The role of CK2β as a mediator of NF-κB pathway in the pathogenesis of osteoarthritis

Sehee Cho

Department of Medical Science

The Graduate School, Yonsei University

The role of CK2β as a mediator of NF-κB pathway in the pathogenesis of osteoarthritis

Directed by Professor Jin Woo Lee

The Master's Thesis
submitted to the Department of Medical Science,
the Graduate School of Yonsei University
in partial fulfillment of the requirements for the degree of
Master of Medical Science

Sehee Cho

December 2021

This certifies that the Master's Thesis of
Sehee Cho is approved.

Thesis Supervisor : Jin Woo Lee

Thesis Committee Member#1 : Sahng Wook Park

Thesis Committee Member#2 : Sung-Hwan Kim

The Graduate School
Yonsei University

December 2021

ACKNOWLEDGEMENTS

2020년 시작된 짧고도 길었던 석사 학위 과정을 마치며 졸업을 맞이하게 되었습니다. 학위 과정 동안 도움을 주셨던 많은 분들에게 감사의 말씀을 전하고자 합니다.

먼저 연구를 시작할 수 있도록 배움의 기회를 주신 저의 지도 교수님 이진우 교수님께 감사의 말씀 드립니다. 교수님께서 주신 훌륭한 가르침과 교훈들 항상 잊지 않고 살아가겠습니다. 바쁘신 와중에도 저의 부족함을 채워 주시기 위해 아낌 없이 조언해주시고 도움을 주신 박상욱 교수님, 그리고 김성환 교수님께 감사드립니다. 학위 과정 동안 가장 가까이에서 저의 연구를 위해 토론해주시고 세심한 조언을 해주신 박광환 교수님께도 정말 감사드립니다.

연구실에서 함께 생활하며 많은 도움을 준 LJWOS 실험실 식구들, 특히 제 연구의 방향을 잡아주시고 제가 올바른 연구자로 성장 할 수 있게 도와주신 제 사수 윤동석 박사님 정말 감사드립니다. 연구실을 이끌어 가시며 항상 편하게 지낼 수 있게 해주셨던 이경미 박사님, 실험실 생활부터 실험에 이르기까지 따뜻하게 챙겨 주셨던 은애 쌤, 그리고 지금은 멋진 사회인으로 성장하였지만 학위 과정 중 나에게 가장 큰 힘이 되어준 수진이, 동기로 생활했던 유지언니, 긍정적인 에너지로 마지막 학기 내내 나를 웃음짓게 해준 승은이와 은지에게도 모두 감사하다는 말씀 전하고 싶습니다.

학위 과정 동안 항상 저를 지지해주며 응원을 보내준 15학번 동기들, 세동지, 혜선, 은채 외에 일일이 언급하지 못하더라도 나의 소중한 친구들 모두 너무 고맙고, 대학원 생활과 첫 독립을 성공적으로 보내게 해준 내 룸메 경은이에게도 진심으로 고마움을 전합니다.

마지막으로, 변함없이 내 곁을 묵묵히 지켜주는 하준이, 나의 든든한 버팀목이자 가장 존경하는 우리 부모님과 언니에게 너무 감사하고 사랑한다고 전하고 싶습니다. 가족이 있었기에 힘든 일을 헤쳐나갈 수 있는 용기를 낼 수 있었고, 기쁜 마음을 나눌 줄 아는 큰 사람이 될 수 있었습니다.

앞으로 이 감사한 마음 간직하며 학위 과정 중 배운 지식들과 깨달음을 한 명의 지성인으로써 사회에 베푸는 삶을 실천하며 살겠습니다. 감사합니다.

조세희 올림

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

ABSTRACT

The role of CK2β as a mediator of NF-κB pathway in the pathogenesis of osteoarthritis

Sehee Cho

Department of Medical Science

The Graduate School, Yonsei University

(Directed by Professor Jin Woo Lee)

Casein kinase 2 (CK2) is a constitutively active Ser/Thr protein kinase that is composed of the catalytic subunits CK2α and/or CK2α' and two CK2β regulatory subunits. CK2 is implicated in various cellular processes, such as cell cycle control, apoptosis, and DNA synthesis. It also regulates various signaling pathways related to inflammation by phosphorylating various substrates. Inflammatory response is a major factor that causes osteoarthritis (OA) progression, so elucidation of the mechanism of CK2 regulation in osteoarthritis would be important for understanding and development of therapeutic modality. OA also causes the articular chondrocytes to undergo a phenotypic shift, leading to

the disruption of homeostasis and ultimately to the aberrant expression of proinflammatory and catabolic genes. However, the role of CK2 in chondrocytes remains unclear, so this study was conducted to determine whether regulating CK2 activity can help maintain chondrocyte homeostasis in OA environments. The results showed that the upregulation of CK2β is associated with OA progression and that CK2β binds to NF-kB p65 and IkB, inducing inflammation responses in osteoarthritic chondrocytes. These results indicate that downregulating CK2 activity by suppressing the regulatory domain CK2β, which is elevated in osteoarthritic environments, can prevent NF-kB-mediated OA pathogenesis in chondrocytes. This study is the first to show that CK2 is important for mediating the NF-κB pathway in chondrocytes.

Key words: Osteoarthritis, inflammation, NF-κB signaling, Chondrocyte, Casein kinase 2β

The role of CK2β as a mediator of NF-κB pathway in the pathogenesis of osteoarthritis

Sehee Cho

Department of Medical Science
The Graduate School, Yonsei University

(Directed by Professor Jin Woo Lee)

I. INTRODUCTION

Osteoarthritis (OA) is the most prevalent joint disease worldwide, causing chronic disability in older people. Various factors are associated with its pathogenesis, including aging, obesity, joint instability, and joint inflammation. [1] OA involves the entire synovial joint, cartilage, synovium, and underlying bone. The cells in each of these tissues have independent capacities to initiate and respond to injury in the joint. As a result, these responds ultimately cause degeneration of cartilage. In the degenerative cartilage in OA, chondrocytes, the cells resident in cartilage, attempt to repair the degenerated extracellular matrix

and the activity of enzymes produced by the chondrocytes digests the matrix, matrix synthesis is inhibited, and the consequent erosion of the cartilage is accelerated.[2] Also, it is well known that the involvement of an inflammatory component, which is marked by symptoms such as joint pain, swelling and stiffness, is occurred in OA. Inflammatory cytokines, chemokines, and other inflammatory mediators are produced by the synovium and chondrocytes.[3] These inflammatory mediators are implicated in OA pain process and in the degradation of the deep layer of cartilage. Since that, initially considered driven, OA is a much more complex disease with inflammatory mediators released by cartilage, bone and synovium.[4-6]

The family of nuclear factor-kappaB (NF-κB) transcription factor is intimately involved in the regulation of expression of numerous genes in a series of the inflammatory response. NF-κB is present in the cytoplasm of almost all mammalian cells in an inactive form associated with the inhibitory κB proteins (IκB), which include IκBα, IκBβ, IκBε and IκBγ. A variety of stimuli such as the cytokines, TNF-α or IL-1β, chemokines, UV radiation and free radicals can trigger the phosphorylation of IκB, resulting in ubiquitin-dependent IκBα degradation in the proteasome. Then, NF-κB signaling is activated by rapid and transient nuclear translocation of canonical NF-κB dimers (p50/RelA and p50/c-Rel).[2,7,8]

In OA, many of inflammatory cytokines such as TNF-α and IL-1 are produced by the synovium and the chondrocytes. Since inflammatory process play a crucial role in the OA progression, many studies have examined the contribution of components of the NF-κB signaling pathways to pathogenesis of

OA.[1] As a master regulator of genes which initiate inflammation associated with osteoarthritis (OA), NF-κB complex which is activated by these cytokines induces the expression of various pro-inflammatory genes in osteoarthritic cartilage.[9] Thus, it would be worthwhile finding upstream regulators involved in this pathway for OA treatment.[9-12]

Casein kinase 2 (CK2) is very conserved serine/threonine kinase that is a constitutively active in eukaryotic organisms. CK2 is a tetrameric protein kinase that is composed of two catalytic subunits (CK2α and/or CK2α') and two CK2β regulatory subunits dimer.[13-17] Since CK2 phosphorylates hundreds of substrates, regulation of CK2 controls cellular processes such as cell cycle control, DNA synthesis, and inflammation. Also, CK2 is implicated in a broad range of human diseases by controlling several signaling pathways. [18-20] Recently, it has been well-defined that CK2 contributes to the dysregulation of cell growth, invasion, survival, and senescence via PI3K/Akt pathway, JAK2/STAT3 pathway and NF-κB pathway in tumorigenesis or carcinogenesis. [21-23]

Not only CK2 is a protein kinase that participates in the transduction of signals that promote cell growth and survival but has been implicated in NF-κB activation. CK2 has been shown to directly phosphorylate the COOH-terminal PEST domain of IκBα and also promotes transactivation by phosphorylation of the RelA(p65) subunit of NF-κB.[23-25] Since NF-κB is a major factor causing inflammation in osteoarthritis, it might be very meaningful to study the mechanism of action of the upstream regulators responsible for modulating NF-κB activity. In this regard, regulation of CK2 activity may be a plausible therapeutic target for OA.[26-29] However, there are no reports of the role of CK2

in OA to date. Despite reports that CK2 regulates NF-kB at a post-translational level, its role in OA remains unclear. Thus, identification of a potential relationship and a role for CK2 in upstream signaling and activation of NF-κB in chondrocytes is needed. For that reason, the aim of this study is to reveal mechanism of CK2 - NF-κB axis in chondrocytes and to test whether CK2 can be a possible therapeutic target for OA.

In this study, I showed that both CK2α and CK2β subunits were detected lower in preserved cartilage tissues, whereas CK2β was highly detected in degenerated cartilage tissues while the protein level of CK2α was almost undetectable. Therefore, I revealed that regulation of CK2β which is elevated in osteoarthritic environment can alleviated TNF-α mediated inflammation responses by modulating IkB-mediated NF-kB activity in chondrocytes. This study will suggest the role of CK2β and elucidate a related mechanism in osteoarthritic inflammatory environment.

