Anatomical basis and physiological and biochemical changes of knee joint meniscus injury

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Abstract

The meniscus is a meniscus-shaped fibrocartilage tissue located between the femur and tibia， it is mainly composed of meniscus cells and related extracellular matrix.The synovial limbus area of the knee joint capsule near the meniscus is divided into red areas with rich blood vessels and white areas with less blood supply according to the distribution of blood vessels,there is a transition zone called the red and white zone between the two;Red zone has better self-repair ability,The injury in this area can be treated by conservative treatment or surgical suture;Once the white area of the meniscus is torn and involves the free edge area,It is often necessary to partially remove the damaged meniscus.When most of the entire meniscus is severely torn and involved,not only the course of the disease is very long, but it cannot be repaired by sutures,Often a subtotal or total meniscus resection is required,whether it is a partial meniscus resection, a subtotal meniscus resection or a complete resection.In the later period, it may cause quadriceps atrophy and osteoarthritis (OA）.OA is a refractory multi-system disease,involve the patient’s peripheral joints,it has high disability and teratogenicity, and is very harmful to human health.Chondrocyte pyrolysis, degradation, and inflammation play a vital role in the destruction of OA articular cartilage and chondrocyte apoptosis.Meniscus stem cells have strong proliferation and differentiation ability,has become one of the hot spots in the field of meniscus repair,this article studies the role of meniscal stem cells in the development of OA.

Keywords Meniscal injury, anatomical basis, physiological changes, biochemical changes

Introduction

Meniscus is the fibrous cartilage plate in the knee joint,the inside is a “C”shape and the outside is an “O”shape.The outer edge of the meniscus is thick, the inner edge is thin and concave, which is composed of collagen fibers, is an important part of the structure and function of the knee joint.It has the properties of relieving vibration,

lubricating joints, reducing friction, increasing stress area, conducting load and maintaining the stability of knee joint[1-4].Trauma or natural aging can make meniscus appear different degrees of deformation, and then cause surrounding tissue edema, proliferation, make the knee joint lose normal function and stability, accelerate the degeneration of articular cartilage, and further lead to the occurrence of OA[5].Meniscus injury is closely related to the occurrence and development of OA.Meniscus injury can be divided into degenerative disease and tear，the long time of degeneration will lead to tear under the action of external force. Tear will also promote the degeneration of meniscus tissue, form a vicious circle, and induce knee osteoarthritis;Meniscus injury is also common in OA patients[6-7].The meniscus is mainly composed of meniscus cells and related extracellular matrix （ ECM ）[8].Meniscus can be divided into red area, red white area and white area according to whether there are blood vessels[9]： there are blood vessels in the outer edge of meniscus, which is called red area and has good healing ability.There are a few blood vessels in the red and white areas, which are located between the outer and inner edge areas, and have a certain healing ability.There is no vascular distribution in the inner margin of meniscus, which is called white area. Because of lack of blood supply, nutrition comes from synovial fluid of joint[10]， therefore, it is difficult to heal after injury and prone to degeneration , which is one of the important inducements of knee osteoarthritis[11].The catabolism of meniscus stem cells (me SCs) is a major cause of OA induced by meniscus injury.As meniscus derived mesenchymal stem cells, Me SCs can produce a large number of extracellular matrix and meniscus fibrochondrocytes, enhance the regeneration ability of injured tissue, and inhibit the process of OA[12,19].The metabolic balance of extracellular matrix is mainly regulated by catabolic enzymes produced and released by chondrocytes, including matrix metalloproteinases (mmp_s) and polyprotein polysaccharide enzymes (adamts).Me SCs catabolism plays a vital role in OA pathogenesis [13].

Inflammation is another major cause of OA induced by meniscus injury.Studies have shown that [14], compared with normal cartilage,in Me SCs cells，

The expression of IL-1、IL-1β、IL-6、TNF-α、HIF-2α、caspase-1 and MMPs and ADAMTS mRNA were significantly up-regulated, which accelerated cartilage degeneration.

