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miR-152 Attenuates Apoptosis in Chondrocytes and Degeneration of Cartilages in Osteoarthritis Rats via TCF-4 Pathway

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Abstract

Introduction: Osteoarthritis (OA) is associated with deregulation of various miRNAs (miRs). The present study reported protective effect of miR-152 in osteoarthritis.

Methods: Tissue cartilage tissues of OA and normal subjects were used, rat model of anterior cruciate ligament transection (ACLT) was developed. Cartilage study was done by Safranin O-fast green, histological and immunostaining. The chondrocytes were isolated from tissues and were treated with IL-1β and infected with miR-152 or TCF-4 cloned lentiviral vectors. MTT assay was done for cell viability, apoptosis by Annexin-V-FITC staining. Expressions of proteins by western blot assay. Collagen-II assay was done by immunofluroscent assay. Luciferase activity by dual luciferase reporter assay.

Results: Upregulation of miR-152 improved viability of chondrocytes, decreased apoptosis and balanced the catabolic and anabolic factors of extracellular matrix in vitro. Injecting miR-152 lentivirus in rats improved articular cartilage in osteoarthritis ACLT rats. Bioinformatics analysis suggested TCF-4 as favorable target gene of miR-152, having binding site on the 3'UTR region of TCF-4 mRNA and inhibited the expression of TCF-4. Osteoarthritis tissue cartilage both from humans and rats showed expression of miR-152 inversely linked with expression of TCF-4.

Conclusion: Present study concludes miR-152 diminished the progression of osteoarthritis partially by inhibiting the expression of TCF-4.

Keywords
miR-152, TCF-4, osteoarthritis, chondrocytes, lentivirus

Background

Osteoarthritis (OA) is age related disorder of joints featuring degradation of articular cartilage, thickening of subchondral bone, inflammation of synovial joints, improper catabolism and anabolism of articular cartilages.[1] Multiple factors contribute in development of OA which include age, injury, infection and adiposity.[2] OA not only promotes disability in elderly people but also lays socioeconomic burden on them.[3] Nevertheless, the current therapeutic approaches for OA are limited to only reducing the pain and surgical replacement of joint at the end stage of this disease. Due to limited knowledge about the pathogenesis of OA efficient disease modifying treatments are unavailable.

miRNAs (miRs) are endogenously produced small non-coding RNAs which alter the expression of target genes at post-transcriptional level via binding to the 3'UTR region of target mRNAs, which degrades these mRNA and associated

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protein synthesis.[4] Recent studies have attracted function of miRs in treating OA. Number of studies have indicated deregulation of miRs in human inflammatory diseases such as OA and have been reported to play important role homeostasis of joints and development of arthritis.[5-7] miR-218 has been reported to promote the process of chondrogenesis of mesenchymal stem cells and also inhibited the later chondrocyte maturation by involving the HPGD pathway.[8] miR-365 has been found to target histone deacetylase- 4 and increases the process of chondrogenesis.[9] miR-145 have been reported to regulate the differentiation of chondrocytes in stem cells via the SOX9 pathway.[10] miR-146a promotes OA via modulating the homeostasis of cartilage by CAMK2D and PPP3R2 signaling.[6] These studies show various mechanisms involved in development and progression of OA. Therefore, a better understanding of the involved mechanisms of miRs in the progression of OA could be a logical approach in improving the therapeutic efficacy in treating OA.

miR-152 belongs to miR-148/miR-152 family of miRNAs having aberrant expression in many diseased conditions including cancer.[11] Recently it has been reported that miR-152-3p is over-expressed in postmenopausal females with bone conditions such as osteoporosis and fracture, fragile suggesting a link between miR-152 and osteoporosis affecting the osteoblasts differentiation.[12] In a report miR-152 has been found to inhibit the levels of cytokines such as IL-6, IL-12 and the tumor necrosis factor (TNF-α).[13] Also, miR-152 has been evidenced to block the expression of pro-inflammatory cytokines in Rheumatoid arthritis.[14] However studies investigating role of miR-152 in bone abnormalities remains unexplored. Looking into the involvement of miR-152 in inflammatory and in some bone abnormalities, we investigated the role of miR-152 in osteoarthritis.

Materials and Methods

Tissue Cartilage

For the study cartilage tissues of OA patients were collected from subjects that were submitted to knee joint replacement surgery, about 25 patients were enrolled for the study among which 15 were females and 10 were males aging between 60 to 70 years of age. The isolated tissues do not included subchondral bone, and were not completely degenerated or fibrous. The study also included partial patients termed as control (total 10, 6 females and 4 males), from these patients normal cartilage were also isolated showing undamaged areas. The extent of damage caused to the cartilage Safranin O-fast green staining was done, the damage was graded on basis of Mankin scale of gradation; grade 1: score 0 to 5, grade 2: score 6 to 10, grade 3: score 11 to 14.[5,6] The study was approved by the Human ethical review board of Shanghai Jiao Tong University School of Medicine, China. All the subjects were educated about the study; informed consent was obtained for each subject.

Animal Studies

For animal studies we used Sprague-Dawley rats aging about 10 weeks (n = 24). The rats were housed under controlled conditions, submitted to 12 hours dark/light cycle and were housed in polypropylene cages. The rats were provided free access to pellet diet and water ad-libitum. The animal studies were approved by the animal ethical review board of Tongji University School of Medicine, Shanghai, China. The rats were distributed into 4 groups with 6 rats in each group viz, Control group, osteoarthritis group (OA), OA+ Negative control group, OA+miR-152 mimic group. The experimental animals were submitted to anterior cruciate ligament transection (ACLT) as reported earlier.[15] Briefly, the rats were submitted to Kitamine anesthesia (30 mg/kg), a parapatellar skin incision was made in the right knee on the medial side of joints, the patella was dislocated and the anterior cruciate ligament was transected. The sham operated rats received anesthesia, incision in the joint without ACLT. The rats were injected with miR-152 lentivirus expressing vector or control lentivirus or vehicle in the operated knee area exactly after 7 days of surgical procedure. The rats received 20 µl of 1 × 109 plaque-forming units 2 times/week for total period of 7 weeks. The rats were sacrificed after total period of 8 weeks post operating them, the surgery performed knee joints were removed and evaluated.

