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Genetic and Epigenetic Insights into Osteoarthritis Pathogenesis; Spotlighting STAT3 and miR-452-3p as Illuminating Examples

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Abstract

Osteoarthritis (OA) is a chronic degenerative joint disease, characterized by progressive degradation and loss of articular cartilage, subchondral bone sclerosis, inflammation, and osteophyte formation. Edema, chronic pain, and limited joint movement are the major signs of OA. Recent studies have shown that it affects 240 million aged people globally, and about 10% of males and 18% of women over the age of 60. Because of this, it is now the main factor in pain, disability, and decreased adult work performance throughout the world. OA can be regarded as a multifactorial disease, with several factors including aging, genetic predisposition, epigenetic control, inflammation, obesity, and trauma being involved in its pathophysiology. Signal transducer and activator of transcription 3 (STAT3) has been described as one of the critical factors involved in the initiation and development of inflammatory responses in OA. Previous studies have provided evidence that microRNAs (miRNAs) play a major role in the regulation of STAT3 expression through binding to the 3′ untranslated region (3′UTR) of STAT3 mRNA.

Keywords: Osteoarthritis, STAT3, miR-452-3p.

1. Introduction

Osteoarthritis is a multifactorial disease characterized by progressive degeneration and eventual failure of the synovial joint functionality. Although it has been traditionally considered as an exclusive disease of the articular cartilage, nowadays it is considered a whole joint disease. Therefore, the progression of the disease involves articular cartilage degeneration, osteochondral bone sclerosis and synovial membrane hypertrophy (**He et al., 2020**).

Any joint from spinal vertebrae to the feet can be affected by OA although it is more frequent in the hand, hip, and knee. Symptoms vary depending on the grade and the location of the disease; however, most common symptoms are pain, stiffness, or loss of movement, swelling and heat around the affected joint. The etiology of OA can be classified as "primary OA" (idiopathic or spontaneous), when the cause is not apparent or" secondary OA" when there is an obvious triggering cause (**Duruöz et al., 2023**).

Previous epidemiological studies have evidently shown that OA susceptibility has a heritable component. However, the specific genetic factors that lead to OA are currently largely unknown. It therefore remains a challenge to identify candidate

genes or risk alleles that contribute to OA pathogenesis. SNPs in the COL-11A1, VEGF, growth/differentiation factor-5 (GDF-5), IL-8 genes have been linked to OA. Some polymorphisms may be specific to certain OA subtypes: e.g.: hip (COL-11A1, VEGF) and knee (COL-9A3, GDF5) or ethnic groups **(Aubourg et al., 2022)**. Identification of these gene SNPs and their relationship with OA would enhance our knowledge of the molecular mechanisms underlying the pathogenesis of OA, so that better diagnostics and more effective treatment can be created for OA in its earliest stages.

2. Definition and prevalence of osteoarthritis

In order to identify and grade OA, Kellgren and Lawrence (K&L) described radiological criteria for classification of knee OA in 1957 and later accepted by the World Health Organization (WHO) in 1961 as the radiological definition of OA for the purpose of epidemiological studies **(Schiphof et al., 2011; Cueva et al., 2022) (Table 1)**.

Table 1: Update of K&L standardized radiographic grading of knee OA **(Schiphof et al., 2011)**.

Grade 0 (none)	Definite absence of x-ray changes of osteoarthritis.
Grade 1 (doubtful)	Doubtful joint space narrowing and possible osteophytic lipping.
Grade 2 (minimal)	Definite osteophytes and possible joint space narrowing.
Grade 3 (moderate)	Moderate multiple osteophytes, definite narrowing of joint space and some sclerosis and possible deformity of bone ends.
Grade 4 (severe)	Large osteophytes, marked narrowing of joint space, severe sclerosis, and definite deformity of bone ends.

Osteoarthritis is the most frequent form of arthritis and a leading cause of pain and disability worldwide. While there are estimated to be more than 100 types of arthritis, osteoarthritis (OA) is the most common form of arthritis, affecting 32.5 million US adults **(Barbour et al., 2017)**. The high prevalence of arthritis manifests in enormous societal and personal costs. The researchers found that from 1990 to 2019, there was a 113.25 percent increase in prevalent cases of OA globally, from 247.51 to 527.81 million, respectively **(Long et al., 2022)**.

Hand OA is the most prevalent form of OA, but knee and hip present the highest burden. Large weight-bearing joints present higher pain and stiffness that often lead to significant problems with mobility and disability requiring expensive surgical treatments. Costs associated with OA include costs for adaptive aids and devices, medicines, surgery, and time off at work. Several studies have shown that the burden of OA is not only physical but also psychological as the loss of mobility is associated with loss of autonomy that ultimately affects the mental health of the patient **(Litwic et al., 2013)**.

3. Risk factors of osteoarthritis

3.1. Systemic risk factors

3.1.1. Ethnicity

Epidemiologic studies have shown that OA prevalence varies between ethnic groups. However, it is important to note that ethnic differences are not only related to genetic factors but also to other variables including lifestyle, nutrition and probably the healthcare disparities between populations. For example, Chinese women rarely have hip OA while Caucasian women are predisposed to have hip OA due to anatomic abnormalities. On the other hand, Chinese women present more prevalent knee OA than the American women, what might be due to physical activities culturally more common for Chinese women such as manual labor and squatting **(Zhang et al., 2001; Nevitt et al., 2002)**.

