SHORT COMMUNICATION

Down-regulated in OA cartilage, SFMBT2 contributes to NF-κB-mediated ECM degradation

Safdar Hussain1,2 | Mengyao Sun1 | Zixin Min1 | Yuanxu Guo1 | Jing Xu1 | Nosheen Mushtaq3 | Lisong Heng4 | Huang Huang1 | Yitong Zhao1 | Ying Yuan1 | Nazim Hussain2 | Fujun Zhang1 | Yan Han1 | Peng Xu4 | Jian Sun1,5 | Shemin Lu1,5

1Department of Biochemistry and Molecular Biology, School of Basic Medical Sciences, Health Science Center, Xi’an Jiaotong University, Xi’an, China
2Centre for Applied Molecular Biology (CAMB), University of the Punjab, Lahore, Pakistan
3Department of Microbiology, School of Basic Medical Sciences, Health Science Center, Xi’an Jiaotong University, Xi’an, China
4Department of Orthopedics and Traumatology, Honghui Hospital, Health Science Center, Xi’an Jiaotong University, Xi’an, China
5Key Laboratory of Environment and Genes Related to Diseases, Ministry of Education, Xi’an, China

Correspondence: Jian Sun and Shemin Lu, Department of Biochemistry and Molecular Biology, School of Basic Medical Sciences, Xi’an Jiaotong University, Health Science Center, Yanta West Road 76, Xi’an, Shaanxi 710061, China (sunjian1@mail.xjtu.edu.cn; lushemin@mail.xjtu.edu.cn).

Funding information
The National Natural Science Foundation of China, Grant/Award Number: 81371986, 81772410, 81301598; The Shaanxi province Natural Science Foundation, Grant/Award Number: S2016YFJM1171; The Fundamental Research Funds for the Central Universities, Grant/Award Number: xj2015073

Abstract
The interplay between anabolic and catabolic factors regulates cartilage matrix homoeostasis. In OA, this balance is disrupted which results in cartilage degradation involving a plethora of inflammatory factors. Here, we identify a novel gene “Scm-like with four MBT domains protein 2” (SFMBT2) negatively regulated in OA cartilage. Articular cartilage from human OA patients undergoing knee arthroplasty surgery exhibited significantly decreased levels of SFMBT2 compared to the normal controls. Down-regulation of SFMBT2 by specific siRNA disturbed the metabolic homoeostasis and led to decreased expression of anabolic genes (SOX9, COL2A1) while increasing the expression of catabolic genes (MMP13 and ADAMTS4), in human chondrocytes. Finally, we revealed that SFMBT2 intervention by siRNA contributed to the catabolic phenotype of human chondrocytes mediated by NF-κB pathway.

KEYWORDS
SFMBT2, NF-κB, ECM metabolism, cartilage, OA

1 | INTRODUCTION

Osteoarthritis (OA) is a painful chronic inflammation of the joint associated with cartilage degeneration. It is the most prevalent form of arthritis affecting millions of persons worldwide, imposing a considerable socio-economic burden. Despite the multifactorial nature of its aetiology, the experimental data provided in many of the studies suggest that OA is linked with alterations of the genes...
expressions rather than modification in the genetic code itself. Reports on chondrocytes behaviour in OA suggest that at different stages and/or locations within articular cartilage, extracellular matrix (ECM) metabolism is regulated through coordinated mechanisms that are not fully understood. In this study we demonstrate down-regulation of SFMBT2 in OA contributes to ECM degradation via activation of the NF-κB signalling pathway.

2 MATERIALS AND METHODS

Cartilage samples were collected from 10 human OA patients (diagnosed with Kellgren and Lawrence grade IV osteoarthritis), undergoing knee arthroplasty surgery (6 women and 4 men; age range 45-69 years). Normal cartilage specimens (controls) were obtained from five donors (2 women and 3 men; age range 38-66 years) at autopsy. All the samples were collected from Honghui Hospital, Xi’an, Shaanxi, China. Accordingly, the study was performed with approval of the Ethical Committee of Xi'an Jiaotong University, Health Science Center, and all individuals provided full written informed consent.

In this study, we employed C28/I2 and SW1353 human chondrocytes and transfected them with specific small-interfering RNA (siRNA) sequence against SFMBT2 (si-SFMBT2) or a scrambled (negative control) siRNA (si-NC) sequence (Table S1), with Lipofectamine™ 2000 (Thermo Fisher Scientific, Shanghai, China). TNF-α (Sigma, Shanghai, China) and IL-1β (Sigma, Shanghai, China; see Figure S2) were used to induce inflammation in the human chondrocytes, and BAY 11-7082 (Abcam, Shanghai, China) was used to block the NF-κB signals (specific details are depicted in the respective figure legends).