II. MATERIALS AND METHODS

1. Cell Culturing

A TC28a2 cell line (Sigma-Aldrich, St.Louis, MO, USA), which is widely used for studying normal and pathological cartilage repair mechanisms related to chondrocyte biology and physiology, was established by transfecting primary cultures (day 5) of costal cartilage taken from a 15-year-old female with a retroviral vector expressing simian virus SV40 large T antigen. The cells were cultured with Dulbecco′s modified Eagle′s medium with high glucose (Gibco, Carlsbad, CA, USA) containing 10% fetal bovine serum (Gibco), 1% (v/v) antibiotic antimycotic solution (Gibco), and 1% Insulin-Transferrin-Selenium-A (Gibco). The cells were harvested at 80% confluence using 0.05% trypsin-EDTA (Gibco). Cells were washed, centrifuged, resuspended, and seeded in new plates. The medium was replaced every 2−3 days with fresh medium.

2. High-density Mass Culturing of the TC28a2 Cell Line

High-density culturing was conducted by first dotting $1x10^5$ cells on the center of each well of a 24-well plate. A single drop of 1X phosphate-buffered saline (PBS) (Thermo Scientific, Logan, UT, USA) was placed in between each well to prevent the cells from drying out. The cells were placed in a 37°C incubator with an atmosphere of 5% CO_2 for 2 hours to stimulate the adherence of the

cells to the plate. They were maintained for 5 days in medium with inflammatory cytokines. The medium with cytokines was replenished every day.

3. Western Blotting

Cell pellets were washed with PBS, lysed with 100 μl PRO-PREP protein extraction solution (iNtRON Biotechnology, Seongnam, Korea), and centrifuged at 12,000rpm for 10min. The total protein concentrations were measured using BCA protein assay kit (Thermo Scientific) and 30 μg of protein. Samples were loaded into 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) gels. Then proteins were transferred onto the polyvinylidene difluoride (PVDF) membranes (Invitrogen, Carlsbad, CA, USA). Once all the proteins were transferred onto the membrane, the membrane was blocked with 5% skim milk (BD Biosciences, San Jose, CA, USA) for 1 hour at room temperature. The membranes were incubated for 4 hours at room temperature or over-night at 4°C with primary antibody. The used antibodies were CK2α (1:1000, sc-12738, Santa Cruz Biotechnology, Dallas, TX, USA), CK2β (1:1000, sc-12739, Santa Cruz Biotechnology), COL2A1 (1:1000, sc-518017, Santa Cruz Biotechnology), p-p65(1:1000, 3033, Cell Signaling, Danvers, MA, USA), p65(1:1000, 8242, Cell Signaling), p-IκB (1:1000, 2859, Cell Signaling), IκB (1:1000, 4812, Cell Signaling), β-ACTIN (1:5000, sc-47778, Santa Cruz Biotechnology).

4. Immunohistochemistry

Samples from each group were fixed in a 10% formalin solution for 5–7 days at room temperature. Samples were decalcified in a 0.5 M EDTA solution with a pH of 7.4 for 2 weeks at room temperature. The decalcified samples were embedded in paraffin blocks. Paraffin sections were dehydrated by passing them through an ethanol series, cleared twice in xylene, and embedded in paraffin. The 5-mm sections were cut using a rotary microtome. Decalcified sections were stained with hematoxylin and eosin and with Safranin O.

Immunohistochemistry was conducted by first retrieving antigens using a citrate buffer with a pH of 6.0 for the deparaffinized sections. Sections were blocked with 1% bovine serum albumin (BSA) in PBS for 1 hour at room temperature and the groups of sections were incubated with each antibody. ab64252 AEC substrate (Abcam, Cambridge, UK) was used for detection.

**5. Immunocytochemistry **

High-density cultured cells were washed with PBS and fixed in 4% paraformaldehyde for 10 minutes and washed twice with PBS. The cells were permeabilized by incubating them with 0.25% PBST (PBS + 0.25% Triton-X 100) for 10 minutes and washed twice with PBS. To reduce nonspecific background staining, the cells were incubated in 3% bovine serum albumin in PBST for 1 hour. The cells were then incubated with COL2A1 (1:500, sc-518017, Santa Cruz Biotechnology), ACAN (1:500, sc-33695, Santa Cruz Biotechnology) and IgG (1:500, 555746, BD Biosciences) overnight at 4°C and washed with PBS. Signal detection was performed using an AEC substrate kit

(Abcam).

6. Safranin O Staining

Cartilage tissues were detected by staining with Safranin O, which is a cationic dye that stains both proteoglycans and glycosaminoglycans. Inflammation was induced and after 5, high-density dot-cultured cells were washed with PBS and fixed in 3.7% formaldehyde for 1 day. Then, 0.5 ml of 1% Safranin O solution (Sigma) was added to the solution which was then incubated for 15 minutes. Safranin O-stained cells were de-stained with 10 % cetylpyridinium and their absorbance at 450 nm was measured.

7. Quantitative real-time PCR

Total RNA was isolated from cells using a K-3141AccuPrep Universal RNA Extraction Kit (Bioneer, Daejeon, Korea). The concentration of RNA was measured by Nanodrop 1000 (Thermo Scientific, Waltham, MA, USA). After RNA isolation, the 1 μg of RNA was reverse-transcribed using a AccuPower RT premix (Bioneer). Quantitative real-time polymerase chain reactions were performed using a StepOnePlus Real-Time PCR System instrument (Applied Biosystems, Carlsbad, CA, USA) according to the manufacturers' guidelines. The appropriate amount of the reverse transcription reaction mixture was amplified with specific primers using SYBR green PCR master mix (PCR Biosystems, London, UK). Primer sequences are shown in Table 1.

8. Human Articular Cartilage Specimens

Human cartilage samples were obtained from 5 patients undergoing total knee arthroplasty with OA, with approval from the Institutional Review Board (IRB) (2019-1374-002) of the Yonsei University College of Medicine. A total of 3 samples from osteoarthritis patients were included in the study. Then, the cartilage tissue was divided into two groups: the site of preserved tissues or the site of degenerated tissues for histological analysis.

9. Transfection

Each well of a 6-well plate was seeded with $2x10^5$ TC28a2 cells, cultured for 24 hours, and the cells were transfected with siRNA Negative control (Bioneer) and siCSNK2B (Santa Crus Biotechnology) using CalFectin (Signagen Laboratories, Rockville, USA). Briefly, diluting 200 nM siRNA Negative control and siCSNK2B into 0.25 ml of serum-free DMEM with high glucose. Vortex gently and spin down to bring drops to the bottom of the tube. For each well, adding 4 μl of CalFectin reagent immediately into 0.25ml of serum-free DMEM with high glucose and CalFectin solution was directly added into 0.25 ml diluted siRNA solution. After 20 minutes incubation at room temperature, each well was replaced with 0.5 ml serum-free medium. Adding the 0.5 ml CalFectin/siRNA mixture drop-wise onto the medium in each well and homogenize the mixture by gently swirling the plate. 24 hours after transfection, cells were re-seeded on 24-well culture plate using high density culture method.

CSNK2B overexpression vector was a gift from J.shim (University of Massachusetts Medical School, USA). Also, 1 μg CSNK2B vector was transfected into TC28a2 cells using same methods.

10. Lentivirus Production

Lenti-X^{TM} 293T cells were purchased from Takara Bio. Lenti-X^{TM} 293T cells were transfected with pCMV4 vector as a control and CSNK2B-mGFP vector. Lenti-X^{TM} 293T cells were also co-transfected with the enveloping and packaging vectors pMDLg/pRRE, pRSV/REV and pMD2.G. The medium was replaced with growth medium containing 10% FBS and 0.1% antibiotic antimycotic solution 24 hours after transfection. The supernatant was collected 48 hours and again 72 hours after transfection and filtered with an 0.45-μm sterile filter.

11. CK2 activity measurement

CK2 activity was measured in triplicate using the CycLex CK2 Assay/Inhibitor Screening Kit (MBL International Corporation, Woburn, MA, USA). Plates are pre-coated wigh a substrate corresponding to recombinant p53, which contains a serine residue that are phosphorylated by CK2. For kinetic analysis, cells were lysed and the 10 μl sample containing CK2 was diluted in 90 μl kinase buffer to begin kinase reaction, in the presence of Mg^{2+} and ^{32}P-labeled ATP. After 30 minutes incubation, each well was washed five times with provided washing

buffer which contains 2 % Tween-20. The amount of phosphorylated substrate was measured by binding it with a horseradish peroxidase conjugate of TK-4D4, an anti-phospho-p53 serine46 specific antibody for 30 minutes at room temperature. The conversion of the chromogenic substrate tetra-methylbenzidine (TMB) from a colorless solution to a blue solution was catalyzed by adding 100 μl substrate reagent into the well. The reaction was stopped using 100 μl stop solution containing 1 N H_2SO_4. The color was quantified by spectrophotometry at 450 nm and reflected the relative amount of CK2 activity in the sample.

12. Immunoprecipitation

TC28a2 cells were seeded in 6-well culture plate with 70% confluence and transfected with pCMV, CSNK2B-GFP, siNC, and siCSNK2B using Calfectin (Signagen laboratories, Rockville, USA). After 24 hours of transfection, Dulbecco's modified Eagle's medium with high glucose mixed with 10μM of MG-132 (proteasome inhibitor [Sigma]) and 10ng/ml TNF-α (R&D systems) were added for 6 hours before which the cells were harvested. The harvested cells were lysed using non-denaturing lysis buffer (20 mM Tris-HCl [Ph 8.0], 137 mM NaCl, 0.5% Nonidet P40 with 2 mM EDTA [pH 8.0], 1 mM phenylmethylsulfonyl fluoride, and protease inhibitor). The lysates were precipitated using protein A/G agarose beads (Santa Cruz Biotechnology) and antibodies (Flag [Sigma] and GFP [NOVUS]). A/G agarose beads with the

lysates and antibodies were collected by centrifugation. The supernatants were removed from the beads. The 2X SDS sample buffer was added and the samples were boiled to obtain the beads' complexes. The prepared samples were loaded into the 10% SDS-PAGE and transferred to polyvinylidene difluoride (PVDF) membranes. Immunoblotting was done with antibodies as indicated in Western Blotting section.

13. *Csnk2b* Conditional Knockdown Mouse

C57BL/6 mice were used for the experimental OA studies. *Csnk2b* $^{fl/fl}$; *Col2-CreER* mice (gifted from J. Shim, University of Massachusetts Medical School, Worcester, MA, USA) were crossed with *Csnk2b* $^{fl/fl}$; *Col2-CreER* mice to produce littermates that had same genotypes as their parents. Offspring were genotyped by PCR using *Cre*-specific primers and primers for detecting *Csnk2b* wild type or floxed alleles. *Cre* recombinase was induced by intraperitoneally injecting 8-week-old *Csnk2b* $^{fl/fl}$; *Col2-CreER* mice with 75 mg/kg of tamoxifen in corn oil once every 24 hours for 5 consecutive days.

