Protection from osteoarthritis: targeting mTOR and autophagy

Dr. Yue Zhang

Cartilage scientist, Division of Genetics and Development, The Toronto Western Research Institute, Toronto Western Hospital, University Health Network, Toronto, Ontario, Canada
Telephone: 1-416-603-5800 Ext 4797 Email: yuezhang@unhresearch.ca

Abstract

BACKGROUND and OBJECTIVES: mTOR (a serine/threonine protein kinase) is a major repressor of autophagy, a cell survival mechanism. The specific in vivo mechanism of mTOR signalling in OA pathophysiology is not fully characterised. We determined the expression of mTOR and known autophagy genes in human OA cartilage as well as mouse and dog models of experimental OA. We created cartilage-specific mTOR knockout (KO) mice to determine the specific role of mTOR in OA pathophysiology and autophagy signalling in vivo.

METHODS: Inducible cartilage-specific mTOR KO mice were generated and subjected to mouse model of OA. Human OA chondrocytes were treated with rapamycin and transfected with Unc-51–like kinase 1 (ULK1) siRNA to determine mTOR signalling.

RESULTS: mTOR is overexpressed in human OA cartilage as well as mouse and dog experimental OA. This correlates with increased chondrocyte apoptosis and reduced expression of key autophagy genes during OA. Subsequently, we show for the first time that cartilage-specific ablation of mTOR results in increased autophagy signalling and a significant protection from destabilization of medial meniscus (DMM)-induced OA associated with a significant reduction in the articular cartilage degradation, apoptosis and synovial fibrosis. Further, we show that regulation of ULK1/adenosine monophosphate-activated protein kinase (AMPK) signalling pathway by mTOR may partially be responsible for regulating autophagy signalling and the balance between catabolic and anabolic factors in the articular cartilage.

CONCLUSIONS: This study provides a direct evidence of the role of mTOR and its downstream modulation of autophagy in articular cartilage homeostasis. Thus targeting cellular homeostasis mediators, such as mTOR and its downstream signalling by autophagy pathway may be a promising therapeutic strategy to achieve chondroprotection and correct the imbalance between catabolic and anabolic processes during OA and related disorders.

Keywords: human, dog, mouse, autophagy, mTOR, osteoarthritis, cartilage, rapamycin

Introduction

Osteoarthritis (OA) is the most common form of arthritis whose exact pathophysiology is still unknown. Some recent efforts are directed at defining early changes that predispose or lead to the onset of OA. As the majority patients develop OA as a function of increasing age as “system – failure” disease, it is essential to understand aging-related changes in cell function. The genome-wide gene expression profiles (GEP) would be expected to assess the early prediction, diagnosis, and prognosis for OA. Our preliminary results have identified unique pattern for OA patients compared to the normal (Figure 1). One surrogate for “absolute” bona-fide system of multicellular organisms would be dynamics global gene regulatory network (GRN), which inherently bears characterization of self-organization and it is critical for functionality of organism level systems. The matter of fact is that researchers could hence identify the unique patterns of each stage in the evolution of disease, therefore possibly more cost-effective alternatives than MRI images, which are suitable for its corresponding stage-specific interventions with drug against biomarkers (GEDI may read both system pattern and individual markers inside).
An holistic view on genome-wide gene expression profile (GEP) of OA as system level GRN’s surrogate. In red, represents the high gene expression levels; in blue, the low expression levels. GEDI: gene expression dynamic inspector, one program characterized with self-organization

Figure 1 The GEDI “cloud”s of normal (left 1-5 ) or mice ( right 6-10) with OA .

The major features of OA are cartilage degradation, synovial inflammation and subchondral bone remodeling. OA is currently managed by using treatments such as acetaminophen, opioids, and non-steroidal anti-inflammatory drugs (NSAIDs) including selective cyclooxygenase-2 (COX-2) inhibitors, all of which only provide symptomatic relief. Loss of chondrocyte cellularity within the articular cartilage is one of the prominent events that contribute to its degradation. However, it is still uncertain as to what mechanisms control the fate of chondrocytes within the articular cartilage during normal versus OA conditions.

