SPANDIDOS PUBLICATIONS EXPERIMENTAL AND THERAPEUTIC MEDICINE 15: 2134-2139, 2018

Effectiveness of lentivirus-mediated RNA interference targeting mouse tumor necrosis factor α *in vitro* and *in vivo*

JIBO WANG[1], YINGJIE ZHAO[1], MIAOMIAO XIN[1], LIN PAN[1], LIQIN WANG[1] and KUN YANG[2]

Departments of [1]Rheumatology and Clinical Immunology, and [2]Central Laboratory, The Affiliated Hospital of Qingdao University, Qingdao, Shandong 266003, P.R. China

Received September 14, 2016; Accepted September 7, 2017

DOI: 10.3892/etm.2017.5609

Abstract. The aim of the present study was to identify the effectiveness of lentivirus-mediated RNA interference (RNAi) targeting mouse tumor necrosis factor-α (TNF-α). RNAi lentivirus was used *in vitro* to transfect RAW264.7 cells, and the expression of *TNF*-α, interleukin (*IL*)-*1*β and *IL-6* mRNAs and TNF-α protein in RAW264.7 cells was measured by reverse transcription quantitative polymerase chain reaction (RT-qPCR) and enzyme-linked immunosorbent assay, respectively. *In vivo*, mice with collagen-induced arthritis (CIA) were injected intravenously with RNAi lentivirus, and CIA arthritis scores and the serum levels of TNF-α were detected. Additionally, joint tissues were subjected to pathological examination. In the cells, the expression level of *TNF*-α mRNA in the RNAi lentivirus group was 0.29±0.02, which was significantly lower than that of the lentivirus negative control (0.93±0.01; t=25.4, P<0.001). In the mice, the serum TNF-α level in the RNAi lentivirus group was 249.25±11.22 ng/ml, which was significantly lower than that of the negative control group (381.86±6.28 ng/ml; P<0.05). However, no difference in *IL-1*α and *IL-6* mRNA levels was identified among the groups (t=1.00, P=0.37; t=1.22, P=0.29). The CIA arthritis score in the RNAi lentivirus group was significantly reduced compared with those in the control and negative control groups (P<0.05). Furthermore, the arthritis scores in the RNAi lentivirus and positive control groups continued to decrease for ≥2 weeks, and the serum TNF-α levels in the RNAi lentivirus and positive control groups were 31.58±2.18 and 35.21±2.25 pg/ml, which were significantly lower than those in the negative control group (46.62±3.02 pg/ml; P<0.05). Thus, targeting of the *TNF*-α gene in mice via lentivirus-mediated RNAi *in vitro* and *in vivo* achieved TNF-α gene downregulation, which indicates that lentivirus-mediated RNA interference may be an effective form of gene therapy against rheumatoid arthritis.

Correspondence to: Dr Jibo Wang, Department of Rheumatology and Clinical Immunology, The Affiliated Hospital of Qingdao University, 16 Jiangsu Road, Qingdao, Shandong 266003, P.R. China
E-mail: jibowangdoc@126.com

Key words: RNA interference, effectiveness, *in vivo, in vitro*, tumor necrosis factor-α

Introduction

The applications of gene therapy have been increasing, and RNA interference (RNAi) has become one of the most promising methods for gene therapy (1,2). Lentiviruses are commonly used as viral vectors due to their beneficial characteristics, including high transduction efficiency and the capacity for achieving stable gene expression by genetic integration (3). Rheumatoid arthritis (RA) is a chronic inflammatory disease characterized by inflammation and progressive joint destruction, affecting 0.5-1% of the population worldwide (4). Although RA is considered a disease of the joints, it may cause a variety of extra-articular symptoms (5). These symptoms clearly demonstrate that RA has features of a systemic disease capable of involving a variety of major organ systems (5). Patients with RA suffer from pain and loss of function, often accompanied by a decreasing quality of life and mortality (5). TNF-α is a key cytokine that mediates inflammation in RA (6). Elevated TNF-α has been observed in the synovial fluid and the synovium of patients with RA (6). TNF-α serves a central role in bone degradation, which induces local inflammation and pannus formation and eventually leads to erosion of the cartilage and bone destruction (6). The use of TNF-α inhibitors has revolutionized the treatment of RA (6). In a previous study, an RNAi lentiviral vector targeting the mouse *TNF*-α gene was constructed and its identification was described. This may be integrated into the genome of target cells and is associated with a long expression duration, low immunogenicity and low cytotoxicity (7). Therefore, the aim of the present follow-up study was to investigate the effects of lentiviral RNAi in a mouse arthritis model. In the present study, RNAi lentiviral vector particles targeting the mouse *TNF*-α gene were used to transfect an *in vitro* model of inflammatory cells (murine RAW264.7 macrophages) and to infect an *in vivo* mouse model of collagen-induced arthritis (CIA), and the effects of targeted TNF-α lentivirus-mediated RNAi in these models were observed.

