I, Feng JQ, Liu Y, Crawford R, Xiao Y. 2012. Phenotypic characterization of osteoarthritic osteocytes from the sclerotic zones: A possible pathological role in subchondral bone sclerosis. Int J Biol Sci 8(3):406–417.

Kalaany NY, Sabatini D. 2009. Tumours with PI3K activation are resistant to dietary restriction. Nature 458(7239):725–731.

Killock D. 2012. Osteoarthritis: The influence of obesity on OA-does size matter or is metabolic dysfunction more important? Nat Rev Rheumatol 8(2):61.

Knauper V, Bailey L, Worley JR, Soloway P, Patterson ML, Murphy G. 2002. Cellular activation of proMMP-13 by MT1-MMP depends on the C-terminal domain of MMP-13. FEBS Lett 532(1–2):127–130.

Koskinen A, Vuolteenaho K, Nieminen R, Moilanen T, Moilanen E. 2011. Leptin enhances MMP-1, MMP-3 and MMP-13 production in human osteoarthritic cartilage and correlates with MMP-1 and MMP-3 in synovial fluid from OA patients. Clin Exp Rheumatol 29(1):57–64.

Levine B, Klionsky DJ. 2004. Development by self-digestion: Molecular mechanisms and biological functions of autophagy. Dev Cell 6(4):463–477.

Liacini A, Sylvester J, Li WQ, Zafarullah M. 2002. Inhibition of interleukin-1-stimulated MAP kinases, activating protein-1 (AP-1) and nuclear factor kappa B (NF-kappa B) transcription factors down-regulates matrix metalloproteinase gene expression in articular chondrocytes. Matrix Biol 21(3): 251–262.

Liacini A, Sylvester J, Li WQ, Huang W, Dehnade F, Ahmad M, Zafarullah M. 2003. Induction of matrix metalloproteinase-13 gene expression by TNF-alpha is mediated by MAP kinases, AP-1, and NF-kappaB transcription factors in articular chondrocytes. Exp Cell Res 288(1):208–217.

Lin TH, Tang CH, Wu K, Fong YC, Yang RS, Fu WM. 2011. 15-deoxy-delta(12,14) -prostaglandin-J2 and ciglitazone inhibit TNF-alpha-induced matrix metalloproteinase 13 production via the antagonism of NF-kappaB activation in human synovial fibroblasts. J Cell Physiol 226(12):3242–3250.

Litherland GJ, Dixon C, Lakey RL, Robson T, Jones D, Young DA, Cawston TE, Rowan AD. 2008. Synergistic collagenase expression and cartilage collagenolysis are phosphatidylinositol 3-kinase/Akt signaling-dependent. J Biol Chem 283(21):14221–14229.

Lopez-Armada MJ, Carames B, Lires-Dean M, Cillero-Pastor B, Ruiz-Romero C, Galdo F, Blanco FJ. 2006. Cytokines, tumor necrosis factor-alpha and interleukin-1beta, differentially regulate apoptosis in osteoarthritis cultured human chondrocytes. Osteoarthritis Cartilage 14(7):660–669.

Mathy-Hartert M, Hogge L, Sanchez C, Deby-Dupont G, Crielaard JM, Henrotin Y. 2008. Interleukin-1beta and interleukin-6 disturb the antioxidant enzyme system in bovine chondrocytes: A possible explanation for oxidative stress generation. Osteoarthritis Cartilage 16(7):756–763.

McDonald PC, Oloumi A, Mills J, Dobreva I, Maidan M, Gray V, Wederell ED, Bally MB, Foster LJ, Dedhar S. 2008. Rictor and integrin-linked kinase interact and regulate Akt phosphorylation and cancer cell survival. Cancer Res 68(6):1618–1624.

Mengshol JA, Vincenti MP, Brinckerhoff CE. 2001. IL-1 induces collagenase-3 (MMP-13) promoter activity in stably transfected chondrocytic cells: Requirement for Runx-2 and activation by p38 MAPK and JNK pathways. Nucleic Acids Res 29(21):4361–4372.