All the molecular expressions at mRNA level were determined by RT-qPCR with the specific primers (Table S2) and at protein level by Western blotting (WB) & Immunohistochemistry (IHC) with respective antibodies (Table S3).

3 RESULTS AND DISCUSSION

3.1 SFMBT2 is down-regulated in OA cartilage

SFMBT2 is a member of polycomb group (PcG) of proteins and is known to repress HOXB13 gene expression via its association with methylated histones H3 and H4. However, no study stating its role in OA/cartilage has yet been reported. In a previous study at our department, differentially expressed genes (DEGs) in OA cartilage were screened by suppression subtractive hybridization (SSH). SFMBT2 was identified from the reverse subtraction library by sequence analysis and a similarity search with the BLAST programme. We selected this gene for further investigations in this study.

Western blot analysis of the ‘OA’ cartilage samples from human patients displayed significantly decreased levels of SFMBT2 (P = 0.003) when compared with ‘Normal’ cartilages from the controls (Figure 1B). In contrast, protein expression of catabolic marker gene MMP3, which belongs to the family of matrix metalloproteinases (MMPs), was up-regulated radically (P = 0.026) in ‘OA’ cartilage samples (Figure 1C). IHC staining of the cartilage specimens exhibited a significantly decreased expression of SFMBT2 (P = 0.0023) in ‘OA’ patients as compared with the ‘Normal’ controls (Figure 1D). These data establish a negative association of SFMBT2 with OA, however, mechanism behind the down-regulation of this gene in OA needs further exploration.

3.2 SFMBT2 knockdown dysregulates the metabolic homeostasis of chondrocytes

Three different siRNA sequences targeting SFMBT2 mRNA (Table S1) were synthesized and tested to intervene its expression in C28/I2 cells (Figure S1). Endogenous SFMBT2 was knocked down >70% by 80 nmol/L of the effective siRNA sequence (siR3). SFMBT2 interference altered the expression of key metabolic genes in C28/I2 chondrocytes. SOX9 and COL2A1 were decreased, whereas MMP13 and ADAMTS4 were increased significantly (P < 0.05) by si-SFMBT2, both at mRNA and protein levels, determined by RT-qPCR (Figure 2A) and Western blotting (Figure 2B) respectively. Expression of Aggrecan (ACAN) did not change indicating no immediate effect of si-SFMBT2 on it. SOX9 is a pivotal transcriptional regulator and is essential for chondrocytes phenotypic stability, differentiation and proliferation. Down-regulation of SOX9 may induce angiogenesis, cartilage resorption and formation of bone marrow and endochondral bone trabeculae, which are associated with OA progression. MMPs and ADAMTSs are known to accelerate the catabolic process and are involved in ECM degradation within the cartilage. Our results indicate that certain levels of SFMBT2 are imperative to maintain the normal metabolic homeostasis, and its down-regulation may promote catabolic phenotype of the chondrocytes.

3.3 SFMBT2 intervention by siRNA contributes to NF-κB-mediated ECM degradation

Pro-inflammatory cytokines such as TNF-α and IL-1β can sponsor catabolism and enhance the expression of matrix degrading genes. TNF-α triggers the catabolic process by activating downstream signalling pathways such as Nuclear Factor kappa B (NF-κB) and Mitogen-Activated Protein Kinases (MAPKs). Active NF-κB is involved in the regulation of various target genes, including chemokines, transcription factors, growth factors, enzymes and cell-cycle regulators, immune receptors, regulators of apoptosis, stress response genes and adhesion molecules.

To depict the mechanism behind the up-regulation of catabolic genes (MMP13 and ADAMTS4) by si-SFMBT2, we treated SW1353 cells with TNF-α and detected the downstream inflammatory signals of NF-κB pathway. TNF-α treatment led to the increased expression of MMP13 and ADAMTS4 in a time-dependant manner, determined by RT-qPCR (Figure 2C). Expression of SFMBT2 initially increased (6 hours), but then displayed a gradually decreasing trend (12-48 hours) under the TNF-α treatment.
Next, SW1353 cells were transfected with si-NC or si-SFMBT2 and stimulated with or without TNF-α in the presence or absence of the specific NF-κB inhibitor (BAY 11-7082). Normally, NF-κB dimers are maintained in the cytosol of unstimulated cells, complexed with IκB proteins. Upon activation by a large number of stimuli (including TNF-α), the IκB proteins undergo phosphorylation, ubiquitylation and proteasome-mediated degradation, which results in the liberation of NF-κB dimers followed by their nuclear translocation. As shown in Figure 2D, TNF-α treatment led to the increased phosphorylation of IκB, accompanied by the activation of NF-κBp65 (RelA) (Figure 2F). Interestingly, si-SFMBT2 mimicked the act of TNF-α in the unstimulated chondrocytes. In addition, si-SFMBT2 boosted up the signals of IκB phosphorylation, when used in combination with TNF-α, and subsequently increased the levels of RelA (Figure 2D,F). However, BAY 11-7082 inhibited the IκB phosphorylation and subsequent levels of RelA, both in TNF-α+si-NC- and TNF-α+si-SFMBT2-treated chondrocytes (Figure 2E,G).