Materials and methods

RNAi lentiviral particles. Three different RNAi lentivirus particles targeting the mouse *TNF*-α gene (siRNA1, siRNA2

and siRNA3) were previously constructed, and the siRNA2 lentivirus particles were selected for use in the present study as they exhibited the most effective knockdown effect (7). The lentivirus negative control (negative control shRNA) and enhanced green fluorescent protein (EGFP) were purchased from Jikai Gene Chemical Technology Co., Ltd. (Shanghai, China). EGFP was used as a marker for the localization of lentivirus particles in different tissues that can be detected using reverse transcription-quantitative polymerase chain reaction (RT-qPCR) and visualized using a fluorescence microscope.

Effects of RNAi lentiviral particles on RAW264.7 cells. RAW264.7 cells (Shanghai Cell Bank of the Chinese Academy of Medical Sciences, Shanghai, China) were cultured with Dulbecco's modified Eagle's medium containing 10% fetal bovine serum (Gibco; Thermo Fisher Scientific, Inc., Waltham, MA, USA) at 37˚C in an atmosphere containing 5% CO_2. Cells were seeded at a density of $4x10^4$ cells/well during the logarithmic growth phase in 24-well cell culture plates, and three wells were plated for each group (lentivirus RNAi, lentivirus negative control and blank control groups). At 24 h after cell seeding, RNAi lentiviral vector particles, negative control lentivirus and an equal volume of culture medium were added to the corresponding wells [$1x10^6$ transducing units (TU); multiplicity of infection, 25]. The culture medium in each well was replaced after 24 h, and lipopolysaccharide (LPS) was added to each group at a final concentration of 1 μg/μl after 48 h. The cells and supernatants were collected following exposure to LPS for 2 h and then analyzed using enzyme-linked immunosorbent assays (ELISAs) and RT-qPCR.

Analysis of TNF-α, interleukin (IL)-1β and IL-6 mRNA levels by RT-qPCR. The total RNA of the RAW264.7 cells was extracted using an RNA extraction kit (Takara Bio, Inc., Otsu, Japan) according to the manufacturer's protocol. In total, 1 μg total RNA was subjected to RT to generate cDNA, using a PrimeScript™ RT reagent kit (cat. no. RR037A; Takara Biotechnology Co., Ltd., Dalian, China). The temperature protocol was as follows: 42˚C for 10 min followed by 95˚C for 2 min. The cDNA was then used to perform qPCR, using a Premix Ex Taq kit (cat. no. RR390Q; Takara Biotechnology Co., Ltd.) under the following thermocycling conditions: 94˚C for 2 min (1 cycle), 95˚C for 5 sec and 62˚C for 40 sec (40 cycles) as previously described (7). Primer Express v2.0 software (Thermo Fisher Scientific, Inc.) was used to design mouse primers and TaqMan probes for *TNF-α*, *IL-1β*, *IL-6*, *β-actin* and *EGFP* genes (7). The primers were synthesized by Sangon Biotech Co., Ltd. (Shanghai, China), and the probes were synthesized by Takara Bio, Inc. (Table I). qPCR was performed on a Rotor Gene RG3500 PCR instrument, and Rotor Gene Q software (Version 6.0, Corbett Life Science; Qiagen, Inc., Valencia, CA, USA) automatically recorded Cq values. The $2^{-\Delta\Delta Cq}$ method was used to calculate the relative mRNA levels using the following equation: $\Delta\Delta Cq=(Cq_{\text{gene of interest}}-Cq_{\text{reference gene}})_{\text{test group}}-(Cq_{\text{gene of interest}}-Cq_{\text{reference gene}})_{\text{control group}}$ (8).