Meniscus degeneration is the main pathological change of meniscus injury and OA, which is closely related to the programmed death of Me SCs.Me SCs and its secreted ECM components (such as collagen 、 proteoglycan, etc.) are important components that constitute and maintain the normal function of articular cartilage.Me SCs the formation of new cartilage matrix components, meniscus can maintain structural and functional stability based on the dynamic balance of cartilage matrix synthesis and catabolism.During OA pathology,Me SCs cell pyroptosis and excessive loss of ECM contribute to OA articular cartilage degeneration [15] .Pyroptosis is an inflammatory programmed cell death mode discovered by Cookson et al. [16] in 2001.Caspase-1/11 activation and release of a large number of proinflammatory cytokine IL-1、IL-1β、IL-6、TNF-α are typical features of pyroptosis[17].The aim of this study was to investigate the potential protective effect of Me SCs in rat knee meniscus injury induced OA model (**Figure** *1*).

Materials and Methods

Experimental materials.20 male rats SD 2 months old,weight（250±10）g，Randomly divided into knee meniscus injury group （ MI group,n=10 ） and Healthy control group(HC group,n=10).The experimental rats were provided by the experimental animal center of Yan'an University, and the experiment was approved by the experimental animal ethics committee of Yan'an University.

Model preparation.The experimental animals were anesthetized by intraperitoneal injection of 4% Pentobarbital Sodium (40 mg / kg).After the anesthesia took effect, the experimental rats were supinely fixed on the animal table, the operation area was strictly disinfected with iodophor, the disposable sterile towel was spread, the incision of about 1 cm size was cut along the medial edge of the patellar ligament of the right hind limb of the rat, the knee joint was exposed layer by layer, the medial meniscus of 80% of the right hind limb was removed, and the joint capsule and skin were sutured

layer by layer after careful hemostasis and washing.The experimental animals were raised in cages and the knee joints were not fixed. Selective treatment of anti-infective drugs according to incision, daily observation and detailed record.

Pathological changes of meniscus.HE staining: all rats were all rats were killed, the posterior horn of the medial meniscus of the right hindlimb of each group was cut, the surrounding tissue was carefully removed ,0.9% sodium chloride saline was washed and fixed in the fixed solution for 24 h, after decalcification. Dehydration from low concentration to high concentration.The tissue was completely immersed in paraffin and then cut into sections after condensation. The slices were pasted in warm water for HE staining 、 Safranin O / fast green staining （ SO staining ） 、 Immunohistochemical detection and other related tests.The samples were dewaxed with xylene II and I step by step, then dehydrated with alcohol from high concentration to low concentration, and rinsed with a steamed water.Hematoxylin staining for 5 min,use tap water slow washing back blue 0.5% hydrochloric acid alcohol differentiation 30 s, tap water immersion 0.5% eosin staining 1~4 min, use a steamed water rinse.After dehydration of ethanol and xylene, the film was sealed and observed.Measurement of cartilage thickness: the whole layer cartilage sections of the medial femoral condyle in each group were cut 3 mm, fixed by 10% formaldehyde, dehydrated, decalcified, paraffin fixed, HE stained, and the cartilage thickness was measured under microscope. The average values of each group were calculated.

Micromass culture, Alcian blue and Safranin O staining. 20 ul of cell suspension at a density of $1x10^6$ cells/mL was pipetted into the 4-well culture plate, chondrocytes were placed in the incubator for 3 h to allow adherence. 0.5 ml of fresh complete DMEM medium containing IL-1β (10 ng/mL) and Cur (10, 20 and 50 μM) or Cur (10, 20 and 50 μM) alone to the culture plate. After 14 days, wells were fixed for 20 min with 0.5 ml of 10% neutral buffered formalin. Subsequently, 0.5 ml of 1% Alcian blue and 1% Safranin O solutions were added to each well for 30 min at room temperature. After washes with 1 × PBS, images of the stained cell mass were obtained by a scanner.

Observation on physiological characteristics of animals

Observation of thigh, leg circumference, meniscus thickness and atrophy degree of quadriceps femoris muscle in right hind limb of 2 groups.

The thigh circumference、leg circumference、meniscus thickness and quadriceps atrophy were measured in HC group and MI group.Because the thighs of rats are shorter than those of humans, the circumference of the thighs is measured 5 cm above the patella.Measure the circumference of the calf at the widest position of the calf. These measurements can compare the thickness of the meniscus and the atrophy of the quadriceps femoris muscle.