14. Experimental OA in Mice

Experimental OA was induced in 10-week-old mice half of which received a tamoxifen injection and half of which did not. All experiments were approved by the Institutional Animal Care and Use Committee (IACUC) protocol of the Yonsei University College of Medicine (approval number: 2019-0018). OA was

induced in the right knee joint of the hind limb by destabilization of the medial meniscus (DMM) surgery of (n = 4). Mice in the sham group (n = 4) received the same surgery except that their medial meniscus was not dissected. Each group of mice were sacrificed 8 weeks after DMM surgery and subjected to histological and biochemical analyses.

15. Statistical analysis

Statistical significance between groups were evaluated by using Student's t-tests or one-way ANOVA using Prism software version 6 (GraphPad Software, San Diego, CA, USA). Data were shown as the mean ± standard deviations. Values of $p < 0.05$ were considered statistically significant.

Table 1. A list of primers used for qRT-PCR

Gene	Primer sequence (5′ → 3′)
CSNK2A1	F : GGTGAGGATAGCCAAGGTTCTG R : TCACTGTGGACAAAGCGTTCCC
CSNK2A2	F : CGACCATCAACAGAGACTGACTG R : GTGAGACCACTGGAAAGCACAG
CSNK2B	F : GCCTGATGAAGAACTGGAAGAC R : TAACCAAAGTCTCCTTGCTGGT
IL1B	P314508 (Bioneer)
IL6	P211161 (Bioneer)
PTGS2	P109609 (Bioneer)
MMP13	F : GACGGGGTTTTGCCACACTG R : ATTGGGTGTGGTGGCTCACG
18s-rRNA	F : ACACGGACAGGATTGACAGA R : GCCAGAGTCTCGTTCGTTAT
β-ACTIN	F : CTTCTACAATGAGCTGCGTG R : TCATGAGGTAGTCTGTCAGG

III. RESULTS

1. CK2β is upregulated in OA patients

To demonstrate whether CK2β is upregulated in human OA environments, RNA and protein were extracted from cartilage tissue obtained from OA patients. Human tissues were categorized according to whether they had been collected from the preserved or degenerated site. I first performed a CK2 activity assay to determine the pattern of CK2 in degenerated tissues and found that CK2 activity was upregulated in the degenerated tissues (Fig. 1A). The Results of a qPCR test showed that the mRNA levels of *CSNK2A1* and *CSNK2A2*, which are the CK2α and CK2α' genes, respectively, were the same in both groups whereas the mRNA levels of *CSNK2B*, the CK2β gene, were three times higher in degenerated tissues than healthy tissues (Fig. 1B).

After that, I used Safranin O staining and immunohistochemistry to confirm the correlation of Figure 1 data. Safranin O staining showed that cartilage tissues were actually destroyed in the degenerated tissues. Then, I checked the protein levels of CK2α and CK2β and found that they were present in low levels in the preserved tissues and that CK2β was highly detected in degenerated tissues while CK2α was almost undetectable (Fig. 2). These results indicated that CK2β may play a crucial role in OA progression in articular cartilage chondrocytes.

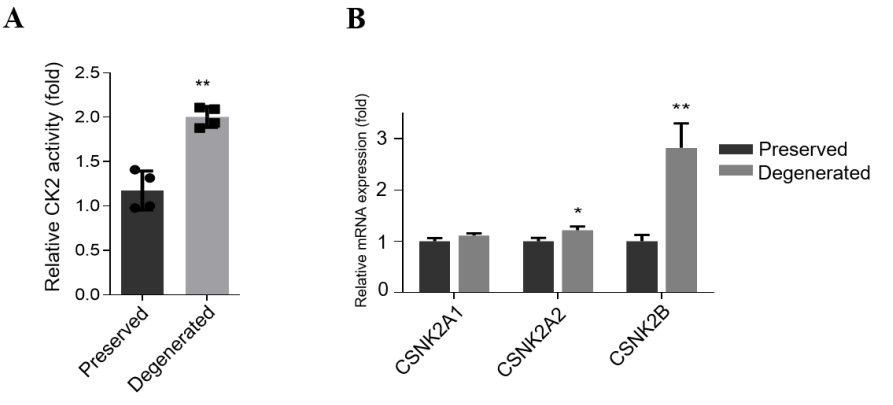

Figure 1. CK2 activity and CK2β mRNA expression levels in OA patients. (A) Chondrocytes from OA patients were lysed, and the amount of phosphorylated substrate was measured by spectrophotometry at 450 nm and normalized by total protein. (B) Relative expression of the indicated genes (*CSNK2A1, CSNK2A2, CSNK2B*) as determined by RT-PCR with mRNA isolated from human OA knee joint cartilage tissues which divided into two groups: preserved and degenerated. Data are shown as means ± SD. * $p< 0.05$, ** $p <0.01$ in a comparison with the preserved group.

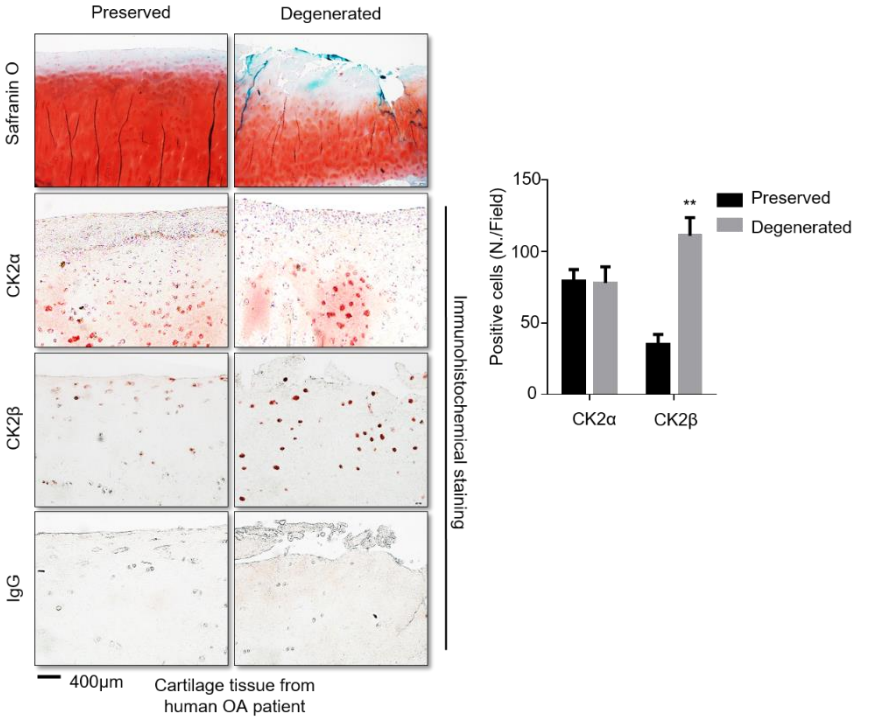

Figure 2. CK2β expression in human OA cartilage tissues. Representative image of Safranin O staining and immunohistochemical staining with CK2α and CK2β in preserved areas and degenerated areas of human OA knee joint cartilage tissues, repectively (n=3 per group). Scale bars: 400μm.

2. Knockdown of CK2β alleviates inflammation responses

I treated TC28a2 human chondrocyte cells with dose-dependent TNF-α, one of the inflammatory cytokines that activate the NF-kB signaling pathway, to induce OA inflammation. CK2 activity assay results showed that a 10 ng/ml concentration of TNF-α was sufficient for increasing CK2 activity in chondrocytes (Fig. 3A), so I chose that as the concentration to be used further in this study.

Under the same conditions, CK2β protein levels were positively correlated with TNF-α stimulation but CK2α protein levels were almost unchanged (Fig. 3B). These results suggest TNF-α stimulation upregulates CK2β expression, which in turn elevates CK2 activity.

Since CK2β is elevated in human OA tissues, so I checked how knocking down CK2β in human chondrocyte TC28a2 cells would affect OA progression. When TC28a2 cells were transfected with siCSNK2B, CK2β protein levels decreased below those of the negative control group (Fig. 4A). I then incubated the transfected TC28a2 cells using a high-density culturing method, with or without TNF-α treatment. CK2 activity increased in the TNF-α mediated inflammatory environment and declined in the CK2β knockdown group despite TNF-α treatment (Fig. 4B).

It is well known that Collagen type II (COL2A1) is a structural component of the cartilage matrix and aggrecan (ACAN) is the major proteoglycan in the articular cartilage. The dense extracellular matrix (ECM) is mainly comprised of COL2A1 and ACAN and gives cartilage its biomechanical properties (Fig.

5A). However, inflammation during OA progression plays an active role affecting both quantity and quality of ECMs meaning that COL2A1 and ACAN are decreased during OA.[30,31] Under TNF-α treatment, Safranin O staining intensity decreased, which also occurred in human OA tissues. This damage was reversed by CK2β knockdown (Fig. 5B). I also showed that COL2A1 and ACAN protein levels were significantly reduced after TNF-α stimulation, but that they significantly recovered after CK2β knockdown (Fig. 5C). Immunocytochemistry also showed that suppressed COL2A1 and ACAN level were restored by CK2β knockdown in inflammatory TC28a2 cells (Fig. 5D). Taken together, these findings suggest that deleting CK2β has a protective role in OA progression by attenuating inflammatory responses.