Analysis of TNF-α contents in the culture supernatants of RAW264.7 cells by ELISA. The cell culture supernatants were subjected to ELISA using a Mouse TNF Alpha PicoKine™ ELISA kit (cat. no. EK0527; Wuhan Boster Biological Technology, Ltd., Wuhan, China) for analysis of the TNF-α content in accordance with the manufacturer's protocol.

Construction of a CIA model and lentivirus distribution in CIA. A total of 50 DBA/1 mice [specific pathogen-free (SPF) grade, female, 6-8 weeks old, 15-19 g] were purchased from Hayes Lake Animal Limited [license number, SCXK (Shanghai) 2007-0005; quarantine no. 0002844] and reared in an SPF animal laboratory. The mice were housed in an environment with a controlled temperature of 18-23˚C and 40-60% humidity. They were maintained in a 12 h light/dark cycle and had access to food and water *ad libitum*. The present study was approved by Medical Ethics Committee of the Affiliated Hospital of Qingdao University (Qingdao, China). Bovine type II collagen (cat. no. C4486; Sigma-Aldrich; Merck KGaA, Darmstadt. Germany) was dissolved in 0.1 M acetic acid to a concentration of 2 mg/ml, stored at 4˚C overnight, and then mixed with an equal volume of Freund's complete adjuvant (cat. no. F5881; Sigma-Aldrich; Merck KGaA) for emulsification. Next, 0.1 ml of the emulsion was intradermally injected into three points of the mouse tail and root. The mice were challenged again on day 21 using the same method (9).

On day 30, four of the mice were intravenously injected with virus negative control containing EGFP into the tail vein ($1x10^7$ TU). On days 3 and 7 after the injection, two mice were sacrificed and intracardiac puncture was performed to extract the blood. Additionally, knee tissues were obtained, and conventional RT-PCR was used to detect EGFP expression for determination of the *in vivo* distribution of the lentivirus.

Effects of lentivirus-mediated RNAi on CIA. The model mice were divided into the lentivirus treatment (n=8), lentivirus negative control (n=8), control (n=8) and positive control (n=9) groups. On day 30, 100 μl RNAi lentiviral vector particles ($1x10^5$ TU/μl), lentivirus negative control or phosphate-buffered saline was injected intravenously into the mice in the lentivirus treatment, lentivirus negative control and control groups followed by a second injection 1 week later. The mice in the positive control group were intraperitoneally injected with 1 mg/kg methotrexate (MTX) three times per week (9). The arthritis scores of the model mice were determined as previously described (9), to determine the success of modeling and evaluate the treatment effects. On day 45, the mice were sacrificed, puncture of the heart was performed to extract blood samples and ELISAs were used to detect the serum levels of TNF-α. Hind knee joint tissues were also obtained for hematoxylin and eosin staining. Joint tissues were stored in 10% EDTA solution (pH 7.4) at 4˚C for 2 weeks to achieve decalcification and subsequently fixed overnight in 4% paraformaldehyde in 10 mM PBS at 4˚C and embedded in paraffin. The paraffin-embedded tissue sections were cut to 5-μm-thick. Staining was performed at room temperature, with hematoxylin for 5 min and eosin for 30 sec. A light microscope was used to observe the stained sections. The objective lens was used at a magnification of x20-40 and the eyepiece was used at a magnification of x10.

Statistical analysis. SigmaStat 11.0 software (Systat Software, Inc., San Jose, CA, USA) was used for data processing. Quantitative data are expressed as the mean ± standard error

Table I. Primers and probes.

Gene	Primers and probes	Annealing temperature (˚C)
TNF-α	Upstream: 5'-TCTTCCCTGAGGTGCAATGC-3'	62
	Downstream: 5'-GCTCCGTTTTCACAGAAAACATG-3'	
	Probe: 5'-(FAM)TGGAGGACCCAGTGTGGGAAGCTGT(TAMRA)-3'	
IL-1β	Upstream: 5'-GGAGCTCCCTTTTCGTGAATG-3'	62
	Downstream: 5'-AGGTAAGTGGTTGCCCATCAGA-3'	
	Probe: 5'-(FAM)CCAAGACAGGTCGCTCAGGGTCACA(TAMRA)-3'	
IL-6	Upstream: 5'-TCCTACCCCAATTTCCAATGC-3'	62
	Downstream: 5'-CCACAGTGAGGAATGTCCACAA-3'	
	Probe: 5'-(FAM)ATCTACTCGGCAAACCTAGTGCGTT(TAMRA)-3'	
β-actin	Upstream: 5'-ATGGTGGGAATGGGTCAGAAG-3'	62
	Downstream: 5'-TCCATGTCGTCCCAGTTGGTA-3'	
	Probe: 5'-(FAM)TGACGAGGCCCAGAGCAAGAGAGGT(TAMRA)-3'	
EGFP	Upstream: 5'-GCTGACCCTGAAGTTCATCTGC-3'	62
	Downstream: 5'-ATGCCCTTCAGCTCGATGC-3'	

