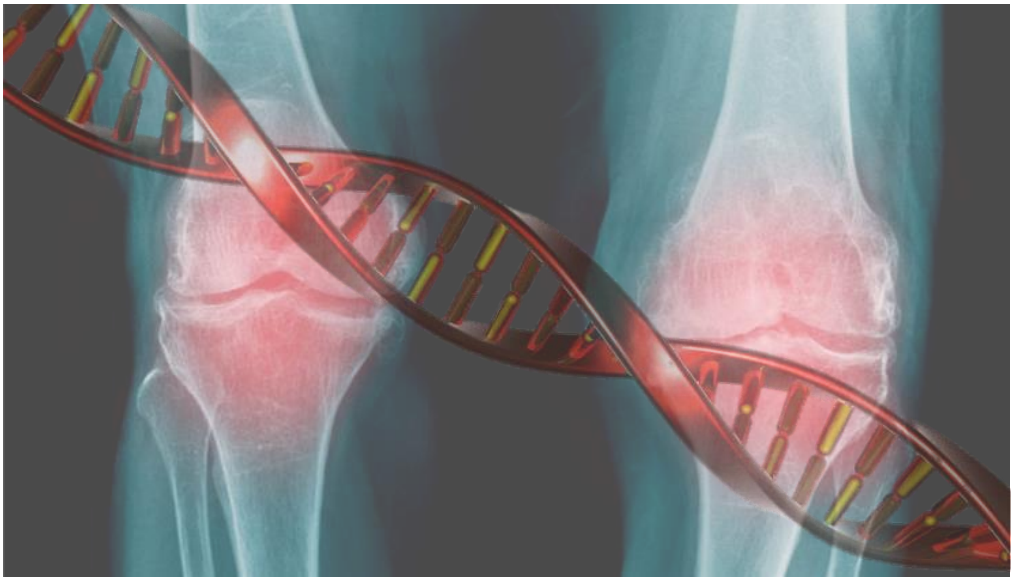
TRABAJO FIN DE GRADO

EPIGENETICS & OSTEOARTHRITIS



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TRABAJO FIN DE GRADO

GRADO EN FARMACIA

EPIGENETICS AND OSTEOARTHRITIS

Revisión bibliográfica

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Sevilla, Julio de 2021

Abstract

Osteoarthritis (OA) is a chronic disease of the joints that affects up to 50% of the population above 65 years old. Despite its elevated prevalence, the mechanism of action of this pathology is yet to be fully elucidated, however, it is known to be influenced by mechanic, metabolic and inflammatory factors. Its main clinical symptoms are disabling pain and loss of function in the affected joint, that significantly deteriorate life quality of the patient.

The main objective of this thesis is to study the alterations in the epigenome of osteoarthritic population, analyze how these alterations correlate with an OA phenotype, and how this data can be used to broaden therapeutic options.

Some studies have brought to light that an important part of OA pathogenesis relies on an aberrant gene expression derived from epigenetic changes, which are the ones that do not modify DNA sequence. This "disease-associated epigenetic control of gene expression" occurs when alterations in the cellular mechanisms of epigenetic regulation change gene expression, and consequently increase the susceptibility of suffering a certain illness. Studying the differences in the epigenome of subjects with OA compared to healthy ones, is useful not only to deeply analyze the pathology itself, but also for its diagnosis, prognostic and even potential treatment.

In this thesis, said differences are evaluated for the main epigenetic marks, which are DNA methylation, posttranslational modification of histones and non-codingRNAs, together with how these correlate with gene expression and its phenotypic consequences in OA. The potential of nutritional therapy as a type of epigenetic therapy, in concomitance with traditional pharmacological therapy, is also studied, as it may play a role in helping improve OA, at least while other more effective disease-modifying drugs are developed that are able to slow, stop or even reverse its progression.

Keywords

Osteoarthritis, DNA methylation, modification of histones, non-codingRNAs, epigenetic therapy

Resumen

La artrosis es una patología crónica articular que afecta hasta al 50% de la población mayor de 65 años. A pesar de su elevada prevalencia, la fisiopatología de la artrosis no se conoce en profundidad, no obstante, se ha demostrado que en ella intervienen diversos factores mecánicos, metabólicos e inflamatorios. Sus manifestaciones clínicas son principalmente dolor inhabilitante y pérdida de función en la articulación afectada, que disminuyen de manera significativa la calidad de vida de aquellos que los sufren.

El objetivo principal de este trabajo es estudiar las alteraciones del epigenoma observadas en la artrosis, analizar como estas se correlacionan con cambios en la fisiopatología, y como esta información puede ser usada para contemplar posibles abordajes terapéuticos

Se ha puesto de manifiesto que una parte de la patogénesis de la artrosis recae sobre una expresión génica anómala derivada de cambios epigenéticos, que son aquellos que no afectan a la secuencia de ADN. Este llamado "control epigenético de la expresión génica asociada a enfermedad" se da cuando alteraciones en los mecanismos celulares de regulación epigenética, al modificar la expresión génica, aumentan la susceptibilidad a una enfermedad. Saber las diferencias en el epigenoma entre sujetos sanos y sujetos con artrosis es útil para el estudio de la propia patología, para su diagnóstico, pronóstico e incluso potencial tratamiento.