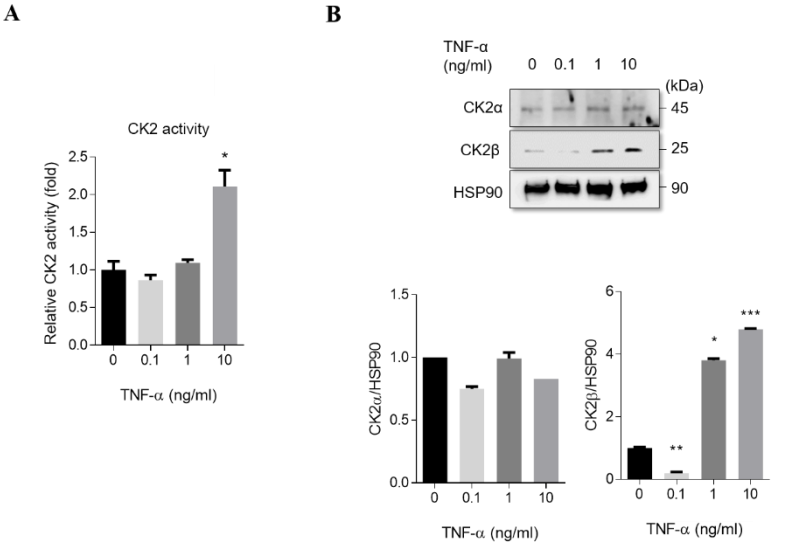

Figure 3. CK2 activity and CK2 subunits beta levels by TNF-α stimulation in dose-dependent manner. (A) TC28a2 cells were treated with either 0, 0.1, 1, or 10 ng/ml of TNF-α for 12 hours. TC28a2 cells were lysed, and the amount of phosphorylated substrate was measured by spectrophotometry at 450 nm and normalized by total protein. (B) Protein levels of CK2α and CK2β were analyzed in TC28a2 treated with 0, 0.1, 1, or 10 ng/ml of TNF-α and being immunoblotted with the indicated antibodies. Band intensities were quantified by Image J software. All error bars indicate S.D. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ compared with the no TNF-α group.

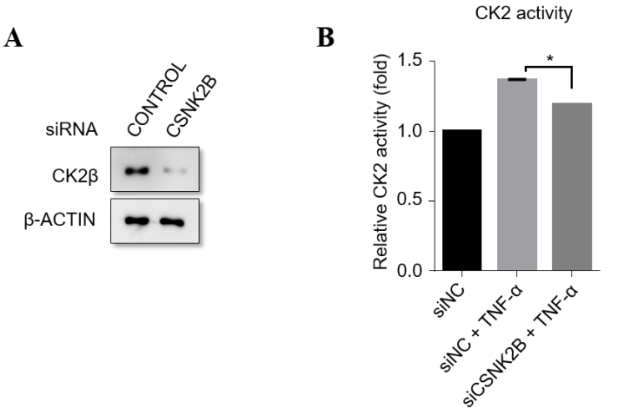

Figure 4. The effect of CK2β knockdown on CK2 activity in chondrocytes. (A) Protein levels of CK2β was analyzed in TC28a2 using western blotting for cells transfected with NC, *CSNK2B* siRNA. (B) TC28a2 cells were transfected with NC, *CSNK2B* siRNA. The cells were maintained under TNF-α (10 ng/ml)-induced inflammatory condition for 6 hours. CK2 activity assay was performed to measure the amount of phosphorylated substrate. The absorbance was measured at 450 nm. * $p < 0.05$ compared to NC siRNA with TNF-α group.

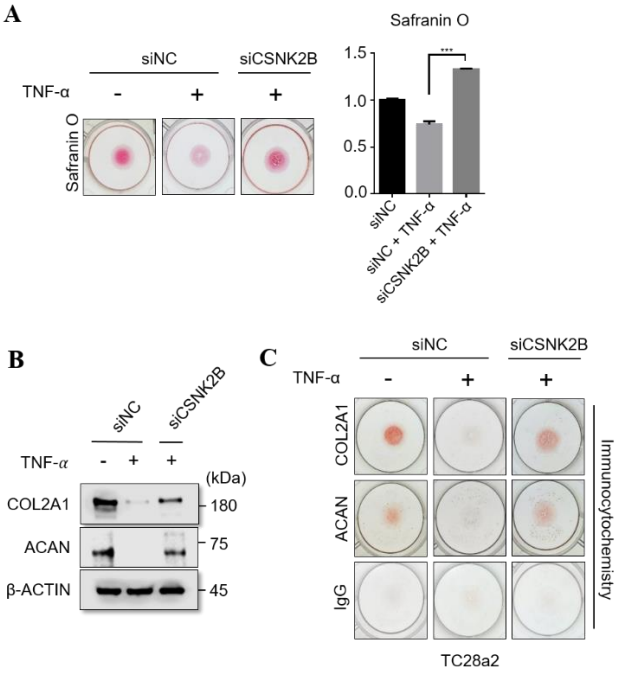

Figure 5. The effect of CK2β knockdown on TNF-α-mediated inflammatory effects in chondrocytes. (A) TC28a2 cells were transfected with NC, *CSNK2B* siRNA. The cells were maintained under TNF-α (10 ng/ml)-induced inflammatory condition in high density mass culture condition for 5 days. Safranin O staining was performed to detect glycosaminoglycans (GAGs). Stained cells were de-stained with 10 % cetylpyridinium for quantitative analysis. The absorbance was measured at 490 nm. *** $p < 0.001$ compared to NC siRNA with TNF-α group. (B) Protein levels of COL2A1 and ACAN in TC28a2 cells transfected with indicated siRNA as shown by western blot analysis. (C) Immunocytochemical staining of COL2A1 and ACAN in high-density mass cultured TC28a2 cells that were incubated under the indicated conditions for 5 days.

3. CK2β phosphorylates p65 and IκB in osteoarthritic chondrocytes

Previous cancer research studies on CK2 showed that it regulates the transcription of several genes by phosphorylating STAT3, AKT, p65, and IκB (Fig. 6A). However, none of them have yet investigated which signaling pathway CK2 regulates in osteoarthritic chondrocytes. Thus, I first examined which signaling pathway is related to inflammatory responses in OA. I induced TNF-α mediated inflammation in TC28a2 cells and found that TNF-a stimulation promoted the phosphorylation of p65 and IκB, which are the components of NF-κB complex, but did not affect or decreased STAT3 and AKT phosphorylation (Fig. 6B). Thus, I assumed that the upregulation of CK2β in osteoarthritic inflammatory environments could be involved in activating the NF-κB signaling pathway by phosphorylating p65 and IκB.

To further understand how CK2β interacts with its expected downstream targets, p65 and IκB, immunoprecipitation was performed and showed that CK2β directly interacted with p65 and IκB in chondrocytes and that the frequency of these interactions was significantly increased in response to TNF-α treatment. Conversely, STAT3 and AKT were not shown to interact with CK2β whether TNF-α treatment occurred or not (Fig. 7A). As a results, these data elucidated that upregulated CK2β may promotes NF-κB activation through phosphorylating p65 and IκB in osteoarthritic chondrocytes (Fig. 7B).

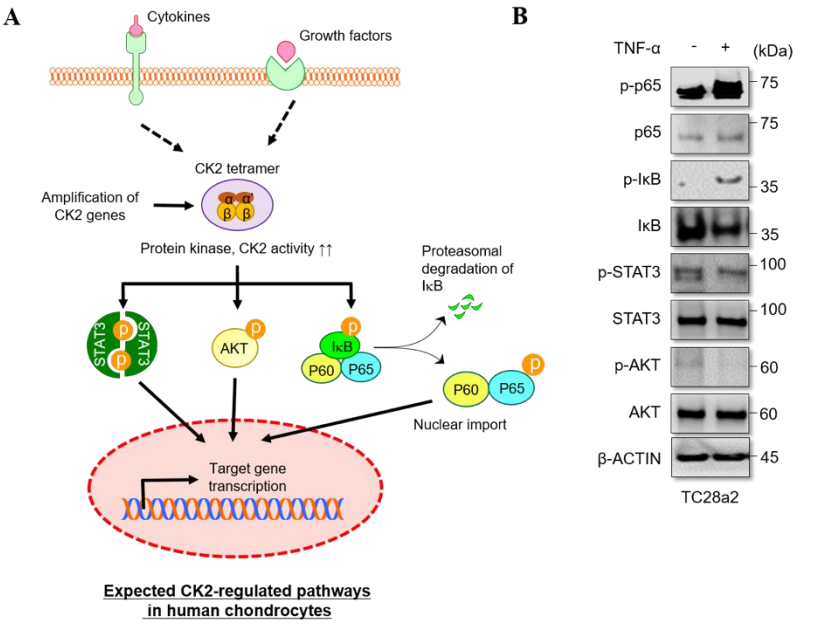

Figure 6. The phosphorylation of IκB in TNF-α-mediated inflammatory environments. (A) Schematic diagram of the likely CK2-related pathways in human chondrocytes. (B) Western blot analysis results of signaling factors such as STAT3, AKT, p65, and IκB. Each target's total and phosphorylated forms were compared. TC28a2 cells were treated with TNF-α (10 ng/ml) for 12 hours.

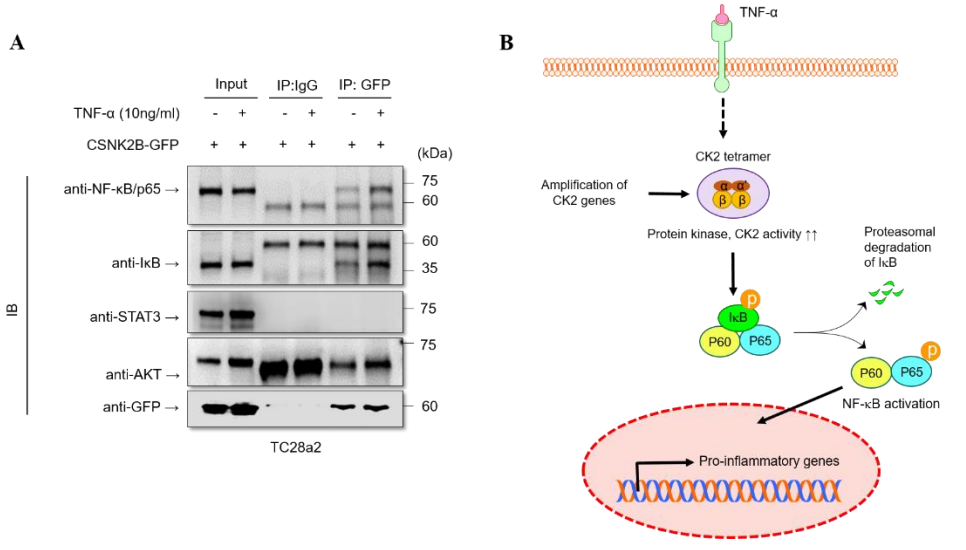

Figure 7. The effect of TNF-α treatment on the interactions of CK2β with NF-κB p65 and IkB. (A) CSNK2B-GFP vector was transfected into TC28a2 cells and cell lysates were immunoprecipitated with anti-GFP and anti-IgG antibodies. Immunoblotting was conducted using anti-NF-κB p65, anti-IκB, anti-STAT3, anti-AKT and anti-GFP antibodies. (B) A schematic diagram of CK2β's downstream pathway for inducing NF-κB activation.