Evaluation of quadriceps atrophy by modified Ashworth (MAS) grading method *(Table 1)*

Maximum flexion of knee joint.The rats were anesthetized and kept in supine position. At this time, the maximum degree of knee flexion is the maximum degree of knee flexion. This measurement can help to observe the changes of knee joint motor function before and after operation.

Front drawer test.Anterior drawer test (ADT test) is a common technique for examining anterior cruciate ligament injury.The anesthetized rats were fixed on the back of the animal table, bent 90 degrees, fixed the distal side of the calf, pulled the proximal side of the calf forward, and the movement score was as follows:(-)， Bilateral tibia moving forward;(1+)， The injury tibia movement is bigger than the healthy tibia movement <5 mm;(2+)，tibial movement 5-10 mm;(3+)，>10 mm，tibial movement.(-) suggested that the stability of ipsilateral knee joint was normal, and other scores suggested that the ipsilateral knee joint was unstable. The higher the score, the greater the degree of instability.

Lachman test.Anesthesia rats were fixed on the back of the animal table and bent 25° for drawer test. The operator grabs the thigh with one hand and the upper tibia with the other. When the muscles relax, pull the tibia forward and pay attention to the scale of moving forward. The scoring system is the same as the front drawer test.

Pivot shift test.Fix the anesthetized rat elevation on the animal table, hip

abduction, tibial rotation, or neutral position, flexion 20°, the doctor holds the upper leg with both hands, muscle relax, The examiner was allowed to bend and reset the knee with axial compression and valgus stress after anterior cruciate ligament injury.the knee joint of the anesthetized rats was fully extended. The examiner held the proximal side of the rat's leg with one hand and the rat's paw with the other hand to rotate the calf. With the gradual rotation, the knee joint bends from 0 degrees, and the knee joint moves from the "lock" position to the subluxation. When the knee joint flexion to 20 degrees, instability appeared as a positive result, indicating instability of the knee joint.After all the above measurements and tests, all rats were kept as usual.

Neurophysiological testing.MI group were measured and detected to observe the changes of anterior cruciate ligament nerve. assessed by electrophysiology including SEPs and MCV. SEPs and MCV. measured by latency and amplitude the latency was prolonged and the amplitude was decreased, suggesting nerve injury and proprioceptive decline.

MI group rats were evaluated three times (1/2 weeks), and HC group also received one time as a contrast. The methods of evaluation are as follows.

Determination of SEPs.Measured at room temperature (26℃) and stimulated the bilateral skin of the ACL attached to the bipolar surface electrode. The stimulation parameter is constant voltage (single wave electric stimulation, wave width 0.1 ms, stimulation intensity 15~20 mA).measured at room temperature (26℃) and stimulated the bilateral skin of the ACL attached to the bipolar surface electrode. The stimulation parameter is constant voltage (single wave electric stimulation, wave width 0.1 ms, stimulation intensity 15~20 mA).

Determination of MCV. The recording electrode was placed in the abdomen of hamstring muscle, and the reference electrode was placed at the recording electrode, 2 cm at room temperature (26 ℃).Then the bilateral areas corresponding to the attached ACL were stimulated with bipolar surface electrodes. The stimulation parameters were constant voltage (single square wave electrical stimulation, wave width 0.1 ms, frequency 2 Hz, stimulation intensity 25 ~ 30 MA).The waveform and latency of MCV were measured and analyzed.The above physiological data were collected and

all rats were tested 3 times. all measurements were performed by the same technician in a rat anesthetic state.