We further detected the expression of MMP13, a target gene of RelA, and observed that TNF-α stimulation led to the increased expression of MMP13 in SW1353 cells. As expected, si-SFMBT2 chondrocytes also exhibited the increased levels of MMP13, treated with or without TNF-α (Figure 2H). BAY11-7082 reduced the
A

Relative mRNA expression

- si-NC
- si-SFMBT2

SFMBT2, SOX9, COL2A1, ACAN, MMP13, ADAMTS4

B

Relative Protein expression

- si-NC
- si-SFMBT2

SFMBT2, SOX9, COL2A1, ACAN, MMP13, ADAMTS4, GAPDH

C

MMP13

ADAMTS4

SFMBT2

TNF-α (h)

Relative mRNA expression

0 3 6 12 24 48

D

p-IκB

GAPDH

TNF-α

- - + +

si-NC

+ - - -

si-SFMBT2

- + - +

E

p-IκB

GAPDH

TNF-α

+ + + +

si-NC

+ - - -

si-SFMBT2

- + - +

BAY (11-7082)

- - + +

F

RelA

Lamin B

GAPDH

TNF-α

- - + +

si-NC

+ - - -

si-SFMBT2

- + - +

G

RelA

Lamin B

GAPDH

TNF-α

+ + + +

si-NC

+ - - -

si-SFMBT2

- + - +

BAY (11-7082)

- - + +

H

MMP13

GAPDH

TNF-α

- - + +

si-NC

+ - - -

si-SFMBT2

- + - +

I

MMP13

GAPDH

TNF-α

+ + + +

si-NC

+ - - -

si-SFMBT2

- + - +

BAY (11-7082)

- - + +
transcription of MMP13; however, the reduction was less in si-SFMBT2 group as compared with the si-NC group (Figure 2). This might be as a result of the other inflammatory factors regulating the MMP13 expression, associated with si-SFMBT2 which needs further explorations.

ACKNOWLEDGEMENTS
This work was supported by grants from The National Natural Science Foundation of China (Project No. 81301598, 81301598), The Shaanxi province Natural Science Foundation (Project No. S2016YJFM1171) and The Fundamental Research Funds for The Central Universities (xj2015073). C28/I2 cell line was provided by Prof. Junling Cao, from Institute of endemic diseases, Xi’an Jiaotong University, Health Science Center.

CONFLICT OF INTERESTS
The authors have no conflict of interest to declare.

AUTHOR’S CONTRIBUTIONS
S.H. designed and executed the experiments, analysed the data and wrote the manuscript. M.S., Y.G. and J.X. helped perform the analysis with constructive discussions. N.M., H.H., Y.Z., Y.Y., N.H., F.Z. and Y.H. helped the experiments. P.X. and L.H. provided the OA and control samples. S.L. and J.S. supervised the study and critically revised the manuscript for important intellectual content.
10. Kobayashi M, Squires GR, Mousa A, et al. Role of interleukin-1 and tumor necrosis factor alpha in matrix degradation of human osteoarthritic cartilage. *Arthritis Rheum*. 2005;52:128-135.

11. Pahl HL. Activators and target genes of Rel/NF-kappaB transcription factors. *Oncogene*. 1999;18:6853-6866.

12. Hayden MS, Ghosh S. Regulation of NF-kappaB by TNF family cytokines. *Semin Immunol*. 2014;26:253-266.

13. Aggarwal BB. Nuclear factor-kappaB: the enemy within. *Cancer Cell*. 2004;6:203-208.

14. Hinz M, Scheidereit C. The IkappaB kinase complex in NF-kappaB regulation and beyond. *EMBO Rep*. 2014;15:46-61.

15. Mitchell S, Vargas J, Hoffmann A. Signaling via the NFκB system. *Wiley Interdiscip Rev Syst Biol Med*. 2016;8:227-241.

**SUPPORTING INFORMATION**

Additional supporting information may be found online in the Supporting Information section at the end of the article.

**How to cite this article:** Hussain S, Sun M, Min Z, et al. Down-regulated in OA cartilage, SFMBT2 contributes to NF-κB-mediated ECM degradation. *J Cell Mol Med*. 2018;22:5753–5758. [https://doi.org/10.1111/jcmm.13826](https://doi.org/10.1111/jcmm.13826)