En este trabajo se analizan dichas diferencias, en los principales marcadores epigenéticos como son metilación del ADN, modificación postraduccional de histonas y ARNs no codificantes, y su relación con la expresión génica y las características fenotípicas de la artrosis. También se estudia el potencial de la terapia nutricional como método de terapia epigenética, que junto con los tratamientos tradicionales pueda mejorar la artrosis, mientras se investigan otros fármacos que modifiquen de forma eficaz la patología, enlenteciendo, deteniendo o revirtiendo su progresión.

Palabras clave

Artrosis, metilación de ADN, modificación de histonas, ARN no codificante, terapia epigenética

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GLOSSARY OF ABBREVIATIONS

OA: osteoarthritis

AC: articular cartilage

SNP: single nucleotide polymorphism

BMI: body mass index

TNF-α: tumor necrosis factor alpha

IL: interleukin

ROS: reactive oxygen species

MMP: metalloproteinase

DM: diabetes mellitus

SF: synovial fluid

ECM: extracellular matrix

NO: nitric oxide

PG: prostaglandin

IGF: insulin growth factor

NF-kB: nuclear factor kappa B

FGF: fibroblast growth factor

TGF: transforming growth factor

PTMH: posttranslational modification of histones

ncRNA: non-coding RNA

miRNA: microRNA

lncRNA: long non-coding RNA

CpG: cytosine-phosphate-guanosine

DNMT: DNA methyltransferases

TET: ten-eleven translocation

HAT: histone acetyltransferase

HDAC: histone deacetylase

TAD: topological associated domain

siRNA: short interfering RNA

piRNA: PIWI-interacting RNA

GWAS: genome-wide association studies

mQTL: methylation quantitative trait loci

DMR: differentially methylated region

GDF5: growth differentiation factor 5

DIO2: Iodothyronine Deiodinase 2

SOX9: SRY-Box Transcription Factor 9

CREB: cAMP responsive element binding protein

ACAN: aggrecan

COL: collagen

HMT: histone methyltransferase

HDMT: histone demethylase

SIRT: sirtuin

KDM: lysine demethylase

NFAT1: nuclear factor of activated T cells 1

DOT1L: disruptor of telomeric silencing 1-like histone methyltransferase

mPGES-1: microsomal prostaglandin E synthase-1

LSD1: lysine-specific histone demethylase

COX: cyclooxygenase

iNOS: inducible nitric oxide synthase

ADAMTS: A disintegrin and metalloproteinase with thrombospondin motif

RISC: RNA-induced silencing complex

IGFBP: insulin-like growth factor binding protein

ceRNA: competing endogenous RNA

iHDAC: HDAC inhibitor

TSA: Trichostatin A

SAM: S-adenosyl methionine

SAH: S-adenosyl homocysteine

EGCG: Epigallocatechin-3-gallate

ALA: alpha-linoleic acid

EPA: eicosapentaenoic acid

DHA: docosahexaenoic acid

1. Introduction

1.1 Concept and epidemiology of Osteoarthritis (OA)

OA is a chronic degenerative disease of the joints, and the most common rheumatic disease in the world (Bortoluzzi et al., 2018; Mobasheri et al., 2017). It is considered a complex, multifactorial condition influenced by mechanical, metabolic and inflammatory components. To date, the pathogenic mechanisms of OA are yet to be fully elucidated. This is probably the reason why there are no effective treatments to cure or even slow the progression of the disease, other than total joint replacement surgery (Xia et al., 2014). Nowadays, the therapeutic approach is a preventive one, by taking action before the onset of the pathology, especially in patients with increased risk of developing it. This method could be even more effective if patients could be screened in advance, bringing to light the importance of the knowledge of biomarkers and genes that affect onset and progression of OA (Sulzbacher, 2013).

OA can be classified as symptomatic and asymptomatic, the latter one being only diagnosable with imaging or laboratory tests. The asymptomatic OA, or radiographic OA, is more prevalent than the symptomatic (probably because it precedes the apparition of symptoms, on an early stage of the disease) (Hunter and Bierma-Zeinstra, 2019).

To adequately establish the epidemiology of OA, three descriptors are to be acknowledged. The definition of OA, the joints analyzed, and the population studied, the last one being because of the demographic, genetic, and environmental differences. (Bortoluzzi et al., 2018; Vina and Kent Kwoh, 2018).

The most prevalently affected joints are knee, hand and hip in that order. It is more prevalent in women (18%) than in men (9.6%), according to WHO scientific group on rheumatic diseases (Fathollahi et al., 2019; Hunter and Bierma-Zeinstra, 2019). The current prevalence globally is around 15%, and could reach 35% by 2035, as the constant increment of aged and obese population will increase the incidence of OA over time.