Me SCs Vitality test

Me SCs separation, culture.In previous studies, the main materials for extraction of mESCs are human meniscus fragments [6] and meniscus of animals (cattle [7], rabbits [8,9], sheep [10]).The steps are as follows:The other tissues adhered to the meniscus surface were first removed, and the meniscus tissue was cut into 1 mm×1 mm after rinsing with a PBS containing double resistance,at 37 ℃ for 4-6 h in PBS solution containing collagenase type I and II,after centrifugation, the supernatant was discarded, counted under the microscope, and then resuspended in the same volume of DMEM medium containing 10% FBS to stop digestion,after counting under microscope, the cells were resuspended in DMEM medium containing 10% FBS to stop digestion,Filter cell suspension with 200 mesh sieve, centrifuge 1,200 r/min for 5 min,, discard supernatant,PBS washed three times,the cells were counted under microscope and then resuspended in complete medium (low sugar DMEM, 10% FBS, 3.7 g / L $NaHCO_3$, 100 U / ml penicillin, 100 μ g / ml streptomycin, 25 ng / ml amphotericin B, 2 mmol / l l L-glutamine) according to 1 × 10^6 / bottle (25 cm^2 medium). The cells were incubated at 37 ℃ in an incubator containing 5% CO_2 and 95% humidity,48~72 h ，wash the unadherent cells with the PBS and replace the new complete medium.When the adherent cells reached 80% ~ 90% of the bottom of the cup, they were digested with 0.25% trypsin and passaged at the ratio of 1:2 or 1:3. When the cell morphology was uniform and slender, MES CS were successfully extracted.

Immunofluorescence microscopy. Chondrocytes (1×10^4/mL) were grown in a 6-well plate containing glass slides and cultured with. complete DMEM medium containing IL-1β (10 ng/mL) and Cur (10, 20 and 50 μM) or Cur (10, 20 and 50 μM), cells were fixed with the 90% ethanol for 30 min, after blocking, chondrocytes were incubated with primary antibody against NF-κB NF-κB (1:200; 8242; CST) and HIF-2α (1:1000; D6T8V ， CST) at 4°C overnight. After incubating the secondary

fluorescein-conjugated goat anti-rabbit antibody (1:200 ， ZF0311 ， OriGene Technologies, Maryland, USA) at room temperature for 1 h, images of stained cells were viewed and obtained using a fluorescence microscope (AXIO Vert.A1 ， magnification, x 400).

Western blotting. Chondrocytes (2×10^5 cells/well) were cultured in 6-well plates, for the time and mode of action of drugs in medium, the protocol of exactly the same as "immunofluorescence" Proteins were lysised determined by BCA method. Proteins (20 μg/lane) were separated by 10 % SDS-PAGE and blotted onto PVDF membranes (Merck). The membranes were blocked with 5% BSA for 1 h at room temperature, the proteins were sequentially probed with primary antibodies SOX9 (1:1000; 82630; CST), NF-κB (1:1000; 8242; CST), pNF-κB (1:1000; 93H1; CST), IκB (1:100; 44D4; CST), pIκB (1:1000; 14D4; CST), HIF-2α (1:1000; D6T8V; CST), MMP9 (1:1000; 13667; CST), ADAMTS5 (1:1000; PAB26036; AmyJet Scientific), iNOS (1:200; 13120S,CST), COX2 (1:200; BA3708; Boster) and GAPDH (1:1000;5174; CST) overnight at 4°C and Anti-rabbit/mouse IgG, HRP-linked antibody (1:3000; 7074/7076; CST). Blots were determined by an chemiluminescence kit (Thermo Fisher Scientific, USA), the images were captured by ChemiDoc XRS Imaging System (Bio-Rad) and analyzed using Image Lab 5.2.1 software.

Real time quantitative-PCR(q-PCR). Cell grouping and settings of "q-PCR" was the same as "Western blotting". Total RNA was extracted from chondrocytes using TRIzol reagent (Thermo Fisher Scientific). 1μg of total mRNA was used to synthesize cDNA in total volume of 20 μl of reaction solution using a PrimeScript RT Master Mix kit (RR036A, TaKaRa). TB Green Premix ExTaq II (RR066A, TaKaRa) was used to detect the mRNA of all genes in qTOWER 2.2 real time PCR sysem (Analytik Jena,German). Primer sequences used were as follows: SOX9 forward, 5'-GTGCAAGCTGGCAAAGTTGA-3'andreverse, 5'- TGCTCAGTTCACCGATGTCC -3'; Col2α forward, 5'-GGTGAGCCATGATCCGCC -3' and reverse, 5'-TGGCCCTAATTTTCGGGCATC-3'; AGG forward, 5'-CGTTGCAGACCAGGAGCAAT-3' and

