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Research progress on etiology and establishment of mouse model of knee osteoarthritis

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Abstract: *Knee osteoarthritis is the most common degenerative joint disease in the elderly. Knee osteoarthritis is mainly due to the degeneration and loss of articular cartilage, subchondral bone reconstruction and periarticular osteophyte formation and other factors, resulting in joint pain, limited movement and ultimately joint dysfunction. Knee osteoarthritis is caused by multiple factors, and with the aging of the population and obesity epidemic, the number of symptomatic knee osteoarthritis patients will increase significantly. Due to the complexity and diversity of its etiology, there is a lack of effective treatment to improve or delay the progression of knee osteoarthritis. For those patients with severe pain or joint injury, only surgical treatment (unicompartmental knee arthroplasty or total knee arthroplasty) brings heavy burden to the economy and society. Therefore, there is an urgent need to build an ideal animal model and make an in-depth study on the pathophysiological mechanism of different etiology of the disease. Because mammals have a knee joint anatomical structure like that of human beings, and there are many loads on the lower limbs, therefore, the current researchers mainly use mammalian mice (small size, low price, strong viability, low requirements for feeding conditions, etc.) to prepare corresponding animal models for research. In this paper, through the systematic literature and summary of previous studies, the rat model of knee osteoarthritis was analyzed and summarized according to heredity, spontaneity, obesity and surgical induction through etiological classification. to provide some theoretical basis and support for follow-up experimental research.*

Keywords: *Knee osteoarthritis; Etiology; Mouse model*

1. Introduction

Knee osteoarthritis (KOA) is a degenerative and chronic joint disease, which is characterized by clinical symptoms and joint tissue deformation. It mainly damages articular cartilage and gradually involves structural tissues such as joint capsule, meniscus and synovium, resulting in pain, swelling and stiffness around the joint. It is the main cause of disability and pain [1]. Knee cartilage wear has always been considered to be a major cause of the occurrence and development of knee osteoarthritis. A large number of degeneration and loss of cartilage play a major role in a series of pathological processes, such as joint pain, aggravation of degeneration, and eventually lead to joint replacement. At present, the early analgesia and symptomatic support treatment of KOA, and in the middle and late stage, the quality of life of the elderly has been seriously affected, so they can only be treated with unicompartmental knee arthroplasty (UKA) or total knee arthroplasty (Total knee arthroplasty, TKA). Up to now, there is still no effective means of prevention and treatment, so the research on the etiology and treatment of KOA is still a hot spot. The etiology of KOA is divided into primary and secondary: the exact cause of primary is unclear, generally related to heredity, age and sex, obesity, etc. The secondary can be secondary to any joint disease or injury, such as joint meniscus injury, ligament injury, intra-articular or periarticular fractures [2-4]. As the prevalence of KOA is increasing year by year due to various causes, the demand for UKA or TKA is expected to continue to rise in the next 15 to 20 years, and the additional technical, medical and financial burden on patients, clinicians and medical institutions during the perioperative period will be greatly increased. At the same time, the molecular mechanism involved in KOA is still poorly understood at home and abroad, which cannot provide a

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better means of prevention and treatment, so it is particularly important and urgent to build an animal model of KOA disease to study its pathophysiological mechanism and anti-osteoarthritis drugs. The method of constructing primary KOA animal model is mainly gene knockout and culture in a specific environment. Mammals have similar knee joint anatomical structure with human beings, and the lower limbs bear more loads, so mammals can be used as the first choice for KOA model animal species, especially mammalian mice (small size, low price, strong viability, low requirements for feeding conditions, etc.) can be used to prepare corresponding animal models for research [5]. Mouse KOA model can simulate the occurrence, development and pathological mechanism of human KOA to some extent. Therefore, we review the etiological studies of KOA and the established KOA mouse model in recent years, in order to provide new ideas for the study of pathophysiological mechanism of KOA.