3.1.2. Age

Age is the strongest risk factor for OA. The presence of radiographic OA rises with age at all joint sites. In a Dutch study, 10–20% of women around 40 years old had evidence of severe radiographic OA of their hands or feet, and by age 70, it increased to approximately 75% **(Litwic et al., 2013)**. This could be explained by several causes. On one side, during aging, chondrocytes, the only cell type of the articular cartilage, do not respond to reparative growth factors and fail to produce extracellular matrix components like aggrecan and COL-II, while they express more MMPs. Furthermore, there is an accumulation of

reactive oxygen species (ROS) that leads to mitochondrial dysfunction and chondrocyte death (Li et al., 2017).

3.1.3. Gender

Women present a higher risk to develop OA, but the risk is site specific (i.e., higher prevalence in knee and hand, higher incidence in knee, hand and hip and higher OA severity in knee) (Srikanth et al., 2005). Importantly, it was also shown that age was a significant contributor to the effect of sex in the observed heterogeneity on OA. It was reported that females over 55 years old have a higher prevalence of knee and hand OA than men of the same age, which can be explained by the hormonal effect of menopause (Prieto-Alhambra et al., 2014).

3.1.4. Nutrition and metabolic disorders

Some epidemiological studies have shown serum cholesterol to be a risk factor for OA development. However, it has been shown that long-chain omega-3 polyunsaturated fatty acids could decrease inflammatory eicosanoids, cytokines, and ROS. Furthermore, diabetes has been described as an independent risk factor for OA, leading to the concept of a diabetes-induced OA phenotype. This may be explained because hyperglycemia causes oxidative stress which induces chondrocyte dysfunction, matrix stiffness and subchondral bone destruction. Finally, Vitamin D deficiency was found to be positively associated with the development and worsening of knee OA, including cartilage loss, and increased joint space narrowing (Wei and Dai, 2022).

3.1.5. Genetics

Although the multifactorial nature of OA is well recognized, genetic factors have been found to be strong determinants of the disease. Evidence of a genetic influence in OA comes from a number of sources, including epidemiological studies of family history and family clustering, twin studies, and exploration of rare genetic disorders (Salzmann et al., 2017). Genome Wide Association Studies (GWAS) have tested the association between thousands of SNPs in the whole genome and OA. It was reported that 17 loci have been associated with hip, knee and hand OA and they seem to be joint-site specific and even gender specific (Van Meurs, 2017) (Figure 1).

Studies have implicated linkages to OA on chromosomes 2q,9q, 11q, and 16p, among others.

Genes implicated in association studies include vitamin D receptor (VDR), Insulin-like growth factor 1 (IGF-1), Estrogen Receptor Alpha (ER-α), Transforming Growth Factor Beta 1 (TGF β1), cartilage matrix protein (CRTM), cartilage link protein (CRTL), and COL- II, IX, and XI. Genes may operate differently in the two sexes, at different body sites, and on different disease features within body sites (Spector and MacGregor, 2004).

3.1.6. Epigenetics

Indeed, epigenetics, a crucial method of gene expression regulation, has been implicated in the start and progression of OA in recent research. It can be defined as changes in gene expression that occur without changes in the DNA sequence. In some cases, epigenetic modifications are stable and passed on to future generations, but in other instances they are dynamic and change in response to environmental stimuli, for example. Three main mechanisms are involved in epigenetic regulation (Figure 2):

1. Modification of histones which alters chromatin conformation.

2. Non-coding RNAs: miRNAs and long non-coding RNAs (lncRNAs) act both transcriptionally and post-transcriptionally in the regulation of mRNA expression.

3. DNA methylation changes that covalently alter DNA structure (Shen et al., 2017).

As a result, it has been proposed that changes in gene expression, rather than changes in the genetic code sequence, are more likely to influence OA development (Cai et al., 2022).

3.1.6.1. DNA methylation

The dynamic DNA methylation process is mediated by DNA methyltransferase (DNMT) enzymes and the demethylation enzymes. Three DNMTs have been reported (DNMT1, 3A, 3B) and they function by catalyzing the addition of a methyl group (CH3) to a cytosine located 5' of a guanine (CpG sites) to form methylated cytosine (5 mC). Recent genome-wide methylation profiling has revealed differentially methylated loci in DNA from cells of OA cartilage and age-matched, non-diseased cartilage (Shen et al., 2017). Taken together, these studies suggest that DNA
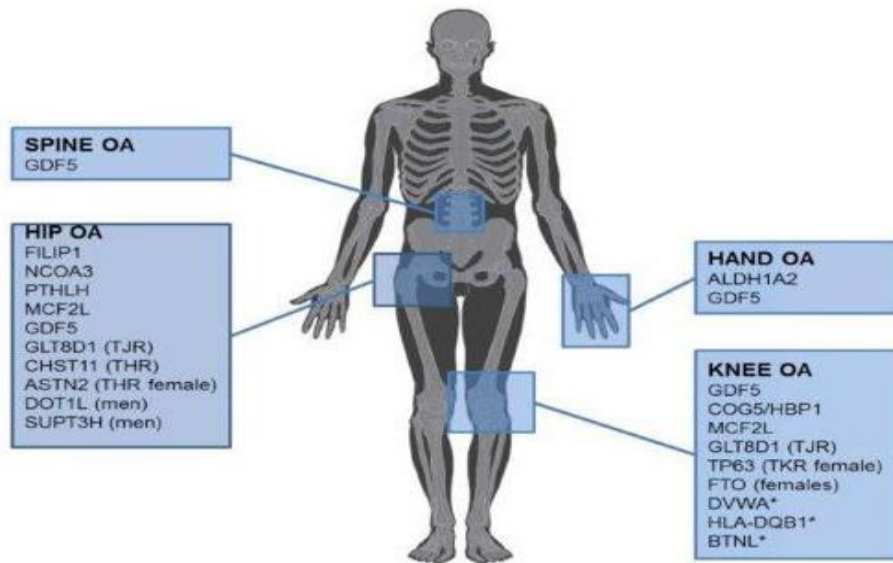
Figure 1: Identified genetic loci for each of the joint sites. OA, osteoarthritis **(Van Meurs, 2017)**
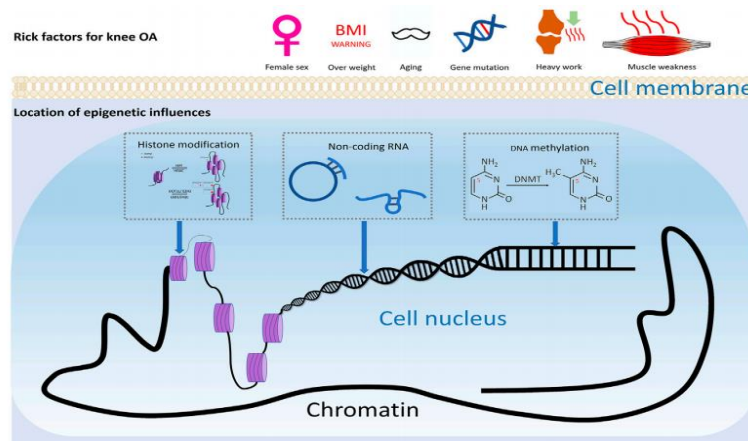