Mammalian target of rapamycin (mTOR), a serine/threonine protein kinase, is a key regulator of cell growth, metabolism, survival and lifespan of organisms. We have previously shown that TOR deficiency in the nematode Caenorhabditis elegans more than doubles its lifespan\(^2\). In mice, treatment with mTOR Complex I inhibitor (rapamycin) extends lifespan of both male and female mice\(^3\), suggesting mTOR indeed as a key mediator of lifespan regulation. Loss of chondrocyte cellularity within the articular cartilage is one of the prominent events that contribute to its degradation during osteoarthritis (OA). However, it is still uncertain as to what mechanisms control the fate of the chondrocytes within the articular cartilage during normal versus OA conditions. One of the key functions of mTOR is the suppression of autophagy, a cell survival mechanism\(^4\). The in vivo role of mTOR in OA pathophysiology is largely unknown. Autophagy is an essential, homeostatic process by which cells break down their own components and is essential for survival, differentiation, development and homeostasis\(^4\). Hypoxia, reactive oxygen species and deprivation of nutrients and energy are among the key inducers of autophagy process and when mTOR is inhibited(Figure 2 ).

Figure 2. The process of autophagy
Recent studies have shown dysregulation in the expression of key autophagy genes in OA pathogenesis. The specific in vivo mechanism of mTOR signalling in OA pathophysiology is not
fully characterised. Our study was designed to first determine the expression of mTOR and autophagy genes in human OA cartilage as well as in mouse and dog models of experimental OA.

**Increased expression of mTOR in human OA as well as mouse and dog models of experimental OA.**

**Human specimens**

Normal human cartilage was obtained from both femoral condyles and tibial plateaus, at autopsy (n = 15, 60.4 ± 15.4 years [mean ± SD]). Human OA cartilage was obtained from patients undergoing knee replacement (n = 32, 65.2 ± 17.6 years).

**Murine model of OA**

OA was surgically induced in 10-week-old control and mTOR KO male mice by DMM or sham surgery (control) in the right knee, as previously described. Histological and biochemical analysis were performed at 5 and 10 weeks post OA surgery. At 5 weeks post OA surgery, right knee joint cartilage from femoral condyles and tibial plateaus was removed and primary chondrocytes were prepared from the mice as previously described.

**Dog model of OA**

Adult mixed breed Mongrel dogs (LAKA, St-Basile-le-Grand, Quebec, Canada) weighing 25 ± 3 kg underwent surgical sectioning of the anterior cruciate ligament of the right knee as previously described and at 8 weeks post surgery, cartilage from femoral condyles and tibial plateaus was removed and processed for RNA extraction as described above. Cartilage from femoral condyles and tibial plateaus of the left (contralateral) knee was used as control cartilage.

Loss of chondrocyte cellularity within the articular cartilage is one of the prominent events that contribute to its degradation during osteoarthritis (OA). However, it is still uncertain as to what mechanisms control the fate of the chondrocytes within the articular cartilage during normal versus OA conditions. mTOR, a serine/threonine protein kinase, is a key regulator of cell growth, metabolism, survival and lifespan of organisms. It has been previously shown that TOR deficiency in the nematode Caenorhabditis elegans is able to double its lifespan. In mice, treatment with mTOR Complex 1 inhibitor rapamycin, extends lifespan of both male and female mice, suggesting mTOR as a key mediator of lifespan regulation. One of the key functions of mTOR is the suppression of autophagy, a cell survival mechanism. The in vivo role of mTOR in OA pathophysiology is largely unknown. Here, for the first time we demonstrate that mTOR is overexpressed in human OA patient cartilage (compared to normal human cartilage) as well as mouse and dog experimental OA cartilage (compared to non-surgery control cartilage) (Figure 3).

We first determined the expression of mTOR in human OA cartilage compared to normal human cartilage. Western blotting and immunohistochemical analysis showed enhanced protein expression of mTOR in human OA cartilage compared to normal human cartilage (Figure 3B).

