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RESEARCH ARTICLE

ALK5 transfection of bone marrow mesenchymal stem cells to repair osteoarthritis of knee joint

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Abstract

Previous studies have suggested that the transforming growth factor-β receptor ALK5 is crucial for articular chondrogenesis by bone marrow mesenchymal stem cells. Here, the wild-type ALK5 plasmids were mutated by overlapping extended PCR and transfected into bone marrow mesenchymal stem cells. The knee joint osteoarthritis mouse model was constructed by cutting off the anterior cruciate ligament and divided into three groups: saline group, bone marrow mesenchymal stem cells and ALK5-transfected bone marrow mesenchymal stem cells group. HE staining showed that the articular cartilage lesions were more serious of saline group compared with that of mesenchymal stem cell group, and this trend was more pronounced as time goes on. Immunohistochemical staining showed that although the expression level of type II collagen in all three groups down-regulated gradually upon time, its expression in ALK5-transfected bone marrow mesenchymal stem cells group was significantly enhanced compared with the other two groups. Micro-CT also suggested that ALK5 transfection of mouse bone marrow mesenchymal stem cells would promote repairing the knee cartilage lesions with arthritis of the mice. Although the osteoarthritis mechanism underlying a variety of factors work together, and the appropriate proportion of ALK5/ALK1 was also emphasized for the treatment of osteoarthritis. This work therefore demonstrated that ALK5 transfection of bone marrow mesenchymal stem cells could be a promising stem cell therapy for repair of cartilage lesions.

Keywords ALK5 transfection · Bone marrow mesenchymal stem cell · Articular cartilage lesion · Osteoarthritis

Introduction

Osteoarthritis is a common clinical degenerative joint disease, which occurs in middle-aged and elderly people, with main pathological characteristics such as osteophyte formation, progressive cartilage degeneration and subchondral bone change [1–3]. Patients with osteoarthritis often suffer from pain and limited function, which seriously reduces their life quality [4]. The incidence of osteoarthritis is the result of a variety of factors, including age, obesity, joint injury, inflammatory factors and genetic factors. In the state of osteoarthritis, the phenotype of the chondrocytes changes, and the synthetic catabolism imbalance eventually leads to the destruction of the cartilage. There are quite minor studies unveiling the molecular pathogenesis of osteoarthritis due to its complexity. Meanwhile, very few effective treatments have been reported to inhibit the degeneration and development of cartilage [5].

Recently, with the rapid development of the technology of gene therapy and tissue engineering and applications, stem cell transplantation brings a new way for the treatment of osteoarthritis. Due to its strong self-renewal and differentiation potential, the bone marrow mesenchymal stem cells (BMSCs) have been widely used [6–8]. As a result, it is more meaningful for chondrocytes to be induced by MSC gene modification with high fabricability [9–12]. Among them, transforming growth factor-β (TGF-β) is a multifunctional growth factor, which plays an important role in the formation, stability and repair of articular cartilage. The signal pathway

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mediated by TGF-β is closely related to osteoarthritis. The receptors of mammalian cells participating in TGF-β signal can be generally divided into two categories: TGF-β receptor I and TGF-β receptor II containing seven and five members, respectively [13]. ALK5, namely, transforming growth factor receptor I (TGF-beta receptor I), belongs to I type receptor. After activating phosphorylation, this intracellular protein kinase will trigger the subsequent biological effects through activating downstream Smad signal molecules [14]. ALK5 induces cartilage differentiation by phosphorylated Smad2/3's TGF-β signaling pathway and inhibits cartilage terminal differentiation [15,35].

The aim of this study is to investigate the therapeutic effect of ALK5-transfected bone marrow mesenchymal stem cells on knee osteoarthritis and to further explore the significance of ALK5 in TGF-β signaling pathway.

Materials and methods

Sample preparation

Forty-six CBL57 mice in spite of male and female, with a body mass of 20–30 g, were provided by the Experimental Animal Center of Ningbo University. All animals were free to act, and strict disinfection of animal feeding in the room. The environment temperature was controlled as 25 °C, and the air humidity was set as 70%. Animals after feeding and water disinfection were sterile. Experiments were completed in the Cell Biology Laboratory of Ningbo University from May 2016 to August 2017. All treatments of animals strictly conform to the ethical requirements of experimental animals [16].

Cell culture

BMSCs were extracted from 6 C57BL mice. The mice were anesthetized and killed by excessive anesthesia. The volume was 75% ethanol, and the skin was sterilized. The skin and muscles of both hind limbs were quickly cut off under sterile condition, and the femur and tibia were soaked in PBS containing streptomycin. After falling off PBS, PBS containing green streptomycin was used to clean the bone marrow cavity for 3 times. A 1-mL disposable syringe was used to extract mesenchymal stem cell culture medium (ScienCell, CA, USA), and the needle was inserted into one end of the bone to wash. Then the other side was then washed to the white color of the bone cavity. In the 60-mm culture dish, the cell growth solution washed out by the bone marrow was placed in the culture box at 37 °C and the volume fraction 5%CO_2 culture box (Forma, USA). After the replacement of cell growth fluid in the second half of 48 h, the fresh medium was replaced every 3 days, and when about 80% of the culture dish was covered by cells, it could be passed on.

Identification of BMSCs

The bone marrow mesenchymal stem cells was identified by CD34, CD44, CD45 and CD105. After the identification of these four kinds of bone marrow mesenchymal stem cell surface antigens, the cultured cells were proved to be mesenchymal stem cells. Take the third generation well growth of stem cells by trypsin (Sigma) centrifugation, supernatant retained cells, adding PBS 500 L pellet suspension clearly marked, join CD34, tube first CD45, joining CD44, tube second CD105, joining CD105, tube third CD45 fourth tube without any anti body. The phenotype of stem cells was identified by flow cytometry (American BD Company).