4. CK2β regulates NF-κB signaling pathway in chondrocyte

To verify CK2β can regulate the NF-κB signaling pathway by phosphorylating IκB, I investigated whether the ubiquitination of IκB is affected by CK2β knockdown. Before performing immunoprecipitation, I identified confirmed the transfection efficiency of IκBα-FLAG-tagged vector to TC28a2 cells via western blotting analysis (Fig. 8A). Then I co-transfected IκBα-FLAG and each siRNA to TC28a2 cells to detect IκB ubiquitination levels changed following CK2β downregulation. I also treated TNF-α with MG132 which inhibits proteasomal degradation after ubiquitination. Only immunoprecipitation with anti-FLAG showed the IκB-FLAG-size protein band, indicating that immunoprecipitation was complete (Fig. 8B). In these same experimental groups, I found that IκB ubiquitination levels were higher in the chondrocytes treated with TNF-α which was treated with MG132 than in the chondrocytes treated with TNF-α only, indicating that IκB goes to the proteasomal degradation pathway via ubiquitination. However, these increased IκB ubiquitination levels were lower in the CK2β knockdown group even though those chondrocytes were treated with TNF-α (Fig. 8C). These results show that CK2β contributes to IκB proteasomal degradation by ubiquitinating IκB in inflammatory chondrocytes.

The NF-κB signaling activation primary mechanism is inducing the degradation of IκBα triggered by its phosphorylation-induced proteasomal degradation.[9,32] Thus, I further investigated CK2β knockdown can suppresses NF-κB activity. In TC28a2 cells, even though TNF-α-mediated inflammation

increased the phosphorylation of p65 and IκB, CK2β's downregulation by siRNA reduced their rates (Fig. 9A). p65 and IκB phosphorylation mediates NF-κB signaling activation, so I measured NF-κB activity using HEK-Blue-hTLR2 cells. Although they were co-transfected by human toll-like-receptor 2 (TLR2) and secreted embryonic alkaline phosphatase (SEAP) genes to detect NF-κB activity, they can also be activated by TNF-α. NF-κB-induced SEAP activity was upregulated by TNF-α but was then downregulated in the siCSNK2B group (Fig. 9B). These results indicate that the NF-κB pathway is activated by CK2β in osteoarthritic chondrocytes through IκB degradation.

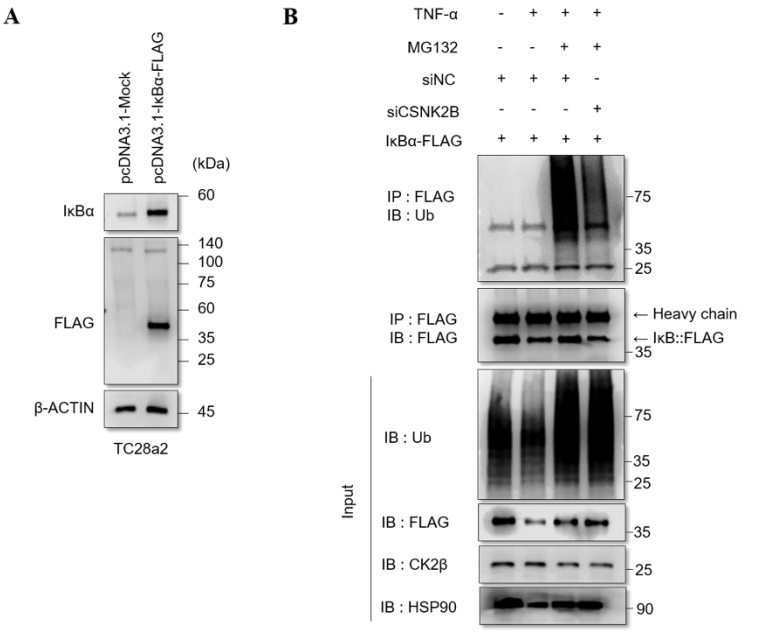

Figure 8. The effect of CK2β knockdown on IκB ubiquitination. (A) Protein levels of IκBα and FLAG were analyzed in TC28a2 cells using western blotting for cells transfected with the pcDNA3.1-MOCK vector control having no insert or the pcDNA3.1- IκBα-FLAG vector. (B) pcDNA3.1-IκBα-FLAG vector and indicated siRNA were co-transfected into TC28a2 cells. TNF-α and MG132 were co-treated for 6 hours. Cell lysates were immunoprecipitated with anti-FLAG and anti-IgG antibodies. Immunoblotting was conducted using anti-FLAG, anti-Ub, anti-CK2β, and anti-HSP90 antibodies.

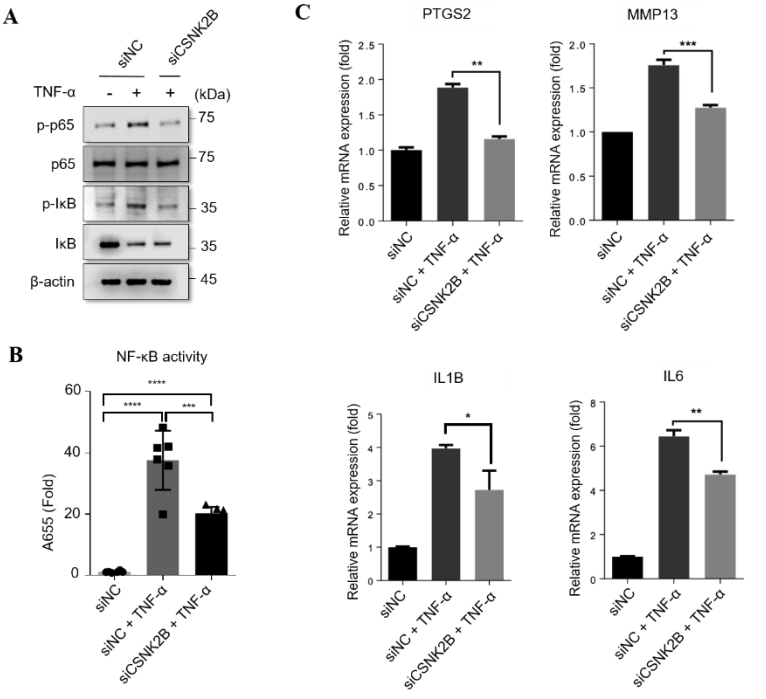

Figure 9. The effect of CK2β knockdown on NF-κB activity. (A) Protein levels of p-p65, p65, p- IκB and IκB were analyzed in TC28a2 cells transfected with NC, *CSNK2B* siRNA under TNF-α-induced inflammatory conditions. (B) NF-κB activity measured using HEK-Blue-hTLR2 cells. NF-κB activity is represented by the absorbance of SEAP activity. n=6 per group. All error bars indicate S.D. Data was analyzed by one-way ANOVA. *** $p < 0.001$, **** $p < 0.0001$ compared between each group indicated. (C) Relative expression of the indicated genes (*PTGS2, MMP13, IL1B, IL6*) as determined by RT-PCR with mRNA isolated from TC28a2 cells transfected with NC, *CSNK2B* siRNA under TNF-α-induced inflammatory conditions. Data are shown as means ± SD. * $p< 0.05$, ** $p <0.01$, *** $p < 0.001$ compared to NC siRNA with TNF-α group.

In addition, CK2β overexpression studies were conducted. CK2β was overexpressed in TC28a2 using lentivirus to western blot analysis (Fig. 10). In consequence, the results showed that the CK2β protein levels of the overexpression and the TNF-α-treated groups were similar (Fig. 11) Also, the results that upregulation of NF-κB activity due to TNF-α stimulation was repeated in CK2β overexpressed chondrocytes. Taken together, these results indicate that the protective effect of CK2β knockdown against the inflammation was related to CK2β-IκB-NF-κB regulation axis.

To further determine the potential of NF-κB signaling as a candidate CK2β target pathway, I would like to determine whether NF-κB signaling is activated in human OA tissues. Immunohistochemistry results revealed that p-p65 and p-IκB were highly expressed in degenerated tissues, indicating that NF-kB-mediated inflammation was activated (Fig. 12A). Western blot analysis indicated that CK2α was almost absent in both preserved and degenerated tissues whereas CK2β was highly expressed in degenerated tissues. p-65 and p-IκB protein levels were higher in preserved tissues than degenerated tissues (Fig. 12B). These results indicate that CK2β upregulation in degenerated cartilage tissues may be associated with IκB-mediated-NF-κB activation, resulting in rapid OA progression in humans.

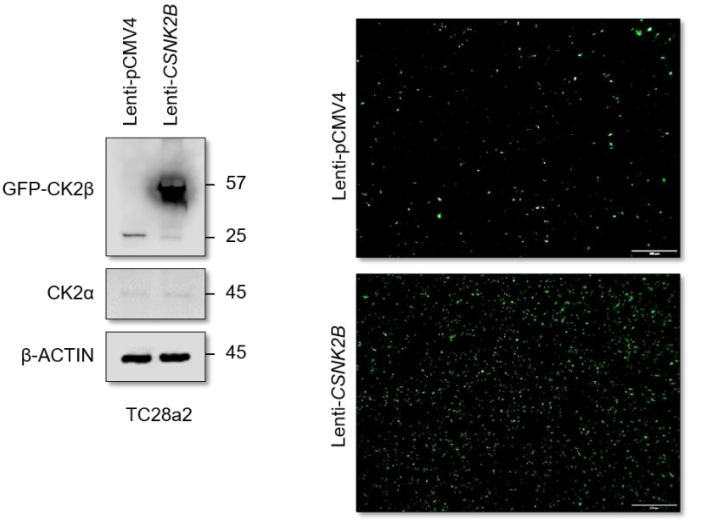

Figure 10. CK2β expression in TC28a2 cells as the result of lentivirus infection. CSNK2B-GFP vector was co-transfected with packaging vectors into Lenti-X^{TM} 293T cells to produce lentivirus. Lenti-CSNK2B infected to TC28a2 cells. Protein levels of CK2β and CK2α were analyzed using western blotting. GFP fluorescent signaling was detected by fluorescence microscopy.