Figure 2: Risk factors of knee OA. Risk factors for knee osteoarthritis include ageing, gender, injury, and joint overloading, etc. Epigenetics may play a considerable role in how these environmental factors lead to altered gene expression and ultimately pathophysiologic manifestations such as cartilage damage and subchondral osteosclerosis. BMI, body mass index; DNMT, DNA metheyltransferase **(Cai et al., 2022)**

methylation changes are highly coordinated with the inflammation response and MMPs activity within the context of OA progression, which is believed to contribute to catabolic responses in chondrocytes. Similarly, the CpG sites within the promoter area of a number of MMPs, including MMP2, MMP9, MMP13, and a disintegrin and metalloproteinase with thrombospondin motifs 4 (ADAMTS4), showed decreased methylation profiles in OA compared to normal cartilage, correlating with elevated gene expression and resulting in ECM degradation. In addition, the MMP13 promoter was found to be specifically demethylated in OA chondrocytes compared to healthy chondrocytes **(Bui et al., 2012)**.

3.1.6.2. Histone modification

Histone modification, including acetylation, methylation, phosphorylation, and ubiquitination within the lysine residues of histone cores, is another epigenetic landmark, which regulates the accessibility of transcriptional machinery to specific DNA loci. Histone acetylation mediated by histone acetyl transferase (HAT) is the major mechanism to de-condense the DNA structure, thereby permitting transcriptional networks to interact with DNA to initiate gene expression. On the other hand, deacetylation mediated by histone deacetylase (HDAC) involves removing the acetylation marker from euchromatin resulting in

inhibition of gene expression (**Clayton et al., 2006**). One study showed that protein levels of HDAC1 and HDAC2 were increased in chondrocytes from OA patients and that this was associated with down-regulation of some cartilage marker genes (e.g., COL-II and aggrecan) (**Hong et al., 2009**).

3.1.6.3. MicroRNAs

Another form of epigenetic regulation involves the small non-coding (miRNAs). Interactions between miRNAs and highly complementary targets lead to mRNA degradation, while incomplete interactions between miRNAs and target transcripts usually lead to translation suppression (**Cai et al., 2022**). As a result, repression of target mRNAs occurs via either inhibition of translation or mRNA degradation (**Malemud, 2018**). From the vast number of published reports on miRNAs, we now know that they are important regulators of many diverse cellular processes such as pluripotency control, differentiation, proliferation, metabolism, and apoptosis. In many disease scenarios, miRNAs have been analyzed as potential biomarkers, and their small size renders them attractive therapeutic targets (**Vegter et al., 2016**).

Several groups of investigators proposed that miRNAs could play a key role in OA. In support of this contention, previously published evidence indicated that miRNAs were regulators of hundreds of genes which were relevant to cartilage development, homeostasis, and OA pathology (**Mirzamohammadi et al., 2014**). Since then, numerous researches have investigated the connection between miRNA expression and effector genes in OA, including inflammation, aging, transcription factors, apoptosis, autophagy, and other pathogenic events in the evolution of the illness (**Wang et al., 2016**).

The intra-articular injection of certain microRNAs has the ability to reverse disease progression, which will revolutionize the treatment of OA (**Cai et al., 2022**). It was reported that the overexpression of miR-146a in chondrocytes could reduce IL-1β induced production of TNF-α. Taken together, these findings suggest that miR-146a could be a promising OA biomarker as well as a potential therapeutic target to regulate inflammatory/catabolic effects in chondrocytes and synoviocytes (**Jones et al., 2009**).

Clearly, many miRNAs have now been reported to induce anti-inflammatory effects in chondrocytes

via regulating different target genes and signaling pathways. With this knowledge, a potentially fruitful strategy moving forward could be to explore a combination miRNA approach to attempt to further inhibit catabolic events and hence stop or slow down OA progression (**Shen et al., 2017**).

3.1.6.4. Long non-coding RNAs

The lncRNAs are generally defined as transcripts of ~200 nucleotides or more in length that do not encode proteins. Like mRNAs, they are primarily transcribed by RNA polymerase II and can be post-transcriptionally processed (i.e., intron removal, alternatively spliced, addition of poly A tails, etc.) (**Shen et al., 2017**). The lncRNAs primarily interact with mRNA, DNA, protein, and miRNA and consequently regulate gene expression at the epigenetic, transcriptional, post-transcriptional, translational, and post-translational levels in a variety of ways (**Cai et al., 2022**). lncRNA and miRNA interaction modes are more unique. Through spatial conformation, the secondary structure created by lncRNA can exert a sponge-like adsorption effect on miRNA, alter the actual contact concentration of miRNA, and the production of inflammatory genes or transcription factors in OA (**Jiang et al., 2021**).