Plasmids construction

Construct ALK5 activated eukaryotic expression plasmid flag-ALK5 T204D [17,18]. PRK-Flag-ALK5 plasmid was used as template, and primers F and Rm, Fm and R were paired for the first-round PCR, and the mutation sites and their sides are obtained, which are about 620 bp and R, respectively. Then, the first-round PCR product with the mole ratio of 1:1 was used as template, and second rounds of PCR were performed with primers F and R, and the full-length ALK5 sequence with mutation site was amplified, which was about 1500 bp. The full-length PCR fragments of PC DNA3 Flag vector and ALK5 T204D mutant were digested with *Hind*III and *Bgl*II, respectively, and then the products were recovered by gel. The two fragments were connected to 2H at room temperature with T4 DNA ligase, and 16 °C connection for overnight. The above linked products were converted to the receptive DH5 of Escherichia coli with 5 μL and 37 °C for overnight culture. Selected monoclonal colonies were seeded in 50 g/mL amphotericin LB medium with overnight oscillation at 37 °C. Plasmid DNA was extracted by alkaline lysis, and the insertion of exogenous gene was identified by *Hind*III and *Bgl*II double enzyme digestion.

Transfection of BMSCs

Mesenchymal stem cells were extracted from the bone marrow of mice of the third generation, and when the cell fusion was 80%, for the transfection of eukaryotic plasmid with Hi gene, the fluorescence expression was observed under the inverted fluorescence microscope (Olympus, Japan), and flow cytometry was used to detect the transfection efficiency. The RT-PCR (Primescript RT Master Mix, Takara, Japan)quantitative analysis after transfection of bone marrow mesenchymal stem cells ALK5 expression and transfection efficiency: RNA were extracted from normal cells and trans-

fected with 3, 7, 14 days cells (TRIzol reagent, Invitrogen, USA), and then reverse-transcribed into cDNA. PCR (instrument bio-rad, USA) amplification analysis to determine the change of ALK5.

Animal grouping and modeling

The establishment of the osteoarthritis model was accomplished through cutting off the mouse anterior cruciate ligament of knee joint [19,20]. In the volume fraction of 3.5% chloral hydrate (10 μL/g) intraperitoneal injection of anesthesia in mice after routine disinfection of surgical skin area, the medial patellar incision was performed, and the skin and joint capsule were cut into the articular cavity, and the patella was pulled out to the lateral side. The flexion of the knee joint revealed the anterior cruciate ligament and medial meniscus anterior horn, and the bilateral anterior cruciate ligament was cut off. The anterior drawer test confirmed that it had been completely cut off. During the operation, the articular cartilage surface was protected. The articular cavity was flushed with saline, and the joint capsule and skin were sutured layer by layer.

After 2 weeks, 36 C57BL mice with successful modeling were randomly divided into 3 groups. Each group consisted of 12 rats and 24 knee joint specimens. The physiological saline group was injected into the knee joint of 0.1 ml physiological saline. The pure bone marrow mesenchymal stem cell group was injected into the knee joint of the bone marrow mesenchymal stem cells with 1×10^7 without ALK5. The ALK5 transfection of bone marrow mesenchymal stem cell group was injected knee joint of the bone marrow mesenchymal stem cells 1×10^7 with flag-alk5 T204D. In addition, 4 mice were injected with Flag-ALK5 T204D to transfect bone marrow mesenchymal stem cells. On the 7th and 14th days, the transfected cells were traced under the fluorescence microscope.

Histological observation

All mice were executed after injection of 4, 8, 12 weeks, and then the bilateral knee specimens were obtained following by HE staining, toluidine blue staining and type II collagen and immunohistochemical detection of rabbit anti-mouse monoclonal antibody and goat anti-rabbit (Abcam, America). Subsequently, scores of cartilage in each group were accomplished according to the modified Mankin score table (Table 1) [21].

Imaging observation

The mice were killed at 4 and 12 weeks after injection. Tissue samples from bilateral paint joints were placed in the scanning bed of micro-CT system (Skyscan1176, Bruker company) and fixed specimens. A continuous micro-CT image was obtained by scanning along the long axis of the specimen. Scanning parameters: resolution 18 m, the rotation angle 360°, the rotation angle increment 0.7°, 65 K tube voltage V, tube current 379 A, scanning time 18min, obtained 500 different sections of 1024 × 1024 pixel images of the same sample [22].

Table 1 Modified Mankin scores for the knee cartilage in mice

Items	Grade
(1) Cartilage structure	
a. Normal	0
b. Irregular surface	1
c. Irregularity and pannus on the surface	2
d. Fracture deep moving layer	3
e. Fractured deep radiation layer	4
f. Fissure deep calcification layer	5
g. Abscission of the cartilage layer	6
(2) Chondrocytes	
a. Normal	0
b. Diffuse increase in the number	1
c. A large number of cluster cell clusters appear	2
d. A significant reduction in the number	3
(3) The content of proteoglycan	
a. Normal	0
b. Mild hypothyroidism	1
c. Moderate hypothyroidism	2
d. Severe hypothyroidism	3
e. Complete disappearance of staining	4
(4) The integrity of tidal lines	
a. Integrity	0
b. Blood vessel crossing	1
Total score	14

Main indexes observation

Histological HE staining and immunohistochemical staining of articular cartilage type II collagen were obtained in groups of 4, 8, and 12 weeks after injection. The micro-CT imaging observation was obtained for three groups at 4 and 12 weeks after injection, and the cell immunofluorescence detection of the cartilage of the bone marrow mesenchymal stem cells after the ALK5 transfection was accomplished at 2 weeks after injection.

Statistical analysis

All values were performed by SPSS 19 software. Data were analyzed by $x \pm s$. LSD t test and one-way ANOVA were utilized to compare the difference of data between groups. The difference was considered significant when $P < 0.05$.