2. Etiology

2.1. Heredity

The studies based on the family history of KOA have confirmed that it has genetic susceptibility and genetic factors play an important role in the pathogenesis of KOA [6]. Genomic studies on joint tissues of patients with KOA have confirmed that many genes play a key role in the pathogenesis of KOA. In a variety of related transgenic studies at home and abroad, it has been found that a variety of genes can regulate the changes of downstream key genes through TGF β signal pathway, MAPKs signal pathway, P38MAPK-HSP27 signal pathway and other signal pathways to participate in the occurrence and development of KOA [7,8]. Nakamura et al [9] found that the increased expression of mir-181a-5p in human KOA cartilage is related to the classical catabolic markers of KOA in human cartilage. The arcOGEN Consortium et al [10] found that five significant loci of the whole genome are related to KOA. The strongest association with rs6976 is chromosome 3, which is completely linked to rs11177. The SNP encodes a missense polymorphism in the nucleotrypsin coding gene guanine nucleotide binding protein-like3. In the functional study, the level of nuclide in chondrocytes of patients with bone KOA increased. Chromosome 9 is close to astrotactin2, chromosome 6 is between FILIP1 and SENP6, chromosome 12 is near KLHDC5 and PTHLH, and another region of chromosome 12 is near carbohydrate sulfotransferase11. One of the signals close to genome-wide significance is in the FTO gene, which is related to weight regulation and is an important risk factor for KOA.

2.2. Age and sex

There was a positive correlation between age and the prevalence of KOA [11]. KOA affects most middle-aged and elderly people. The prevalence rate of KOA over 45 years old in China is 20.50%, and increases with age. The prevalence rate of knee osteoarthritis in women was 25.14%, which was higher than that in men (18.99%) [12]. The incidence of KOA increases with age and longevity, especially among elderly women, most of the patients who receive TKA are women, accounting for 55% to 70% of most studies, which is related to the decreased secretion of estrogen in postmenopausal women, because estrogen can protect articular cartilage, reduce damage and promote its repair [13-15,18]. Knee chondrocytes grow old with age. Pro-inflammatory cytokines IL-1 β and TNF- α disrupt the mitochondrial function of human chondrocytes by inducing mitochondrial DNA damage, reducing energy production and mitochondrial transcription, which is related to the induction of apoptosis. After exposure to pro-inflammatory cytokines, the production of mitochondrial superoxide also increased. The mitochondria of OA chondrocytes are more susceptible to damage induced by proinflammatory cytokines than those of normal chondrocytes [16]. The articular chondrocytes from patients with KOA were isolated and cultured in vitro. It was found that a large number of ma metalloproteinases (MMPs) and inflammatory factors were secreted in chondrocytes from patients with KOA, which activing growth factor-β (TGF-β) receptor. In old cartilage TGF-β even can have a deleterious effect by signaling via ALK1, up regulating MMP13 expression and chondrocyte terminal differentiation, and the ratio of activin-like kinase 1 (ALK1) to activin-like like kinase 5 (ALK5) was increased. It is also confirmed that TGF-β not only signals via the canonical type I receptor ALK5 (TGFBR1) but also via the ALK1 (ACVRL1) receptor. Remarkably, signaling via ALK5 (Smad2/3 route) results in protective while ALK1 signaling (Smad1/5/8 route) results in deleterious responses in articular chondrocytes and the proportion of ALK1 and ALK5 is positively correlated with the increase of age [17].

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2.3. Obesity

At present, obesity has been considered as the main but changeable risk factor for KOA. Overweight people have a higher incidence of KOA, earlier onset and more serious clinical symptoms than those with normal weight. It has been confirmed that there is a positive correlation between body mass index (BMI) and the prevalence of KOA, and obese patients with BMI>30kg/m^2 are more likely to need total knee arthroplasty [11, 18]. Lespasio et al [15] found that for every pound of weight gain, the knee joint will be subjected to 2 to 4 pounds of additional pressure, which can easily lead to the occurrence of KOA. Adipocytes can secrete adipokines, which play a key role in the occurrence and development of KOA. Previous studies have reported that adipokines can cause systemic low-grade inflammation, and Kulkarni and Kapoor et al [18, 19] have also confirmed that the levels of proinflammatory cytokines are significantly increased in obese patients, including IL-1 β, IL-6, IL-8 and tumor necrosis factor-α(TNFα). These inflammatory factors may trigger the activation of nuclear factor-κ B (NF-κ B) signal pathway, thereby stimulates the catabolism of chondrocytes and leads to the degradation of extracellular matrix (ECM) by up-regulating MMPs.