3.2. Local factors

3.2.1. Joint injury

There is a clear association between joint injury and OA. Examples of joint injury include striking a joint with a football helmet or falling so that the joint hits directly on a hard surface. Disruption of the periarticular and articular soft tissues, including joint capsule, ligament, or meniscal injuries, does not directly damage articular cartilage, but the joint instability that occurs as a result of some capsular, ligamentous, and meniscal injuries causes repetitive increased loading of articular cartilage that results in joint degeneration. Studies on both animal and human models convincingly demonstrated that a loss of anterior cruciate ligament integrity, damage to the meniscus, and meniscectomy lead to knee OA (**Werner et al., 2022; Holers et al., 2023**).

3.2.2. Obesity

A high number of studies have shown that obesity represents one of the most important risk factors and it is also a predictor for OA progression, especially in knee joint. The relationship between body mass index (BMI) and OA of the knee is

mainly linear. Consequently, weight loss in obese OA patients slows disease progression and improves symptoms and functional capacity. Some studies have shown an association, suggesting that obesity may predispose to hand OA, perhaps via an inflammatory or metabolic intermediary that has not yet been identified. This means that obesity plays a role not only as a local factor but as a systemic as well (**Nedunchezhiyan et al., 2022**).

3.2.3. Sports and physical activity

The effect of sports on the risk of OA is highly associated with the subject´s joint health and the participation on high-impact sports; sports that cause minimal joint impact and torsional loading by people with a normal joint anatomy and neuromuscular function have minimal effect on the risk of OA. Furthermore, sports with a high joint impact such as running are not associated with OA risk in runners that have been training since young ages and have a low body mass index (BMI). However, people with abnormal joint anatomy or alignment, previous joint injury or surgery, joint instability, articular surface incongruity or dysplasia, disturbances of joint or muscle innervations, or inadequate muscle strength have increased risk of OA during participation in any type of physical activity that include impact and torsion loading (**Butt et al., 2022**).

3.2.4. Joint biomechanics

Mechanical factors play important roles in promoting both the health of a joint and its degeneration. Appropriate loading is required to maintain healthy joint tissues, while aberrant loading contributes to the development and progression of OA. Abnormal joint anatomy or function, such as hip dysplasia or femoroacetabular impingement are long established risk-factors for OA. Similarly, tibial, and femoral bone morphology can predict the development of knee OA (**Postler et al., 2023**).

4. Symptoms of osteoarthritis

Symptoms differ between acute and chronic inflammation and patients with OA may experience both: acute flares may occur either on the background of chronic synovitis or in an otherwise non-inflamed joint. Acute inflammation usually has a sudden onset, becoming apparent over minutes or hours with the classic symptoms of heat, pain, redness and swelling. Chronic inflammation develops over a longer period and may persist for

days, weeks or months. Neutrophils are the most abundant inflammatory cells in acute synovitis, whereas in chronic synovitis in OA, macrophages are most abundant, often with lymphocytic infiltrates (**Tarner et al., 2005**). Pain is one of the classic symptoms of acute inflammation. This is mainly due to the sensitization of fine unmyelinated sensory nerves present in the osteoarthritic joint. However, this is not restricted to acute inflammation and chronic inflammation could also be a source of pain in OA (**Bonnet and Walsh, 2004**).

5. Pathophysiology of osteoarthritis

Inflammation is increasingly recognized as contributing to the symptoms and progression of OA. Inflammatory cytokines (such as IL-1β and TNF-α), chemokines, and other inflammatory mediators are produced by cells from the principal tissues implicated in OA: synovium, subchondral bone, and cartilage (**Figure 3**). In fact, synovial membrane inflammation is one of the principal processes that take place in the development of the disease, along with degradation of the articular cartilage and subchondral bone affectation, mainly by neovascularization, sclerosis, and new bone formation (osteophytes) (**Chow and Chin, 2020**). Therefore, three main processes are implicated in the OA pathophysiology: angiogenesis, inflammation, and degradation. These three processes are also deeply related to the main OA symptomatology: swelling and pain (**Yao et al., 2023**).