Further, we also observed a significant upregulation (p<0.05) in the mRNA expression of mTOR in human OA cartilage compared to normal human cartilage (Figure 3). Similarly, mTOR expression was also significantly upregulated (P<0.05) in both dog OA cartilage as well as mouse OA chondrocytes compared to control dog cartilage and control sham-surgery chondrocytes respectively (Figure 3).
Figure 3 Enhanced expression of mTOR during OA.

Aberrant expression of autophagy genes in human OA cartilage compared to normal human cartilage

Since mTOR is a major repressor of autophagy, we determined the expression of 84 key autophagy genes in normal versus OA cartilage using human autophagy PCR arrays. Autophagy PCR arrays show that in OA cartilage, 20 autophagy-related genes were significantly down-regulated and 5 autophagy-related genes were significantly up-regulated compared to normal human cartilage (Figure 4).

Figure 4. Global Autophagy-focused Genes Expression Pattern in human Cartilage

Normal          OA
**Generation of Inducible cartilage-specific mTOR knockout mice**

Inducible cartilage-specific mTOR KO mice were generated by mating mice containing a mTOR gene flanked by LoxP sites [C57BL/6- mTOR^fl/fl, Jackson Laboratory] with C57BL/6 Col2-rt-TA-Cre transgenic mice. 5 weeks old mTOR^fl/fl Cre mice were fed doxycycline for 7 days. mTOR^fl/fl Cre mice without doxycycline (only PBS) treatment were used as control mice.

Overall, we found that

1. **Enhanced expression of mTOR during OA.**
2. Increased chondrocyte apoptosis and decreased expression of autophagy genes during OA.
3. Inducible cartilage-specific mTOR KO mice exhibit protection from DMM-induced OA (Figure 5).

4. mTOR KO mice exhibit decreased articular chondrocyte apoptosis, increased expression of autophagy markers and decreased expression of MMP-13 during OA. 5. mTOR controls autophagy by modulating ULK1 expression in the articular cartilage.

6. Aberrant expression of autophagy-related genes in OA human cartilage compared to normal human cartilage.

7. Effect of rapamycin treatment on the expression of autophagy markers as well as catabolic and anabolic factors control and mTOR KO mouse OA chondrocytes.
Inducible cartilage-specific mTOR KO mice exhibit protection from DMM-induced OA

In closing, this study for the first time provides a direct evidence of the in vivo role of mTOR in articular cartilage homeostasis. Thus targeting cellular homeostasis mediators, such as mTOR and its downstream signaling by autophagy may be a promising therapeutic strategy to achieve OA protection.

References

Yue Zhang received his PhD degree from University of Fribourg, Switzerland in 2006; he and his colleague reported deficiency of TOR kinase doubles life span in *C. elegans* in Nature. He did his post-doc in University of Pittsburgh Medical Center and established DAF-12/Vitamin D receptor as development capacitor in *C. elegans*. Since 2008, first as research fellow, then promoted as instructor, he pursued cancer and Huntington's diseases research at Beth Israel Deaconess Medical Center, Harvard Medical School, Boston.

In 2012, he joined Osteoarthritis (OA) Unit, University of Montreal Hospital Research Centre. His studies on the role of cartilage homeostasis mediator mTOR and autophagy pathway with patient specimens and model organisms led to novel potentials for OA prevention. Now he works as cartilage scientist in arthritis research center, Toronto Western Hospital, UHN. Recent work includes the identification of OA biomarkers in cartilage and investigation of interrelationships among obesity, OA and autophagy. Current interests include studies on roles of Vitamin D receptor as health capacitor in autoimmune diseases such as rheumatic arthritis and
associated cancers. As founding editor (Current Synthetic and Systems biology) or executive editor (Human Genetics and Embryology), and other several journals, he also has some interests in publishing. He authored/coauthored more than 40 publications. He and his wife have two sons in Toronto.

Yue Zhang, PhD
Cartilage scientist, Division of Genetics and Development, The Toronto Western Research Institute, Toronto Western Hospital, University Health Network, 60 Leonard Avenue, Toronto, Ontario, Canada M5T 2S8 Telephone: 1-416-603-5800 Ext 4797 Email: yuezhang@uhnresearch.ca or zy1001@yahoo.com