TNF-α, tumor necrosis factor-α; IL, interleukin; EGFP, enhanced green fluorescent protein.

of the mean. Quantitative data among groups were compared using one-way analysis of variance, and Dunnett's multiple comparisons tests or Student's t-tests were used for comparisons between groups. $P<0.05$ was considered to indicate a statistically significant difference.

Results

Effects of RNAi lentiviral particles on TNF-α, IL-1β and IL-6 expression in RAW264.7 cells. As depicted in Fig. 1A, the relative expression of *TNF-α* mRNA in the RNAi lentivirus group was 0.29±0.02, and that in the lentivirus negative control group was 0.93±0.01 ($t=25.4$, $P<0.0001$). The relative levels of *IL-1β* mRNA in the RNAi lentivirus and lentivirus negative control groups were 0.97±0.07 and 1.05±0.05, respectively; the difference between these two values was not statistically significant ($t=1.00$, $P=0.37$). The *IL-6* mRNA levels in the RNAi lentivirus and lentivirus negative control groups were 0.94±0.01 and 1.02±0.06, respectively. No significant differences were identified between these groups ($t=1.22$, $P=0.29$).

TNF-α protein expression levels in the RNAi lentivirus, lentivirus negative control and blank control groups were 249.25±11.22, 381.86±6.28 and 393.88±8.38 ng/ml, respectively (Fig. 1B), with statistically significant differences detected among the groups ($F=82.0$, $P<0.0001$). The expression of TNF-α protein was decreased significantly in the RNAi lentivirus group compared with the lentivirus negative control groups ($P<0.05$). However, when the negative control and lentivirus blank control groups were compared, no significant difference was detected ($P>0.05$).

Construction of a CIA model and lentivirus distribution in CIA. Arthritis symptoms first appeared ~28-32 days following immunization and were identified in the footpad, ankle, knee and interphalangeal joint. The arthritis symptoms included erythema, swelling of the joint, individual digit, or the entire paw. Although the majority of the symptoms were observed in the hind legs, the front paws were also involved. The modeling rate was 68.74%. Tail vein injection of lentivirus negative control carrying the *EGFP* gene was performed, and RT-qPCR was used to detect the *EGFP* expression in the peripheral blood and joint tissues of CIA model mice (Fig. 2).

Effects of lentivirus-mediated RNAi on the CIA arthritis scores. The arthritic scores 8 days following the intravenous or intraperitoneal treatment (day 37 following arthritis induction) in the lentivirus treatment, positive control, negative control lentivirus and blank groups were 2.50±0.19, 1.25±0.16, 3.75±0.16 and 3.63±0.18, respectively; statistically significant differences were detected among the groups ($F=42.8$, $P<0.0001$; Fig. 3). In the pairwise comparisons, the scores for the lentivirus treatment and positive control groups were significantly lower than that of the negative control group ($P<0.05$). No significant difference was identified among the negative control and blank control groups ($P>0.05$). The arthritis scores of the lentivirus treatment and positive control groups slowly and continuously decreased for at least 2 weeks following the injection, whereas the arthritis scores of the blank control and lentivirus negative control groups continued to increase.

Effects of lentivirus-mediated RNAi on serum TNF-α in CIA model mice. The serum TNF-α levels of the lentivirus treatment, positive control, lentivirus negative control and blank control groups were 31.58±2.18, 35.21±2.25, 46.62±3.02 and 52.10±3.13 pg/ml, respectively, with statistically significant differences among groups ($F=13.2$, $P<0.001$; Fig. 4). In the pairwise comparisons among groups, the TNF-α levels of the lentivirus treatment and positive control groups were significantly lower than that of the negative control group ($P<0.05$). However, no significant difference ($P>0.05$) was observed between the negative control and blank control groups.