5.1. Angiogenesis and inflammation

In normal joints, oxygen pressure in synovial fluid ranges from 50 mmHg to 60 mmHg (**Lund-Olesen, 1970**). However, during the development of OA, the inflammation of the synovial membrane increases the oxygen demand of this tissue; so that it cannot be compensated by the vascular expansion of the hyperplastic synovium. Under hypoxic situations, the hypoxia-inducible factor 1 (HIF-1) is the major activated transcription factor and it is mainly responsible for the angiogenic switch. In the pathophysiology of OA, inflammation can occur locally, within the synovium, and systemically, with inflammatory agents circulating in the blood. In early OA, as an attempt to repair, the chondrocyte exhibits a transient proliferative response, increasing the synthesis of cartilage matrix, cytokines, and matrix-degrading enzymes (**Zeng et al., 2022**).
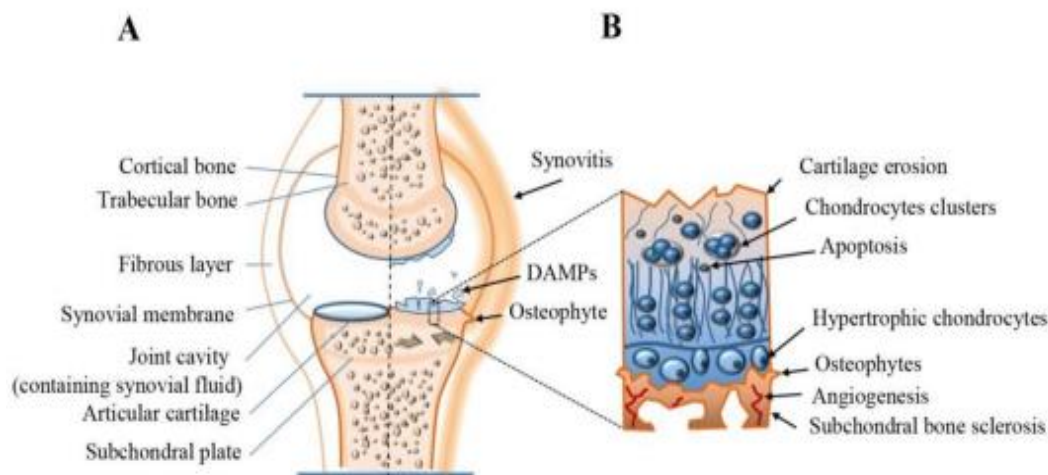
Figure 3: Properties of an OA joint. Hallmarks of OA such as synovial membrane inflammation, cartilage degeneration with the release of danger-associate molecular patterns (DAMPs), osteochondral bone formation (osteophytes) b) magnification of a cross-section of the tissue with evidence of subchondral bone sclerosis, hypertrophic chondrocytes, apoptotic chondrocytes, and chondrocyte clusters (**Martel-Pelletier et al., 1991**).

Elevated levels of inflammatory cytokines have been measured in OA synovial fluid and have been shown to play important roles in the destruction of cartilage, synovitis, and pain. One of these cytokines is IL-1β which suppresses the synthesis of COL-II and aggrecan, induces the production of MMPs and other inflammatory cytokines such as IL-6 and IL-8, and plays an important role in pain sensitivity (**Mabey and Honsawek, 2015**). Also, breakdown products from damaged cartilage act as damage-associated molecular patterns (DAMPs) and are released into the synovial cavity where they initiate synovial inflammation (**Lambert et al., 2020**). Hence, immune cells are attracted into synovium, angiogenesis is increased, and chondrocytes behavior shifts towards a hypertrophic phenotype, characterized by type X collagen synthesis. This process results in a positive feedback, as chondrocytes produce additional inflammatory cytokines and proteolytic enzymes that eventually increase cartilage degradation and induce further synovial inflammation (**Shigley et al., 2023**).

5.2. Degradation of the articular cartilage

Tissue breakdown is a sequential process that begins at the articular surface with the digestion of non-collagenous proteins and continues by degrading the type II collagen fibrillar network (**Grenier et al., 2014**). Even though the chondrocytes produce increased amounts of the proteoglycan components in response to matrix damage, there is limited proteoglycan aggregation and immobilization within the matrix due to the loss of the collagen structural integrity. Several proteases are implicated in the ECM cartilage degradation mainly grouped as serine proteases, cysteine proteases and metalloproteinases that in turn comprise MMPs, a disintegrin and metalloprotease (ADAMs) and ADAMTs (**Troeberg and Nagase, 2012**).

In OA, the larger proteoglycan aggrecan is digested by members of the proteinase family ADAMTS, specially ADAMTS-4 and ADAMTS-5. ADAMTS-4 and -5 are also named aggrecanase-1 and -2, respectively, due to their capacity to cleave aggrecan in an exclusive site between Glu373 and Ala374 (**Tang, 2001**). Cleavage of aggrecan liberates the major part of the molecule from the cartilage which affects the compressive resistant and shock absorbing capability of the tissue under loading. In vivo studies suggest that dual inhibition of ADAMTS-4 and ADAMTS-5 may be a reasonable strategy for inhibiting aggrecanase activity in human OA since the double knock out mice presented a protection against OA without affecting normal physiology (**Song et al., 2007**).

The MMPs are a family of zinc dependent endopeptidases that are able to degrade most of the components of the ECM. There are four subfamilies of MMPs mainly classified by its preferred substrate: collagenases, stromelysins, gelatinases and membrane-type matrix metalloproteinases. MMPs are secreted as latent pro-enzymes and are activated by proteases, such as MMP-14 and MMP-2, or intracellularly by other

mechanisms **(Cabral-Pacheco et al., 2020)**. Collagenases (collagenase-1 [MMP-1], collagenase-2 [MMP-8] and collagenase-3 [MMP-13] have the ability to degrade non-collagenous and non-fibrillar collagen ECM components, as well as to destabilize the fibrillar collagen triple-helix and digest its fibers. Collagenases have the capacity to unwind the triple helix and cleave the native helix of types I, II, and III fibrillar collagens at a single peptide bond, generating fragments approximately 3/4 and 1/4 the size of the original molecule. MMP-8 is the predominant collagenase in healing wounds and its overexpression is involved in the pathogenesis of nonhealing ulcers **(Chung et al., 2004; Troeberg and Nagase, 2012)**.

6. STAT3 and osteoarthritis

Signal transducer and activator of transcriptions (STATs) are a group of proteins that regulate intracellular transcription and therefore control many cellular activities including cell proliferation, cell apoptosis, inflammation, differentiation, and cell migration. The STATs play an essential role during embryo development, immune response, and tumor growth **(Onishi and Zandstra, 2015)**. Therefore, the STATs are regarded as valuable targets in cancer studies and diseases caused by inflammatory disorders **(Kasembeli et al., 2018)**.