Histological Analysis and Immunostaining

The isolated knee cartilage tissues were fixed in paraformaldehyde (4%) and then embedded in paraffin for obtaining sections of 5 µm using a rotary microtome (Leica Biosystems USA). The extent of cartilage destruction was studied by performing the safranin O staining as reported earlier.[16] The guidelines given by Osteoarthritis Research Society International (OARSI) were followed for grading the score of histological analysis.[17] The grading system depicted score 0 to 6 as normal to loss of cartilage (more than 80%). The scoring was done by performing the study at least 5 times for each isolated knee tissue.

Transfection of Cells Treatment of Interlukin-1β (IL-1β)

The isolated tissues were processed and chondrocytes were isolated from them as per the reported method.[18,19] Briefly, the isolated tissues were converted into small pieces and digested in media consisting trypsin (0.1%) and then transferred to DMEM media. The resultant was filtered to remove undigested tissues. The filtrate was centrifuged for isolating the chondrocytes and was maintained in DMEM media supplemented with fetal bovine serum (FBS) (10%) at 25°C with CO_2 (5%). The apoptosis in chondrocytes was induced by treating them with IL-1β (10 ng/ml) in complete media.

Lentivirus Transduction

The lentiviral vector pCDH-CMV-MCS-EF1 was cloned with sequences of miR-152 and TCF-4 precursor separately. The lentiviruses were generated using HEK293 T cells, the stable

infected cells were picked out using Puromycin. The process of transfection of chondrocytes took place by seeding them in 6 well plates and then treating the with recombinant lentivirus units along with Polybrene (8 μg/ml) (SigmaAldrich USA).

Extraction of RNA and Quantitative Reverse Transcription-PCR (qRT-PCR)

For extracting total RNA TRIzol reagent was used (Invivogen, USA). The isolated RNA was submitted for reverse transcription using RT Reverse Transcription Kit (ThermoFisher USA). The cDNA was submitted for qRT-PCR using ABI Prism 7000 sequence detecting system (Biocompare, USA) with the help of SYBR® Green qPCR supermix (Bio-Rad, USA).The fold changes in the expression were evaluated by comparative threshold cycle (Ct) method. GAPDH and U6 were used as controls for mRNA and miR respectively.

MTT Cell Viability Assay

Foe evaluating the cell viability, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay was done. The Lentivirus infected cells (5×103 cells/well) were submitted for seeding in 96 well plates and cultured for 96 hours. To these cells, MTT was added (0.5 mg/ml) and incubated for 4 hours, to the isolated supernatant formazan crystals were added dissolved in dimethyl sulfoxide. The optical density was recorded for the resulting colored solution at 490 nm using a microplate reader.

Apoptosis Rate in Chondrocytes

The rate of apoptosis in IL-1β induced chondrocytes was studied by performing assay with a Annexin-V-FITC apoptosis detection kit (ThermoFisher USA). The assay was performed using fluorescence-activated cell sorting (FACS) flow cytometry (BD Biosciences, USA).

Immunofluorescent Assay

To study the expression of collagen type-II, Immunofluorescence was done in chondrocytes. The isolated chondrocytes were incubated with collagen-II antibody for 12 hours at 4°C. The cells were rinsed using PBS and were then incubated with II^{ry} antibody for 1 hour. The Nuclei of cells were submitted to DAPI staining for 10 minutes and the images were recorded by a digital microscope (Olympus, Japan).

Western Blot Analysis

The samples were submitted for lysis and the total protein contents were estimated protein estimation kit (ThermoFisher, USA). The obtained lysates were centrifuged and submitted for gel electrophoresis on a SDS-PAGE and then loaded to PVDF membranes. The resultant PVDF membranes were blocked for 4 hours using 5% skim milk and then incubated using I^{ry} antibodies for 12 hours at 4°C, followed by addition of horseradish peroxidase labeled II^{ry} antibodies. The proteins were observed using the enhanced chemiluminescence technique. For the experiment the I^{ry} antibodies used were anti-cleaved PARP1 (1:1000) (ab4830), anti–cleaved caspase 3 (1:1000) (ab2302), anti-TCF4 (1:1000) (ab185736) from Abcam, USA. Anti-MMP-3 (1:1000) (ThermoFisher USA), Anti-MMP-13 (1:1000) (#94808) (Cell Signaling USA), Anti-Collagen II antibody (1:1000) (ab34712) (Abcam USA), Anti-aggrecan antibody (1:1000) [6-B-4] (ab3778) (Abcam, USA). For the study Actin was used as loading control.

Luciferase Activity Assay

For studying the luciferase activity, the luciferase vector was manipulated and into it, either mutant (MUT) TCF-4 or the wild type (WT) 3'-UTR bearing the target site for miR-152 fragment was inserted. The cells were seeded in 96 well plates and were co-transfected using a reporter plasmid and a negative control (NC) RNA or miR-152 mimic with the help of a Lipofectamine transfecting reagent (ThermoFisher USA), after 48 hours the luciferase activity was studied using a dual luciferase reporter system. For the activity, the ratio of Renilla and firefly luciferase activities was recorded.

Statistical Analyses

All the statistics was performed using GraphPad Prism 4.0 software. The results were presented as mean $\pm$ SD. Student's t test was performed for comparing the results of 2 groups, one way ANOVA was done for more than 2 groups. The correlation between the expression of TCF-4 and miR-152 was done by Pearson's correlation evaluation. The P value <0.05 was regarded to be statically significant.