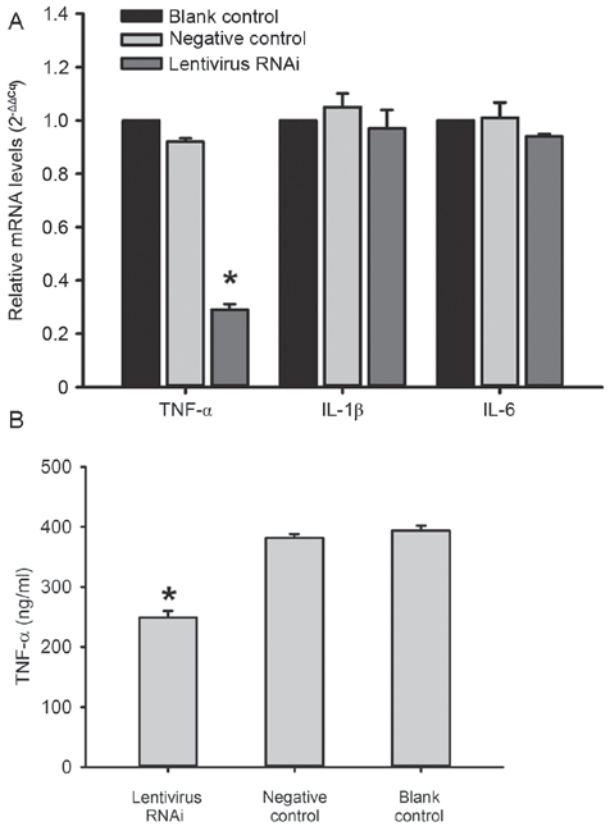

Figure 1. The relative mRNA and protein expression levels of cytokines in RAW264.7 cells. (A) The relative mRNA expression levels of TNF-α, IL-β and IL-6 were detected by reverse transcription-quantitative polymerase chain reaction. (B) The relative protein expression levels of TNF-α were detected by western blot analysis. *P<0.0001 vs. the negative control group. TNF-α, tumor necrosis factor-α; IL, interleukin; RNAi, RNA interference.

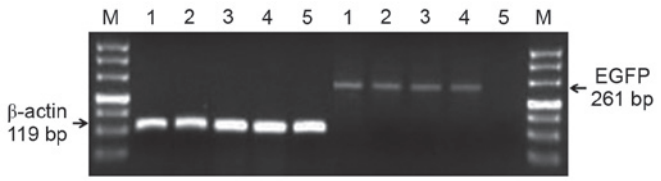

Figure 2. EGFP mRNA expression in the peripheral blood and synovial cells of the mice after lentivirus transduction. Lane M, marker; lane 1, peripheral blood 3 days after lentivirus transduction; lane 2, synovial cells 3 days after lentivirus transduction; lane 3, peripheral blood 7 days after lentivirus transduction; lane 4, synovial cells 7 days after lentivirus transduction and lane 5, peripheral blood 3 days after PBS control treatment. EGFP, enhanced green fluorescence protein.

Effects of lentivirus-mediated RNAi on CIA joint tissue pathology. Synovial pathology revealed that there was significant synovial hyperplasia in the negative control group, along with the appearance of inflammatory cell infiltration. Mild synovial hyperplasia was observed in the lentivirus treatment group; however, inflammatory cell infiltration was not significant (Fig. 5).

Discussion

RNAi involves sequence-specific post-transcriptional gene silencing mediated by double-stranded RNA (1,10) and has become a major gene therapy approach. The key to the implementation of RNAi is carrier selection; lentiviral vectors possess features including high gene transduction efficiency, gene integration, long-term expression, low immunogenicity, low cytotoxicity and effective transfection in a variety of cell lines (11-16). Furthermore, TNF-α is an important pro-inflammatory cytokine involved in the pathogenesis of inflammatory diseases, including RA (6). In the present study, the *TNF*-α gene served as a target in the construction of RNAi lentivirus particles and *in vitro* and *in vivo* were performed studies to observe the effects of lentivirus-mediated RNAi.