The STATs family is composed of 7 members in mammalian cells: STAT1, STAT2, STAT3, STAT4, STAT5a, STAT5b and STAT6. They share similar activities while having different properties as well. For example, STAT1, STAT2 and STAT3 are involved in interferon induced transcription. STAT3, STAT4, STAT5 and STAT6 play an important role in IL induced CD4+ T cell differentiation. The STAT3 protein has the most complex set of functions among the STAT family and is therefore particularly interesting to study **(Onishi and Zandstra, 2015)**.

The genomic location of the STATs is as variable as the functions of this protein family. The genes encoding for STAT1 and STAT4 are located in chromosome 1 while STAT2 and STAT6 are located in chromosome 10 and STAT5a and STAT5b are mapped in chromosome 11 in human cells. The genomic study of the STAT members may also contribute to the understanding of the STATs' functions and mechanisms. The complex functions of STATs can also be revealed by the differences of the protein structure of each STAT family **(Hu et al., 2021a) (Figure 4)**.

6.1. Transcriptional and post-transcriptional regulation of STAT3

STAT3 protein is expressed at a basal level in cells but rapidly increases once activated by specific cytokines. STAT3 is a critical factor in IL-6 induced gene regulation. STAT3 can be phosphorylated by IL-6 signal pathway, whereas IL-6 can also activate STAT3 at the transcriptional level. The level of STAT3 mRNA increases 1 h after IL-6 treatment and reaches to the maximum value at 3 h **(Qi and Yang, 2014)**.

MicroRNAs are endogenously expressed small non-coding RNAs acting at the post-transcriptional level where they promote mRNA degradation and block protein translation. Recent findings suggest that complex transcriptional and post-transcriptional circuits control miRNAs. STAT3 has emerged as an important regulator of their expression and biogenesis and, in turn, STAT3 signaling pathways are controlled by distinct miRNAs **(Bartel, 2004)**.

6.2. Post-translational modification of STAT3

6.2.1. STAT3 phosphorylation

STAT3 protein exists in the cytoplasm as an inactive form until phosphorylation by receptor-associated kinases. Activated JAK kinases phosphorylate STAT3 through binding of the SH2 domain to a phosphorylated tyrosine residue, by which the C-terminus of p-STAT3 triggers its release from receptor and form a homo- or hetero-dimerization of p-STAT3. Dimerized STAT3 translocate to the nucleus and binds to the promoters bearing cognate DNA-binding sequences **(Michels et al., 2013)**. STAT3 can be also phosphorylated by other tyrosine kinases, such as the Src family. However, several articles reported that un-phosphorylated STAT3 can interact with NF-κB. Un-phosphorylated STAT3 (U-STAT3)/NF-κB complex translocates into the nucleus and activates the expression of NF-κB target genes **(Yang and Stark, 2008)**.

6.2.2. STAT3 acetylation

Protein acetylation is a crucial post-translational modification of gene expression and is involved in extensive physiological and pathological processes **(Hohl et al., 2013)**. The inhibition of HDACs can induce the acetylation of STAT3 at Lys-685 **(Sun et al., 2009)**. A significant increase in STAT3

acetylation at Lys-685 was detected in tumor tissues (**Belo et al., 2019**).

6.2.3. STAT3 methylation

STAT3 can be methylated in both its N-terminal domain (K49) and in adjacent coiled-coil domain. As reported, this methylation is a negative regulator for the expression of a specific subset of STAT3 target genes (**Dasgupta et al., 2015**).

6.3. Role of STAT3 in osteoarthritis pathogenesis

The STAT3 pathway is a well-conserved pathway that is closely related to the expression of genes, including cell growth, survival, and apoptosis. In addition, the JAK2/STAT3 signaling pathway is relevant to the initiation and progression of diseases, such as cancer, neurological, and immune-inflammatory conditions. Several studies have shown that the pathogenesis of OA is, at least in part, the result of interactions between STAT3 and multiple signaling pathways (**Zhao et al., 2020**). This indicates that JAK2/STAT3 pathway may be a prospective target for the therapy of OA. The relationship between OA and JAK2/STAT3 signaling pathway is closely related (**Figure 5**) (**Chen et al., 2023**). The specific mechanisms involved are described in the following subsections.

6.3.1 STAT3 signaling pathway and cartilage/synovium in osteoarthritis

6.3.1.1. Cartilage homeostasis

Cartilage homeostasis is a state of equilibrium in the synthesis of ECM that is critical to overall joint health. The progression of cartilage homeostasis is characterized by the upregulation of COL-II and aggrecan levels, along with a decrease ADAMTs and MMPs (**Knäuper et al., 1997**). MMPs family (MMP1, MMP3, MMP-9, MMP13) and ADAMTs family (ADAMTs-4, ADAMTs-5) cause matrix degradation and disrupt cartilage homeostasis (**Vincenti and Brinckerhoff, 2002**).

Previous studies exposed that the expression of the STAT3 pathway was abnormally activated in osteoarthritic cartilage relative to normal cartilage tissue. Inhibition of the STAT3 pathway prevented an increase in the expression of MMPs and further reversed the imbalance in cartilage homeostasis (**Cao et al., 2018**). Another study detected the expression of STAT3 in different groups of cartilage weight-bearing areas by immunohistochemistry and found that the

expression levels of STAT3 in OA cartilage tissue were significantly higher than those in normal cartilage. It was further confirmed that high expression of STAT3 decreased COL-II levels and caused cartilage matrix damage. Collectively, inhibition of STAT3 could promote ECM anabolism and maintain cartilage homeostasis (**Shao et al., 2021**).