reverse,5'-CTCGGTCATGAAAGTGGCG-3'; and MMP9 forward, 5'-GTACTCGACCTGTACCAGCG -3' and reverse, 5'-AGAAGCCCCACTTCTTGTCG -3'. ADAMTS5 forward, 5'-AAGAGGAGGAGGAGGAGGAGGAG-3' and reverse, 5'-AATGGTTGTGAGCTGCCGTATGG-3'; β-actin forward, 5'-GTTGTCGACGACGAGCG-3' and reverse, 5'-GCACAGAGCCTCGCCTT-3'. COX2 forward, 5'- AACCGAGTCGTTCTGCCAAT -3' and reverse, 5'-CTAGGGAGGGGACTGCTCAT -3'; iNOS forward, 5'- GCCCAGCCAGCCCAAC -3' and reverse, 5'- GCAGCTTGTCCAGGGATTCT -3'. The $2^{-\Delta\Delta Ct}$ method using β-actin as the control was used to analyze the expression of genes (24).

Statistical analysis. Data from more than 3 sets of repeated trials of multiple groups were presented by means ± standard deviation (SD) and analyzed by One-way ANOVA. $P < 0.05$ was considered statistically significant.

Rusults

Histopathological observation of meniscus

The general morphology and pathological changes of meniscus, international society for osteoarthritis research (ICRs, II) score of the 2 groups were observed.

HC group： HE staining: meniscus showed dense tissue, normal shape of chondrocytes, no inflammatory cells, collagen fiber bundles arranged orderly, dense connective tissue characteristics(*Figure 2A*)； ***OS staining:***There was no abnormal change of meniscus, the anatomical structure of meniscus was complete, because of the existence of synovial fluid, the surface of meniscus was bright white, very smooth and moist, the surface was flat and had high elasticity(*Figure 2B*)，thickness is normal（*Table 2-1*).

MI group： HE staining:The surface structure of meniscus is rough and uneven, the smoothness is obviously reduced, the local subsidence occurs, the collagen fiber is loose, the tissue sequence is damaged, the thickness is uneven, and a large number of inflammatory cells infiltrate(*Figure 2C*)； ***OS staining:***There are obvious wear marks on the free edge of meniscus, and the surface of meniscus is rough and uneven, the

color is yellow, dark yellow, the toughness and elasticity are very poor, the touch is broken, and the degree of tension decreases obviously(*Figure 2D*) ， loss of cartilage thickness is evident（*Table 2-1*).

The difference between MI group cartilage thickness and HC group cartilage thickness was statistically significant($P<0.05$ or $P<0.01$).

HC group and MI group rat body weight、 thigh and calf circumference、 maximum knee flexion、 anterior drawer、 Lachman and pivotal displacement test、 meniscus thickness and quadriceps atrophy are all significant difference($P<0.05$ or $P<0.01$)（*Table 2-1*、*2-2*、*2-3*）.

Neuroelectrophysiological test of 2 groups of rats.

Comparison with MI group， MI group SEPs and MCV latency increased significantly(P<0.05 or P<0.01)， the amplitude is significantly reduced(*P<0.05 or P<0.01*)， the results suggest that knee joint nerve injury, functional impairment and proprioceptive decline(*Table 3*).

Biochemical results

Vitality of meniscus stem cells（Me SCs） in two groups of rats、Me SCs cell IL-1、IL-1β 、 IL-6 、 Tumor necrosis factor-α(TNF-α) 、 hypoxia-inducible factor 2a（HIF-2α）、caspase-1 mRNA and protein expression.

MTS assessment Me SCs vitality,the results show that:Comparison with HC group， MI group Me SCs activity declined significantly,Me SCs concentrations are:HC group>MI group(*Figure 3A*，P<0.05).

The mRNA expression of IL-1、IL-1β、IL-6、TNF-α、HIF-2α in Me SCs cells was detected by ELISA and Western-blot. The results showed that:IL-1 concentrations are:HC group<MI group(*Figure 3B*);IL-1β concentrations are:HC group<MI group(*Figure 3C*)；IL–6 concentrations are:HC group<MI group(*Figure 3D*)；TNF-α concentrations are:HC group<MI group（*Figure 3E*)；HIF-2α concentrations are:HC group<MI group(*Figure 3F*);caspase-1 concentrations are:HC group<MI group(*Figure 3G*);The above results are all P<0.05, and the differences are all statistically significant.

mRNA and protein expression of Me SCs extracellular matrix metalloproteinases (MMPs)、ADAMTS.