According to the most recent Global Burden Disease study, knee OA is the eleventh highest contributor to global disability (measured by years lived with disability). (Bortoluzzi et al., 2018; He et al., 2020)

This pathology can be fragmented into what are called phenotypes. Phenotypes are the combination of attributes of the disease, that describe differences between patients. OA is a very heterogenous and complex pathology, and it is not odd that different pathogenic mechanisms derive into different phenotypes. There are six variables that represent the clinical phenotypes: chronic pain, inflammation, metabolic syndrome, metabolism of bone and cartilage, mechanical overload and minimal joint disease. The last one classifies patients according to disease progression (Vina and Kent Kwoh, 2018).

1.2 Risk factors for OA

The risk factors can be classified as non-modifiable (age, gender, genetics, etc.); and modifiable (obesity, diet, mechanical overload, etc.), that can potentially mitigate the risk of developing OA (Mobasheri and Batt, 2016).

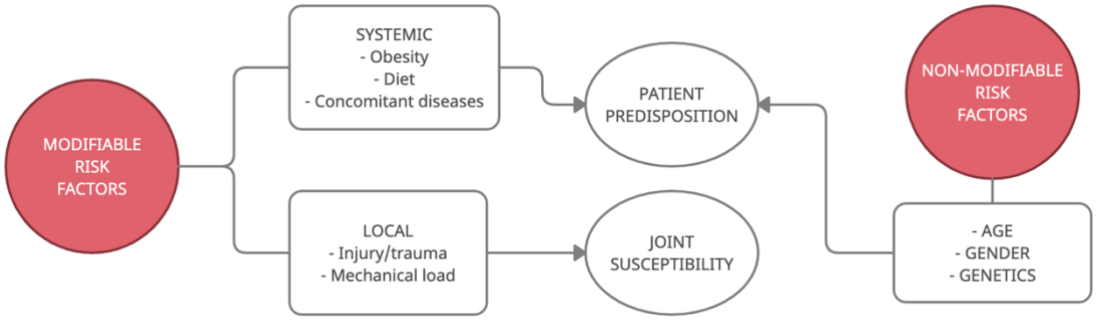

Figure 1. Risk factors for OA (Mobasheri and Batt, 2016)

- **Age**: it is the biggest risk factor. 50% of people above 65 years old suffer from OA. Aging and inflammation are important factors in the development and progression of musculoskeletal diseases in general. The low-grade inflammation related to age is called "inflammaging". With age, tissue repairment and bone plasticity decrease and chondrocyte apoptosis and bone fragility increase. Aging also correlates with long-time effects of mechanical load, changes in the

composition of cartilage (it becomes more rigid with time) and cumulative exposure of various risk factors, as well as with physiological changes such as mitochondrial dysfunction, oxidative stress, increase of pro-inflammatory cytokines and unrepaired molecular damage to DNA, proteins and lipids. Cellular senescence is another age-related phenomenon that consists in irreversible cell growth arrest. This event is normal with age, but in people with OA, chondrocyte senescence or "chondrosenescence" is even more significant (and risk factors increase its probability). In this situation chondrocytes demonstrate a deviant behavior. Chondrosenescence along with immunosenescence and the inflammatory microenvironment in the articular joint further allow age related degradation of articular cartilage (AC) and other tissues (Bortoluzzi et al., 2018; He et al., 2020; Hunter and Bierma-Zeinstra, 2019; Mobasheri and Batt, 2016; Sulzbacher, 2013).

- **Gender:** female gender is more susceptible of suffering OA (except for cervical and shoulder OA that are more prevalent in men), specifically, post-menopause women. There is evidence of the presence of estrogen receptors in the majority of joint tissues, which may indicate that hormones play a role in the pathogenesis of disease. This fact together with the different composition of the female body (with higher percentage of fat) may explain why OA is more prevalent in female population, and therefore makes being a woman a risk factor (Bortoluzzi et al., 2018; Vina and Kent Kwoh, 2018).

- **Genetics:** there are several genes that affect both prevalence and progression of the disease, and the subpopulations of OA are linked to different genetic variations (phenotypes). Mutations in genes can have a predictive value on characteristics of the disease. OA-associated variants are represented by common single nucleotide polymorphisms (SNPs) with minor allele frequencies, that have moderate to small effect size. These monogenic mutations associate with early onset. Late onset on the other hand is associated with multifactorial causes (genetic variants added to other risk factors) (He et al., 2020; Hunter and Bierma-Zeinstra, 2019; Sulzbacher, 2013; Vina and Kent Kwoh, 2018).

