Fibroblast growth factor-8(FGF-8) upregulates gelatinases expression in chondrocytes through nuclear factor-κB p65

Hongcan Huang
Sichuan University Huaxi College of Stomatology: Sichuan University West China College of Stomatology

Jing Xie
Sichuan University West China Hospital of Stomatology: Sichuan University West China College of Stomatology

Jieya Wei
Sichuan University West China Hospital of Stomatology: Sichuan University West China College of Stomatology

Siqun Xu
Sichuan University West China Hospital of Stomatology: Sichuan University West China College of Stomatology

demao zhang (demao.zhang666@scu.edu.cn)
Sichuan University https://orcid.org/0000-0002-4437-3496

Xuedong Zhou
Sichuan University West China Hospital of Stomatology: Sichuan University West China College of Stomatology

Research Article

Keywords: FGF-8, Chondrocytes, MMP-2, MMP-9, NF-κB signal

Posted Date: April 15th, 2022

DOI: https://doi.org/10.21203/rs.3.rs-1546078/v1

Abstract

Background Fibroblast growth factor 8 (FGF-8), as a member of the fibroblast growth factor (FGFs), exerts pleiotropic effects in bone development and homeostasis. Studies have reported that FGF-8 is expressed in osteoarthritis and promotes disease progression. Here I attempt to investigate the effect of FGF-8 on the gelatinase (MMP-2 and MMP-9) expression of cultured chondrocytes.

Methods and Results Gelatin zymography was performed to evaluate the activation of gelatinases. Western blot was employed to investigate the expression of the gelatinases and also NF-κB p65 signaling pathways, and the activation of NF-κB p65 signaling wa confirmed by immunofluorescence staining. According to our findings, recombinant FGF-8 increased the production and activation of gelatinases in primary chondrocytes. The activation of NF-κB signaling, which resulted in acetylated p65 accumulating in the cell nucleus, regulated FGF-8-induced gelatinase production. We further found that NF-κB inhibitors, BAY 11-7082, could inhibit up-regulation of gelatinase induced by FGF-8.

Conclusions FGF-8 enhanced the expression and activation of gelatinases from cultured chondrocytes of mouse. FGF-8 may participate in the degradation of cartilage and exacerbation of osteoarthritis by promoting expression of gelatinases.

Introduction

Fibroblast growth factors (FGFs) are protein ligands that are released and operate in a paracrine or endocrine manner to perform pleiotropic activities in development, tissue homeostasis, and metabolism[1–3]. The human–mouse FGF family consists of 24 members, and according to phylogenetic analysis, these genes may be divided into seven subfamilies. FGF-8 subfamily consists of FGF-8, FGF-17 and FGF-18[4]. It has been reported that in osteoarthritis (OA), FGF-8 induced the release of matrix metalloproteinase-3 (MMP-3) and prostaglandin E2 (PGE2) and led to degradation of extracellular matrix (ECM). In addition, FGF-8 and IL-1 synergistically accelerated ECM degradation, and low levels of IL-1 significantly increased ECM degradation in the presence of FGF-8[5]. Under experimental OA conditions, FGF-8 had pathological effects on rat and rabbit articular cartilage[5]. Therefore, FGF-8 might be involved in the deterioration of cartilage tissue and aggravates the symptoms of osteoarthritis. Osteoarthritis (OA) is a common degenerative joint disease characterized by inflammation and major structural changes of the joint, causing pain and functional disability[6–8]. Osteoarthritis is now known to be a complex condition affecting the whole joint, in which activation of matrix proteases has a pivotal role[9]. OA process is characterized by changes of chondrocytes. Chondrocytes in OA had been shown to produce ECM-degrading proteins, including Matrix metalloproteinases (MMPs)[10, 11].

Matrix metalloproteinases (MMPs) are zinc-dependent endopeptidases and have been divided into six groups: collagenases, gelatinases, stromelysins, matrilysins, membrane-type MMPs, and other non-classified MMPs[11]. As one part of the family, genatinases include two members, genatinase A and gelatinase B, namely MMP-2 and MMP-9 respectively. Genatinases feature a fibronectin type II motif

inside the active domain, which leads to binding and degrading gelatin (denatured collagen). Except ECM remodeling, MMP-9 also transformed some cytokines and chemokines into active or inactive (for example pro-IL-1b)[12]. Deletion of MMP-9 resulted in defects of growth plate and human MMP-2 mutants showed an osteolytic syndrome[13, 14]. Previous data showed an over-release of gelatinases in the injured joint which might result in exacerbation of cartilage degradation in osteoarthritis[15, 16]. Furthermore, MMP-2 and MMP-9 could activate other MMPs and amplified catalytic reaction[17]. It is obvious that gelatinases play significant roles in joints in normal and pathological conditions. The changes of gelatinase expression in chondrocytes after FGF-8 treatment can further help us understand the role of FGF-8 and gelatinases in cartilage and explore new potential treatment for OA.

Nuclear factor kappa beta (NF-κB) is an ubiquitously-expressed transcription factors family involved in broad cell behavior, like cell death, immunity and inflammatory. The mammalian NF-κB subfamily is composed of five proteins: RelA/p65, RelB, c-Rel, NF-κB1/p50 and NF-κB2/p52. Under unstimulated conditions, NF-κB proteins are bound to inhibitory NF-κB proteins (IkB) as the form of hetero-dimers in the cytoplasm. After stimulated, IkB is phosphorylated by IκB kinases (IKKs) and then degradated by the ubiquitin–proteasome system. Subsequently, the dimers are released to transfer into the nucleus and trigger the expression of target genes[18]. NF-κB signaling played the central role in triggering the up-regulation of MMPs expression and translating into extracellular matrix (ECM) damage of cartilage[19–21]. The NF-κB pathway was significant for expressing inflammation-related genes in chondrocytes, such as MMP-1,3, and 13[20, 22].

In this study, we explored the expression of gelatinases in cultured articular chondrocytes under FGF-8 stimulation. We provided evidence that p65 was involved in FGF-8-mediated induction of gelatinases. Furthermore, we showed that the secretion of MMP-2 and MMP-9 was up-regulated by FGF-8, which might imply that FGF-8 was catabolic with pathological effects in OA cartilage. We hope to provide a new direction for further understanding of the role of FGF-8 in physiology and pathology of cartilage.

Materials And Methods

Cell Culture

Chondrocytes were isolated from knee joints of 1-day-old mice as described previously [15]. Briefly, the mouse was first sterilized and sacrificed to collect the knee joints. The epidermis was removed and the joints were cut into small pieces with trypsin (0.25%) for 30 minutes (min). Then the supernatant was replaced by 0.5% collagenase type II for 12 hours (h). The suspension was collected and mixed 1:1 (V/V) with Dulbecco's modified Eagle's medium (DMEM) (high-glucose DMEM, 4 mmol/L L-glutamine, 0.1 mmol/L non-essential amino acids and 1% penicillin–streptomycin solution) containing 10% fetal bovine serum (FBS). Following centrifuging the mixed suspension at 1 000 r/min for 5 min, the supernatant was replaced by 10% FBS DMEM to resuspend the cells, which were then seeded into plates or flasks and cultured in a humidified atmosphere containing 5% CO_2 at 37°C.

FGF-8 Treatment

A concentration of 5 ×10^5 cells per well was seeded into six-well plates (85–95% confluence) and cultured in DMEM with 10% FBS as previously described[23]. After 12h, the cells were starved in 2% FBS DMEM for 12h to equilibrate all cells. After that, the cells were incubated in DMEM medium with 1% FBS (control: 0 ng/ml FGF8), DMEM with with 1% FBS and different concentrations (1,5,10,50 and 100 ng/ml) of recombinant FGF-8 (Peprotech, #100 – 25). For western blot, the cell lysates were collected after 6, 12, and 24 h and for zymography the supernatant samples after 12, 24, 48, and 72 h.

NF-κB Inhibitor Treatment

BAY 11-7082 (20mg/mlx0.1ml, Beyotime, China), a NF-κB inhibitor, was used to check effects of NF-κB on gelatinase products in cultured chondrocytes. After cell seeding, 12h starvation, we exchanged the media with fresh 1% FBS culture media with or without BAY 11-7082 pre-treated for 1h, then we added FGF-8 into the media.