Results

Expression of miR-152 Was Suppressed in OA Tissue Cartilage and IL-1β Treated Chondrocytes

The Hematoxylin and Eosin (H&E) was done and the articular cartilage tissue showed appearance of defects, structural breakage and damage and also decrease in number of cartilage cells of OA group whereas the control group showed normal morphology (Figure 1A). For investigating the involvement of miR-152 in OA, the levels of miR-152 in cartilages of 25 OA and 20 normal subjects was investigated by qRT-PCR, the outcomes suggested that the expression level of miR-152 was significantly lower in OA patients against normal (Figure 1A). We found that the expression level of miR-152 was inversely correlated when observed for Mankin score (Figure 1B). On comparing the expression of miR-152 between male and female rats, showed no significant change in expression (Figure 1C), similarly no significant changes were found compared between expression of miR-152 and age (Figure 1D). Further comparing the expression of miR-152 between human tissues and animals showed agreement (Figure 1E). The chondrocytes showed normal morphology having slabstone shape appearing spherical and fusiform in shape, upon

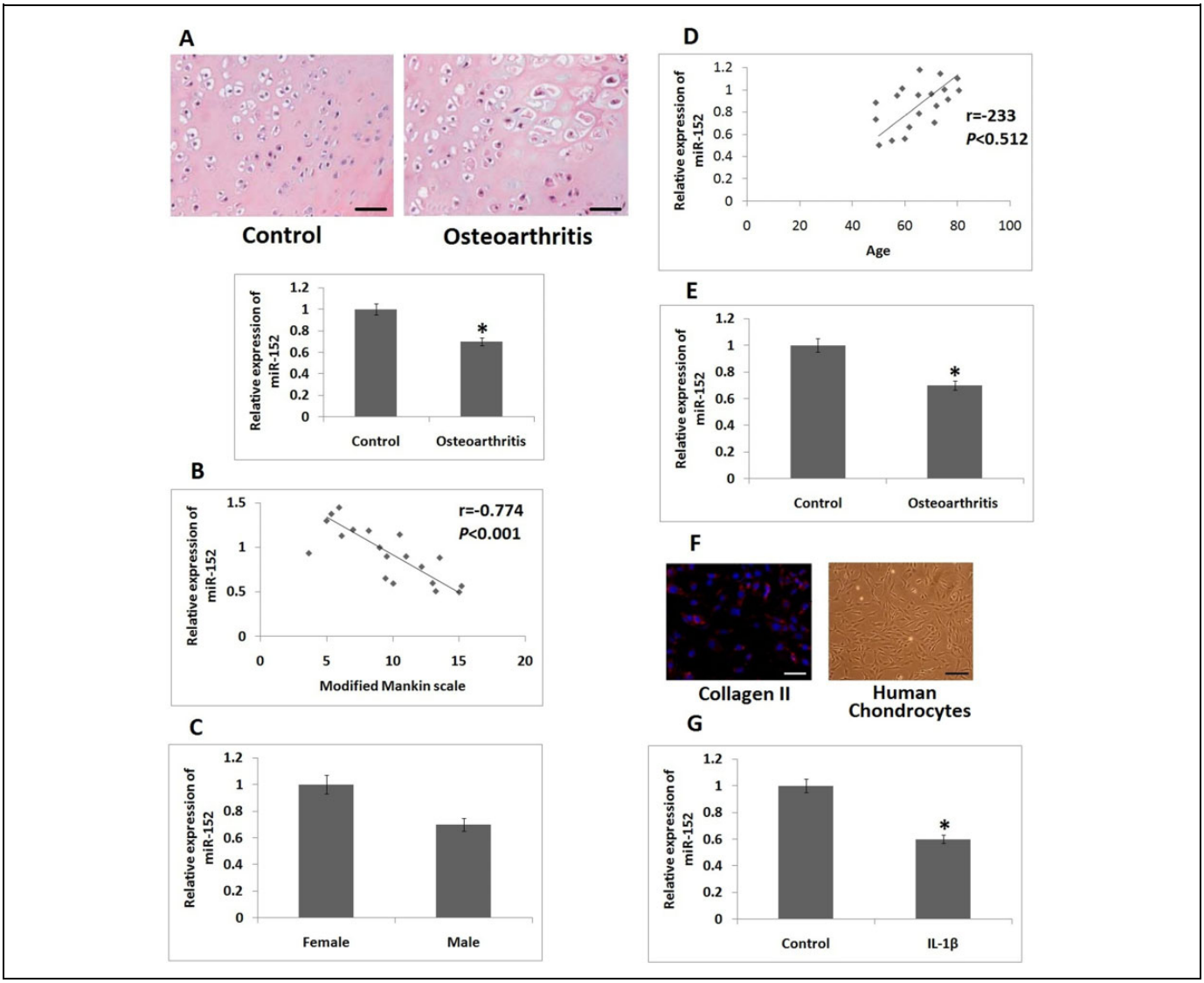

Figure 1. Expression levels of miR-152 in osteoarthritis cartilage tissues and chondrocytes treated with IL-1β. A: qRT-PCR analysis for expression of miR-152 in osteoarthritis and healthy human cartilage tissue samples. B: Results of modified Mankin scale showed negative correlation with levels of miR-152. C: Results of qRT-PCR assay for expression of miR-152 in cartilage samples in male and female subjects. D: Quantitative analysis showing no association between expression of miR-152 and age. E: qRT-PCR assay showing expression of miR-152 in tissues of rats submitted for osteoarthritis with cartilages of normal rats serving as control. F: Immunofluorescence staining was done for collagen II in chondrocytes. G: Results of qRT-PCR showing expression of miR-152 in chondrocytes exposed to IL-1β (10 ng/ml) for 12 hours. *P < 0.05.