- **Obesity:** it is the most important modifiable risk factor. Taking into consideration that there is a clear association between body mass index (BMI) and hand OA, it cannot be said that the relation between obesity and the pathology occurs solely because of mechanical overload. It must also have a metabolic and pro-inflammatory component to it, that actually correlates better with percentage of fat and waist circumference than with BMI. Adipose tissue releases adipokines which are tissue-specific cytokines that promote the inflammatory cascade, inducing the production of other inflammatory cytokines such as tumor necrosis factor (TNF)-α and interleukin (IL)-6. Moreover, mechanical overload causes apoptosis of chondrocytes, fissures and microcracks, with the consequent inflammatory response. Finally, weight loss has been associated with improved symptoms in a dose-response manner, and slower cartilage degeneration (Bortoluzzi et al., 2018; He et al., 2020; Sulzbacher, 2013; Vina and Kent Kwoh, 2018).

- **Diet:** high levels of glucose in the bloodstream accumulate in chondrocytes, increasing reactive oxygen species (ROS) and expression of metalloproteinases (MMPs). Likewise, high levels of LDL cholesterol present in the bloodstream oxidize, then deposit in the cartilage and produce inflammation, ROS and mitochondrial dysfunction (Bortoluzzi et al., 2018)

- **Mechanical load:** abnormal load can come with occupations that stress one joint in particular or with extreme sport activity, for example. Physiological mechanical load is normal, expected and necessary in order to maintain the equilibrium of anabolic and catabolic mechanisms. However, supraphysiological mechanical load is not normal, and has been proved to deteriorate AC and make it more susceptible to lesions. Furthermore, there are signs that indicate that substantial mechanic stimuli can transform into pro-inflammatory signals (Bortoluzzi et al., 2018; He et al., 2020; Mobasheri and Batt, 2016).

- **Injury/trauma:** trauma implies an immediate loss of cells through necrosis and apoptosis which can lead to post-traumatic OA (He et al., 2020).

- **Concomitant diseases**: metabolic syndrome (obesity, hypertension, hyperglycemia, dyslipidemia and insulin resistance), diabetes mellitus (DM) and other rheumatic diseases have shown to be associated with increased probabilities of developing OA (Bortoluzzi et al., 2018; Mobasheri and Batt, 2016).

1.3 Etiopathogenesis of OA

Healthy joints (Figure 2) are composed by two or more articulating bones or subchondral bones, ligaments, capsule, synovial membrane, cartilage and periarticular muscles. Inside the capsule, the synovial membrane which is conformed by two layers: the internal synovial intima, with macrophages and dendritic cells, and the outer synovial intima with fibroblast-like synoviocytes (this layer is supplied with vasculature, nerves and lymphatics). The synovial fluid (SF), made of lubricin and hyaluronan, is secreted by the synovial cells, and fills the joint cavity. The SF has many functions such as lubricating the joint, supplying nutrients and oxygen and eliminating cellular waste, and also secreting pro-inflammatory molecules. The cartilage of the joint also has layers (Figure 3). Superficial zone, middle or

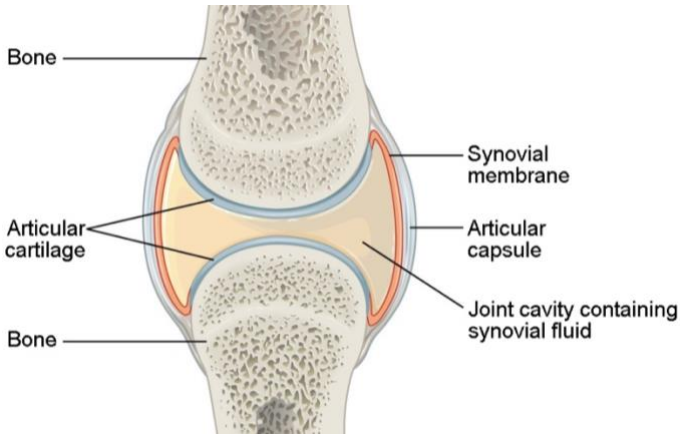

Figure 3. Structure of the joint. (TeachmeAnatomy, 2021)

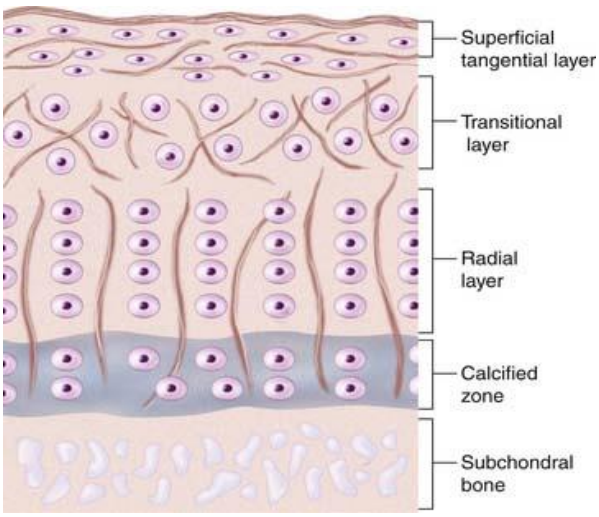

Figure 2. Layers of the cartilage (Clinicalgate, 2015)