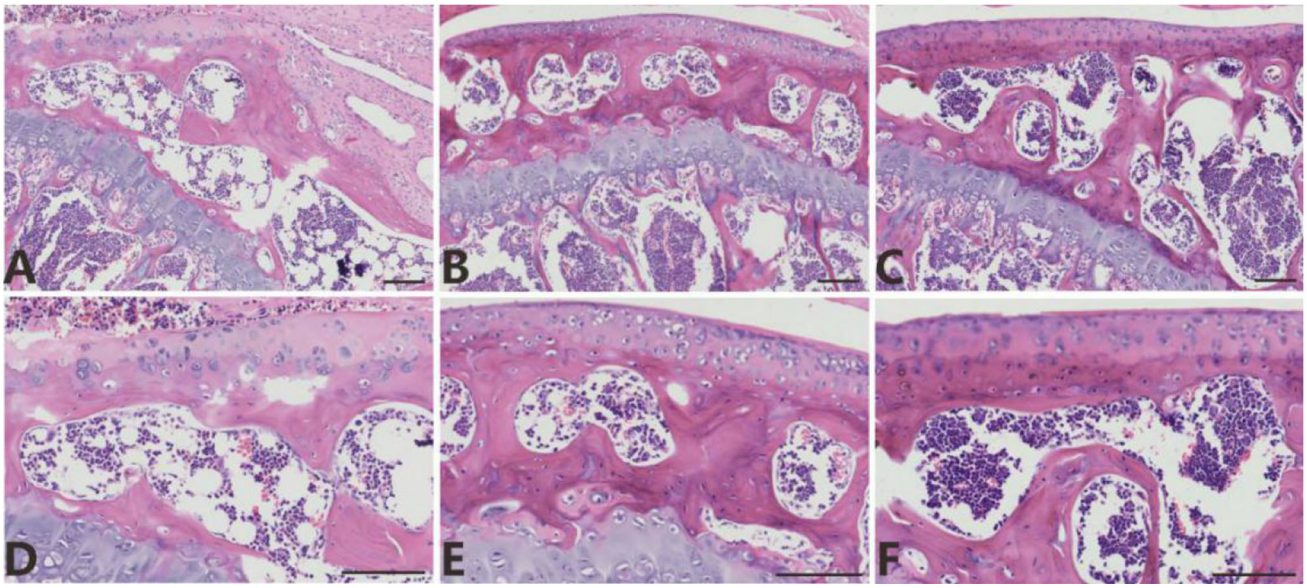

Fig. 1 HE staining of the mice's articular cartilage in each group after 4 weeks of injection (scale bar = 100 μm), **a**, **d** are the low and high magnifications of articular cartilage in the normal saline group, with cracks in the surface of the cartilage. **b**, **e** are the results of low and high magnifications of articular cartilage in the bone marrow mesenchymal stem cell group, and the cartilage cells increased and the cartilage layer was thickened. **c**, **f** show that there is no significant difference between the normal articular cartilage and bone marrow mesenchymal stem cells

Results

The recombinant plasmid Flag LK5 T2 04 D was digested with *Bgl*II and *Hind*III, and two bands of ~ 6000 and 1500 bp were obtained. The length of the recombinant plasmid corresponded to that of the empty carrier and ALK5, respectively. Identification of recombinant plasmid showed that plasmid was successfully constructed. The sequencing results were consistent with the expected results compared in NCBIBlast with the purpose of mutation, 204th amino acid from ACT (T) GAT (D) mutation.

Identification of ALK5 gene transduced bone marrow mesenchymal stem cells. Based on the flow cytometry results, the markers CD34 (−), CD45 (−), CD44 (+) and CD105 (+) were identified, suggesting that the cultured cells were relatively pure. Evaluation of gene transfection efficiency: After transfection of bone marrow mesenchymal stem cells for 3 days, green fluorescent protein-positive cells could be observed under fluorescence microscope. The transfection efficiency of flow cytometry was 65%, and ALK5 expression was positive by immunohistochemistry. RT-PCR results showed that the expression rate of ALK5 in bone marrow mesenchymal stem cells transfected by Flag-ALK5 T204D was highest at 1 week after transfection, and still remained at a high level in the next 3 weeks.

As shown in Fig. 1, HE staining results appeared to be different among the three groups after 4 weeks of injection. The cartilage cells arranged in disorder, and HE staining is weak, there are occasional loss of staining matrix. In contrast, the cartilage cells in bone marrow mesenchymal stem cell group were arranged neatly and the cartilage layer thickens at the same specifications. In terms of ALK5-transfected bone marrow mesenchymal stem cells group, we did not observe a significant difference with normal the articular cartilage. After 8 weeks of injection (Fig. 2), cartilage cells in saline group decreased and disorganized with obvious HE staining, while they proliferated significantly with the decreasing HE staining in bone marrow mesenchymal stem cells group. However, the cartilage cells ALK5 transfected bone marrow mesenchymal stem cell group were homogeneous distributed, slightly shallation of HE staining and thinning of cartilage layer. Upon 12 weeks after injection (Fig. 3), the cartilage cells in saline group decreased significantly with the cartilage layer stripping and subchondral bone exposure. As a result, HE staining almost disappeared. Meanwhile, HE was also severely impaired with the full-thickness articular surface fracture in bone marrow mesenchymal stem cells group. Also cartilage cell reduced in ALK5-transfected bone marrow mesenchymal stem cell group, but its distribution was more uniform, and cartilage surface crack increased.

The results of improved Mankin scores at each time point of each group are shown in Table 2. The cartilage injury in saline group was much more serious compared with that of the other two groups. And the trend was more obvious upon time. However, the cartilage injury of bone marrow mesenchymal stem cells group was repaired to some extent,