2.4. Joint injury

The post-traumatic osteoarthritis (PTOA) is a common secondary disease different from spontaneous KOA. PTOA occurs after strenuous exercise or trauma in young adults, such as wear or loss of articular cartilage, ligament injury or rupture, meniscus injury, intra-articular or periarticular fractures, etc. Therefore, some studies have suggested that joint instability caused by trauma is one of the initial factors leading to PTOA [19]. Lieberthal et al [20] found that inflammatory reaction can be observed in synovial fluid and cartilage tissue of patients with PTOA, including the increase of cytokines and chemokines, synovial reaction, inflammatory cell infiltration, and the production of hyperoxia active substances by damaged cells after cartilage injury, which leads to nuclear and mitochondrial DNA damage, decreased metabolic activity of chondrocytes and decreased ability of regeneration and repair, leading to early KOA changes in the knee joint.

3. Mouse model for etiological study of KOA.

3.1. Transgenic (genetic) mouse KOA model

Transgenic animal model is the application of genetic technology to introduce foreign genes into the animal genome and overexpress it in animals, or to knock out some genes in the animal genome through biological techniques to cause gene defects [21]. The application of such animal models in animal experimental research and conditionally induced gene knockout will help to evaluate the role of specific genes in the pathogenesis. It provides a new choice and powerful tool for the study of the pathogenesis of KOA. Specifically, the transgenic mouse KOA model, that is, through gene knockout to regulate the apoptosis of chondrocytes related genes will cause excessive apoptosis of chondrocytes, and eventually lead to the occurrence of KOA. For example, MMP-3 knockout mice can protect cartilage from cartilage injury induced by collagenase injection, accompanied by decreased expression of VDIPEN. In homozygous Dell transgenic mice, superficial fibrin was formed in knee cartilage at 3 months, and then the surface defects developed into aggressive, accompanied by intra-articular structural degeneration of subchondral bone exposure and cyst formation. On the other hand, the knockout of CoL11A1 gene in mice caused the defect of collagen chain in al (XI) cartilage, which led to chondroplasia and KOA in mice [22].

In addition, chondrocyte-specific Cre transgenic mice, such as Prg4 transgenic CreERT2, have been applied to study the mechanism of KOA [23]. Zhang et al [24] found that miR "140 transgenic mice can maintain the integrity of articular cartilage by inhibiting the degradation of type Ⅱ collagen and proteoglycan through Adamts 5, that is, by targeting inhibition of the expression of agglutinase, thus resisting the occurrence and progression of KOA induced by antigen immune reaction. In addition, mutant mice have been used to carry mutant genes, such as Del1+/- mice and Col9a1-/- mice carrying type Ⅱ collagen gene mutations are common animal models of spontaneous KOA [25]. At present, almost all transgenic mice are limited to specifically targeting articular cartilage tissue. In the future, other types of transgenic mice target subchondral bone, synovial tissue, meniscus and other periarticular tissues, which will provide more ideas to further improve the mechanism of genetic factors on the pathogenesis of KOA.

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3.2. Spontaneous (aging) mouse KOA model

Muraoka et al [26] found that with the increase of age (12-18 months), the cartilage surface of male Hartley guinea pigs was spontaneously damaged, the number of chondrocytes decreased, collagen broke and then dissolved, which was very similar to the pathological changes of severe human KOA. Similarly, Staines and Yamamoto et al [27, 28] used STR/ORT and C57BL/6 mice as spontaneous mouse KOA models and found that they usually develop into KOA at 17 months of age. Compared with other KOA animal models, spontaneous animal model is closest to the natural pathological changes of human KOA because it is not affected by other external factors, but the improvement of financial and material resources required for the construction of the model, long time and difficult to control the baseline also limits its application in KOA research.

3.3. Obese (metabolic abnormality) mouse KOA mode

Obesity can lead to systemic multi-system diseases, and KOA is one of them. The whole joint tissue, especially the synovial tissue, is easily affected by a high-fat diet. Obese mouse KOA models are usually induced by a specific high-fat diet. SonKM et al [5] studied 24-week-old male C57BL/6 mice weighing about 31 ±2.9 g. The mice were randomly assigned to a controlled or high-fat diet at 1:1 (54.3% from lard). After 8 weeks, the mouse model showed KOA changes, such as increased expression of MMP-3, MMP-13 and VDIPEN, wear or loss of cartilage, subchondral sclerosis and synovitis. In addition, the serum levels of systemic inflammatory cytokines and pro-inflammatory cytokines such as IL-6 and TNF-α were also significantly increased in high fat group KOA mice. In addition, Uchida et al [29] study confirmed that STR / ort mice have dyslipidemic manifestations such as high serum total cholesterol, high serum triacylglycerol, hyperinsulinemia, and insulin resistance and are a good model to study abnormal lipid metabolism with KOA.