Immunofluorescence staining for analysis of collagen II, the chondrocytes were identified to have normal morphology (Figure 1F). It was observed that upon exposure of chondrocytes to IL-1β resulted in suppressed levels of miR-152 (Figure 1G). Altogether, the outcomes indicated that suppression of miR-152 may be involved in progression of OA.

miR-152 Encouraged Cell Proliferation and Inhibited Apoptosis and Matrix Synthesis in Chondrocytes

For studying the involvement of miR-152 in the pathogenesis of osteoarthritis, we infected the human chondrocytes with lentivirus in vitro for overexpression of miR-152 (Figure 2A). The results of MTT assay suggested that, inducing overexpression of miR-152 encouraged proliferation of normal and IL-1β exposed chondrocytes (Figure 2B- G). Results of western blot analysis suggested that overexpression of miR-152 suppressed the expression of cleaved PARP, cleaved caspase-3, MMP-13 and MMP-3, however the results also suggested that miR-152 overexpression caused increased expression levels of collagen-II and aggrecan in normal cells (Figure 2C-2F). Apoptosis study revealed that, the treatment of IL-1β increased the number of apoptotic cells and the overexpression of miR-152 caused the number of apoptotic cells to be decreased (Figure 2 H).

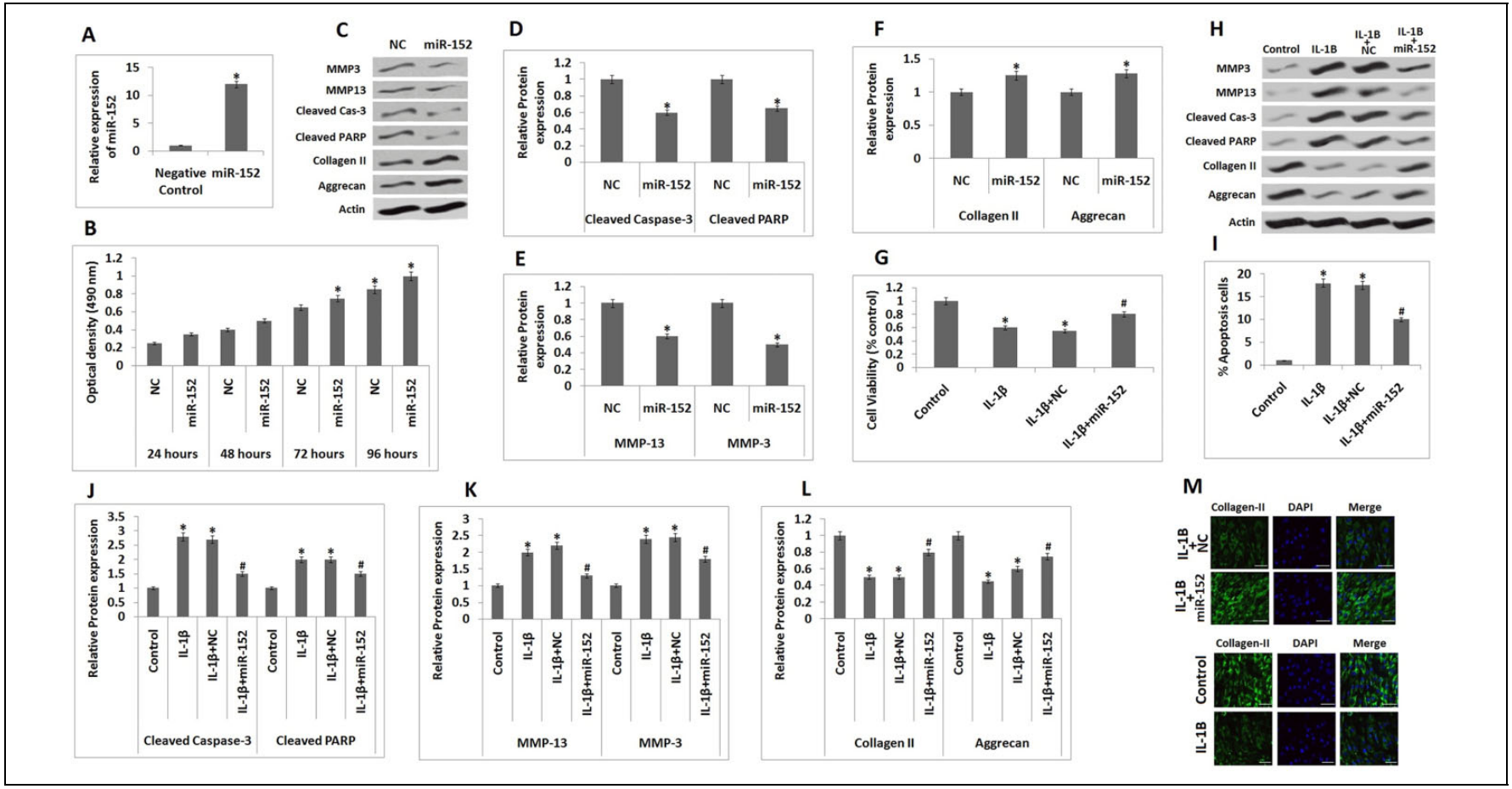

Figure 2. miR-152 is involved in regualting the proliferation, apoptosis and functioning of chondrocytes. A: qRT-PCR assay results of miR-152 expression in human chondrocytes treated with NC or miR-152 expression lentivirus. B: MTT assay results showing viability of chondrocytes at defined time points post infection with NC or miR-152 expressing lentivirus. C: Western blot analysis showing expression of MMP-3, MMP-13, cleaved caspase-3, cleaved PARP, Aggrecan and collagen type II. D-F: Densitometric analysis of MMP-3, MMP-13, Cleaved caspase-3, PARP, Aggrecan and collagen type II. G: Results of cell viability by MTT assay in chondrocytes treated with NC or miR-152 expressing lentivirus. H: Results of western blot analysis showing expression of MMP-3, MMP-13, Cleaved caspase-3, PARP, Aggrecan and collagen type II. I: Results of flow cytometry for apoptosis analysis. J-L: Results of densitometric analysis showing expression of MMP-3, MMP-13, Cleaved caspase-3, PARP, Aggrecan and collagen type II. M: Results of immunofluoroscent analysis showing collagen type II positive cells. *$P < 0.05$ against control or NC, $^{\#}P < 0.05$ against IL-1β+NC or IL-1β treated group.