The *in vitro* observation of RNAi lentivirus particles revealed that the *TNF*-α gene was effectively silenced in inflammatory cells (murine RAW264.7 macrophages). Additionally, following RNAi lentiviral particle transfection, the TNF-α mRNA and protein expression levels in LPS-stimulated RAW264.7 cells were significantly lower than those in the lentivirus negative and blank control groups, indicating that *in vitro* transfection with the constructed RNAi lentivirus particles effectively silenced *TNF*-α gene expression. Notably, these RNAi lentivirus particles targeting the *TNF*-α gene did not affect *IL-1*β and *IL-6* mRNA expression, providing support for the specificity of RNAi. However, due to technical challenges, the

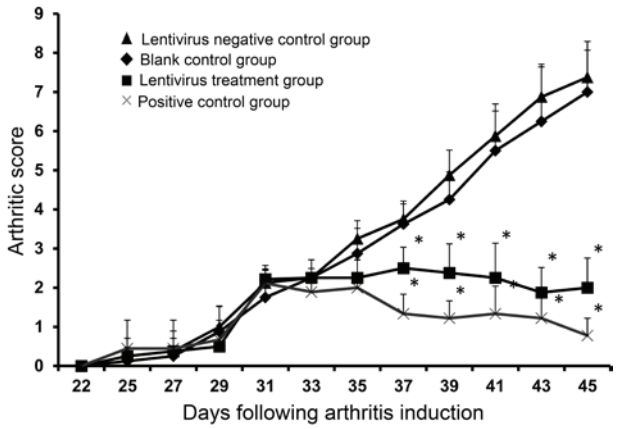

Figure 3. Arthritis scores for mice with collagen-induced arthritis. The arthritis scores of the mice were measured following the induction of arthritis. *P<0.05 vs. the blank control group.

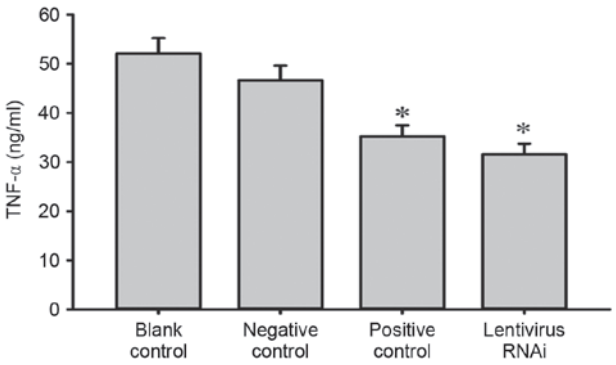

Figure 4. Serum TNF-α content levels following treatment. *P<0.05 vs. the negative control group. TNF-α, tumor necrosis factor-α.