RNA sequencing

RNA sequencing was performed as previously described[24]. Briefly, primary chondrocytes were seeded into 35 mm single well at the concentration of 1×10^6 cells per plate, and incubated in 10% FBS DMEM for 12h. The cells were then starved in DMEM containing 2% FBS for another 12h to equilibrate all cells. Then the cells were cultured with fresh DMEM with 1% FBS (control group with 0 ng/ml FGF-8) or DMEM containing of 1% FBS and recombinant FGF-8 (100 ng/ml). Using Trizol (No. 15596-026, Thermo Fisher Scientific), we collected lysates from cells at 95% confluency. The experiment was performed three times independently using different chondrocytes from different mice. The transcriptome analysis of cell samples was performed by Shanghai Life Gene Biotechnology Co., Ltd. (Shanghai, China). The Bioanalyzer 2100 System (Agilent Technologies, CA, USA) was used to assess RNA integrity prior to transcriptome sequencing. The manufacturer's instructions specified that 1.5 μg of total RNA was utilized as the input material for RNA sample detection. The index-coded samples were clustered using the HiSeq 4000 PE Cluster Kit (Illumia, San Diego, CA, USA) on a cBot Cluster Generation System. Initially, an internal perl script was used to process raw data in fast q format to obtain clean data (clean reads) through removing reads containing adapters, reads containing ploy-N, and low-quality reads. HISAT2 v2.1.0 was used to align paired-end clean reads to the reference genome. Counting reads mapped to each gene was performed with HTSeq v0.6.1. Gene FPKMs were calculated by adding up the FPKMs of transcripts associated with each gene group. Based on GO (Gene Ontology) and KEGG enrichment analysis, differentially expressed genes were identified. Differentially expressed genes were deemed significantly enriched by GO terms or KEGG terms with a P-value less than 0.05. The DESeq2 R package (1.26.0) was used for differential expression analysis (control group vs FGF-8 group). P value < 0.05 and |FoldChange| ≥1.5 were set as the threshold for significantly differential expression.

Western Blot

The Western Blot procedure was followed as reported earlier[25]. The antibodies that were employed are as follows: 1:1000 primary antibody MMP-2 (ab37150, Abcam, USA), MMP-9 (ab52915, Abcam, USA), NF-κB p65 (250060,ZEN BIO, China), NF-κB p65 (Acetyl K310, ab19870, Abcam, USA), β-actin (sc-47778,

SANTA CRUZ, USA), and 1:5000 secondary antibody (m-IgGκ BP-HRP, sc-516102, Cruz Marker, Shanghai, China; mouse anti-rabbit IgG-HRP, sc-2357, Cruz Marker, Shanghai, China). Then a Western Blotting Luminol Reagent kit (Santa Cruz Biotechnology, Santa Cruz, USA) was used to visualize the membranes and Image J software was employed to quantify the protein bands.

Gelatin Zymography

The gelatin zymography was performed as previously described[25]. Briefly, 10% SDS-polyacrylamide gels containing 0.05% gelatin (Thermo-Fisher Scientific, Waltham, MA USA), were utilized to separate the proteins. Then three times of wash was carried out with renaturing buffer (2.5% Triton X-100 diluted in water). Then, the renaturing buffer was replaced by proteolysis buffer (50mM CaCl2, 0.5M NaCl, 50mM Tris, pH 7.8) to incubate the gels at 37°C for 16 ~ 18h. Next, the gels were washed with renaturing buffer before adding coomassie blue. Two hours later, coomassie blue was replaced by destain buffer (40% methanol, 7.5% acetic acid, 52.5% H2O). There was no coomassie blue staining in the regions of enzymatic activity (white bands). Image J software was used to quantify the band densities.

Immunofluorescence staining

Immunofluorescence staining was carried out as described before[23]. The cells were cultured with fresh 10% FBS DMEM with 100 ng/ml FGF-8 or without FGF-8 (as control) for 6h. Firstly, the cells were fixed by 4% cold paraformaldehyde for 15 min and permeabilized with 0.5% Triton X-100. Then the cells were blocked with 5% bull serum albumin (BSA) (Sigma-Aldrich, MO) for 1h before incubation at 4°C overnight with 1:200 primary antibody NF-κB p65 (acetyl K310, ab19870, Abcam, USA) or total NF-κB p65 (250060, ZEN BIO, China). Then the primary antibody was replaced by Alexa Fluor 488-conjugated fluorescent secondary antibody (ab150113, Abcam, USA) for 2h. All samples were counterstained with FITC-phalloidin (Invitrogen, Carlsbad, CA, USA) overnight and DAPI (D9642; Sigma, St. Louis, MO, USA) for 10 minutes. A confocal laser scanning microscope (CLSM, Olympus, FV3000, Japan) was used to take the pictures of the stained cells.

Statistics

The data were showed by mean ± standard deviation (SD) of at least three independent experiments. When comparing the differences between the groups, one-way analysis of variance (ANOVA) was employed. $P < 0.05$ ($\alpha = 0.05$) was set as the critical value for the significance level.

Results

High MMP-2 and MMP-9 activity of chondrocytes induced by FGF-8 in vitro.

Through RNA sequencing, we revealed the changes of the selected 25 genes in the extracellular matrix (Fig. 1A). We found that the genes of MMP-2 and MMP-9 showed changes of expression. Then the gelatin zymography assays showed increased activity of MMP-2 and MMP-9 after FGF-8 treatment in a time-dependent manner (Fig. 1B). FGF-8 enhanced MMP-2 and MMP-9 activity in primary chondrocytes in

a time-dependent manner, according to the quantification (Fig. 1C). The activity of MMP-2 increased by up to 1.85 times and the activity of MMP-9 up to 2.45-fold in primary chondrocytes at 72h after treatment with 100 ng/ml FGF-8 (Fig. 1D). Then we performed western blot to examine the production of MMP-2 and MMP-9 after stimulation of FGF-8 with different concentration of 0, 1, 10, 100 ng/ml at 24h (Fig. 1E). The expression of MMP-2 and MMP-9 was increased with FGF-8 treatment, and MMP-2 was increased by up to 1.10-fold and MMP-9 up to 1.29-fold in primary chondrocytes at 100 ng/ml FGF-8 compared to control group (Fig. 1F).

The activation of NF-κB p65 in cultured chondrocytes with FGF-8 treatment in vitro.

In order to explore the involvement of p65 in the FGF-induced gelatinase expression in cultured chondrocytes, western blot was performed to detect the activation of p65 (Fig. 2A). Both the signaling factor p65 and its acetylation (acetyl p65) were significantly enhanced following FGF-8 treatment at 6h and 12h (Fig. 2A). And the activation of p65 following FGF-8 treatment at 6 and 12h was validated by quantitative analysis. The production of p65 was raised by up to 1.24-fold and acetyl p65 up to 1.71-fold in primary chondrocytes compared to control cells at 6h at 100 ng/ml FGF-8, with the expression of p65 increased by up to 1.15-fold and acetyl p65 up to 1.40-fold at 12h (Fig. 2B).

FGF-8-induced gelatinase expression can be recovered by NF-κB inhibitor.

To learn more about the involvement of p65 in the production of gelatinase in chondrocytes induced by FGF-8, a NF-κB inhibitor, BAY 11-7082, was used. Firstly, the western blot assay showed that BAY 11-7082 significantly inhibited the up-regulation of both p65 and acetyl p65 induced by FGF-8 in primary chondrocytes (Fig. 2C, 2D). Then we detected the expression of MMP-2 and MMP-9 (Fig. 2E), the result showed that the expression of MMP-2 and MMP-9 stimulated by FGF-8 was significantly inhibited by BAY 11-7082 (Fig. 2E, 2F).

FGF-8-induced p65 translocation into nuclear can be recovered by NF-κB inhibitor.