Similarly, the outcomes of western blot suggested that overexpression of miR-152 significantly ameliorated the overexpression of cleaved PARP and capase-3 mediated by IL-1β (Figure 2I and 2 J). Further in the study, the effect of miR-152 on synthesis of matrix biomarkers. The outcomes suggested that the increased expression levels of MMP-13 and MMP-3 and decreased levels of both collagen-II and aggrecan which are the ECM components after exposure to IL-1β were attenuated by overexpression of miR-152 (Figure 2I, 2 K and 2 L). The results of collagen-II were further confirmed by performing immunofluorescent staining of lentivirus transfected chondrocytes (Figure 2 M). The results indicated that miR-152 promoted proliferation of cells and synthesis of matrix biomarkers and suppressed the apoptosis in chondrocytes.

miR-152 Halts the Degradation of Cartilage Matrix in the Rat Model of Osteoarthritis

Accounting into the inhibitory effect of miR-152 on the IL1β mediated apoptosis and inducing effect on matrix degradation enzymes in vitro, we studied the effect of miR-152 on development of osteoarthritis in vivo using a rat model of osteoarthritis. The outcomes of qRT-PCR suggested that the levels of miR-152 in the rat cartilage tissues was significantly elevated post injecting the rats with miR-152 expressing lentivirus (Figure 3A). The results also suggested that the infection of lentivirus caused a significant increased destruction of cartilage tissues post ACLT surgery (Figure 3B), decreased the proteoglycan loss, protected cartilage destruction and decreased cellularity of articular chondrocytes. The results also suggested that, overexpression of miR-152 decreased the elevated catabolic as well as apoptotic responses and reduced the ECM components in rats (Figure 3D). These outcomes in total confirmed that miR-152 halts the degradation of cartilage matrix in rats submitted to osteoarthritis.

TCF-4 Is a Potential Target of miR-152

For predicting potential target of miR-152 Bioinformatics was used for predicting the target, TargetScan and miRanda algorithms were utilized. The results of analysis suggested that TCF-4 3'UTR had a specific binding site for miR-152 sequence (Figure 4A). The outcomes of qRT-PCR along with western blot analysis suggested that increased expression level of miR-152 resulted in decreased the mRNA as well as protein levels of TCF-4 in chondrocytes significantly (Figure 4B and C). To find if TCF-4 was a favorable targeted of miR-152, luciferase assay was done for which a luciferase reporter plasmid carrying mutant (MUT) or wild type (WT) TCF-4 3'UTR transfected with miR-152 expressing or negative control lentivirus. The outcomes demonstrated that overexpression of miR-152 resulted in significant decrease of luciferase activity in WT TCF-4 3'UTR but not in (MUT) TCF-4 3'UTR (Figure 4D). Treatment of IL-1β enhanced the expression of TCF-4 at both protein and mRNA levels in chondrocytes, miR-152 reversed these changes (Figure 4E and F). In addition to this, the protein levels of TCF-4 were on the upper side in the tissues from rats induced to osteoarthritis compared to sham control rats, the levels were attenuated in osteoarthritis rats treated with miR-152 (Figure 4G). The levels of TCF-4 were also found to be increased in human cartilage tissues having osteoarthritis compared to normal subjects, the findings correlated positively with Mankin score (Figure 4H and I). We also found a inverse correlation between expression levels of TCF-4 and miR-152 in osteoarthritis tissues (Figure 4J). The results confirmed that TCF-4 is potential target of miR-152, also we found that expression of TCF-4 is decreased by miR-152 in chondrocytes treated with IL-1β or in case of osteoarthritis.

miR-152 Protected Apoptosis and Inhibited Expression of TCF-4 in IL-1β–Induced Chondrocytes

To study if TCF-4 was involved in the miR-152 regulated apoptosis of chondrocytes functions, lentiviral vectors loaded with TCF-4 gene were constructed and infected in chondrocytes. Western blot analysis confirmed overexpression of TCF-4 in chondrocytes infected with TCF-4 lentiviral vectors (Figure 5A). The results also showed that, overexpression of TCF-4 blocked the cell proliferation and enhanced cell apoptosis. Additionally, when TCF-4 and miR-152 was overexpressed in chondrocytes, the results of functional assay suggested miR-152 negative regulated apoptosis and miR-152 s beneficial effects on functions of chondrocytes in presence of TCF-4 (Figure 5B-5G). These outcomes suggested that miR-152 shows its protective action on chondrocytes by suppressing expression of TCF-4.

Discussion

Studies recently have been inclined toward role if miRs in osteoarthritis. Here in the present investigation, we showed that expression level of miR-152 is downregulated remarkably in the tissue cartilages of patients diagnosed for osteoarthritis as well as in isolated chondrocytes exposed to IL-1β. We found that, upregulation of miR-152 improved or assuaged osteoarthritis in rats via enhancing the activity of chondrocytes and inhibiting the degradation of matrix and apoptosis. It was also evidenced that TCF-4 was the potential target of miR-152 also the expression of TCF-4 was negatively linked with expression of miR-152 in human tissue cartilage samples from osteoarthritis subjects. Further it was also observed that increased expression of TCF-4 inhibited the effects of miR-152 on apoptosis of isolated chondrocytes and also suppressed the levels of catabolic as well as anabolic factors.