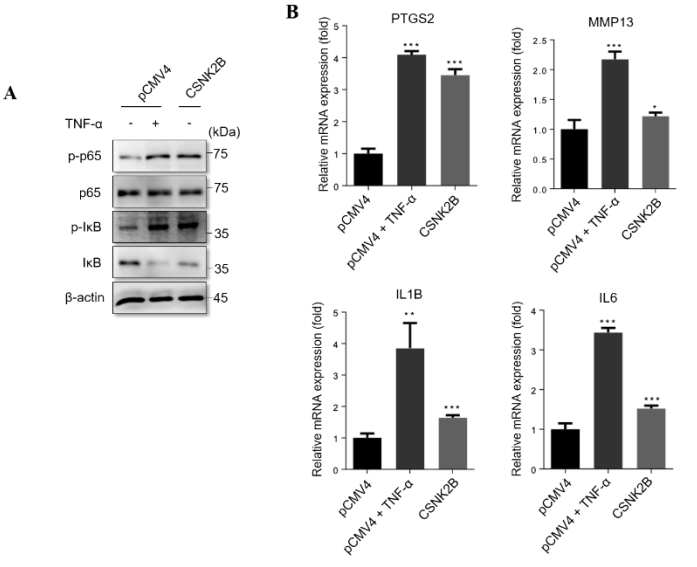

Figure 11. Overexpression of CK2β induces NF-κB activation in human chondrocytes. (A) The results of western blot analyses of NF-κB-related genes, namely p-p65, p65, p-IκB, and IκB, in TNF-α-mediated inflammatory or CK2β-overexpressed TC28a2 cells. (B) Relative expression of the indicated genes (*PTGS2*, *MMP13*, *IL1B*, *IL6*) as determined by RT-PCR with mRNA isolated from TC28a2 cells infected with lenti-pCMV4 under TNF-α-induced inflammatory conditions and with lenti-*CSNK2B*. Data are shown as means ± SD. * $p< 0.05$, ** $p <0.01$, *** $p < 0.001$ in comparison with pCMV4 control group.

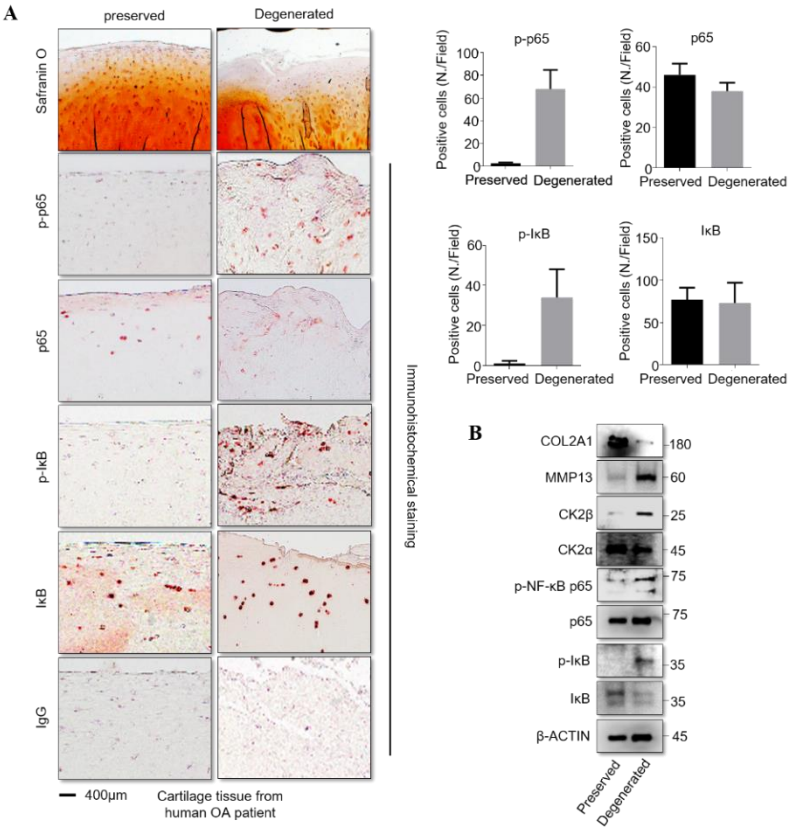

Figure 12. IκB-mediated NF-κB signaling in human OA cartilage tissues. (A) Representative images of immunohistochemical staining to detect p-p65, p65, p- IκB, and IκB in preserved and degenerated human OA knee joint cartilage tissues (n = 3 per group). Scale bars: 400 μm. (B) Western blot analysis results for NF-κB related genes. Protein was extracted from preserved and degenerated human OA knee joint cartilage tissue samples.

5. *Csnk2b* conditional knockdown mice show a protective effect on OA-like phenotype

The results above suggested that CK2β might play a role in OA progression, so I conducted an in vivo study to investigate the in vivo function of *Csnk2b* in OA progression. I used 8-week-old C*snk2b*$^{fl/fl}$; *Col2-CreER* mice for cartilage-specific knockdown of *Csnk2b*. They received proper concentrations of tamoxifen via intraperitoneal injections daily for 5 consecutive days (Figs. 13A and B). After 1 week, the mice were sacrificed to evaluate the knockdown efficiency through histology and western blot analysis. Following these validations, I confirmed that *Csnk2b* was deleted in knee-joint cartilage (Figs. 13C and D).

Next, well-established surgical destabilization of the medial meniscus (DMM) model of OA was used to examined whether the knockdown of *Csnk2b* had an impact on articular cartilage in the DMM-induced OA. 8 weeks after surgery, Safranin O staining was performed in sham-operated, DMM-operated mice and DMM-operated *Csnk2b* KO mice. DMM-operated C*snk2b*$^{fl/fl}$ mice exhibited significantly more cartilage erosion than the sham-operated mice. However, more of the DMM-operated *Csnk2b* knockdown mice's cartilage thickness was preserved than the DMM mice, indicating that the *Csnk2B* knockdown mice were better protected from DMM-induced OA than the control mice (Fig. 14).

Activation of NF-κB signaling pathway via degradation of IκB after phosphorylation was detected in DMM-operated control mice, as determined by immunohistochemical staining. As I expected, knockdown of *Csnk2b* in knee-joint cartilage tissues of DMM-operated mice significantly suppressed

NF-κB activation consistent with the inhibition of OA cartilage destruction. These results show that *Csnk2b* knockdown can have an anti-inflammatory effect and protect against cartilage destruction in DMM-induced OA (Fig. 15).

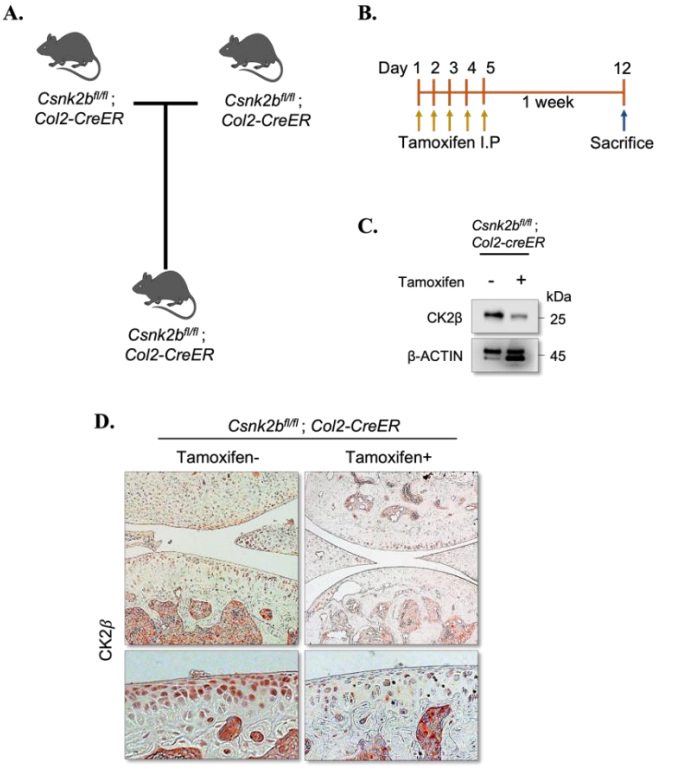

Figure 13. Induction of cartilage specific *Csnk2b* conditional knockdown. (A) *Csnk2b* $^{fl/fl}$; *Col2-CreER* mice were crossed with *Csnk2b* $^{fl/fl}$; *Col2-CreER* mice to produce *Csnk2b* $^{fl/fl}$; *Col2-CreER* mice littermates. (B) Experimental timelines. C57B6 mice at 8 weeks old were treated with 5 consecutive intraperitoneal injections of tamoxifen. (C) Protein levels of CK2β was analyzed using western blotting in knee joint cartilage tissues obtained from *Csnk2b* $^{fl/fl}$; *Col2-CreER* mice with tamoxifen injection. (D) Representative images of immunohistochemical staining results of CK2β in knee joint cartilage tissues obtained from *Csnk2b* $^{fl/fl}$; *Col2-CreER* mice with tamoxifen injection.

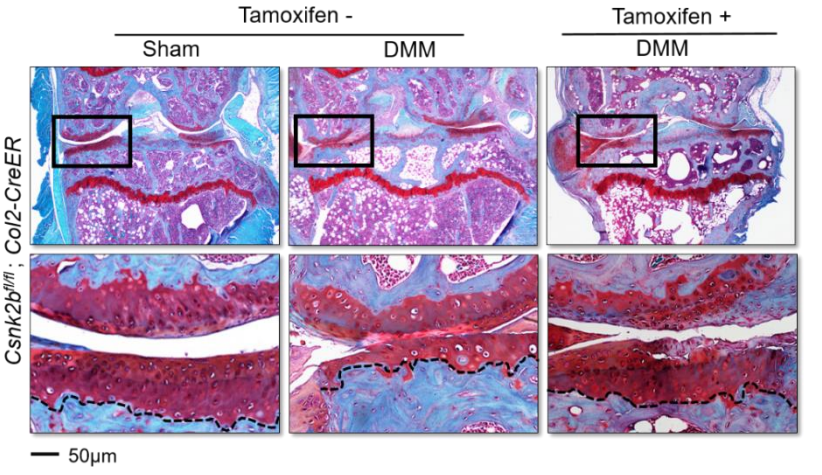

Figure 14. The effect of *Csnk2b* knockdown on DMM-induced cartilage destruction. Safranin O and fast green staining of articular cartilage tissues from *Csnk2b* $^{fl/fl}$; *Col2-CreER* mice that underwent sham or DMM surgery is indicated (n = 4 per group). Scale bar = 50μm. Safranin O and fast green staining demonstrated OA progression through the 8-week time course in the medial tibial plateau.