The expression of p65 and translocation of it into the nucleus following treatment with 100 ng/ml FGF-8 for 6h were then examined using immunofluorescence staining. We found that FGF-8 promoted the expression of p65 primarily in nuclear regions which could be recovered by NF-κB inhibitor (Fig. 3A). In comparison to the control group, measurement of the fluorescence intensity distribution of total p65 in one chondrocyte revealed a propensity for p65 to be transported into nuclear (Fig. 3B). Phosphorylated p65 (p-p65) was shown to bind to CBP/p300 complexes to translate into acetylation of p65, which then triggered transcription[26]. Following that, we noticed that the FGF-8-treated group had a higher amount of acetyl p65 in their nuclei than the control group (Fig. 4A). When the fluorescence intensity distribution of acetyl p65 in one chondrocyte was quantified, it revealed a propensity for acetyl p65 to accumulate in the nucleus, which could be retrieved using NF-κB inhibitor (Fig. 4B). This was consistent with the WB data, indicating that nuclear p65 played an essential part in gelatinase regulation.

Discussion

Gelatinases belongs to the matrix metalloproteinase family. MMP-2 and MMP-9 were discovered in the early 1970s [27] and early 1980s [28], respectively, and named gelatinase A and gelatinase B in 1991 to distinguish the molecular mass of the two (gelatinase A, 72kDa; gelatinase B, 92kDa) [29]. Based on the naming rules established by the International MMP Conference held in Florida in 1989, gelatinases were renamed in the form of MMP abbreviation with number, namely MMP-2 (gelatinase A) and MMP-9 (gelatinase B) [30]. As an important regulator of cell activity, gelatinases not only affected reproduction, angiogenesis and tissue remodeling, bone development, wound healing, neuronal network learning, memory and other broad sides of life, but also participated in periodontitis, allergy, osteochondral condition, cardiovascular disease, diabetes and other pathological processes [31–34]. Studies had reported that MMP-2-deficient mice developed normally and were fertile without any serious anatomical abnormalities [35]. Human MMP-2 deficiency which resulted from inactivating autosomal recessive mutations in MMP-2 gene, manifested as severe osteolysis and arthritis [13, 36]. MMP-2-deficient mice also showed similar symptoms, but relatively mild [37]. Winchester syndrome was another condition related to changes in MMP-2 activity. Due to decreased activation of MMP-2, Winchester syndrome was characterized by osteolysis-related symptoms [38]. MMP-9 deficiency was non-lethal in mice, but abnormal development of growth plates in the long bones and delayed endochondral ossification were observed in MMP-9-null mice. This abnormality seemed to be caused by abnormal vascular invasion. MMP-9 was considered to be a key regulator of growth plate angiogenesis and hypertrophic chondrocyte apoptosis [14]. Consistent with this, later studies in chickens also found tibial chondrodysplasias related to the decreased expression of the MMP-9 gene. During the development of mice, it has been reported that there was high expression of MMP-2 in osteoclasts [39]. Indeed, MMP-2 and MMP-9 played a significant role in maintaining homeostasis in joints. In adult tissues, the basic expression of MMP-2 has been observed in normal articular cartilage, suggesting that MMP-2 might be involved in the physiological ECM turnover of articular cartilage, and the expression of MMP-9 was relatively low and limited to some chondrocytes in the very superficial layer. However, gelatinase expression was elevated in many pathologies such as tumor metastasis and osteoarthritis. And in this work, FGF-8 promoted gelatinase expression and activity by cultured articular chondrocytes. We suggested that this increased expression of gelatinases might have negative effects on cartilage, such as promoting cartilage matrix degradation and activating various pro-inflammatory factors. As has been reported previously, the cartilage section of OA patients showed that the expression and distribution of MMP-2 and MMP-9 were increased compared with the normal control group [40–42]. However recent studies showed that the increase of MMP-2 in joint synovial fluid might have a protective effect. MMP-2 deficient mice had been found with increased arthritis grades [43, 44], and injection of fibroblasts that secreted MMP-2 into the joints could reduce the response. So, it requires further studies that the effect of FGF-8-induced gelatinase expression on cartilage.

The functions of FGF-8 on developing tissues has been widely reported. FGF-8 was expressed during gastrulation[45]. Researches on embryogenesis in mice and chicks found that FGF-8 played a very important role in the formation of central nervous system, craniofacial organs, heart system, limbs and urogenital system [46–49]. FGF-8-deficient mouse could not develop through gastrulation [50]. In the

early limb ectoderm, the inactivation of FGF-8 resulted in a noteworthy reduction in the size of limb buds [51]. The skull was formed through intraperiosteal osteogenesis. Schmidt and colleagues found that moderately increased FGF-8 expression led to craniosynostosis, while higher FGF-8 level shifted the fate of mesenchymal cells from ossification to abnormal cartilage formation [50]. FGF-8 might also be involved in ectopic bone and cartilage formation in breast cancer cells that produced large amounts of FGF-8 [52]. But its functions on adult tissues were less studied. Uchii and his colleague provided new findings on the role of FGF-8 in joint inflammation. They found that FGF-8 could promote the destruction of articular cartilage by inducting catabolic factors such as MMP in joints [5]. Consistently, Liu and his colleague found that FGF-8 and FGF receptor-3 (FGFR-3) were all increased in the cartilage of children with Kashin-Beck disease, one type of OA [53]. The role of FGF-8 in cultured chondrocytes was studied in this work. Through western blot, zymography and immunofluorescence, we found that recombinant FGF-8 could increase gelatinase expression of culture chondrocytes. Given what we found, we hypothesized that FGF-8 might participate in the degradation of cartilage and exacerbation of osteoarthritis by enhancing expression of gelatinases. But other MMPs, like MMP-1, MMP-3 and MMP-13, were not measured.

In addition, FGF-8-induced expression of gelatinases was regulated through activation of NF-κB p65 signaling with acetylated p65 accumulating in the nucleus of the cell (Fig. 5). And we further found that NF-κB inhibitor could inhibit up-regulation of gelatinase induced by FGF-8. Activation of NF-κB signaling by FGFs has been reported, like FGF19 in hepatocytes [54], FGF1 In LX-2 cell [55], FGF2 in vascular smooth muscle cells [56]. It has also been reported that FGF-8 mediated NF-κB signaling in the nervous system [57]. This paper demonstrated for the first time that FGF-8 activated the NFkB signaling pathway in mouse knee chondrocytes. However the details by which FGF-8 activated p65 remained to be explored.

Our work provided a further insight into the regulation of FGF-8 in the expression of MMP-2 and MMP-9 in cultured chondrocytes. It indicated the potential role of FGF-8 in participating in OA. Most of studies focused on the pivotal role of FGF-8 in embryogenesis and morphogenesis, while it was less reported about its role in adult tissues, especially in joints. Uchii and his colleagues reported FGF-8 was expressed low in normal knees but higher in OA models, which indicated that FGF-8 might act as a catabolic mediator of cartilage[5]. Further studies are required for the role of FGF-8 in articular chondrocytes.

In conclusion, FGF-8 enhanced the expression and activity of MMP-2 and MMP-9 of cultured chondrocytes of mouse through upregulating NF-κB p65 signaling.

Declarations

Acknowledgements The authors thank all investigators and supporters involved in this study.

Data availability All data generated or analyzed during this study are included in this published article.

Funding This study was supported by the National Natural Science Foundation of China [No. 81870754], [No. 81771047] and [No. 82001062].

Declaration of Interests The authors declare that no competing interests exist.

Ethical statement All animal samples utilized in this investigation were collected and used in conformity with ethical principles, and the Institutional Review Board evaluated and approved the agreement (IRB, Institutional Review Board at the West China Hospital of stomatology, WCHSIRB-D-2017-029).