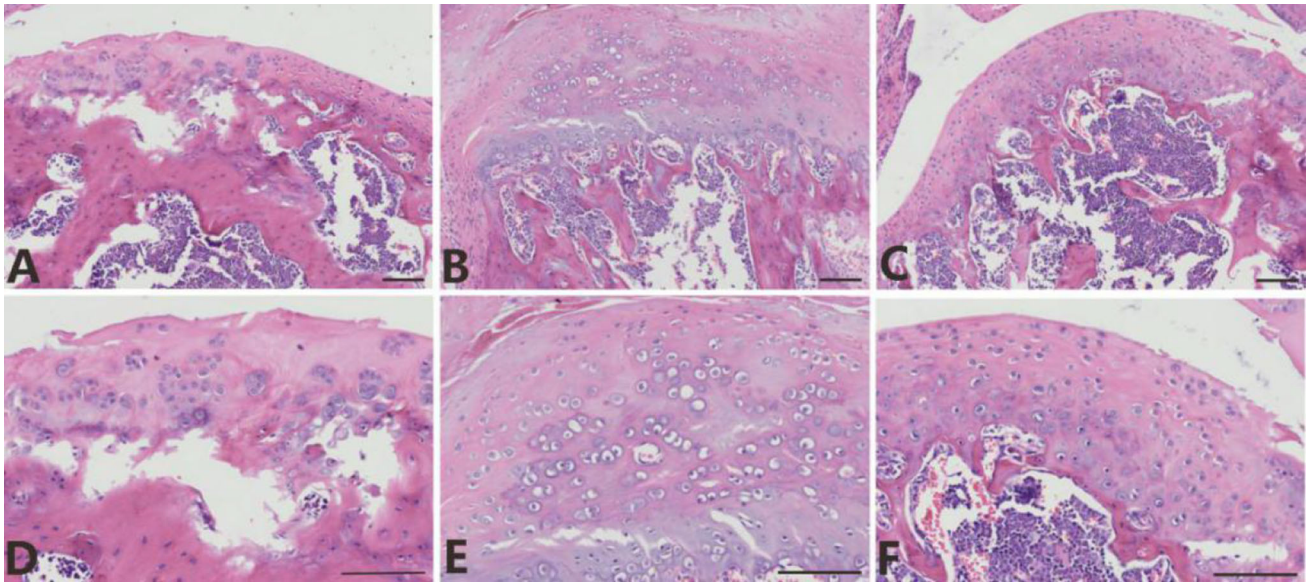

Fig. 2 HE staining of the mouse articular cartilage in each group after 8 weeks of injection (scale bar = 100 μm). **a**, **d** are the low and high magnifications of the articular cartilage in the saline group, and the chondrocytes were arranged in clusters. **b**, **e** are the results of low and high magnifications of articular cartilage in the bone marrow mesenchymal stem cell group. The cartilage cells decreased, the cartilage layer became thinner, and the staining became shallow. **c**, **f** show that the cartilage cells were slightly reduced and the cartilage layer was thinner

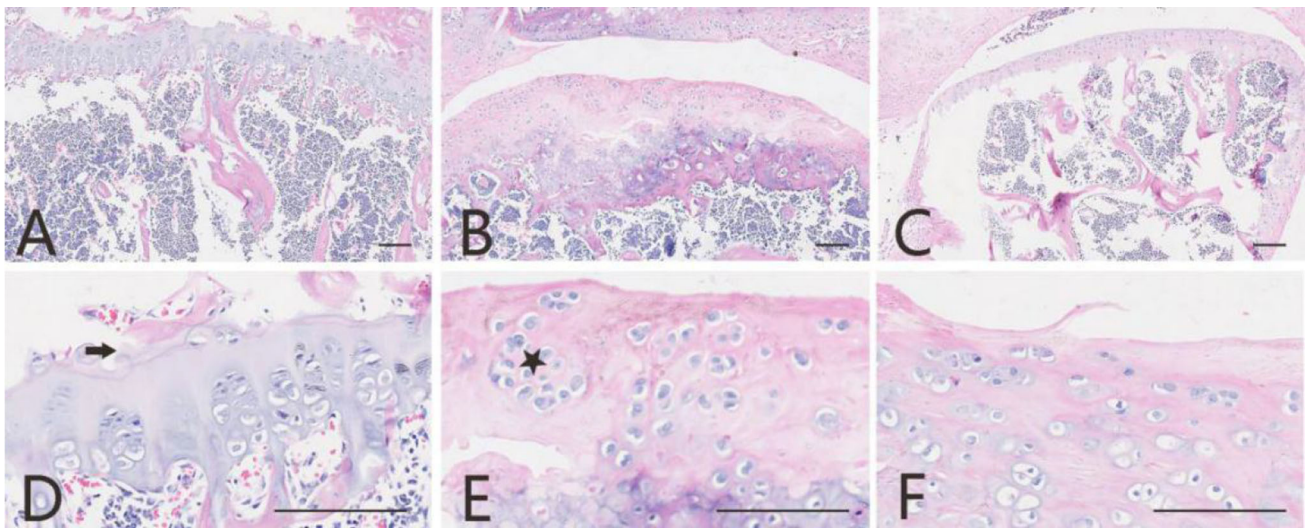

Fig. 3 HE staining of the mouse articular cartilage in each group after 12 weeks of injection (scale bar = 100 μm). **a**, **d** are the low and high magnifications of the articular cartilage in the saline group, and the cartilage layer was removed. **b**, **e** are the low and high magnifications of articular cartilage in the bone marrow mesenchymal stem cell group, and the whole layer of chondral cartilage was found. **c** is the result of the staining of articular cartilage in the bone marrow mesenchymal stem cell group of ALK5 transfection, and the surface fissure of the cartilage increased, while the chondrocytes proliferated

while that of ALK5-transfected bone marrow mesenchymal stem cells group was obviously repaired. It was also worth mentioning that the statistically significant difference gradually decreased upon injection time.

We further evaluated the repair performance by using toluidine blue staining. As shown in Fig. 4, the cartilage cells appeared to be a long fusiform or polygon morphology with a smooth membrane. There was occasional loss of staining matrix, and cartilage surface local cracks were observed. And the cell number in bone marrow mesenchymal stem cell group was up-regulated and the cartilage layer thickened compared with saline group. In contrast, the cartilage cells

Table 2 Modified Mankin scores of the mouse knee samples by HE staining

Group	4 weeks after injection	8 weeks after injection	12 weeks after injection
Saline group	3.85 ± 0.15	7.60 ± 0.30	10.20 ± 0.36
Bone marrow mesenchymal stem cell group	1.7 ± 0.46^{a}	6.11 ± 0.46^{a}	7.10 ± 0.76^{a}
ALK5-transfected bone marrow mesenchymal stem cell group	1.80 ± 0.35^{a}	3.50 ± 0.43^{ab}	3.73 ± 0.52^{ab}

Compared with physiological saline group, $^{a}P < 0.05$; compared with bone marrow mesenchymal stem cell group, $^{b}P < 0.05$