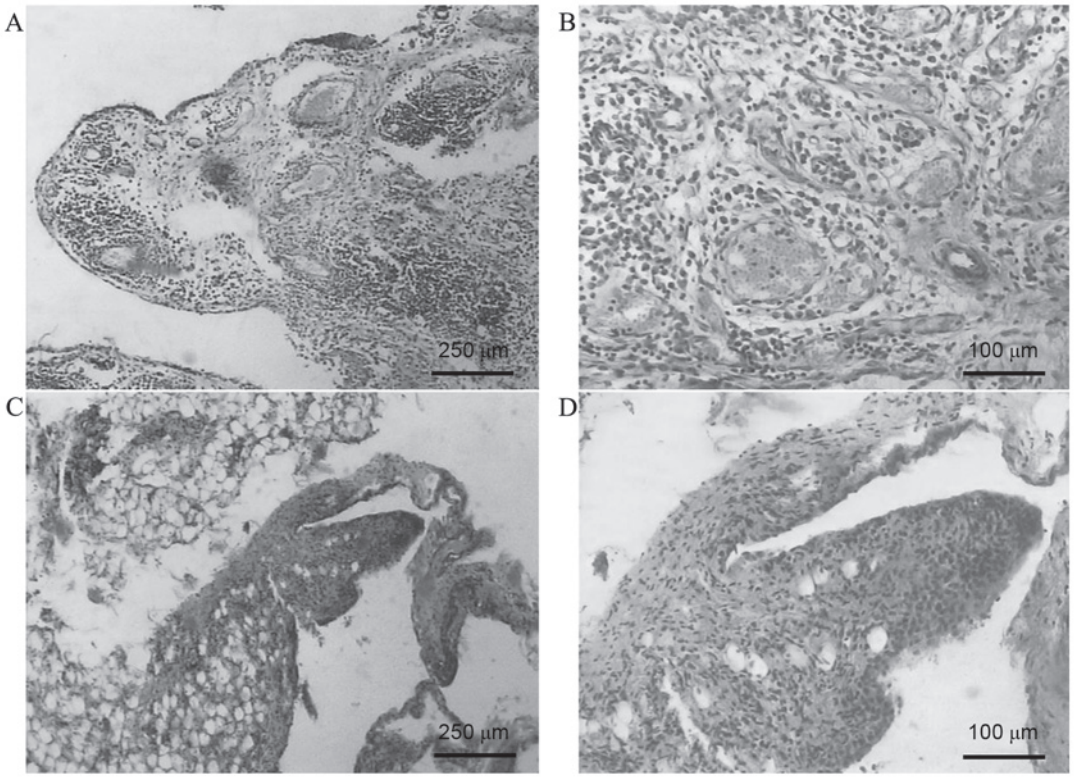

Figure 5. Effects of lentivirus-mediated RNA interference on joint pathology in mice with collagen-induced arthritis. Hematoxylin and eosin staining. (A) The negative control. (B) Magnified area of inflammation from the negative control; (C) The lentivirus treatment group. (D) Magnified area of the lentivirus treatment group.

duration of RNAi efficacy in the *in vitro* experiments was not evaluated.

CIA is capable of simulating the characteristics of RA (17). Thus, the *in vivo* RNAi experiment in the present study used the CIA mouse model. Tail vein injection with lentivirus carrying the *EGFP* gene was performed for negative controls, and expression of the *EGFP* gene was measured in the peripheral blood and joint tissues of the CIA model mice on days 3 and 7 by RT-qPCR. The results indicated that lentivirus particles were distributed in the lesions and effectively expressed *EGPF*, with expression lasting until at least day 7 after transfection.

On day 8 following the injection of RNAi lentivirus particles through the tail vein or the intraperitoneal injection of MTX, the joint scores of the affected joints in the lentivirus treatment and positive control groups were significantly lower than those of the control and lentivirus negative control groups. The joint scores slowly decreased over time and continued to decrease for ≥2 weeks, whereas the joint scores of the control and lentivirus negative control groups continued to increase. Serum TNF-α levels in the lentivirus treatment group were also significantly lower than those in the negative control lentivirus and blank control groups. Observation of the pathological inflammation of the inflammatory joints revealed that the number of inflammatory cell infiltrates and the degree of synovial hyperplasia in the lentivirus treatment group were lower than those in the negative and blank control groups. Furthermore, the analysis of physical symptoms, cytokine expression and joint involvement in arthritis demonstrated the effectiveness of the *in vivo* therapy by lentivirus-mediated RNAi.

The application of RNAi for clinical use remains difficult and complicated, with several limitations. Firstly, RNAi is known to exhibit off-target effects (18,19); current biotechnology can only predict a small portion of the off-target effects of RNAi (20). Secondly, RNAi may also exhibit non-specific effects, including interferon effects (21). In addition, as an RNAi vector, lentivirus can yield interference fragments for the target gene by integrating into the genome, which can inhibit target gene expression long-term; however, this may cause genetic damage, necessitating further studies. Thus, RNAi is currently only in the experimental gene therapy stage. A number of studies have evaluated the utility of RNAi experimental gene therapy using different approaches, i.e., varying target gene selection, transfection methods and carriers (3,22), and have reported meaningful results.

In the present study, RNAi lentiviral particles targeting the mouse *TNF*-α gene were constructed and *in vitro* and *in vivo* experiments were performed to demonstrate the effectiveness of these particles. The results indicate the feasibility and effectiveness of lentivirus-mediated RNAi experimental gene therapy.

Acknowledgements

The present study was funded by the Scientific Research Award Fund of Outstanding Young Scientist in Shandong Province (grant no. 2006BS03005), the Shandong Provincial Natural Science Foundation (grant no. ZR2016HM56) and the National Natural Science Foundation of China (grant no. 81671603).