One of the main features of osteoarthritis is alterations in behavior of chondrocytes resulting in increased production of proteolytic enzymes along with destruction of cartilage and joint function. Hence studies about the molecular pathways and involved mechanisms responsible for decrease in number of chondrocyte cells and destruction of ECM in the tissue

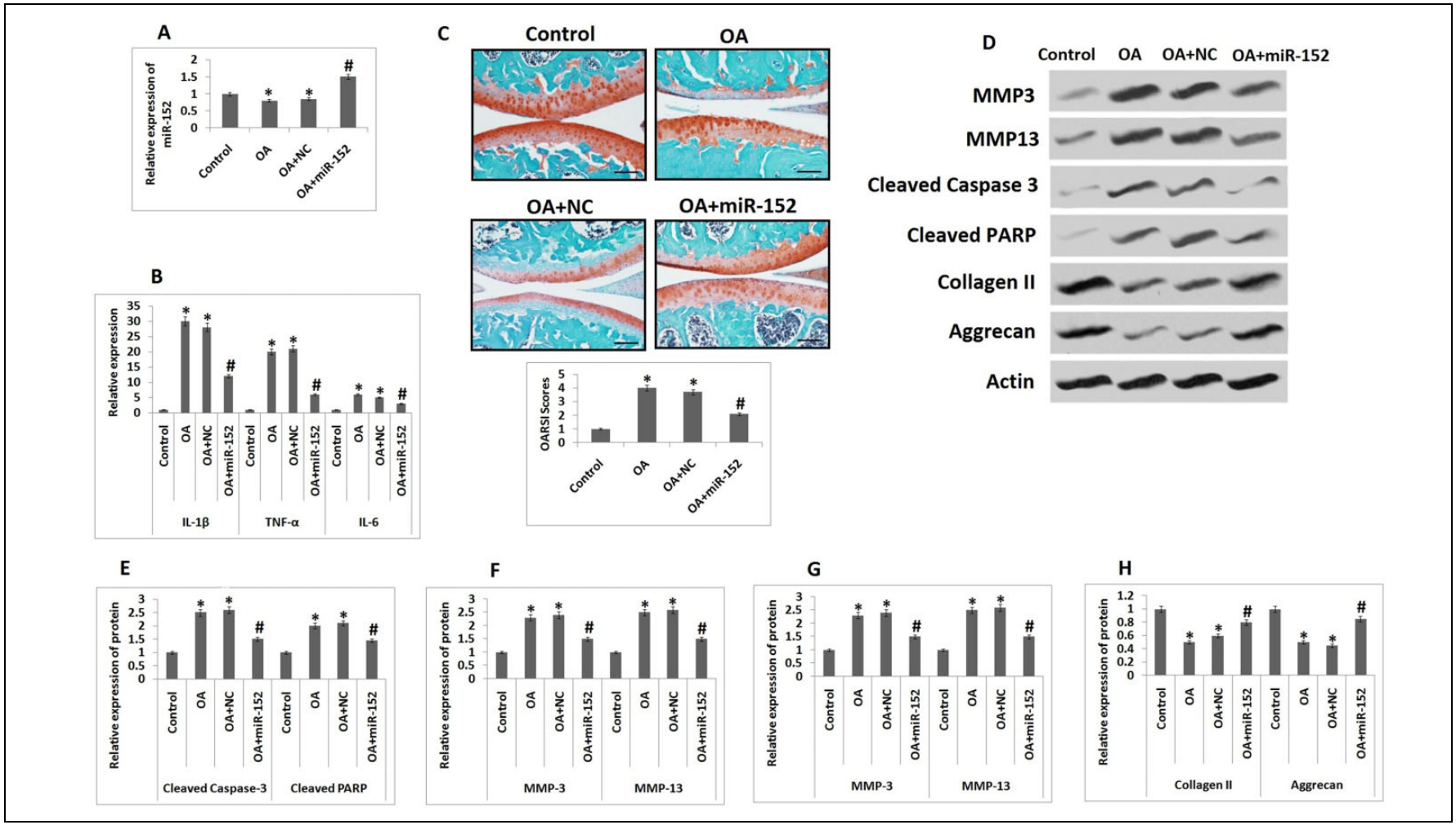

Figure 3. MiR-152 eases the degradation of cartilage matrix in vivo. A: Expression of miR-152 in cartilage of control, osteoarthritis, osteoarthritis and miR-152 lentivirus or NC lentivirus treated rats. B: Safranin O staining and OARSi grading in ACLT and sham rats injected with miR-152 expressing lentivirus or NC RNA. C: The mRNA levels of inflammtory cytokines in osteoarthritis rats treated with in NC or miR-152 expressing lentivirus. D: Western blot analysis for expression of MMP-3, MMP-13, Cleaved caspase-3, PARP, Aggrecan and collagen type II. E-H: Densitometric analysis of MMP-3, MMP-13, Cleaved caspase-3, PARP, Aggrecan and collagen type II. *P < 0.05 against control group, #P < 0.05 against osteoarthritis and osteoarthritis+NC treated group.