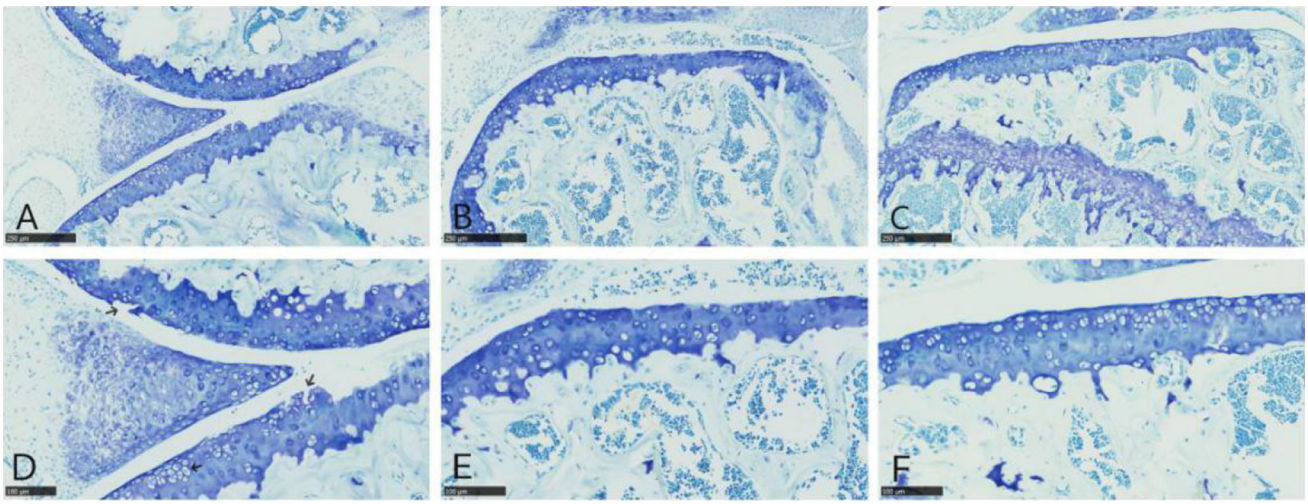

Fig. 4 Toluidine blue staining of the mouse articular cartilage in each group after 4 weeks of injection (scale bar = 100 μm). **a**, **d** are the low and high magnifications of articular cartilage in the normal saline group, with cracks on the surface of the cartilage. **b**, **e** are the results of low and high magnifications of articular cartilage in the bone marrow mesenchymal stem cell group, and the cartilage cells increased and the cartilage layer was thickened. **c**, **f** show no significant difference between the normal articular cartilage and the normal articular cartilage, with the results of ALK5 transfection of bone marrow mesenchymal stem cells

in ALK5-transfected bone marrow mesenchymal stem cell group appeared to be normal morphology with deeply stained nucleus and homogeneous cytoplasm. After 8 weeks of injection (Fig. 5), the number of cartilage cells in saline group was reduced and their nucleus was unclear. And multiple fractures of the cartilage layer appeared. The chondrocytes of the mesenchymal stem cells of the bone marrow were clumped together, arranged in disorder and colored. However, the cartilage cells in ALK5-transfected bone marrow mesenchymal stem cell group appeared to be of more normal morphology, the density was less, and their stain was slightly lighter. Upon 12 weeks after injection (Fig. 6), the cartilage cells in saline group were significantly reduced. And the subchondral bone was exposed as well as the staining color almost disappeared. While the cartilage cells in bone marrow mesenchymal stem cell group were appeared to be disorder and dysplasia. In contrast, the chondrocytes in ALK5-transfected bone marrow mesenchymal stem cell group was of tufted distribution with decreased coloration. And the cartilage layer was thin with shallow fissure in the surface.

The results of improved Mankin scores at each time point of each group are shown in Table 3. The cartilage injury in saline group was much more serious compared with that of the other two groups. And the trend was more obvious upon time. However, the cartilage injury of bone marrow mesenchymal stem cells group was repaired to some extent, while that of ALK5-transfected bone marrow mesenchymal stem cells group was obviously repaired. It was also worth mentioning that the statistically significant difference gradually decreased upon injection time. These results were consistent with the HE staining observation.

Type II collagen immunohistochemical staining: The positive effect of ALK5 transfection on bone marrow mesenchymal stem cells was stronger than that of the other two groups. With the injection time prolonged, the positive staining was weaker in each group (Fig. 7).

Furthermore, we observed a lot of positive differentiation of good morphology of cartilage cells even after 2 weeks of intra-articular injection of ALK5-transfected bone marrow mesenchymal stem cells into articular cartilage, suggesting

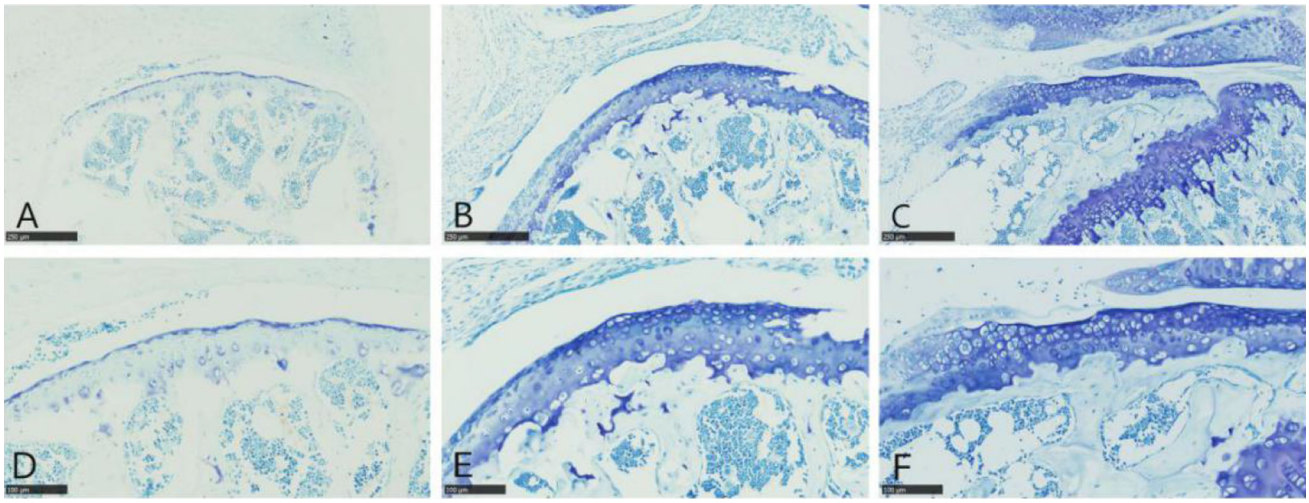

Fig. 5 Toluidine blue staining of the mouse articular cartilage in each group after 8 weeks of injection (scale bar = 100 μm). **a**, **d** show low and high magnifications of the articular cartilage in the saline group, and the chondrocytes were arranged in clusters. **b**, **e** are the bone marrow mesenchymal stem cell group. The cartilage cells decreased, the cartilage layer became thin, and the staining became shallow. **c**, **f** are the results of the staining of articular cartilage in the bone marrow mesenchymal stem cells with ALK5 transfection. The chondrocytes decreased slightly, and the cartilage layer became thinner