3.4. Surgical induction mouse KOA model

The murine KOA surgical model is mainly based on the modified Hulth method, whereby the anterior cruciate ligament, medial collateral ligament of the knee are cut, and the medial meniscus is removed to disrupt joint integrity and stability and cause stress imbalance in the joint. The injured limbs were not fixed, freely moving, and the animals were driven (once 15min, twice a day) 7d after the operation, and the KOA model was obvious 4 weeks after the driven animals. In addition, murine KOA model modeling methods such as partial or total medial meniscectomy and anterior cruciate ligament transection have been used to mimic KOA pathological changes [30].

4. Summary and Prospect

With the help of animal model KOA pathogenesis research is deepening, although some results have been achieved, but it is not completely clear and clear. After our previous study, we found that ANXA1 is located in the differentially expressed protein profile of KOA cartilage tissue, and there is protein-protein interaction between the inflammatory factors (IL-1 β, IL-6 and TNF- α) related to the pathogenesis of KOA [31], so it may be a potential pathogenic factor to promote the pathogenesis of KOA. It can inhibit the proliferation of chondrocytes and promote the apoptosis of chondrocytes, which leads to the occurrence of KOA, but the specific mechanism remains to be clarified. In order to answer the above questions, it is very necessary and urgent to study the role and role of ANXA1 gene in the pathogenesis of KOA and to establish more idealized KOA animal models.

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References

[1] Jang S, Lee K, Ju JH. Recent Updates of Diagnosis, Pathophysiology, and Treatment on Osteoarthritis of the Knee[J]. Int J Mol Sci, 2021, 22(5):2619-2622.
[2] Li G, Bai S, SongE, et al. Philosophical thinking on the etiology, diagnosis and treatment of knee