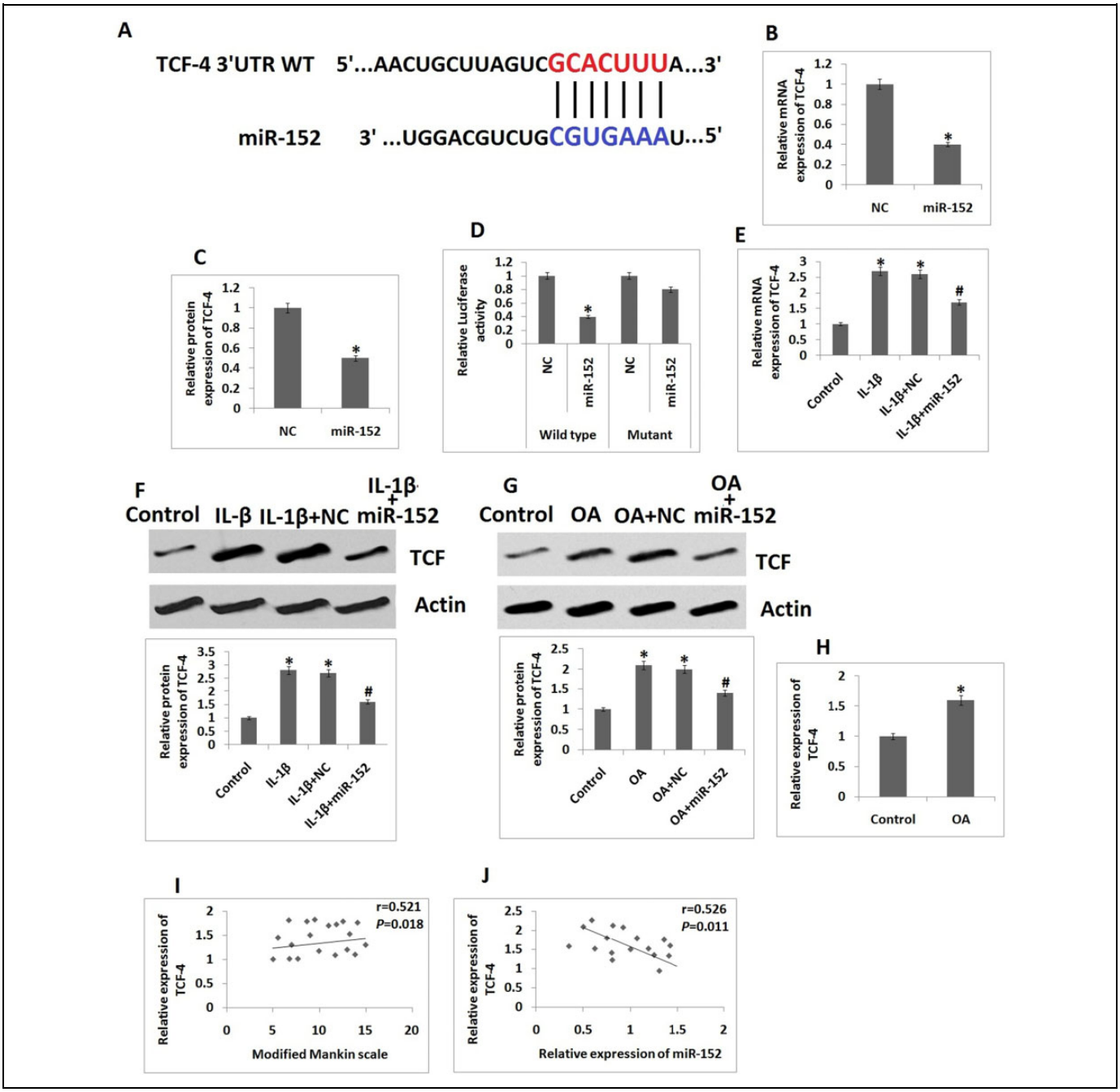

Figure 4. miR-152 inhibits expression of TCF4 by directly binding to the 3'UTR site. A: The binding site for miR-152 in TCF4 mRNA. B-C: qRT-PCR and western blot analysis of mRNA and protein levels respectively of TCF4 in chondrocytes transfected with NC or miR0152 expressing lentivirus. D: The results of luciferase activity in chondrocytes transfected with wild type, mutant TCF4 along with negative control or miR-152 RNA. E-F: The protein as well as mRNA levels were estimated by western blot and qRT-PCR analysis in IL-1β treated chondrocytes transfected with negative control or miR-152 expressing lentivirus. G: protein expression of TCF4 in tissue cartilage of control, osteoarthritis, osteoarthritis+ negative control and osteoarthritis+ miR-152 treated group. H: qRT-PCR assay of mRNA levels of TCF4 osteoarthritis and healthy human cartilage tissues. I: Expression of TCF-4 and modified Mankin scale in tissues of osteoarthritis subjects. J: Expression of TCF4 and expression of miR-152 in human osteoarthritis cartilage tissues of human subjects. *P < 0.05 against control or negative control groups, #P < 0.05 against IL-1β, IL-1β+NC, osteoarthritis or osteoarthritis +negative control groups.

cartilage in progression of osteoarthritis may be helpful in developing treatment therapies of osteoarthritis.[20] Multiple studies have reported involvement of miRs in the pathogenesis of osteoarthritis such as miR-218, miR-365, miR-145 and miR-146a.[6,8-10] miR-152-3p is over-expressed in postmenopausal females reported with osteoporosis and fracture, fragile suggesting a link between miR-152 and osteoporosis affecting the osteoblasts differentiation.[12] miR-152 exerts inhibitory effect