transitional zone, deep or radial zone and calcified cartilage. All of them harbor chondrocytes in various stages of maturation. These chondrocytes are the only cell type in the cartilage, and they are responsible of secreting anabolic components such as aggrecan and collagen type II (this is the principal type of collagen in AC, though there are more), that make the extracellular matrix (ECM), and catabolic components such as MMPs, that degrade the ECM. These substances maintain the homeostasis of the cartilage and form a specific architecture that makes it very flexible and resistant. These cells have limited contact with vasculature, so they receive most of the nutritional supplies from the SF. In fact, the lack of blood vessels, makes this tissue very susceptible to minimal changes in its composition and any variation in its components can affect homeostasis (Fathollahi et al., 2019; Grandi and Bhutani, 2020; He et al., 2020; Xia et al., 2014).

The pathogenesis of OA involves the imbalance between destruction and production of joint tissues (Hunter and Bierma-Zeinstra, 2019).

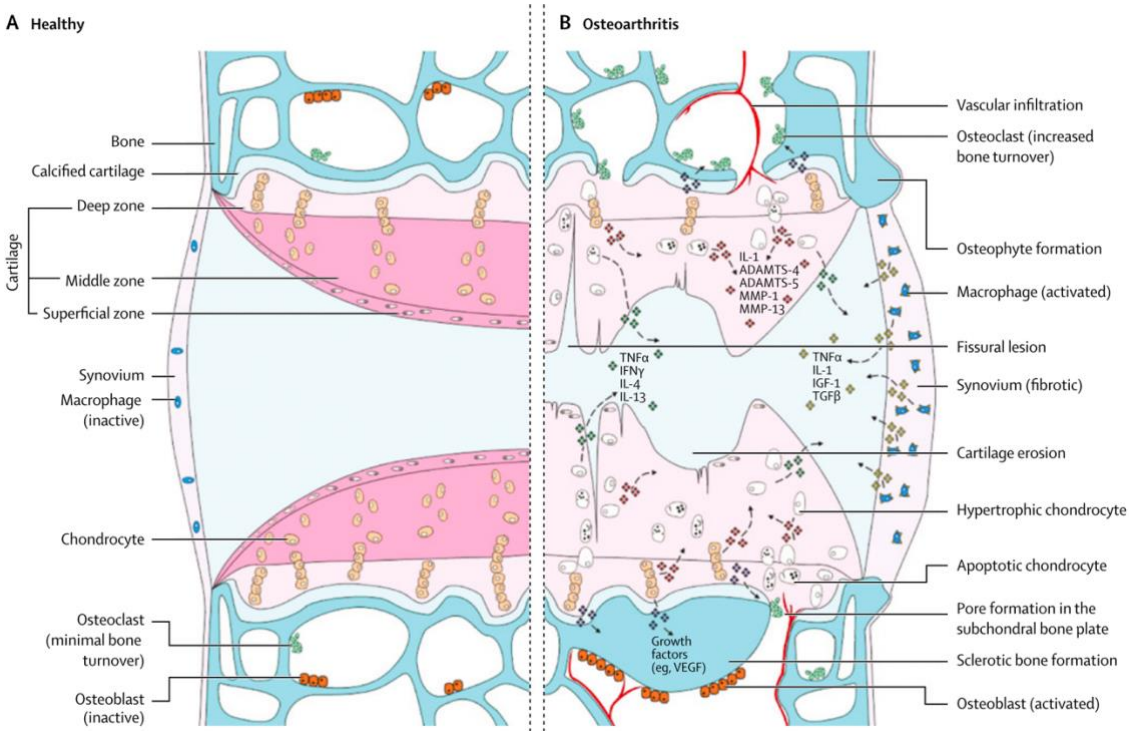

Figure 4. Changes in AC during OA progression (Hunter and Bierma-Zeinstra, 2019).

In the early stages of OA, the cartilage is intact. The first altered component is the ECM which loses its integrity (this can be due to cumulative effect of risk factors, and multiple signaling pathways), and chondrocytes respond to the damage by synthetizing matrix components and trying to repair it. But this situation is only temporary, as it leads to early

maturation of chondrocytes into hypertrophic chondrocytes. These chondrocytes suffer a premature senescence and lose their ability to synthesize matrix. They show an increased secretion of ECM catabolic products and pro-inflammatory mediators that break apart the collagen net and act on the adjacent synovium to promote the inflammatory response. The final outcome is cartilage erosion, and over time, deep fissures in the cartilage and lesion of subchondral bone. AC keeps degenerating, until there is no more, and the bones start to friction (Hunter and Bierma-Zeinstra, 2019; Mobasheri and Batt, 2016; Xia et al., 2014).

Synoviocytes start secreting proinflammatory molecules such as IL1β, IL-1α, TNF-α, nitric oxide (NO) and prostaglandins (PGs) as a result of chondrocyte activation of inflammatory pathways. More structural changes take place due to catabolic activity performed by ECM degrading enzymes of which expression is increased in this state. In consequence, the infiltration of inflammatory and immune cells into the synovium (synovitis) followed by hyperplasia and vascularization of the zone becomes patent (Fathollahi et al., 2019; Grandi and Bhutani, 2020; Hunter and Bierma-Zeinstra, 2019; Sulzbacher, 2013).