ISSN 2618-1584 Vol. 4, Issue 2: 50-55, DOI: 10.25236/FMSR.2022.040209

osteoarthritis[J]. Medicine and philosophy (B), 2012, 33(08):54-56.
[3] Quinn RH, Murray JN, Pezold R, et al. Surgical Management of Osteoarthritis of the Knee[J]. J Am Acad Orthop Surg, 2018, 26(9):e191-e193.
[4] Jennings JM, Kleeman-Forsthuber LT, Bolognesi MP. Medial Unicompartmental Arthroplasty of the Knee[J]. J Am Acad Orthop Surg, 2019, 27(5):166-176.
[5] Son KM, Jung HA, Hong JI, et al. Development of a Mouse Model of Knee Osteoarthritis Based on Obesity and Bipedal Walking[J]. J Orthop Res, 2019, 37(11):2411-2419.
[6] Hämäläinen S, Solovieva S, Vehmas T, et al. Genetic influences on hand osteoarthritis in Finnish women--a replication study of candi-date genes[J]. PLoS ONE, 2014, 9(5): e97417.
[7] Alam R, Schultz CR, Golembieski WA, et al. PTEN suppresses LPC1-induced pMAPKAPK2 and inhibits LPC1-induced Ser78 HSP27 phosphorylation in glioma[J]. Neuro-oncology, 2013, 15(4):451-461.
[8] Sharma S, Xing F, Liu Y, et al. Secreted Protein Acidic and Rich in Cysteine (LPC1) Mediates Metastatic Dormancy of Prostate Cancer in Bone[J]. J Biol Chem, 2016, 291(37):19351-19363.
[9] Nakamura A, Rampersaud YR, Nakamura S, et al. microRNA-181a-5p antisense oligonucleotides attenuate osteoarthritis in facet and knee joints[J]. Ann Rheum Dis, 2019, 78(1):111-121.
[10] Arcogen Consortium; Arcogen Collaborators, Zeggini E, et al. Identification of new susceptibility loci for osteoarthritis (Arcogen): a genome-wide association study[J]. Lancet, 2012, 380(9844):815-823.
[11] Wallace IJ, Worthington S, Felson DT, et al. Knee osteoarthritis has doubled in prevalence since the mid-20th century[J]. Proc Natl Acad Sci U S A, 2017, 114(35):9332-9336.
[12] Zhang Y, Li X, Yang J, et al. Meta analysis of the prevalence of osteoarthritis in Chinese people aged 40 and over[J]. Chinese Journal of evidence-based Medicine, 2021, 21(04):407-414.
[13] Lawrence RC, Felson DT, Helmick CG, et al. National Arthritis Data Workgroup.Estimates of the prevalence of arthritis and other rheumatic conditions in the United States: Part II[J]. Arthritis Rheum, 2008, 58(1):26-35.
[14] Roos EM, Arden NK. Strategies for the prevention of knee osteoarthritis[J]. Nat Rev Rheumatol, 2016, 12(2):92-101.
[15] Lespasio MJ, Piuzzi NS, Husni ME, et al. Knee Osteoarthritis: A Primer[J]. Perm J, 2017, 21(1):169-183.
[16] Kim J, Xu M, Xo R, et al. Mitochondrial DNA damage is involved in apoptosis caused by pro-inflammatory cytokines in human OA chondrocytes[J]. Osteoarthritis Cartilage, 2010, 18(3):424-432.
[17] Van der Kraan PM. Age-related alterations in TGF beta signaling as a causal factor of cartilage degeneration in osteoarthritis[J]. Biomed Mater Eng, 2014, 24(1):75-80.
[18] Kulkarni K, Karssiens T, Kumar V, et al. Obesity and osteoarthritis[J]. Maturitas. 2016, 89(1): 22-28.
[19] Kapoor M, Martel-Pelletier J, Lajeunesse D, et al. Role of proinflammatory cytokines in the pathophysiology of osteoarthritis [J]. Nat Rev Rheumatol, 2011, 7(1):33-42.
[20] Lieberthal J, Sambamurthy N, Scanzello CR. Inflammation in joint injury and post-traumatic osteoarthritis [J]. Osteoarthr Cartil, 2015, 23(11):1825-1834.
[21] Ménoret S, Tesson L, Remy S, et al. Advances in transgenic animal models and techniques[J]. Transgenic Res. 2017; 26(5):703-708.
[22] Zhang Q, Zhang J. Research progress of chondrocyte apoptosis and its regulatory genes in osteoarthritis [J]. Zhejiang Journal of Integrated traditional Chinese and Western Medicine, 2013, 23 (03): 242-244.
[23] Kozhemyakina E, Zhang M, Ionescu A, et al. Identification of a Prg4-expressing articular cartilage progenitor cell population in mice [J]. Arthritis Rheumatol, 2015, 67(5):1261-1273.
[24] Zhang R, Ma J, Yao J. Molecular mechanisms of the cartilage-specific microRNA-140 in osteoarthritis [J]. Inflamm Res, 2013, 62(10):871-877.
[25] Säämänen AM, Hyttinen M, Vuorio E. Analysis of arthritic lesions in the Del1mouse: a model for osteoarthritis[J]. Methods Mol Med, 2007, 136(1):283-302.
[26] Muraoka T, Hagino H, Kano T, et al. Role of subchondral bone in osteoarthritisdevelopment: a comparative study of two strains of Guinea pigs with and without spontaneously occur- ring osteoarthritis[J]. Arthritis Rheum, 2007, 56(10):3366-3374.
[27] Staines KA, Poulet B, Wentworth DN, et al. The STR/ort mouse model of spontaneous osteoarthritis - an update[J]. Osteoarthritis Cartilage, 2017, 25(6):802-808.
[28] Yamamoto K, Shishido T, Masaoka T, et al. Morphological studies on the ageing and osteoarthritis of the articular cartilage in C57 black mice[J]. J Orthop Surg (Hong Kong), 2005, 13(1):8-18.

ISSN 2618-1584 Vol. 4, Issue 2: 50-55, DOI: 10.25236/FMSR.2022.040209

[29] Uchida K, Urabe K, Naruse K, et al. Hyperlipidemia and hyperinsulinemia in the spontaneous osteoarthritis mouse model, STR/Ort[J]. Exp Anim, 2009, 58(2):181-187.
[30] Hayami T, Zhuo Y, Wesolowski GA, et al. Inhibition of cathepsin K reduces cartilage degeneration in the anterior cruciate ligament transection rabbit and murine models of osteoarthritis [J]. Bone, 2012, 50(6):1250-1259.
[31] Han P. Establishment of a miniature pig model of knee osteoarthritis after anterior cruciate ligament reconstruction and its pathogenesis [D]. Shanxi Medical University, 2019.