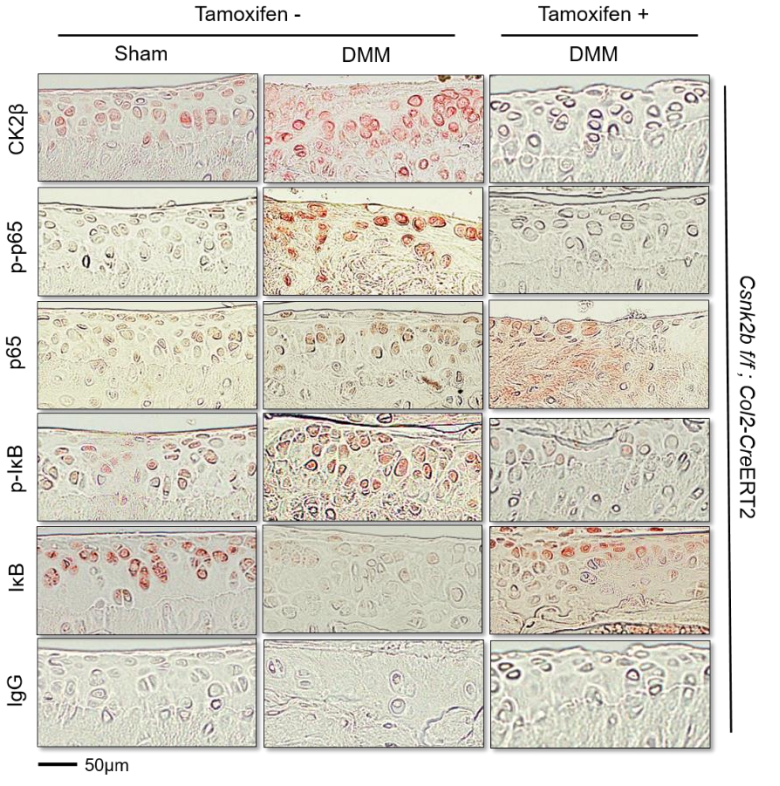

Figure 15. The effect of *Csnk2b* knockdown in cartilage on NF-κB activity. Representative images for p-p65, p65, p-IκB, and IκB (all red) immunostaining in cartilage tissues obtained from *Csnk2b* $^{fl/fl}$; *Col2-CreER* mouse knees with sham or DMM surgery. Scale bar=50μm.

IV. DISCUSSION

Articular cartilage consists of chondrocytes surrounded by ECM. Chondrocytes in the articular cartilage engage in metabolic activities including synthesizing a large number of ECM components, such as collagen, glycoproteins, proteoglycans, and hyaluronan. These metabolic activities are affected by many factors, such as pro-inflammatory cytokines and growth factors that have anabolic and catabolic effects. Normally chondrocytes are in a state of physiological and metabolic homeostasis, but their metabolic homeostasis and cartilage remodeling activities in the osteoarthritic environment can be disrupted under inflammatory conditions. In addition, inflammatory cytokines, such as IL-1b, TNF-a, and IL-6, are secreted by chondrocytes and synoviocytes. The upregulation of these inflammatory cytokines plays a role in disrupting cartilage homeostasis and MMP-mediated cartilage degradation during OA progression. Thus, it is important to regulate chondrocytes' homeostatic changes that occur during the progression of OA. I hypothesized that finding a molecular target that can regulate inflammatory responses in OA would inhibit the progression of OA by allowing for the repair of adjacent tissues.[33]

Numerous recent studies have reported that CK2 activity is implicated in signaling pathways by phosphorylating various substrates, such as STAT3, AKT, NF-κB p65, and IkB. CK2 is a key part of cancer biology and has been identified as a promising anticancer drug target. Although CK2 has been well investigated in cancer and tumor therapy research and related signaling

pathways are also associated with OA progression, there have not yet been any reports about the role that CK2 plays in osteoarthritic chondrocytes.

To understand the expression patterns of CK2 subunits in human OA tissue, mRNA expression and protein levels were compared in human OA tissues. CK2β mRNA expression and protein levels were both higher in degenerated tissues than preserved tissues, but CK2α expression levels were nearly identical (Fig. 1). Next, histological analysis was conducted to identify the expression levels of the CK2 alpha and beta subunits, CK2α and CK2β, respectively, in human OA tissues. The results showed high levels of CK2β were present only in degenerated OA tissues but in low levels in healthy tissues where similar levels of CK2α were detected in both types of tissues (Fig. 2). Thus, an in vitro OA-like inflammatory environment in TC28a2 human chondrocyte cells was established by stimulating them with TNF-α to identify the link between CK2 and OA environments. CK2 activity and the protein levels of CK2 subunit beta, CK2β, were elevated in a TNF-α dose-dependent manner whereas the protein levels of CK2 subunit alpha, CK2α, barely changed (Fig. 3). These results showed that TNF-α-mediated inflammation upregulates CK2 activity largely by upregulating CK2β levels in chondrocytes.

This study's results suggest that the upregulation of CK2β is associated with OA progression, so the role that CK2β plays in OA progression and the mechanism of how it regulates inflammation in chondrocytes should be identified. First, I examined whether the downregulation of CK2 activity by suppressing the regulatory domain CK2β that is elevated in osteoarthritic

environment could prevent NF-kB-mediated OA pathogenesis in chondrocytes. The results showed that CK2 activity was increased by TNF-α treatment and then decreased when transfected with siCSNK2B (Fig. 4). TNF-α treatment also decreased the levels of COL2A1 and ACAN, components of healthy cartilage matrix. However, CK2β knockdown by siRNA significantly attenuated inflammation in TC28a2 cells (Fig. 5). The results clearly indicated that downregulating CK2β reduces inflammatory responses in chondrocytes.

A cancer biology study of CK2 showed that it directly phosphorylates STAT3, AKT, NF-kB p65, and IkB as a regulator of each signaling pathway.[34] Accordingly, I hypothesized that it would regulate these pathways in OA chondrocytes as well. Among them, phosphorylation of p65 and IkB was enhanced by TNF-α stimulation (Fig. 6). The CK2β would be interacted with NF-kB p65 and IkB, inducing inflammation responses in osteoarthritic chondrocytes. Thus, immunoprecipitation analysis was conducted to determine whether CK2β and interacts with the signaling factors STAT3, AKT, NF-kB p65, and IkB. The results showed that CK2β directly interacted with NF-kB p65 and IkB in chondrocytes and that it did so more with TNF-α treatment than without it. These results indicate that CK2β binds frequently with NF-kB p65 and IkB, resulting in greater activation of the NF-kB signaling pathway for transcript downstream pro-inflammatory genes in chondrocytes (Fig. 7).

Furthermore, the result that CK2β-mediated phosphorylation can induce IkB ubiquitination was confirmed, resulting in the proteasomal degradation of IkB. To identify the role of CK2β in the ubiquitination of IkB, the IkBα-FLAG

vector and siRNAs were co-transfected to TC28a2 cells and then performed immunoprecipitation with anti-FLAG antibody. Under TNF-α treatment, no ubiquitinated protein bands were detected because of the ubiquitin-proteasome-pathway. Numerous intracellular proteins are degraded by the ubiquitin-proteasome-pathway in all tissues. IkB, which inhibits the nuclear translocation of NF-kB complex, is also degraded through the ubiquitin-proteasome-pathway and this process is triggered by its phosphorylation.[32,35,36] After the ubiquitination and rapid degradation of IkB, freed NF-kB translocates to the nucleus to accelerate the inflammatory response. For that reason, when the proteasome inhibitor MG132 was co-treated with TNF-α, the ubiquitination levels of IkB increased. However, CK2β knockdown decreased the ubiquitination rate of IkB despite TNF-α stimulation. These results show that IkB ubiquitination-induced proteasomal degradation occurred as a result of CK2β-mediated phosphorylation in osteoarthritic chondrocytes (Fig. 8). The NF-kB signaling pathway is activated by the degradation of IkB to promote the nuclear translocation of NF-kB complex. In the same manner, phosphorylation of NF-kB p65 also leads to NF-kB activation by importing NF-kB dimer to the nucleus. Thus, western blot analysis showed that the phosphorylation of p65 and IkB increased in the group that was treated with TNF-α only while it decreased in the TNF-α-treated CK2β knockdown group. This result indicates that CK2β knockdown suppresses NF-kB activity in chondrocytes. The HEK-Blue-hTLR2 (InvivoGen) was used to quantify NF-kB activity. HEK-Blue-hTLR2 cells are also stimulated by TNF-α because it is a NOD agonist, so I used them to measure SEAP activity. I found that siCSNK2B

downregulated NF-kB activity during TNF-α treatment (Fig. 9). Additionally, to determine whether CK2β overexpression without TNF-α could activate NF-κB signaling, such as TNF-α stimulation, CK2β overexpression experiments were conducted. CK2β-overexpressing lentivirus was transduced to TC28a2 human chondrocytes. A control group that was treated with TNF-α and one that was not were compared to a CK2β-overexpressing group that was not treated with TNF-α. As I hypothesized, the phosphorylation of p65 and IkB was elevated by both TNF-α stimulation and CK2β overexpression. Also, the mRNA expression levels of NF-kB downstream targets were elevated in a similar manner (Fig. 11). This study was conducted to determine how NF-kB signaling is activated in human OA tissues and to determine whether it can be used as a potential downstream signaling target of CK2β. Similar to the results of the in vitro inflammatory chondrocyte experiment (Fig. 6), both histological analysis and western blot results indicated that p-p65 and p-IκB were present in large quantities and the protein levels of p-p65 and p- IκB were higher in degenerated human OA tissues in which where CK2β was upregulated (Fig. 12). This result indicates that the CK2β-IκB-NF-κB axis is a potential therapeutic target for OA.

For further study, a conditional knockdown mouse experimental model was established for the cartilage-specific deletion of the *Csnk2b* gene (Fig. 13). I tried to determine whether the *Csnk2b* conditional knockdown can serve as a potential clinical intervention to prevent OA progression using mouse OA experimental models. The *Csnk2b* knockdown mouse model with DMM surgery exhibited a protective effect against DMM-induced cartilage erosion and

suppression of the NF-κB signaling pathway, which was consistent with the in vitro results (Figs. 14 and 15). These results indicate suppressing *Csnk2b* may be used to treat OA. However, not only progressive cartilage erosion in subchondral bone but also osteophyte formation, subchondral bone remodeling, and synovial membrane inflammation also play roles in the progression of OA. Although I focused on the inflammatory responses in chondrocytes alone in this study, some of these other factors may also be influenced by CK2β regulation. For that reason, other manifestations of OA should be identified to determine whether CK2β can be a therapeutic target for treating OA.