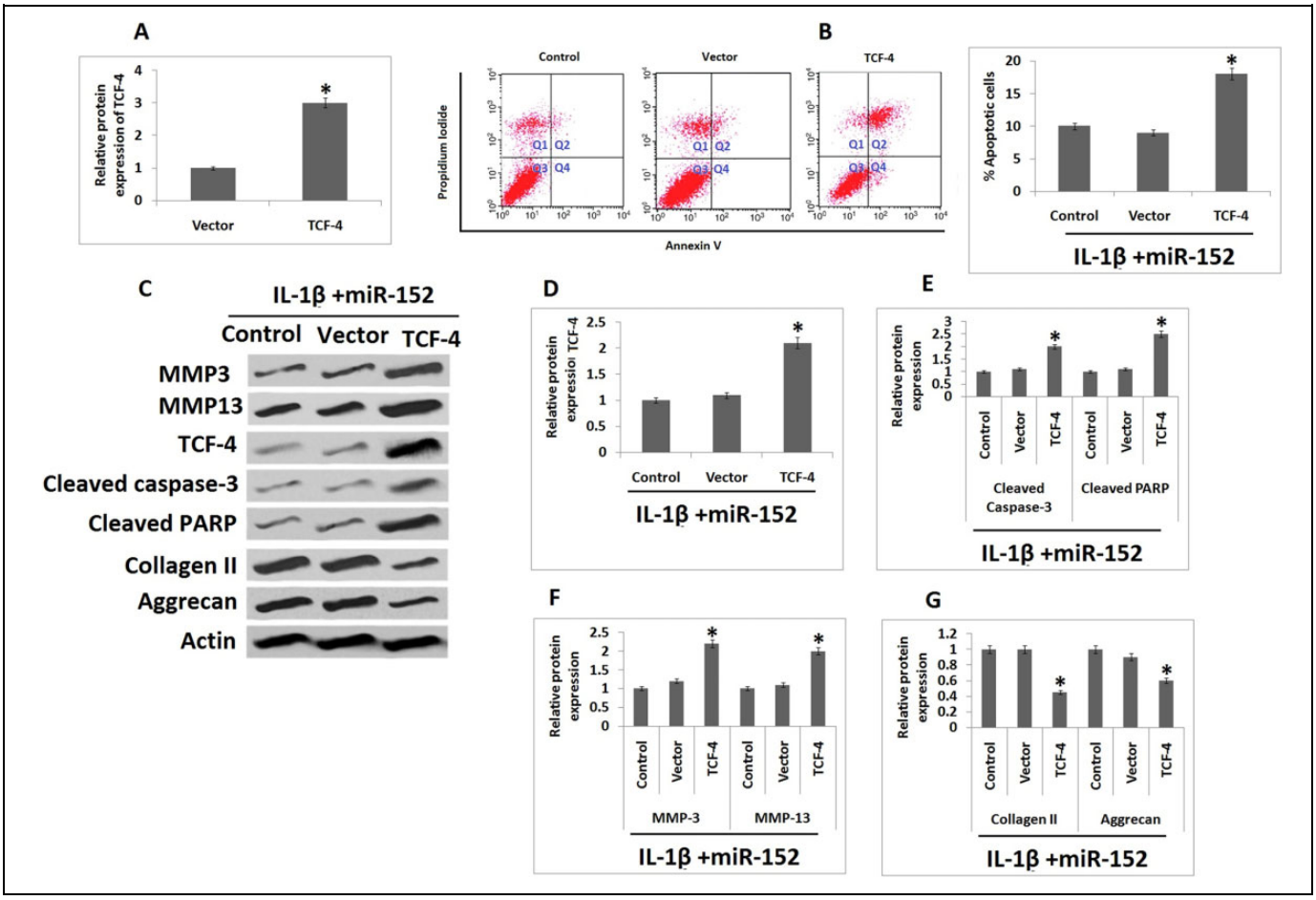

Figure 5. TCF4 attenuated the inhibitory effect of miR-152 on apoptosis and degradation of ECM on IL-1β induced apoptosis of chondrocytes. A: Results of western blot showing expression of TCF4 in human chondrocytes transfected with TCF4 or control (vector) expressing lentivirus. B: Flow cytometry analysis for apoptosis in chondrocytes post Annexin V and Propidium iodide staining. C: Western blot analysis for expression of protein levels of MMP-3, MMP13, TCF4, cleaved caspase 3, cleaved PARP, collagen type II and Aggrecan. D-G: Densitometric analysis for expression of MMP-3, MMP13, TCF4, cleaved caspase 3, cleaved PARP, collagen type II and Aggrecan. *P < 0.05.

on the levels of cytokines like IL-12, IL-6 and the TNF-α,[13] in addition to this miR-152 blocks the expression of pro-inflammatory cytokines in Rheumatoid arthritis.[14] Studies for role of miR-152 in various other bone abnormalities remains poorly studied. As miR-152 was involved in some inflammatory and bone abnormalities, the present work was undertaken to screen the role of miR-152 in a inflammatory and joint disorder i.e osteoarthritis. Here it was evidenced that, expression levels of miR-152 is suppressed in chondrocytes and tissues of patients with osteoarthritis. IL-1β is member of Interlukin family, it can cause various pathogenic changes in chondrocytes as it is found to play important role in progression if osteoarthritis.[21,22] In the present work it was found that expression of miR-152 was suppressed in IL-1β exposed normal chondrocytes. Also it was found that; upregulation of miR-152 expression caused a significant inhibition of IL-1β mediated apoptosis in chondrocytes. It is reported that, IL-1β increases the levels of MMP-3 and MMP-13 these are the matrix destruction enzymes responsible for degradation of ECM, leading to synovial inflammation and generation of pro-inflammatory cytokines causing destruction of cartilages.[23,24] The outcomes of our study suggested that, up-regulation of miR-152 attenuates the balance between catabolic and anabolic processes of articular cartilage of the joints.

Transcription factor-4 (TCF-4) is a protein responsible for various physiological processes.[25,26] TCF-4 has been found to act as oncogene and regulates the growth of tumor and its metastasis.[27,28] Overexpression of TCF-4 blocks the cell proliferation and also causes overexpression of p27.[29] In addition to this TCF4 has been found to act on NF-κB cascade in Chondrocytes and the C20/A4 Chondrocyte cell lines.[30] However, role of TCF-4 in osteoarthritis still remains unexplored. In the present work, bioinformatics algorithms were used for predicting the potential targets of miR-152, looking into the outcomes TCF-4 was targeted in our further research. It was observed that TCF-4 was the direct target of miR-152 and was markedly downregulated by overexpression of miR-152 in chondrocytes. In addition to this, TCF-4 was overexpressed in rat as well as human cartilage tissues affected by osteoarthritis and TCF-4 expression levels were inversely correlated with expression of

miR-152. It was observed that upregulation of TCF-4 resulted in significant blockade of effects of miR-152 on apoptosis in chondrocytes and also on expression levels of catabolic and anabolic factors applicable for cartilage ECM.

Conclusion

Altogether, the present work demonstrated that miR-152 is downregulated in humans well as rat osteoarthritis affected tissue cartilage and also in IL-1β induced normal chondrocytes. Upregulation of miR-152 eases osteoarthritis by suppressing degradation of cartilage matrix and also the apoptosis of chondrocytes by blocking the expression of TCF-4 both in vivo and in vitro. The present results increase our knowledge about the involvement of miR-152 into the pathogenesis of osteoarthritis which would be useful in developing novel therapeutic approaches in treating osteoarthritis.

Authors' Note

Daqian Wan and Yang Qu contributed equally to this work. DW and YQ contributed equally to the work. DW, YQ, SA and LC planed the work together. DW and YQ preformed the animal studies. SA and LC performed the rest of the experiments. All the authors prepared the manuscript and reviewed it. All the data is presented in paper. The supporting study data for work is under ethics laws of university and is hence not presented here. Here by we declare that our institutes are aware of the work and declare consent for publication of the manuscript. The animal studies were approved by the animal ethical review board of Tongji University School of Medicine, Shanghai, China.

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Declaration of Conflicting Interests

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