References

1. Goetz R, Mohammadi M (2013) Exploring mechanisms of FGF signalling through the lens of structural biology. Nat Rev Mol Cell Biol 14(3):166–180. https://doi.org/10.1038/nrm3528
2. Pu J, Wang R, Zhang G, Wang J (2019) FGF-7 facilitates the process of psoriasis by inducing TNF-alpha expression in HaCaT cells. Acta Biochim Biophys Sin (Shanghai) 51(10):1056–1063. https://doi.org/10.1093/abbs/gmz095
3. Zhu S, Ma L, Wu Y, Ye X, Zhang T, Zhang Q, Rasoul LM, Liu Y, Guo M, Zhou B, Ren G, Li D (2014) FGF21 treatment ameliorates alcoholic fatty liver through activation of AMPK-SIRT1 pathway. Acta Biochim Biophys Sin (Shanghai) 46(12):1041–1048. https://doi.org/10.1093/abbs/gmu097
4. Itoh N, Ornitz DM (2004) Evolution of the Fgf and Fgfr gene families. Trends Genet 20(11):563–569. https://doi.org/10.1016/j.tig.2004.08.007
5. Uchii M, Tamura T, Suda T, Kakuni M, Tanaka A, Miki I (2008) Role of fibroblast growth factor 8 (FGF8) in animal models of osteoarthritis. Arthritis Res Ther 10(4):R90. https://doi.org/10.1186/ar2474
6. Dieppe PA, Lohmander LS (2005) Pathogenesis and management of pain in osteoarthritis. The Lancet 365(9463):965–973. https://doi.org/10.1016/s0140-6736(05)71086-2
7. Vina ER, Kwoh CK (2018) Epidemiology of osteoarthritis: literature update. Curr Opin Rheumatol 30(2):160–167. https://doi.org/10.1097/BOR.0000000000000479
8. Wang K, Ren Y, Lin S, Jing Y, Ma C, Wang J, Yuan XB, Han X, Zhao H, Wang Z, Zheng M, Xiao Y, Chen L, Olsen BR, Feng JQ (2021) Osteocytes but not osteoblasts directly build mineralized bone structures. Int J Biol Sci 17(10):2430–2448. https://doi.org/10.7150/ijbs.61012
9. Glyn-Jones S, Palmer AJR, Agricola R, Price AJ, Vincent TL, Weinans H, Carr AJ (2015) Osteoarthr Lancet 386(9991):376–387. https://doi.org/10.1016/s0140-6736(14)60802-3
10. Hartl FU, Bracher A, Hayer-Hartl M (2011) Molecular chaperones in protein folding and proteostasis. Nature 475(7356) 324 – 32. https://doi.org/10.1038/nature10317
11. Vandenbroucke RE, Libert C (2014) Is there new hope for therapeutic matrix metalloproteinase inhibition? Nat Rev Drug Discov 13(12) 904 – 27. https://doi.org/10.1038/nrd4390
12. Opdenakker G, Van den Steen PE, Van Damme J (2001) Gelatinase B: a tuner and amplifier of immune functions. Trends Immunol 22(10):571–579. https://doi.org/10.1016/s1471-4906(01)02023-3

13. Martignetti JA, Aqeel AA, Sewairi WA, Boumah CE, Kambouris M, Mayouf SA, Sheth KV, Eid WA, Dowling O, Harris J, Glucksman MJ, Bahabri S, Meyer BF, Desnick RJ (2001) Mutation of the matrix metalloproteinase 2 gene (MMP2) causes a multicentric osteolysis and arthritis syndrome. Nat Genet 28(3):261–265. https://doi.org/10.1038/90100
14. Vu TH, Shipley JM, Bergers G, Berger JE, Helms JA, Hanahan D, Shapiro SD, Senior RM, Werb Z (1998) MMP-9/gelatinase B is a key regulator of growth plate angiogenesis and apoptosis of hypertrophic chondrocytes. Cell 93(3):411–422. https://doi.org/10.1016/s0092-8674(00)81169-1
15. Xie J, Fu N, Cai LY, Gong T, Li G, Peng Q, Cai XX (2015) The effects of interleukin-1beta in modulating osteoclast-conditioned medium's influence on gelatinases in chondrocytes through mitogen-activated protein kinases. Int J Oral Sci 7(4):220–231. https://doi.org/10.1038/ijos.2015.39
16. Xie J, Wang CL, Yang W, Wang J, Chen C, Zheng L, Sung KLP, Zhou X (2018) Modulation of MMP-2 and MMP-9 through connected pathways and growth factors is critical for extracellular matrix balance of intra-articular ligaments. J Tissue Eng Regen Med 12(1):e550–e565. https://doi.org/10.1002/term.2325
17. Jablonska-Trypuc A, Matejczyk M, Rosochacki S (2016) Matrix metalloproteinases (MMPs), the main extracellular matrix (ECM) enzymes in collagen degradation, as a target for anticancer drugs. J Enzyme Inhib Med Chem 31(sup1):177–183. https://doi.org/10.3109/14756366.2016.1161620
18. Rigoglou S, Papavassiliou AG (2013) The NF-kappaB signalling pathway in osteoarthritis. Int J Biochem Cell Biol 45(11):2580–2584. https://doi.org/10.1016/j.biocel.2013.08.018
19. Goldring MB, Marcu KB (2009) Cartilage homeostasis in health and rheumatic diseases. Arthritis Res Ther 11(3):224. https://doi.org/10.1186/ar2592
20. Roman-Blas JA, Jimenez SA (2006) NF-kappaB as a potential therapeutic target in osteoarthritis and rheumatoid arthritis. Osteoarthritis Cartilage 14(9):839–848. https://doi.org/10.1016/j.joca.2006.04.008
21. Saklatvala J (2007) Inflammatory signaling in cartilage: MAPK and NF-kappaB pathways in chondrocytes and the use of inhibitors for research into pathogenesis and therapy of osteoarthritis. Curr Drug Targets 8(2):305–313. https://doi.org/10.2174/138945007779940115
22. Scanzello CR, Plaas A, Crow MK (2008) Innate immune system activation in osteoarthritis: is osteoarthritis a chronic wound? Curr Opin Rheumatol 20(5):565–572. https://doi.org/10.1097/BOR.0b013e32830aba34
23. Wang Q, Zhou C, Li X, Cai L, Zou J, Zhang D, Xie J, Lai W (2019) TGF-beta1 promotes gap junctions formation in chondrocytes via Smad3/Smad4 signalling. Cell Prolif 52(2):e12544. https://doi.org/10.1111/cpr.12544
24. Zhou C, Zhang D, Du W, Zou J, Li X, Xie J (2020) Substrate mechanics dictate cell-cell communication by gap junctions in stem cells from human apical papilla. Acta Biomater 107:178–193. https://doi.org/10.1016/j.actbio.2020.02.032
25. Zhang L, Cui Y, Yang Y, Wei J, Liu W, Cai L, Wang L, Zhang D, Xie J, Cheng L (2021) The virulence factor GroEL promotes gelatinase secretion from cells in the osteoblast lineage: Implication for direct

crosstalk between bacteria and adult cells. Arch Oral Biol 122:104991. https://doi.org/10.1016/j.archoralbio.2020.104991