ELISA and Western-blot detection of mRNA and protein expression in extracellular MMPs、ADAMTS,the results show that:MMPs concentrations are:HC group<MI group(*Figure4A*);ADAMTS concentrations are:HC group<MI group(*Figure 4B*).

Comparison of pyrolysis of Me SCs cells in two groups of rats

Flow cytometry the results show,Comparison with MI group， MI group **Me SCs**Cellular pyroptosis increased significantly(*Figure5A,P<0.01*)；qRT-PCR detects Me SCs pyroptosis,Comparison with HC group， MI group Me SCs cell pyroptosis increased significantly(*Figure5B,P<0.01*).

Discussion

Meniscus is a kind of fibrocartilage board, which has various complex functions such as relieving stress, reducing shock, stabilizing joints, lubricating joints, reducing friction and knee joint proprioception[18].The degeneration, destruction and hyperosteogeny of articular cartilage are the main pathological features of meniscus injury.The HE staining in this study showed that: The cartilage surface is smooth and flat, the structure is clear, the tide line is neat, the cells of each layer are arranged neatly and evenly, and there is no obvious inflammatory cell infiltration of HC group;The cartilage surface is rough and thin, cracks appear, the tide line is broken and irregular, the number of cells in each layer is significantly reduced and the arrangement is disordered, accompanied by a large amount of inflammatory cell infiltration of MI group The cartilage surface is rough and thin, cracks appear, the tide line is broken and irregular, the number of cells in each layer is significantly reduced and the arrangement is disordered, accompanied by a large amount of inflammatory cell infiltration, which is consistent with previous studies[20].

Studies have shown that in the acute phase of OA inflammation, inflammation leads to the local release of pain-causing inflammatory mediators, such as bradykinin, 5-OH, histamine, H^+, K^+, purines, prostaglandins, leukotrienes and neuropeptides,

which may change the damage Ion conduction at the outer end of the receptors directly or indirectly enhances the sensitivity of nociceptors, causing corresponding changes in the physiology and electrophysiology of the knee joint [21].This experiment shows:A lot of inflammatory mediators in the Me SCs,Stimulation causes atrophy of quadriceps femoris， this causes the circumference of the rat's thigh and calf to decrease、Maximum flexion of knee joint（+）、 Front drawer test（+）、Lakman test（+）、pivot shift test（+）and SEPs extension and MCV reduction，Knee motor dysfunction、nerve damage、Proprioception drops and other changes.

The above results suggest that： OA inflammatory pain caused by meniscus injury,Causes of physiological and electrophysiological changes in the knee joint of rats may be that inflammation promotes the expression of peripheral glial nerve growth factor (NGF),there may be a role of NGF, which is worthy of our further study.

Pyrolysis is a newly discovered mode of cell death,cell swelling and lysis,cell membrane dissolution (extracellular release of cytoplasmic contents)，at the same time, chromosomal DNA fragmentation is characteristic of pyroptosis,it is also a new death pattern different from apoptosis and cell necrosis.Cell membrane dissolution leads to the release of a large number of inflammatory factors, such as IL-1、IL-1β、IL-6、TNF-α、HIF-2α, to the extracellular,these factors activate cytokines caspase-1, induce Caspase-1- dependent classical pyroptosis [21]。

Study shows [22],IL-1β play an important role in the pathogenesis of OA,IL-1β play an important role in the pathogenesis of OA,promote cartilage fibrosis and so on.IL-1β stimulates chondrocytes and synovial cells to produce MMPs and ADAMTS,MMPs and ADAMTS reduce collagen II and proteoglycan in the matrix,accelerating the destruction of articular cartilage;MMPs can be divided into six categories:collagenase、 gelatinase、 matrix lysates、 MMPs、 membrane matrix metalloproteinases and other types.Major include： MMP-1 、 -8 、 -13and -18 etc.MMP-13 also known as collagenase 3， studies have shown,the high expression of MMP-13 in patients with OA suggests that increased MMP-13 is related to cartilage

degradation [23,24].This study found that,MMPs-13 outside Me SCs is highly expressed in MI group, suggesting that MMPs-13 is involved in tissue matrix homeostasis and repair, and has corresponding temporal and spatial activity in tissue growth, repair and remodeling.