6.3.1.2. Inflammatory response

The inflammatory response usually occurs in conjunction with OA pathogenesis and OA-related symptoms. During the progression of OA, large amounts of inflammatory factors (IL-1β, IL-6, TNF-α, and IL-8) that are produced in chondrocytes or synovial cells can accelerate cartilage degradation (**Kapoor et al., 2011**). Particularly, IL-1β can cause intense inflammatory responses by activating complex pathway networks. Numerous studies identified that STAT3 was rapidly phosphorylated under the stimulation of IL-1β. In addition to IL-1β, IL-6 also induces an inflammatory response in chondrocytes (**Zhang et al., 2015b**).

The synovium consists of synovial cells, fibroblasts, and macrophages. Synovitis is a joint lesion in which the synovial membrane is irritated and becomes inflamed, resulting in an imbalance in fluid secretion. STAT3 signaling pathway effectively mediates inflammation in OA synovial cells. It was found that JAK2 inhibition reduced inflammation in synovial cells by inhibiting STAT3 phosphorylation (**Gyurkovska et al., 2014**).

6.3.1.3. Programmed cellular death

Aggregation of inflammatory responses may lead to programmed cell death, mainly including the aberrant levels of cell proliferation, apoptosis, and autophagy that exerted a disruptive influence on cartilage integrity and progression (**Hwang and Kim, 2015**). JAK2/STAT3 signaling pathway plays an important regulatory role in chondrocyte survival and apoptosis. A Previous study showed that parathyroid hormone (PTH) could suppress chondrocyte apoptosis by downregulating the expression of caspase-3 via inhibiting the JAK2/STAT3 pathway (**Shao et al., 2021**).

Additionally, numerous studies have shown that IL-1β treatment could induce apoptosis of chondrocytes and trigger the activation of STAT3

signaling pathway. Therefore, the STAT3 pathway positively mediates chondrocyte apoptosis, and the inhibition of STAT3 pathway may protect against OA by reducing apoptosis and enhancing the proliferation of chondrocytes **(Shao et al., 2020; Shao et al., 2021)**.

Recently, we found that increased STAT3 expression levels were associated with increased proinflammatory cytokines, upregulated expression of degradative MMPs, and degradation of cartilage components, which collectively intensified OA inflammatory symptoms and decreased joint performance **(Wahba et al., 2023)**.

6.3.2. STAT3 signaling pathway and subchondral bone in osteoarthritis

Subchondral bone, the bone component under calcified articular cartilage, protects articular cartilage from external mechanical loads by distributing the loads evenly over the joint surface **(Hu et al., 2021b)**. Microstructural alterations in subchondral bone are responsible for cartilage instability and lead to cartilage degeneration over time. The subchondral bone and osteochondral junction may be subjected to inappropriate external mechanical loading, thereby compromising their integrity. Some studies have shown that the STAT3 signaling pathway played an important role in subchondral bone remodeling **(Zhang et al., 2020)**.

The activity of osteoclast and osteoblast played an important regulatory role in bone modeling, reconstruction, and dynamic homeostasis. Previous study showed that HIF-1 enhanced the level of RANKL by activating STAT3 pathway. This facilitation of osteocyte-mediated osteoclastogenesis by HIF-1 via STAT3 regulation may be a mechanism for enhancing bone resorption in OA (Zhu et al., 2019). In consistent with these reports, it was found that STAT3 inhibitors could suppress RANKL-induced osteoclastogenesis and prevent bone loss **(Latourte et al., 2017)**. Collectively, the beneficial effects of STAT3 inhibition on cartilage include an increase in subchondral bone mass and further protection of the subchondral microarchitecture from deterioration **(Chen et al., 2023)**.

7. microRNAs and osteoarthritis

MicroRNAs are small, single-stranded, non-coding RNAs, consisting of 20-25 nucleotides, whose role is post-transcriptional regulation of gene expression. MiRNAs are partially complementary and bind to

3'-UTR of their target mRNA. They then inhibit the translation of their target mRNA or cause its degradation. Thus, miRNAs inhibit the expression of their target gene at post-transcriptional level **(Bartel, 2004)**. The miRNAs can act as a biomarker and provide a potential tool for the diagnosis and prognosis of human diseases. MicroRNAs have attracted a lot of attention lately, since they have the potential to be used as biomarkers or as novel therapeutic agents. Numerous studies have shown that miRNA expression is altered in OA and these alterations possibly contribute to OA pathogenesis **(Panagopoulos and Lambrou, 2018)**.

7.1. MicroRNAs are involved in cartilage matrix degradation and joint inflammation in osteoarthritis

Dysregulation of the expression of several miRNAs in OA results in increased production of cartilage matrix degrading enzymes (e.g., MMPs, ADAMTS proteases). Downregulation of miR-24, miR-27b, miR-148a, miR-210, miR-222, miR-370, miR-373 and miR-488 in OA cartilage leads directly or indirectly to an increase in the production of MMPs and ADAMTS proteases and downregulation of matrix components (COL-II, aggrecan) **(Matsukawa et al., 2013; Vonk et al., 2014; Panagopoulos and Lambrou, 2018)**.

Besides, other miRNAs are involved in the production of proinflammatory cytokines like TNF-α, IL-1β, IL-6 and IL-8 in OA. Downregulation of miR-130a and miR-149 in OA chondrocytes results also in an increase in the production of TNF-α, IL-1β and IL-6 **(Li et al., 2015)**. Moreover, upregulation of miR-381a-3p in OA chondrocytes inhibits nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, alpha (IκBα), resulting in an increase of the production of TNF-α, cyclooxygenase-2 (COX-2), Inducible nitric oxide synthase (iNOS), IL-6 and IL-8 **(Xia et al., 2016)**.