Osman B, Akool el S, Doller A, Muller R, Pfeilschifter J, Eberhardt W. 2011. Differential modulation of the cytokine-induced MMP-9/TIMP-1 protease-antiprotease system by the mTOR inhibitor rapamycin. Biochem Pharmacol 81(1):134–143.

Otero M, Plumb DA, Tsuchimochi K, Dragomir CL, Hashimoto K, Peng H, Olivotto E, Bevilacqua M, Tan L, Yang Z, Zhan Y, Oettgen P, Li Y, Marcu KB, Goldring MB. 2012. E74-like factor 3 (ELF3) impacts on matrix metalloproteinase 13 (MMP13) transcriptional control in articular chondrocytes under proinflammatory stress. J Biol Chem 287(5):3559–3572.

Pitsillides AA, Beier F. 2011. Cartilage biology in osteoarthritis–lessons from developmental biology. Nat Rev Rheumatol 7(11):654–663.

Prasadam I, Friis T, Shi W, van Gennip S, Crawford R, Xiao Y. 2010a. Osteoarthritic cartilage chondrocytes alter subchondral bone osteoblast differentiation via MAPK signalling pathway involving ERK1/2. Bone 46(1):226–235.

Prasadam I, van Gennip S, Friis T, Shi W, Crawford R, Xiao Y. 2010b. ERK-1/2 and p38 in the regulation of hypertrophic changes of normal articular cartilage chondrocytes induced by osteoarthritic subchondral osteoblasts. Arthritis Rheum 62(5):1349–1360.

Prasadam I, Crawford R, Xiao Y. 2012a. Aggravation of ADAMTS and matrix metalloproteinase production and role of ERK1/2 pathway in the interaction of osteoarthritic subchondral bone osteoblasts and articular cartilage chondrocytes—possible pathogenic role in osteoarthritis. J Rheumatol 39(3): 621–634.

Prasadam I, Mao X, Wang Y, Shi W, Crawford R, Xiao Y. 2012b. Inhibition of p38 pathway leads to OA-like changes in a rat animal model. Rheumatology (Oxford) 51(5):813–823.

Runhaar J, Koes BW, Clockaerts S, Bierma-Zeinstra SM. 2011. A systematic review on changed biomechanics of lower extremities in obese individuals: A possible role in development of osteoarthritis. Obes Rev 12(12):1071–1082.

Sabers CJ, Martin MM, Brunn GJ, Williams JM, Dumont FJ, Wiederrecht G, Abraham RT. 1995. Isolation of a protein target of the FKBP12-rapamycin complex in mammalian cells. J Biol Chem 270(2):815–822.

Sasaki H, Takayama K, Matsushita T, Ishida K, Kubo S, Matsumoto T, Fujita N, Oka S, Kurosaka M, Kuroda R. 2012. Autophagy modulates osteoarthritis-related gene expressions in human chondrocytes. Arthritis Rheum 64:1920–1928.

Scherz-Shouval R, Shvets E, Fass E, Shorer H, Gil L, Elazar Z. 2007. Reactive oxygen species are essential for autophagy and specifically regulate the activity of Atg4. EMBO J 26(7):1749–1760.

Schulze-Tanzil G, Mobasheri A, Sendzik J, John T, Shakibaei M. 2004. Effects of curcumin (diferuloylmethane) on nuclear factor kappaB signaling in interleukin-1beta-stimulated chondrocytes. Ann N Y Acad Sci 1030:578–586.

Shakibaei M, John T, Schulze-Tanzil G, Lehmann I, Mobasheri A. 2007. Suppression of NF-kappaB activation by curcumin leads to inhibition of expression of cyclo-oxygenase-2 and matrix metalloproteinase-9 in human articular chondrocytes: Implications for the treatment of osteoarthritis. Biochem Pharmacol 73(9):1434–1445.

Shigemitsu K, Tsujishita Y, Hara K, Nanahoshi M, Avruch J, Yonezawa K. 1999. Regulation of translational effectors by amino acid and mammalian target of rapamycin signaling pathways. Possible involvement of autophagy in cultured hepatoma cells. J Biol Chem 274(2):1058–1065.

Troeberg L, Nagase H. 2012. Proteases involved in cartilage matrix degradation in osteoarthritis. Biochim Biophys Acta 1824(1):133–145.