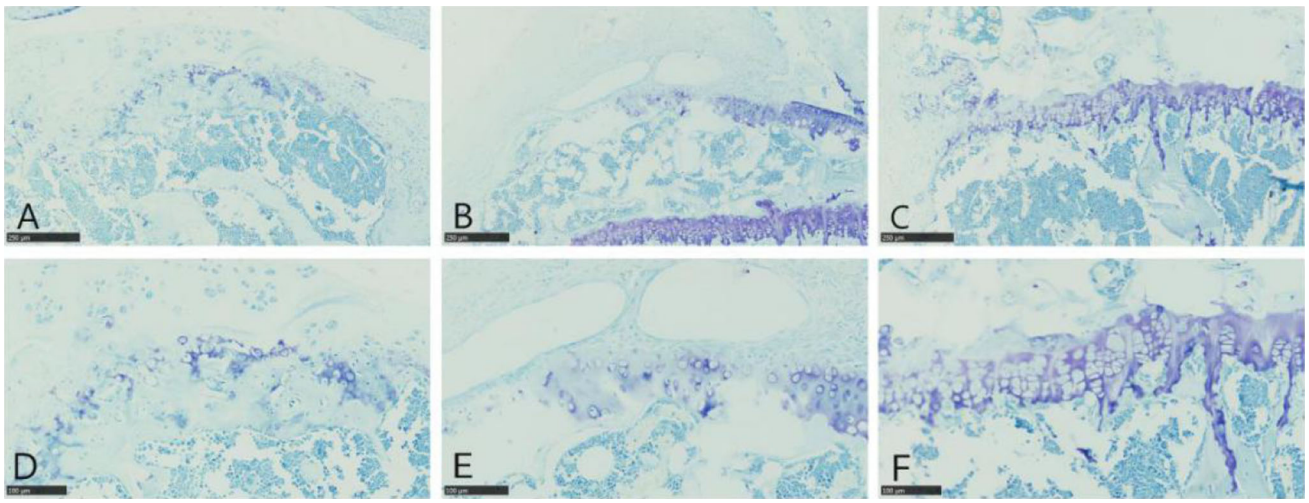

Fig. 6 Toluidine blue staining of the mouse articular cartilage in each group after 12 weeks of injection (scale bar = 100 μm). **a**, **d** are the saline group, and the cartilage layer was removed. **b**, **e** are the articular cartilage in the bone marrow mesenchymal stem cell group, and the whole layer of chondral cartilage was found. **c**, **f** are the bone marrow mesenchymal stem cells with ALK5 transfection and show a better proliferation of chondrocytes

Table 3 Modified Mankin scores of the mouse knee samples by toluidine blue staining

Group	4 weeks after injection	8 weeks after injection	12 weeks after injection
Saline group	3.27 ± 0.18	7.60 ± 0.24	12.20 ± 0.26
Simple bone marrow mesenchymal stem cell group	1.90 ± 0.46^{a}	5.91 ± 0.46^{a}	8.10 ± 0.76^{a}
ALK5-transfected bone marrow mesenchymal stem cell group	1.10 ± 0.35^{a}	3.10 ± 0.43^{ab}	6.73 ± 0.52^{ab}

Compared with physiological saline group, $^{a}P < 0.05$; compared with bone marrow mesenchymal stem cell group, $^{b}P < 0.05$