26. Perkins ND (2007) Integrating cell-signalling pathways with NF-kappaB and IKK function. Nat Rev Mol Cell Biol 8(1):49–62. https://doi.org/10.1038/nrm2083
27. Harris ED Jr, Krane SM (1972) An endopeptidase from rheumatoid synovial tissue culture. Biochim Biophys Acta 258(2) 566 – 76. https://doi.org/10.1016/0005-2744(72)90249-5
28. Murphy G, Cawston TE, Galloway WA, Barnes MJ, Bunning RA, Mercer E, Reynolds JJ, Burgeson RE (1981) Metalloproteinases from rabbit bone culture medium degrade types IV and V collagens, laminin and fibronectin. Biochem J 199(3):807–811. https://doi.org/10.1042/bj1990807
29. Masure S, Proost P, Van Damme J, Opdenakker G (1991) Purification and identification of 91-kDa neutrophil gelatinase. Release by the activating peptide interleukin-8. Eur J Biochem 198(2):391–398. https://doi.org/10.1111/j.1432-1033.1991.tb16027.x
30. Nagase H, Barrett AJ, Woessner JF Jr (1992) Nomenclature and glossary of the matrix metalloproteinases. Matrix Suppl 1:421–424
31. Henriet P, Emonard H (2019) Matrix metalloproteinase-2: Not (just) a "hero" of the past. Biochimie 166:223–232. https://doi.org/10.1016/j.biochi.2019.07.019
32. Katono T, Kawato T, Tanabe N, Suzuki N, Yamanaka K, Oka H, Motohashi M, Maeno M (2006) Nicotine treatment induces expression of matrix metalloproteinases in human osteoblastic Saos-2 cells. Acta Biochim Biophys Sin (Shanghai) 38(12):874–882. https://doi.org/10.1111/j.1745-7270.2006.00240.x
33. Poudel B, Lee YM, Kim DK (2015) DDR2 inhibition reduces migration and invasion of murine metastatic melanoma cells by suppressing MMP2/9 expression through ERK/NF-kappaB pathway. Acta Biochim Biophys Sin (Shanghai) 47(4):292–298. https://doi.org/10.1093/abbs/gmv005
34. Vandooren J, Van den Steen PE, Opdenakker G (2013) Biochemistry and molecular biology of gelatinase B or matrix metalloproteinase-9 (MMP-9): the next decade. Crit Rev Biochem Mol Biol 48(3):222–272. https://doi.org/10.3109/10409238.2013.770819
35. Itoh T, Ikeda T, Gomi H, Nakao S, Suzuki T, Itohara S (1997) Unaltered secretion of beta-amyloid precursor protein in gelatinase A (matrix metalloproteinase 2)-deficient mice. J Biol Chem 272(36):22389–22392. https://doi.org/10.1074/jbc.272.36.22389
36. Bhavani GS, Shah H, Shukla A, Gupta N, Gowrishankar K, Rao AP, Kabra M, Agarwal M, Ranganath P, Ekbote AV, Phadke SR, Kamath A, Dalal A, Girisha KM (2016) Clinical and mutation profile of multicentric osteolysis nodulosis and arthropathy. Am J Med Genet A 170A(2):410–417. https://doi.org/10.1002/ajmg.a.37447
37. Mosig RA, Dowling O, DiFeo A, Ramirez MC, Parker IC, Abe E, Diouri J, Aqeel AA, Wylie JD, Oblander SA, Madri J, Bianco P, Apte SS, Zaidi M, Doty SB, Majeska RJ, Schaffler MB, Martignetti JA (2007) Loss of MMP-2 disrupts skeletal and craniofacial development and results in decreased bone mineralization, joint erosion and defects in osteoblast and osteoclast growth. Hum Mol Genet 16(9):1113–1123. https://doi.org/10.1093/hmg/ddm060

38. Evans BR, Mosig RA, Lobl M, Martignetti CR, Camacho C, Grum-Tokars V, Glucksman MJ, Martignetti JA (2012) Mutation of membrane type-1 metalloproteinase, MT1-MMP, causes the multicentric osteolysis and arthritis disease Winchester syndrome. Am J Hum Genet 91(3):572–576. https://doi.org/10.1016/j.ajhg.2012.07.022
39. Reponen P, Sahlberg C, Munaut C, Thesleff I, Tryggvason K (1994) High expression of 92-kD type IV collagenase (gelatinase B) in the osteoclast lineage during mouse development. J Cell Biol 124(6):1091–1102. https://doi.org/10.1083/jcb.124.6.1091
40. Duerr S, Stremme S, Soeder S, Bau B, Aigner T (2004) MMP-2/gelatinase A is a gene product of human adult articular chondrocytes and is increased in osteoarthritic cartilage. Clin Exp Rheumatol 22(5):603–608
41. Lipari L, Gerbino A (2013) Expression of gelatinases (MMP-2, MMP-9) in human articular cartilage. Int J Immunopathol Pharmacol 26(3):817–823. https://doi.org/10.1177/039463201302600331
42. Soder S, Roach HI, Oehler S, Bau B, Haag J, Aigner T (2006) MMP-9/gelatinase B is a gene product of human adult articular chondrocytes and increased in osteoarthritic cartilage. Clin Exp Rheumatol 24(3):302–304
43. Itoh T, Matsuda H, Tanioka M, Kuwabara K, Itohara S, Suzuki R (2002) The role of matrix metalloproteinase-2 and matrix metalloproteinase-9 in antibody-induced arthritis. J Immunol 169(5):2643–2647. https://doi.org/10.4049/jimmunol.169.5.2643
44. Xue M, McKelvey K, Shen K, Minhas N, March L, Park SY, Jackson CJ (2014) Endogenous MMP-9 and not MMP-2 promotes rheumatoid synovial fibroblast survival, inflammation and cartilage degradation. Rheumatology (Oxford) 53(12):2270–2279. https://doi.org/10.1093/rheumatology/keu254
45. Mahmood R, Bresnick J, Hornbruch A, Mahony C, Morton N, Colquhoun K, Martin P, Lumsden A, Dickson C, Mason I (1995) A role for FGF-8 in the initiation and maintenance of vertebrate limb bud outgrowth. Curr Biol 5(7):797–806. https://doi.org/10.1016/s0960-9822(95)00157-6
46. Blunt AG, Lawshe A, Cunningham ML, Seto ML, Ornitz DM, MacArthur CA (1997) Overlapping expression and redundant activation of mesenchymal fibroblast growth factor (FGF) receptors by alternatively spliced FGF-8 ligands. J Biol Chem 272(6):3733–3738. https://doi.org/10.1074/jbc.272.6.3733
47. Crossley PH, Minowada G, MacArthur CA, Martin GR (1996) Roles for FGF8 in the induction, initiation, and maintenance of chick limb development. Cell 84(1):127–136. https://doi.org/10.1016/s0092-8674(00)80999-x
48. Heikinheimo M, Lawshe A, Shackleford GM, Wilson DB, MacArthur CA (1994) Fgf-8 expression in the post-gastrulation mouse suggests roles in the development of the face, limbs and central nervous system. Mech Dev 48(2):129–138. https://doi.org/10.1016/0925-4773(94)90022-1
49. Ohuchi H, Yoshioka H, Tanaka A, Kawakami Y, Nohno T, Noji S (1994) Involvement of androgen-induced growth factor (FGF-8) gene in mouse embryogenesis and morphogenesis. Biochem Biophys Res Commun 204(2):882–888. https://doi.org/10.1006/bbrc.1994.2542

50. Schmidt L, Taiyab A, Melvin VS, Jones KL, Williams T (2018) Increased FGF8 signaling promotes chondrogenic rather than osteogenic development in the embryonic skull. Dis Model Mech 11(6). https://doi.org/10.1242/dmm.031526
51. Lewandoski M, Sun X, Martin GR (2000) Fgf8 signalling from the AER is essential for normal limb development. Nat Genet 26(4):460–463. https://doi.org/10.1038/82609
52. Valta MP, Hentunen T, Qu Q, Valve EM, Harjula A, Seppanen JA, Vaananen HK, Harkonen PL (2006) Regulation of osteoblast differentiation: a novel function for fibroblast growth factor 8. Endocrinology 147(5):2171–2182. https://doi.org/10.1210/en.2005-1502
53. Liu H, Fang Q, Wang M, Wang W, Zhang M, Zhang D, He Y, Zhang Y, Wang H, Otero M, Ma T, Chen J (2018) FGF8 and FGFR3 are up-regulated in hypertrophic chondrocytes: Association with chondrocyte death in deep zone of Kashin-Beck disease. Biochem Biophys Res Commun 500(2):184–190. https://doi.org/10.1016/j.bbrc.2018.04.023
54. Pan Q, Zhang X, Zhang L, Cheng Y, Zhao N, Li F, Zhou X, Chen S, Li J, Xu S, Huang D, Chen Y, Li L, Wang H, Chen W, Cai SY, Boyer JL, Chai J (2018) Solute Carrier Organic Anion Transporter Family Member 3A1 Is a Bile Acid Efflux Transporter in Cholestasis. Gastroenterology 155(5):1578–1592e16. https://doi.org/10.1053/j.gastro.2018.07.031
55. Wang C, Li Y, Li H, Zhang Y, Ying Z, Wang X, Zhang T, Zhang W, Fan Z, Li X, Ma J, Pan X (2020) Disruption of FGF Signaling Ameliorates Inflammatory Response in Hepatic Stellate Cells. Front Cell Dev Biol 8:601. https://doi.org/10.3389/fcell.2020.00601
56. Sigala F, Savvari P, Liontos M, Sigalas P, Pateras IS, Papalampros A, Basdra EK, Kolettas E, Papavassiliou AG, Gorgoulis VG (2010) Increased expression of bFGF is associated with carotid atherosclerotic plaques instability engaging the NF-kappaB pathway. J Cell Mol Med 14(9):2273–2280. https://doi.org/10.1111/j.1582-4934.2010.01082.x
57. Garcia-Hernandez S, Potashner SJ, Morest DK (2013) Role of fibroblast growth factor 8 in neurite outgrowth from spiral ganglion neurons in vitro. Brain Res 1529:39–45. https://doi.org/10.1016/j.brainres.2013.07.030