Osteophytes begin to form on the edges of the bone creating cysts and bone spurs, due to altered endochondral ossification, which is a process involved in the growth of long bones in which hypertrophic chondrocytes, at their last stage of maturation, mediate cartilage calcification in what is called the growth plate, then undergo apoptosis and leave room for osteoblasts to fill the space with new bone (Hunter and Bierma-Zeinstra, 2019; Sulzbacher, 2013).

In terms of molecular mechanisms, chondrocyte differentiation, endochondral ossification, and overall joint development are tightly regulated by a variety of growth factors, transcription factors and signaling pathways such as nuclear factor kappa B (NF-kB), transforming growth factor-beta (TGF-β), fibroblast growth factor (FGF) and Wnt/β-catenin pathway, which play an important part in OA pathogenesis (Xia et al., 2014).

NF-kB is a family of inducible transcription factors that mediates the expression of genes related to immune and inflammatory responses, and therefore plays a crucial role in chronic inflammatory illnesses (Liu et al., 2017).

TGF-β is a family of proteins that are involved in chondrocyte hypertrophy and maturation. The proteins included in this family, that act as ligands (like bone morphogenetic protein (BMP)-7) cause a signaling cascade when they interact with their receptors. This cascade activates SMAD proteins (transcription factors) that form a complex and translocate to the nucleus. Loss of this signaling pathway is associated with cartilage damage and OA progression. However, increased levels of TGF-β in subchondral bone can cause aberrant bone remodeling and actually worsen OA, demonstrating that this pathway has a dual activity, with both protective and catabolic roles (Xia et al., 2014).

FGF is a family of growth factors that can have anabolic or catabolic activity in the joint, depending on the type. FGF-2 for example, is increased in response to injury of the cartilage matrix, and causes further catabolism of it, by upregulating the expression of ECM degrading proteins. FGF-18 on the other hand, is involved in the development, regeneration and repair of functional cartilage (Xia et al., 2014).

Wnt is a family of extracellular glycoproteins with multiple biological functions, including the regulation of developmental processes such as cartilage homeostasis. β-catenin is a transcription factor located in the nucleus of which levels are regulated by Wnt signaling. It is in charge of acting upon the expression of Wnt target genes. The increased Wnt activity with the consequent accumulation of β-catenin, promotes chondrocyte hypertrophy and cartilage degradation via upregulation of ECM degrading proteins. Nevertheless, this signaling pathway also has a dual role in OA pathogenesis as it has been observed that when using beta-catenin inhibitors in animal models, there was an enhanced destruction of cartilage as a result (He et al., 2020; Xia et al., 2014).

1.4 Signs, symptoms and diagnosis of OA

The main clinical symptoms of OA are pain in the affected joint, morning stiffness, functional limitations with reduced range of motions, crepitus, joint instability, swelling, muscle weakness and fatigue. The main signs (identified with radiographic and laboratory methods) are joint deformity, joint space narrowing, subchondral sclerosis, bone eburnation and cyst formation, osteophyte formation, synovial distension and inflammation, thinned and rough AC, reactive bone hyperplasia and hypertrophy of the joint capsule (He et al., 2020; Hunter and Bierma-Zeinstra, 2019; Xia et al., 2014).

The pain is intermittent and specifically associated with movement, and is the most disabling of the symptoms. The cause of the pain is not only mechanical (because of friction), but there is also an increased responsiveness of peripheral nociceptors, due to inflammation and tissue damage. Additionally, neuropathic pain occurs in 23% of patients, because of inadequate innervation of the joint tissues (Hunter and Bierma-Zeinstra, 2019).

Clinical diagnosis of OA is based on the symptoms, and physical examination, following a set of criteria established by rheumatology entities such as the American College of Rheumatology or the European League Against Rheumatism. Imaging or laboratory tests are not needed but can be taken into consideration if other diagnoses are suspected. There is no correlation between radiographic severity or disease duration with pain sensitization. However, this correlation does exist with synovitis and pain sensitization (Hunter and Bierma-Zeinstra, 2019).

There is more to it than physical manifestations, nonetheless. A variety of psychological problems are associated with the disease. Evidence indicates that OA patients have increased risk of depression and suicidal ideation, accompanied by memory loss and mood impairments due to poor sleep quality (Bortoluzzi et al., 2018; Hunter and Bierma-Zeinstra, 2019).

1.5 Conventional therapies for OA

Therapies for OA that stop the progression of the disease or cure it do not exist in the market as of now. For that reason, management of the disease focuses on alleviating the symptoms and bettering the life quality of the patient (Fisterra, 2016).

OA management is a combination of pharmacological and non-pharmacological therapies, planned ahead individually according to the needs of each patient (Kolasinski et al., 2020).