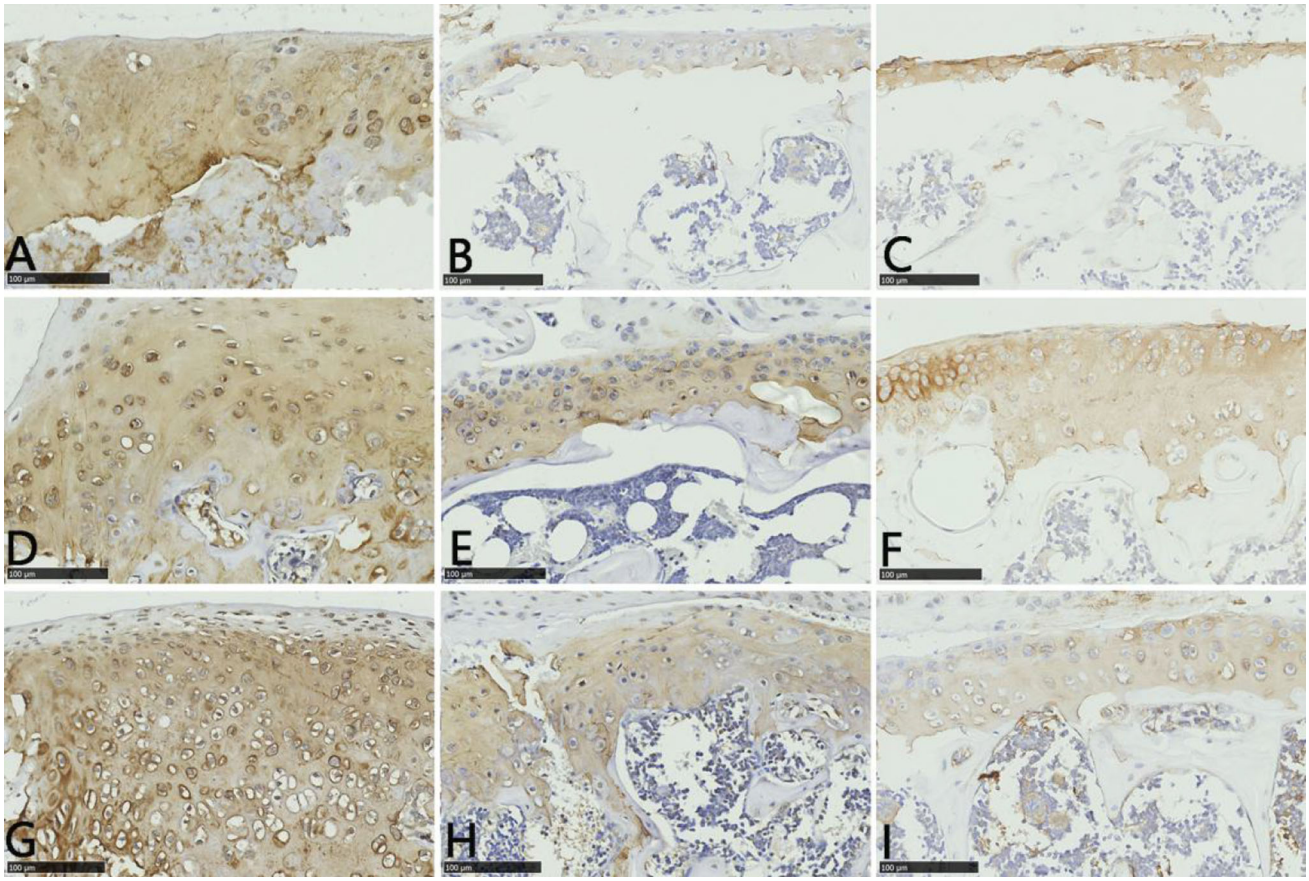

Fig. 7 Collagen II immunohistochemical staining of the articular cartilage sections after 4, 8 and 12 weeks of injection (scale bar = 100 μm). **a–c**, respectively, show the collagen staining of the articular cartilage after 4, 8 and 12 weeks of injection of saline group, and **d–f** are the results of the collagen staining of the articular cartilage after 4, 8 and 12 weeks of injection with bone marrow mesenchymal stem cells. **g–i** show the collagen staining results of ALK5-transfected group after 4, 8 and 12 weeks of injection into the articular cartilage. With the increase in injection time, the positive colorization of each group decreased, but ALK5-transfected cells decreased slower compared with the other two groups

the continuous differentiation capacity of ALK5-transfected bone marrow mesenchymal stem cells into cartilage cells (Fig. 8).

The trabecular bone in saline group appeared to be not uniform, wide and dense as well as "fusion." In contrast, the subchondral trabecular bone texture in bone marrow mesenchymal stem cells group and ALK5-transfected bone marrow mesenchymal stem cells group was clear. And there even were obvious reticular cross sections in ALK5-transfected bone marrow mesenchymal stem cells group, as shown in Fig. 9. After 12 weeks of intra-articular injection, the trabecular bone in saline group was dense and disorganized. And the joint medial edge showed different degrees of oval or circular osteophyte formation. As to the bone marrow mesenchymal stem cells group, the subchondral bone trabeculae increased and the dense mesh structure appeared. In terms of ALK5-transfected bone marrow mesenchymal stem cells group, the subchondral bone trabecular bone was irregular in shape, and the distribution became inhomogeneous, widened and dense as well as "fusion."

Discussion

The introduction of tissue engineering to the treatment of osteoarthritis is a hot topic at present, in which the acquisition of seed cells and the maintenance of physiological characteristics are a difficult problem. Bone marrow mesenchymal cells have the characteristics of strong self-renewal and multidirectional differentiation and are easy to obtain, stable in methods, and easy to be cultured and amplified in vitro. The results of the existing experimental data indicated that intraventricular injection of bone marrow mesenchymal stem cells could promote the regeneration of chondrocytes [23–27]. At the same time, using bone marrow mesenchymal stem cells to prevent osteoarthritis cannot only affect tissue repair in

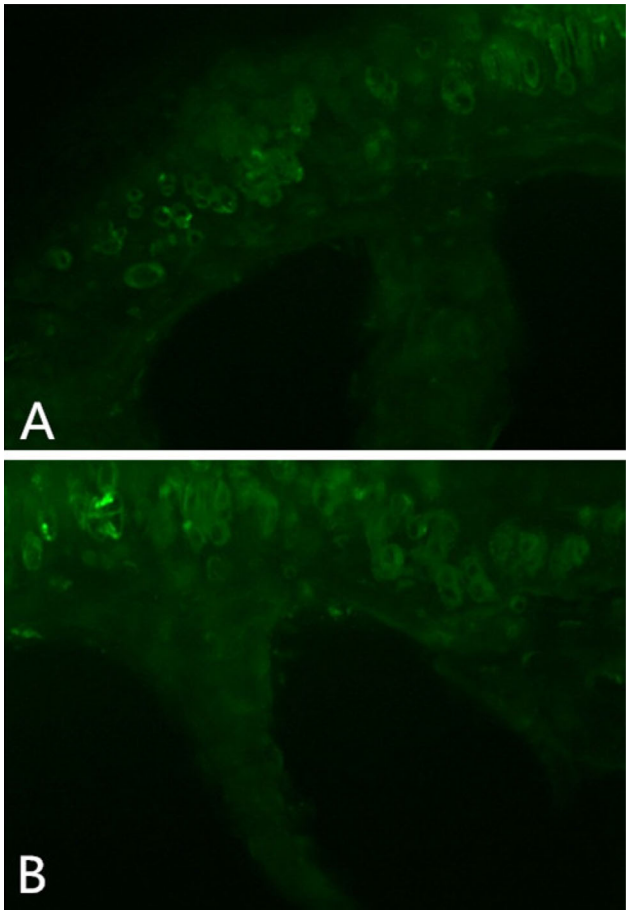

Fig. 8 Morphologies of ALK5-transfected bone marrow mesenchymal stem cells are shown by immunofluorescence staining after 1 and 2 weeks of injection (× 200); positive chondrocytes could be seen at 1 and 2 weeks after injection of ALK5-transfected bone marrow mesenchymal stem cells

structure, but also perform effective immune modification and anti-inflammatory.

Transgenic mice research shows that the TGF-β signaling pathway plays an important role in OA evolution. It owns the ability to promote the differentiation of osteoblasts into osteoblasts and angiogenesis in subchondral bone, stimulating synovial-associated cell proliferation and synovial [28]. TGF-β signaling pathway is a superfamily containing many members, which regulates cell growth, proliferation, differentiation, migration and apoptosis. However, some negative effects of TGF-β on cartilage have already been reported by other reports [29–34]. Injection of high-dose TGF-β1 into the joint can induce chemotaxis and activation of inflammatory cells, leading to typical cartilage defects, such as fibrosis and osteophyte formation. Also, in vivo experimental studies showed that TGF-β in chondrocytes mediates Smad2/3 signal through classical type I receptor ALK5, as well as Smad1/5/8 pathway through ALK1 receptor [35]. With the growth of age, the decrement of ALK5 was much faster as compared with ALK1, resulting in the differentiation of chondrocytes into the hypertrophic phenotype of osteoarthritis [36–38]. These studies might indicate that the decrease in ALK5/ALK1 ratio had broken the signal balance of TGF-β, so TGF-β signal was biased toward Smad1/5/8 signaling pathway, which made chondrocyte terminal differentiation and aggravated the process of cartilage arthritis.