Figures

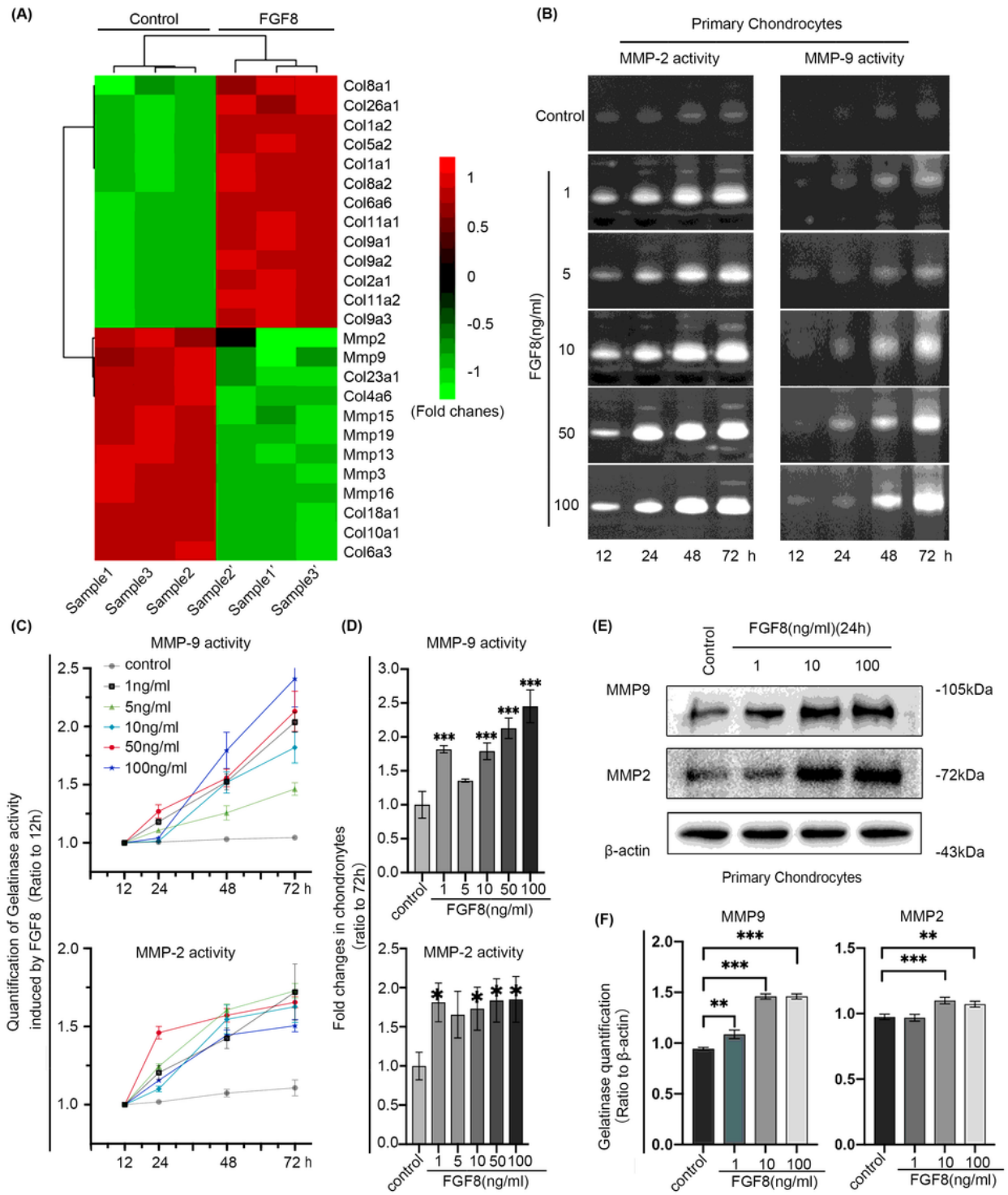

Figure 1

FGF-8 promotes the activity and expression of MMP-2 and MMP-9 in cultured chondrocytes from mice

(A) RNA sequencing results revealed differences of the chosen 25 genes in the extracellular matrix between the control and FGF-8 groups. Whole cell lysates from control and FGF-8 treatment were analyzed by characterizing the mRNA transcripts. After treatment for 72h, cell samples were obtained.

The data were presented as log2(1+FPKM) and formatted by the online Rpackage. FPKM: Fragments Per Kilobase of transcript per Million fragments mapped. Three pairs of lysate samples were taken from three independent cell isolates (n=3), namely Sample 1 and1', Sample 2 and2', and Sample 3 and 3'. Each pair of cells came from the same mother.

(B) Zymography images indicating a time-dependent increase in activity of MMP-2 (left) and MMP-9 (right) in primary chondrocytes treated with FGF-8 after 12, 24, 48 and 72h. The gels depicted were based on 3 separate experiments (n = 3).

(C) Image J software was used to do a quantitative analysis of the bands in (A). The data were the mean of three different experiments (n = 3).

(D) Quantitative analysis of gelatinase activity promoted by FGF-8 at 72h in (A). The data were presented in the form of the mean of three different experiments (n = 3). Statistical significance was considered when $*p < 0.05$, $**p < 0.01$ or $***p < 0.001$.

(E) The expression of MMP-2 and MMP-9 induced by FGF-8. The gels shown were representative of three different experiments (n = 3).

(F) Histogram showing the fold change of MMP-2 and MMP-9 expression induced by FGF-8 in (D). The data were the mean of three different experiments (n = 3). Statistical significance was considered when $*p < 0.05$, $**p < 0.01$ or $***p < 0.001$.