V. CONCLUSION

In this study, I found that increased levels of CK2β in human OA is related to OA progression. Also, I identified the roles that CK2β plays as an upstream regulator of the NF-kB mediated inflammatory response in chondrocytes. My study sheds light on the mechanism of CK2β-IκB-NF-κB axis in chondrocytes and provides preliminary evidence that the downregulation of CK2β can contribute to chondrocyte homeostasis in osteoarthritic inflammatory environment.

REFERENCES

1. Saito T, Tanaka S. Molecular mechanisms underlying osteoarthritis development: Notch and NF-kappaB. Arthritis Res Ther 2017;19:94.
2. Sandell LJ, Aigner T. Articular cartilage and changes in arthritis. An introduction: cell biology of osteoarthritis. Arthritis Res 2001;3:107-13.
3. Goldring MB, Otero M. Inflammation in osteoarthritis. Curr Opin Rheumatol 2011;23:471-8.
4. Berenbaum F. Osteoarthritis as an inflammatory disease (osteoarthritis is not osteoarthrosis!). Osteoarthritis Cartilage 2013;21:16-21.
5. Sinusas K. Osteoarthritis: diagnosis and treatment. Am Fam Physician 2012;85:49-56.
6. Neogi T, Zhang Y. Osteoarthritis prevention. Curr Opin Rheumatol 2011;23:185-91.
7. Roman-Blas JA, Jimenez SA. NF-kappaB as a potential therapeutic target in osteoarthritis and rheumatoid arthritis. Osteoarthritis Cartilage 2006;14:839-48.
8. Choi MC, Jo J, Park J, Kang HK, Park Y. NF-kappaB signaling pathways in osteoarthritic cartilage destruction. Cells 2019;8.
9. Liu T, Zhang L, Joo D, Sun SC. NF-kappaB signaling in inflammation. Signal Transduct Target Ther 2017;2.
10. Lian C, Wang X, Qiu X, Wu Z, Gao B, Liu L, et al. Collagen type II suppresses articular chondrocyte hypertrophy and osteoarthritis progression by promoting integrin beta1-SMAD1 interaction. Bone Res

2019;7:8.

11. Wang Y, Fan X, Xing L, Tian F. Wnt signaling: a promising target for osteoarthritis therapy. Cell Commun Signal 2019;17:97.
12. van der Kraan PM, van den Berg WB. Chondrocyte hypertrophy and osteoarthritis: role in initiation and progression of cartilage degeneration? Osteoarthritis Cartilage 2012;20:223-32.
13. Graham KC, Litchfield DW. The regulatory beta subunit of protein kinase CK2 mediates formation of tetrameric CK2 complexes. J Biol Chem 2000;275:5003-10.
14. Meggio F, Boldyreff B, Marin O, Pinna LA, Issinger OG. Role of the beta subunit of casein kinase-2 on the stability and specificity of the recombinant reconstituted holoenzyme. Eur J Biochem 1992;204:293-7.
15. Bragdon B, Thinakaran S, Moseychuk O, King D, Young K, Litchfield DW, et al. Casein kinase 2 beta-subunit is a regulator of bone morphogenetic protein 2 signaling. Biophys J 2010;99:897-904.
16. Gietz RD, Graham KC, Litchfield DW. Interactions between the subunits of casein kinase II. J Biol Chem 1995;270:13017-21.
17. Bibby AC, Litchfield DW. The multiple personalities of the regulatory subunit of protein kinase CK2: CK2 dependent and CK2 independent roles reveal a secret identity for CK2beta. Int J Biol Sci 2005;1:67-79.
18. Litchfield DW. Protein kinase CK2: structure, regulation and role in cellular decisions of life and death. Biochem J 2003;369:1-15.
19. Borgo C, D'Amore C, Sarno S, Salvi M, Ruzzene M. Protein kinase CK2: a potential therapeutic target for diverse human diseases. Signal

Transduct Target Ther 2021;6:183.

20. Dubois N, Willems M, Nguyen-Khac MT, Kroonen J, Goffart N, Deprez M, et al. Constitutive activation of casein kinase 2 in glioblastomas: Absence of class restriction and broad therapeutic potential. Int J Oncol 2016;48:2445-52.

21. Rabalski AJ, Gyenis L, Litchfield DW. Molecular pathways: emergence of protein Kinase CK2 (CSNK2) as a potential target to inhibit survival and DNA damage response and repair pathways in cancer cells. Clin Cancer Res 2016;22:2840-7.

22. Dominguez I, Sonenshein GE, Seldin DC. Protein kinase CK2 in health and disease: CK2 and its role in Wnt and NF-kappaB signaling: linking development and cancer. Cell Mol Life Sci 2009;66:1850-7.

23. Yu M, Yeh J, Van Waes C. Protein kinase casein kinase 2 mediates inhibitor-kappaB kinase and aberrant nuclear factor-kappaB activation by serum factor(s) in head and neck squamous carcinoma cells. Cancer Res 2006;66:6722-31.

24. Romieu-Mourez R, Landesman-Bollag E, Seldin DC, Sonenshein GE. Protein kinase CK2 promotes aberrant activation of nuclear factor-kappaB, transformed phenotype, and survival of breast cancer cells. Cancer Res 2002;62:6770-8.

25. Romieu-Mourez R, Landesman-Bollag E, Seldin DC, Traish AM, Mercurio F, Sonenshein GE. Roles of IKK kinases and protein kinase CK2 in activation of nuclear factor-kappaB in breast cancer. Cancer Res 2001;61:3810-8.

26. Gibson SA, Benveniste EN. Protein Kinase CK2: an emerging regulator of immunity. Trends Immunol 2018;39:82-5.

27. Buontempo F, McCubrey JA, Orsini E, Ruzzene M, Cappellini A, Lonetti A, et al. Therapeutic targeting of CK2 in acute and chronic leukemias. Leukemia 2018;32:1-10.

28. Borgo C, Ruzzene M. Role of protein kinase CK2 in antitumor drug resistance. J Exp Clin Cancer Res 2019;38:287.

29. Kim JM, Yang YS, Park KH, Ge X, Xu R, Li N, et al. A RUNX2 stabilization pathway mediates physiologic and pathologic bone formation. Nat Commun 2020;11:2289.

30. Maldonado M, Nam J. The role of changes in extracellular matrix of cartilage in the presence of inflammation on the pathology of osteoarthritis. Biomed Res Int 2013;2013:284873.

31. Shi Y, Hu X, Cheng J, Zhang X, Zhao F, Shi W, et al. A small molecule promotes cartilage extracellular matrix generation and inhibits osteoarthritis development. Nat Commun 2019;10:1914.

32. Viatour P, Merville MP, Bours V, Chariot A. Phosphorylation of NF-kappaB and IkappaB proteins: implications in cancer and inflammation. Trends Biochem Sci 2005;30:43-52.

33. Calich AL, Domiciano DS, Fuller R. Osteoarthritis: can anti-cytokine therapy play a role in treatment? Clin Rheumatol 2010;29:451-5.

34. Zheng Y, McFarland BC, Drygin D, Yu H, Bellis SL, Kim H, et al. Targeting protein kinase CK2 suppresses prosurvival signaling pathways and growth of glioblastoma. Clin Cancer Res

2013;19:6484-94.

35. Christian F, Smith EL, Carmody RJ. The regulation of NF-kappaB subunits by phosphorylation. Cells 2016;5.

36. Lecker SH, Goldberg AL, Mitch WE. Protein degradation by the ubiquitin-proteasome pathway in normal and disease states. J Am Soc Nephrol 2006;17:1807-19.

ABSTRACT (IN KOREAN)

골 관절염 발병 기전에서 NF-κB 신호전달체계 매개자로서의 CK2β의 역할

<지도교수 이 진 우>

연세대학교 대학원 의과학과

조 세 희

Casein Kinase 2 (CK2) 는 진핵 생물체에 존재하는 매우 보존된 세린/트레오닌 단백질 인산화 효소이다. 두개의 촉매 역할을 하는 α와 α' 소단위체와 두개의 조절 역할을 하는β 소단위체가 사량체를 이루며 활성을 가지며 세포 내에서 세포 회로 조절, DNA 합성, 염증 등과 같은 다양한 반응에 관여한다. 특히, 다양한 기질들을 인산화함으로써 염증반응을 촉진하는 신호전달체계를 조절한다는 것이 잘 알려져 있다. 이러한 염증반응은 골 관절염을 일으키는 주된 요인 중 하나이기 때문에 이를 조절하는 상위 조절자의 매커니즘을

밝히는 것은 매우 중요하다. 즉, CK2 활성의 조절은 골 관절염의 새로운 치료 타겟으로써 가능성이 있다고 할 수 있다. 그럼에도 불구하고 골 관절염 내 CK2 역할에 대해서는 규명 된 바 전무하다. 본 연구에서는 인간 골 관절염 무릎 연골 조직에서 CK2의 β 소단위체의 발현이 증가 되어있는 것을 확인하였고, 이에 따른 CK2 활성의 증가를 확인하였다. 따라서 *in vitro* 내 염증성 사이토카인인 TNF- α 를 처리하여 염증 환경을 조성 한 인간 연골세포주에 CK2β 발현을 억제하여 효과를 관찰하였다. 그 결과, CK2β 발현이 억제된 연골세포에서 염증으로부터 유도되는 반응들이 감소하는 것을 확인하였다. 또한, TNF- α 환경 내 연골세포에서 CK2β가 NF-κB p65와 IκB 와 직접적으로 결합하여 인산화 시킴으로써 하위의 NF-κB 매개 염증반응을 촉진한다는 것을 규명하였다.

이를 통해, 증가된 CK2β를 억제함으로써 CK2의 활성을 낮추는 것은 골 관절염 내 NF-κB 염증 환경을 완화시킬 수 있다는 가능성을 시사하였다. 즉, CK2β-IκB-NF-kB 경로의 조절이 골 관절염의 새로운 치료 타겟이 될 수 있음을 의미한다.

핵심되는 말: 골 관절염, 염증반응, NF-κB 신호전달체계, 연골세포, Casein kinase 2β