TGF-β plays a role in organisms in multiple groups [36]. Previous studies have shown the expression of MMP13 was increased with the transfection of chondrocytes, leading to the overexpression of ALK1. As a result, the expression of cartilage cells on collagen-induced degeneration of cartilage cells could be inhibited. Meanwhile, the experimental results by inhibiting the expression of ALK5 at the target site have also been reported. The current study has not yet been used to observe the therapeutic effect of high expression of ALK5 gene in the body of osteoarthritis in the body.

The ALK5-activated ALK5 T204D eukaryotic expression plasmid Flag was constructed in the current work, and it was further transfected into bone marrow mesenchymal stem cells. Afterward, through the combination of good seed cell and gene engineering technology, the transfected cells were injected into the knee joint cavity. This strengthens target growth factor expression in cells, and the concentration reached a stable value [37]. And finally, the observation in cartilage repair effect was accomplished. Firstly, the effects of ALK5 gene transfection on bone marrow mesenchymal stem cells were evaluated by HE staining, toluidine blue staining of articular cartilage and improved Mankin score. The results showed that the bone marrow mesenchymal stem cells after the overexpression of ALK5 gene were better than bone marrow mesenchymal stem cells for the repair of diseased cartilage. But later, this advantage decreases. It suggested that excessive expression of ALK5 inhibits the terminal differentiation of chondrocytes and causes the dysfunction of cartilage function. Based on the immunohistochemical results, it showed that the expression of type II collagen in ALK5-transfected bone marrow mesenchymal stem cell group was stronger than that without modification. This indicated the overexpression of ALK5 gene after bone marrow mesenchymal stem cells in the specific effect on the repair of articular defects and effect. And these results were well consistent with the previous results [35,36]. It was worth mentioning that articular cartilage lesions gradually increased even in ALK5 bone marrow mesenchymal stem cells-transfected group, as demonstrated in micro-CT results. These results suggested that the osteoarthritis mechanism underlying a variety of factors work together. And the appropriate proportion of ALK5/ALK1 was also emphasized for the treatment of osteoarthritis.

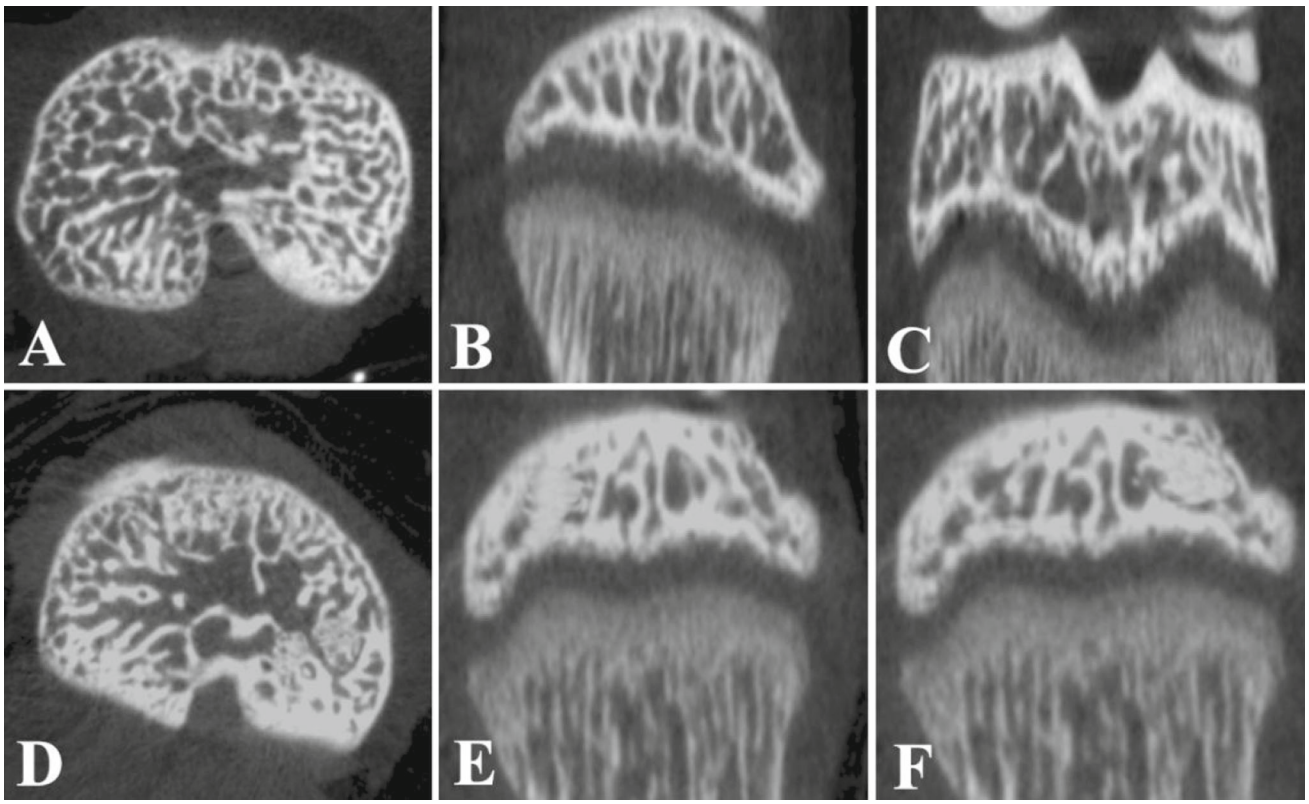

Fig. 9 Micro-CT of articular cartilage after 4 and 12 weeks of injection, **a**, **d** are the micro-CT cross-sectional images after 4 and 12 weeks of injection with bone marrow mesenchymal stem cells of ALK5 transfection. **b**, **e** are the micro-CT coronal images of bone marrow mesenchymal stem cells after 4 and 12 weeks of injection. **c**, **f** show the coronal plane image of micro-CT after 4 and 12 weeks of injection of saline

Conclusion

In summary, we demonstrated that ALK5 transfection of bone marrow mesenchymal stem cells could be a promising stem cell therapy for repair of cartilage lesions. And the positive relationship with age ALK5 degeneration through TGF-β signaling pathway can be used as an important way to prevent senile OA.

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