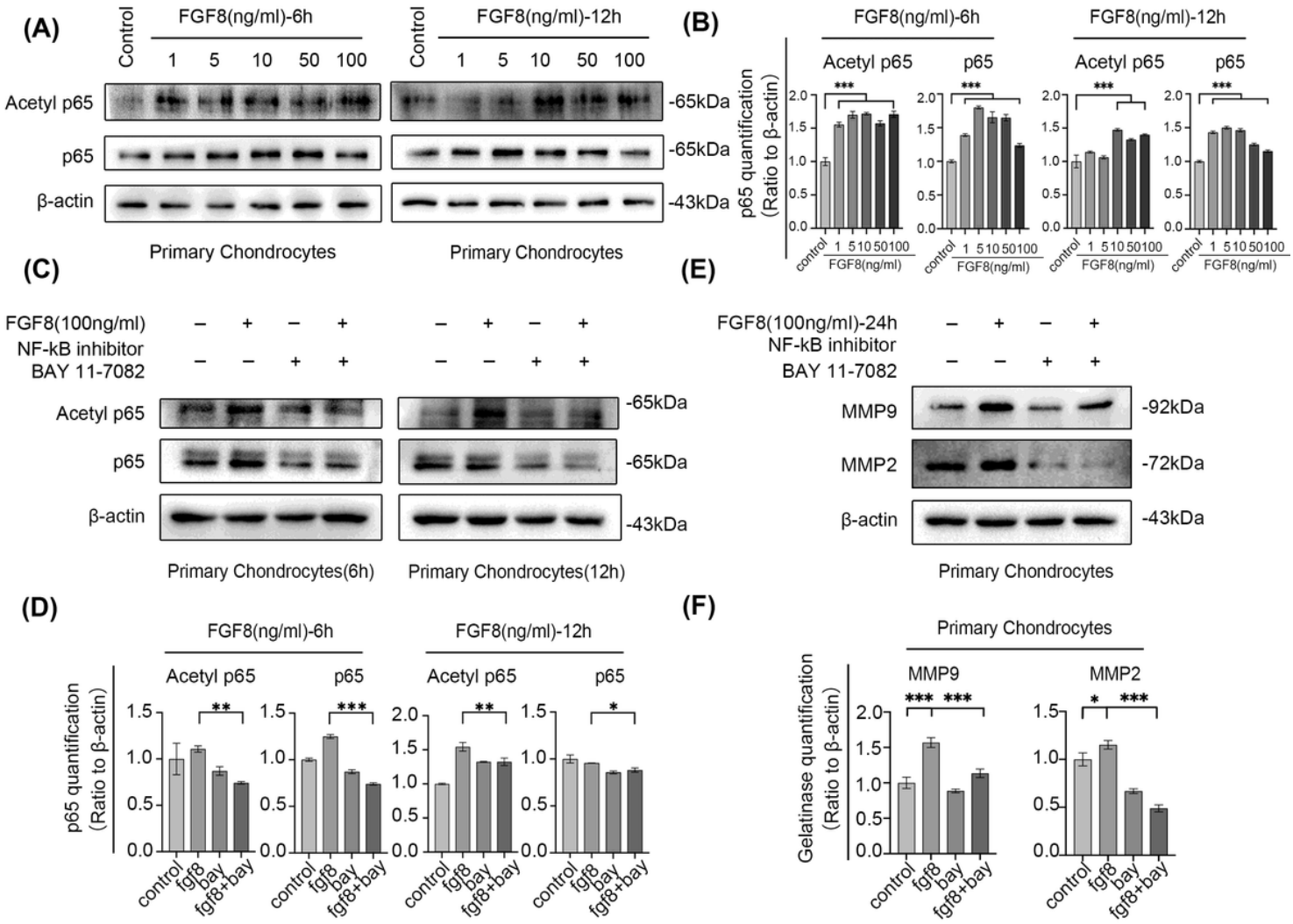

Figure 2

FGF-8 activates p65 in primary chondrocytes, and NF-κB inhibitor BAY 11-7082 can suppress the up-regulation of gelatinases induced by FGF-8

(A-B) The expression of p65 and acetyl p65 detected by WB (A) and quantitative analysis of (A) in (B). beta-actin was used as internal reference. The gels shown were representative of three different experiments (n = 3). Statistical significance was considered when $*p < 0.05$, $**p < 0.01$ or $***p < 0.001$.

(C-D) The expression of p65 and acetyl p65 with treatment of FGF-8 and BAY 11-7082 detected by WB(C) and quantitative analysis of (C) in (D). beta-actin was used as internal reference. The gels shown were representative of three different experiments (n = 3). Statistical significance was considered when $*p < 0.05$, $**p < 0.01$ or $***p < 0.001$.

(E-F) The expression of MMP-2 and MMP-9 with treatment of FGF-8 and BAY 11-7082 detected by WB(E) and quantitative analysis of (E) in (F). beta-actin was used as internal reference. The gels shown were

representative of three different experiments (n = 3). Statistical significance was considered when *$p <$ 0.05, **$p < 0.01$ or ***$p < 0.001$.

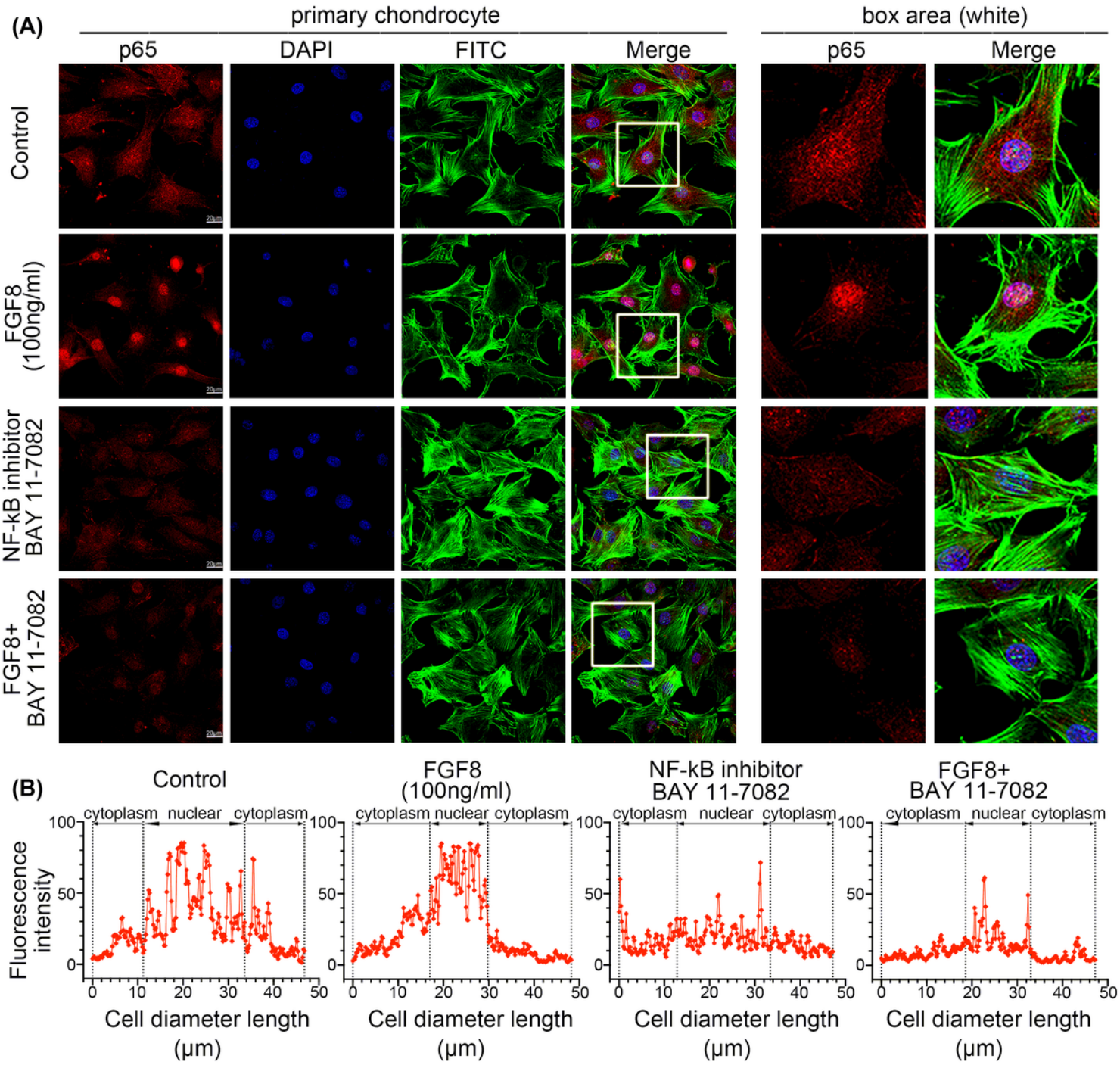

Figure 3

FGF-8 activates p65 signaling in the primary chondrocytes by Immunofluorescence

(A) Representative immunofluorescence staining by CLSM exhibiting the production of p65 in articular chondrocytes treated with FGF-8 (100 ng/ml, 6h), BAY 11-7028 (5 μM, pre-treatment for 1h), FGF-8 and BAY 11-7028, respectively.