Stablishing an adequate pharmacological treatment for OA consists in part of using, if possible, the drug with least systemic effect, at the minimal possible dose and the least duration necessary. Local administration is preferable over systemic. It is also important to considerate individual physiological and pathological situations (gastrointestinal affections, cardiovascular conditions, etc.). In Table 1 there is a summary of pharmaceutical recommendations according to the American College of Rheumatology (Kolasinski et al., 2020).

Therapeutic group	**Drug**	**Recommendation**	**References**
Nonsteroidal anti-inflammatory drugs (NSAIDs)	Paracetamol	Recommended for all OA forms	(Kolasinski et al., 2020).
	Other oral NSAIDs	Strongly recommended for all OA forms	
	Topical NSAIDs	Strongly recommended for knee OA	
Corticoids	Intraarticular corticoid injection	Strongly recommended for knee and hip OA	
Opioids	Tramadol	Recommended for all OA forms	
	Other opioids	Recommended against for any OA	
Selective serotonin reuptake inhibitors (SSRIs)	Duloxetine	Recommended for all OA forms	
Components of the cartilage	Glucosamine	Strongly recommended against for any OA	

	Chondroitin sulfate	Strongly recommended against for any OA	
Others	Topical capsaicin	Recommended for knee OA	

Table 1. Pharmacotherapeutic recomendations for OA (Kolasinski et al., 2020).

Paracetamol is less effective than other oral nonsteroidal anti-inflammatory drugs (NSAIDs), however an assessment of the risk-benefit ratio is needed considering the fewer adverse effects of paracetamol. Intraarticular corticoid injection is useful in patients that did not respond to oral analgesics. (Hunter and Bierma-Zeinstra, 2019; Fisterra, 2016).

In end-stages of the disease, when patient quality of life is severely reduced, joint replacement surgery is a cost-effective treatment for OA (and the only therapeutic possibility for restoration of the joint).

Disease-modifying OA drugs are being investigated. These are targeted therapies mostly administered via intra-articular injections that can stop or even reverse the progression of OA. As of now, none are approved, but some are in late stages of development such as "Sprifermin", "Human PRP", "teriparatide", "TPX-100" and "ABT-981", all in phases 2 or 3 clinical trials (Hunter and Bierma-Zeinstra, 2019).

Among the non-pharmacological therapies, the main approach is educating the patient, giving information about the disease and about therapeutic possibilities and their advantages and disadvantages. Exercise is essential in order to better the pain and function of the joint. Some examples are walking or tai chi, both strongly recommended for any form of OA. In case of patients with overweight or obesity, weight loss is strongly recommended for all forms of OA (evidence indicates a dose-response association between weight loss and the effect on pain and function). Cane use is recommended in patients with significant limitations in ambulation, joint stability and pain. Lastly, in some patients, if needed, cognitive behavioral therapy has been proven to better the psychological effects of the pathology (Hunter and Bierma-Zeinstra, 2019; Kolasinski et al., 2020).

1.6 Introduction to epigenetics

The modern definition of epigenetics is "information-containing factors, other than DNA sequence that cause stable changes in gene expression and are maintained during cell divisions". These factors are principally, DNA methylation, posttranslational modifications of histones (PTMH), changes in chromatin structure and non-coding RNAs (ncRNAs), such as microRNA (miRNA) and long non-coding RNA (lncRNA). However, the specific definition of epigenetics and its factors is still a matter of controversy (van Meurs et al., 2019).

DNA methylation refers to the addition of a methyl group to a cytosine in the DNA (Figure 5), especially when it is part of a cytosine-phosphate-guanosine (CpG) dinucleotide sequence (this type of sequence appears with high frequency in regions of the DNA called CpG islands, and in regions upstream of a target gene). These methylations are carried out by a family of enzymes called DNA methyltransferases (DNMTs). The most known DNMTs are DNMT1 which copies the preexisting methylation pattern of the mother strand in the new DNA strand during replication, and DNMT3a and DNMT3b which are in charge of the *de novo* methylation (Fathollahi et al., 2019; van Meurs et al., 2019; Simon and Jeffries, 2018).

The objective (or consequence) of methylation depends on the regions in which it occurs. In somatic cells, 80% of CpGs are methylated, particularly in repetitive sequences in intergenic regions and introns. Additionally, gene promoters can be methylated or not. This methylation is associated with repression in gene expression because the methylation itself blocks the binding of transcription factors to the DNA. Methylation in other parts of the gene body, like enhancers, has a more unpredictable effect (Fathollahi et al., 2019; van Meurs et al., 2019).
Methylcytosine can turn into hydroxymethylcytosine (intermediate of the demethylation process) by the action of the ten-eleven translocation (TET) family of enzymes. This modification is related to transcriptional activation (Fathollahi et al., 2019)