7.2. MicroRNAs are involved in apoptosis in osteoarthritis

Aberrant expression of miRNAs facilitates apoptosis of articular chondrocytes, thus enhancing the degradation of articular cartilage. The involvement of miR-9, miR-26a, miR-139 and miR-146 in increased apoptosis of chondrocytes in osteoarthritic cartilage has already been discussed

(**Panagopoulos and Lambrou, 2018**). Another miRNA involved in chondrocytes apoptosis in OA is miR-210 which is also involved in increased apoptosis of osteoarthritic chondrocytes. The expression of miR-210 is inhibited in OA chondrocytes, resulting in upregulation of its target gene, death receptor 6 (DR6). Increased expression of DR6 leads to increased activation of the NF-κB signaling pathway and facilitates the apoptosis of the chondrocytes (**Zhang et al., 2015a**).

7.3. Extracellular vesicles and microRNAs in osteoarthritis

The miRNAs may be packaged in extracellular vesicles such as exosomes, secreted from one cell and transferred to another cell, to act as gene expression regulators. They participate in OA pathogenesis by mediating cell to cell communication in osteoarthritic joints (**Chen et al., 2012**). It was shown that exosomes derived from IL-1β-stimulated OA cartilage upregulated the expression of MMP13, IL-1β, TNF-α and COX-2 in OA synovium (**Zhou et al., 2020**).

7.4. MicroRNAs as biomarkers in osteoarthritis

MicroRNAs may be detected in peripheral blood and synovial fluid incorporated in extracellular vesicles or bound to lipoproteins and RNA-binding proteins. Their stability, ease of measurement and different expression in the blood and synovial fluid of OA patients offer the opportunity of using them to predict the prognosis or even measure disease activity or predict response to treatment. **Murata et al. (2010)** showed that plasma levels of miR-16 and miR-132 differentiated OA patients from healthy controls since they were significantly lower in the former. Moreover, synovial fluid concentrations of miR16, miR-146a, miR-155 and miR-223 were significantly lower in patients with OA compared to patients with rheumatoid arthritis and could differentiate those two groups of patients (**Murata et al., 2010**).

7.5. Therapeutic potential of microRNAs in osteoarthritis

Current treatment of OA includes drugs such as NSAIDs for alleviating symptoms and total joint arthroplasty in cases of severe OA. There are no drugs that halt the progress of the disease, like disease-modifying drugs do in rheumatoid arthritis. miRNAs represent a promising target for the treatment of OA. A remarkable number of miRNAs

participate in the pathogenesis of OA. Inhibition of these miRNAs with antisense oligonucleotides (anti-miRs) or administration of miRNAs that silence genes participating in OA pathogenesis could be a novel approach for arresting the progress of OA. An advantage of this approach is that synovial joints are an isolated environment and intra-articular administration of miRNAs would not have systemic effects. However, an important issue is the delivery method of the miRNAs or the anti-miRs. Several solutions have been proposed, including extracellular vesicles (exosomes), nanoparticles and antibodies (**Li and Rana, 2014**).

An example of targeting miRNAs for the treatment of OA is miR-140. **Karlsen et al. (2016)** studied the protective effect of miR-140 in an in vitro model of OA. They transfected miR-140 into IL-1β-treated articular chondrocyte and mesenchymal stem cell cultures and they demonstrated that miR-140 upregulated the synthesis of cartilage matrix components and downregulated the production of cartilage degradation enzymes (**Karlsen et al., 2016**).

7.6. miR-452-3p and osteoarthritis

MicroRNA 452-3p (miR-452-3p, also known as has-miR-452-3p) is encoded by the chromosomal region Xq28 in humans and clustered together with miR-224 within the gamma-aminobutyric acid A receptor epsilon subunit gene. miR-452 is considered one of the important members of the miRNA family, divided into two subtypes: miR-452-5p and miR-452-3p (**Karimi et al., 2023**).

Our recent research has provided compelling evidence for the involvement of miR-452-3p in the regulation of inflammatory processes associated with OA pathogenesis. We found that the observed association between decreased miR-452-3p levels and heightened expression of key inflammatory mediators, including plasmin, STAT3, TNF-α, and MMP-3, sheds light on the regulatory influence this microRNA exerts over critical pathways implicated in joint inflammation and tissue degradation. Significantly, OA patients consistently demonstrate downregulated miR-452-3p levels compared to their normal healthy counterparts, establishing a compelling correlation between miR-452-3p dysregulation and the disease state (**Wahba et al., 2023**). This molecular signature not only underscores the diagnostic potential of miR-452-3p as a biomarker but also positions it as a promising therapeutic target for interventions aimed at

the inflammatory milieu and alleviating the destructive processes associated with OA. Moving forward, further exploration of the intricate interactions between miR-452-3p and its target molecules holds the key to unlocking novel avenues for more targeted and effective therapeutic strategies in the management of OA.

8. Conclusion

Osteoarthritis is a chronic degenerative joint disease, characterized by progressive degradation and loss of articular cartilage, subchondral bone sclerosis, inflammation, and osteophyte formation. OA is the most prevalent type of arthritis accounting for most of the pain, disability, and impaired adult work performance globally. Interestingly, ECM proteins, signaling pathways, transcriptional factors, inflammatory mediators, and other factors that are commonly linked to OA are all to differing degrees susceptible to genetic and epigenetic regulation. It was demonstrated that the decreased miR-452-3p and increased STAT3 expression levels were associated with increased proinflammatory cytokines, upregulated expression of degradative MMPs and degradation of cartilage component which collectively intensified OA inflammatory symptoms and decreased joints performance.

Conflict of interest

None of the authors have any conflicts of interest.

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