(B) Quantification of p65 fluorescent intensity in (A) to describe the distribution of p65 cross the cell. The data were the representative of three different experiments (n = 3)

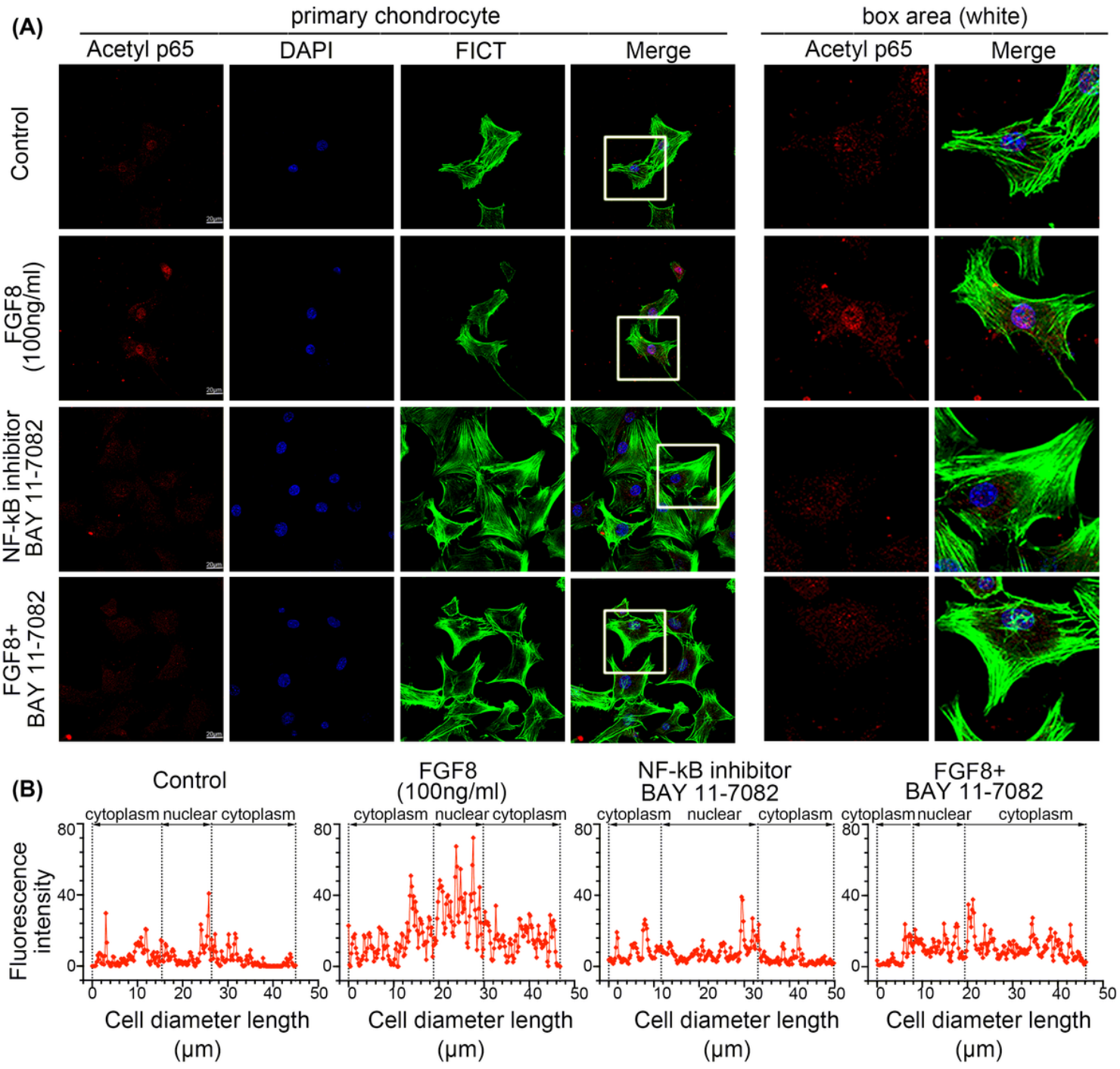

Figure 4

FGF-8 activates acetyl p65 in the primary chondrocytes by Immunofluorescence.

(A) Representative immunofluorescence staining by CLSM exhibiting the production of acetyl p65 in chondrocytes treated with FGF-8 (100 ng/ml, 6h), BAY 11-7028 (5 μM, pre-treatment for 1h), FGF-8 and BAY 11-7028, respectively.

(B) Quantification of acetyl p65 fluorescent intensity in (A) to describe the distribution of acetyl p65 cross the cell. The data were the representative of three different experiments (n = 3)

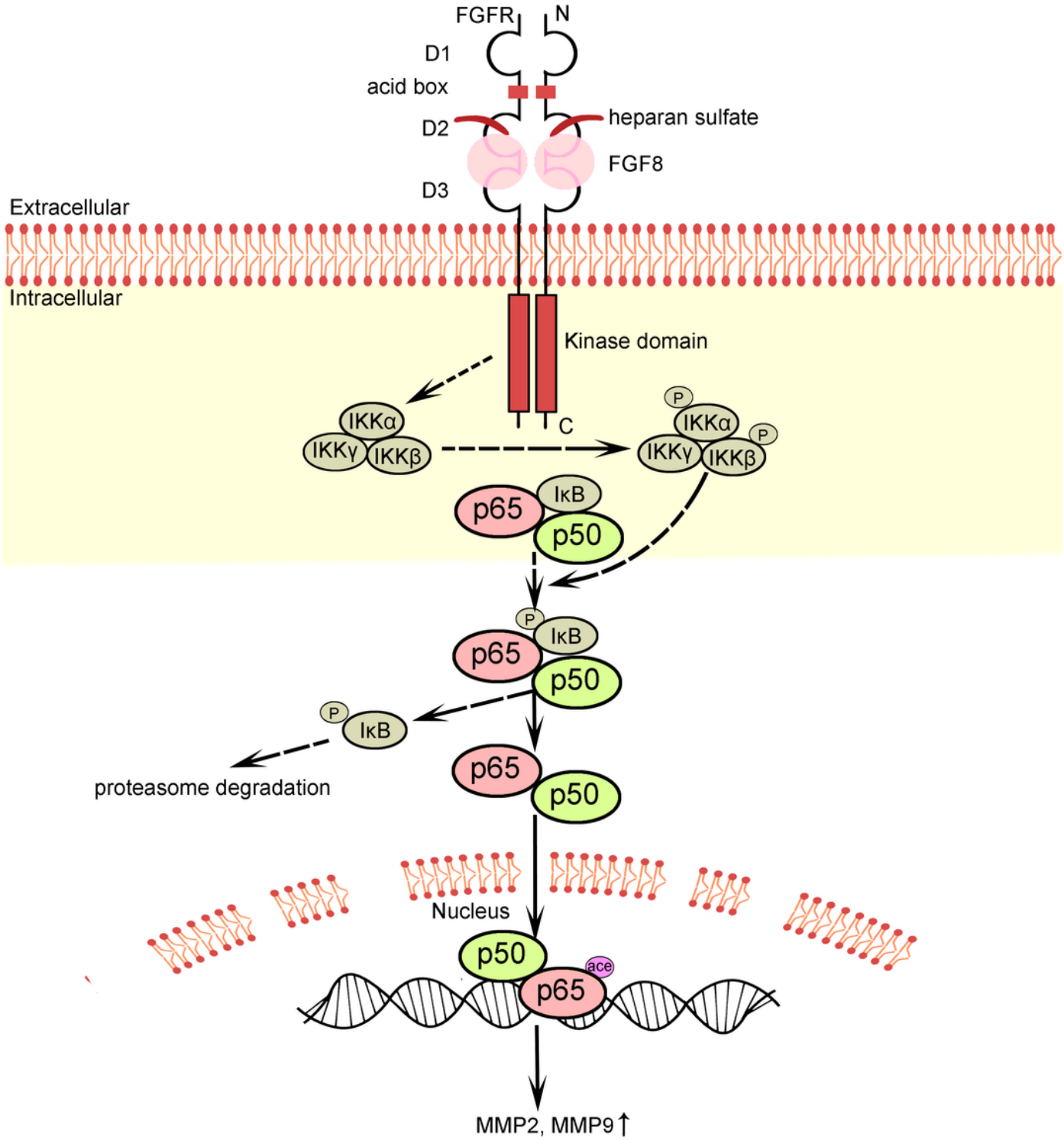

Figure 5

Schematic diagram elucidates the mechanism of upregulation of MMP-2 and MMP-9 by FGF-8 through NF-κB p65 signaling in cultured chondrocytes.

The mechanism that FGF-8 promoted the expression of gelatinases through p65 is indicated by solid arrows, and what are not included in this study is indicated by dashed arrows according to previous reports. FGF-8 induced p65 of the canonical NF-κB signalling pathway, then activated p65 was translocated into cell nucleus and acetylated, then started expression of gelatinases. FGFR, fibroblast growth factor receptor; D1-D3, the extracellular ligand-binding domain of FGFR is composed of three immunoglobulin (Ig) like domains, designated D1–D3; NF-κB, nuclear factor kappa-light-chain-enhancer of activated B cells; IκB, Inhibitors of NF-κB; IKK, IκB kinases; P, phosphate; ace, acetyl.