Studies have shown that [25] ADAMTS family also plays an important role in the pathogenesis of OA.In particular, MMP-13, like ADAMTAS-5, plays an important role in the degradation of cartilage extracellular matrix [26] .ADAMTS-5 is one of the most important enzymes to degrade proteoglycan,it plays an important role in the degradation of cartilage proteoglycan.This study found that,ADAMTS-5 outside Me SCs is highly expressed in MI group,it is suggested that ADAMTS-5 plays an important role in the degradation of ECM of articular cartilage.

Cartilage tissue consists of chondrocytes and ECM,the specific composition is shown in the figure below（*Figure 6*）.ECM synthesis and degradation under normal physiological conditions,during the occurrence and development of OA,ECM dynamic equilibrium is broken,the living environment of cartilage cells is destroyed [26,27] .Meniscus stem cells have colony formation,“Dry”features such as self-proliferation and multi-directional differentiation potential,and expressed multiple mesenchymal stem cell markers,has become one of the research hotspots in the field of meniscus repair [28] .Me SCs can divide into meniscus chondrocytes,this experiment found that:MI group Me SCs the number of cells decreased and the arrangement was disordered,accompanied by a large number of inflammatory cell infiltration in Me SCs cells,at the same time, Me SCs extracellular MMP-13 and ADAMTAS-5 are highly expressed,cause the ECM dynamic equilibrium state to be broken.It suggests whether we can consider using Me SCs as intra-articular injection cells or seed cells to treat meniscal tears as a research focus.

But the damage caused by meniscus injury is greater than that caused by repair, the study needs to be further refined, including: ①Clarify the specific mechanism of Me SCs inhibiting local inflammation, protecting joint surfaces, and effectively preventing OA;②All experimental rats are male, and the influence of sex hormones is indeed uncertain.In order to ensure the scientificity of the experimental results, the

next step is to select rats of two sexes for research;③Further study the mechanism of OA occurrence and development, and provide objective experimental basis for better treatment of osteoarthritis.

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Declaration of interest statement

None.

Ethics statement

The Ethics Committee of Yan'an University (Shaanxi , China) approved this study.

Data Availability Statement

The datasets used during the current study are available from the corresponding author on reasonable request.

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Figure Legends

Figure 1. Mechanism pattern of osteoarthritis induced by meniscus injury. (From:Lewinson RT, Madden R, Killick A, et al. Foot structure and knee joint kinetics during walking with and without wedged footwear insoles. J Biomech. 2018;73:192-200. doi:10.1016/j.jbiomech.2018.04.006）

Figure2.Gross morphology of meniscus in 2 groups of rats.（×200）（A）.Normal meniscus HE staining；（B）.Normal meniscus OS staining；（C）.Damage meniscus HE staining；(D).Damage meniscus OS staining.

Figure3.The viability of rat Me SCs, the mRNA and protein expression of IL-1、IL-1β 、 IL-6 、 TNF-α 、 HIF-2α within the cells.(A).MTS test Me SCs vitality;(B).ELISA and Western-blot detection Me SCs intracellular IL-1 expression;(C).ELISA and Western-blot detection Me SCs intracellular IL-1β expression;(D).ELISA and Western-blot detection Me SCs intracellular IL-6 expression;(E).ELISA and Western-blot detection Me SCs intracellular TNF-α expression;(F).ELISA and Western-blot detection Me SCs intracellular HIF-2α expression;(G).ELISA and Western-blot detection Me SCs intracellular caspase-1 expression.

Figure4.Me SCs MMPs 、 ADAMTSexpression.(A).ELISA and Western-blot detection Me SCs extracellular MMPs expression;(B).ELISA and Western-blot detection Me SCs extracellular ADAMTS expression.

Figure5. Flow cytometry and qRT-PCR detection of Me SCs cell pyroptosis. (A). Flow cytometry detection of Me SCs cell pyroptosis；(B).qRT-PCR detection of Me SCs cell pyroptosis. (n=10, ANOVA followed by Turkey's multiple comparison test, $^{*}p < 0.05$，$^{**}p < 0.01$).

Figure6. Physical and histomorphologic structure of meniscus of knee joint.