References

1. Hannon GJ: RNA interference. Nature 418: 245-251, 2002.
2. Schiffelers RM, Xu J, Storm G, Woodle MC and Scaria PV: Effects of treatment with small interfering RNA on joint inflammation in mice with collagen-induced arthritis. Arthritis Rheum 52: 1314-1318, 2005.
3. Kato K, Miyake K, Igarashi T, Yoshino S and Shimada T: Human immunodeficiency virus vector-mediated intra-articular expression of angiostatin inhibits progression of collagen-induced arthritis in mice. Rheumatol Int 25: 522-529, 2005.
4. Firestein GS: Etiology and pathogenesis of rheumatoid arthritis. In: Kelley's textbook of rheumatology. 9th edition. Saunders, Philadelphia, PA, pp1059-1108, 2013.
5. Sweeney SE, Harris ED Jr and Firestein GS: Clinical features of rheumatoid arthritis. In: Kelley's Textbook of Rheumatology. 9th edition. Saunders, Philadelphia, PA, pp1109-1136, 2013.
6. Blüml S, Scheinecker C, Smolen JS and Redlich K: Targeting TNF receptors in rheumatoid arthritis. Int Immunol 24: 275-281, 2012.
7. Wang J, Liang H, Zhao Y, Liu X, Yang K and Sui A: Construction and identification of an RNA interference lentiviral vector targeting the mouse TNF-α gene. Exe Ther Med 10: 2283-2288, 2015.
8. Livak KJ and Schmittgen TD: Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) method. Methods 25: 402-408, 2001.
9. Apparailly F, Verwaerde C, Jacquet C, Auriault C, Sany J and Jorgensen C: Adenovirus-mediated transfer of viral IL-10 gene inhibits murine collagen-induced arthritis. J Immunol 160: 5213-5220, 1998.
10. Sharp PA: RNA interference-2001. Genes Dev 15: 485-490, 2001.
11. Gouze E, Pawliuk R, Pilapil C, Gouze JN, Fleet C, Palmer GD, Evans CH, Leboulch P and Ghivizzani SC: In vivo gene delivery to synovium by lentiviral vectors. Mol Ther 5: 397-404, 2002.
12. Lewis PF and Emerman M: Passage through mitosis is required for oncoretroviruses but not for the human immunodeficiency virus. J Virol 68: 510-516, 1994.
13. Naldini L, Blömer U, Gallay P, Ory D, Mulligan R, Gage FH, Verma IM and Trono D: In vivo gene delivery and stable transduction of nondividing cells by a lentiviral vector. Science 272: 263-267, 1996.
14. Wilson AA, Kwok LW, Porter EL, Payne JG, McElroy GS, Ohle SJ, Greenhill SR, Blahna MT, Yamamoto K, Jean JC, *et al*: Lentiviral delivery of RNAi for in vivo lineage-specific modulation of gene expression in mouse lung macrophages. Mol Ther 21: 825-833, 2013.
15. Wollebo HS, Woldemichaele B and White MK: Lentiviral transduction of neuronal cells. Methods Mol Biol 1078: 141-146, 2013.
16. Houbracken I, Baeyens L, Ravassard P, Heimberg H and Bouwens L: Gene delivery to pancreatic exocrine cells in vivo and in vitro. BMC Biotechnol 12: 74, 2012.
17. Pietrosimone KM, Jin M, Poston B and Liu P: Collagen-induced arthritis: A model for murine autoimmune arthritis. Bio Protoc 5: pii:e1626, 2015.
18. Jackson AL, Bartz SR, Schelter J, Kobayashi SV, Burchard J, Mao M, Li B, Cavet G and Linsley PS: Expression profiling reveals off-target gene regulation by RNAi. Nat Biotechnol 219: 635-637, 2003.
19. Fedorov Y, Anderson EM, Birmingham A, Reynolds A, Karpilow J, Robinson K, Leake D, Marshall WS and Khvorova A: Off-target effects by siRNA can induce toxic phenotype. RNA 129: 1188-1196, 2006.
20. Birmingham A, Anderson EM, Reynolds A, Ilsley-Tyree D, Leake D, Fedorov Y, Baskerville S, Maksimova E, Robinson K, Karpilow J, *et al*: 3'UTR seed matches, but not overall identity, are associated with RNAi off-targets. Nat Methods 3: 199-204, 2006.
21. Svoboda P: Off-targeting and other non-specific effects of RNAi experiments in mammalian cells. Curr Opin Mol Ther 9: 248-257, 2007.
22. Jiang LD, Wang HZ, Zhao FD, Xu RY, Guo JS, Bao CD and Wang JY: Effect of RNAi on the expression of COX-2 in human rheumatoid arthritis synovial fibroblasts. Chin J Rheumatol 11: 275-279, 2007.