Histones are alkaline proteins that form nucleosomes by wrapping DNA around octamers composed by two of each histone (H2a, H2b, H3 and H4), 147 bp and a linker (H1)(HE

et al., 2020; Khan and Haqqi, 2019). Regarding the PTMH, it consists of the addition of chemical groups to the N-terminal tail of histones, such as methyl, acetyl, phosphoryl and ubiquitin groups (Figure 5). The chemical group added, together with the type of amino acid changed, will lead to a specific result. The pattern of combination of histone modification forms what is called the "histone code", that regulates the expression of certain genes by altering the chromatin structure and thus, the DNA's accessibility for transcriptional proteins. As an example, it has been observed that acetylation, mediated by histone acetyltransferases (HATs), and deacetylation, mediated by histone deacetylases (HDACs), of certain amino acids activate and silent gene expression, respectively. However, a single modification is not enough to predict the transcriptional behavior of the genetic sequence (Fathollahi et al., 2019; van Meurs et al., 2019; del Real et al., 2018).

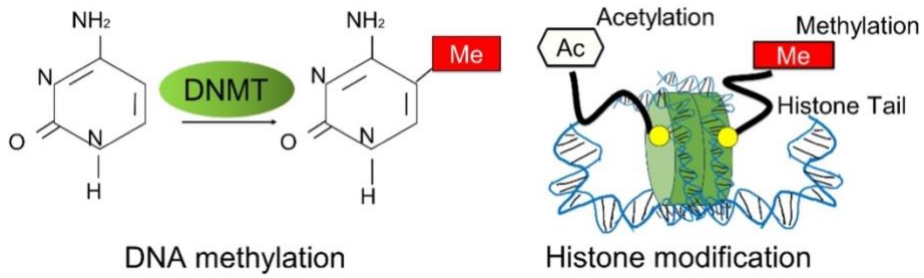

Figure 5. DNA methylation and histone modification. (Zhuang et al., 2020)

The spatial organization of chromatin also has an important role in regulating gene expression. It is known that the genome is segmented into what are called "topological associated domains" (TADs), which are regions that are not necessarily near each other, but interact with each other more frequently than with other regions of the genome (this explains, for example, why enhancers are not always close to a gene body). There are some binding factors that help the chromatin to loop, so the regions of a TAD can interact with each other. Generally, these regions will have diverse functions, and depending of which ones are interacting, the outcome will be different (van Meurs et al., 2019).

Another layer of epigenetic regulation is the presence of ncRNAs, which do not code proteins, but carry out multiple functions in the regulation of gene expression at a both transcriptional and posttranscriptional level. They can be classified into two groups, long

noncoding RNAs (lncRNAs) with more than 200 nucleotides, and small ncRNAs with less than 200 nucleotides. This last group can equally be divided into subgroups that include short interfering RNAs (siRNAs), micro RNAs (miRNAs) and PIWI-interacting RNAs (piRNAs). All ncRNAs can be transcribed from noncoding genes, introns and untranslated regions of protein-coding genes (Fathollahi et al., 2019; van Meurs et al., 2019).

miRNAs and siRNAs are principally in the cytoplasm and bind to the 3'-UTR regions of messenger RNA (mRNAs) to inhibit protein synthesis. But they can also be present in the nucleus and mediate DNA methylation and histone modification. The major role of lncARNs is in the nucleus, where they induce DNA modifications such as loop formation and X chromosome inactivation, and histone modifications. However, they can also bind to miRNAs to inhibit their function and regulate siRNAs formation. lncRNAs are also involved in mRNA splicing, maturation, transport, localization and translation, and protein stability (Fathollahi et al., 2019; van Meurs et al., 2019).

2. Objectives

In the last decade the amount of information available about the pathogenesis of certain diseases has increased considerably. And specifically, one of the novel aspects being studied is the epigenetic changes that occur, that can worsen the pathology or that are a consequence of it. It has been found that an appropriate balance of epigenetic processes is fundamental for a healthy functioning of the organism, and that deregulated epigenetic modifications are involved in the development of several diseases (such as inflammatory diseases). Therefore, it can be said that epigenetics is a factor just as important as genetics in order to predict the progression and prognostic of a disease, and also to study possible therapeutic approaches.

This thesis focuses on analyzing the current epigenomic data, applied to a specific disease, osteoarthritis, and its objectives are:

- To stablish the main differences of epigenetic marks between healthy population and population with OA.
- To stablish how epigenetic changes correlate with characteristics, development and progression of OA.
- To bring to light the potential use of epigenetics to delay the onset and better the course of OA.

3. Methodology

This project consists of a bibliographic review of a number of scientific articles related to the matter of study, in this case, epigenetics and osteoarthritis. For that, a comprehensive research has been done in various databases such as Medline, Elsevier and Web of Science where several free-access articles where consulted, as well as in official webpages such as Fisterra.

The search strategy is based on the use of an advanced search tool, PubMed, that filters the results in accordance with the information needed. Thus, several searches have been made using key words such as “pathogenesis AND osteoarthritis”, “epidemiology AND osteoarthritis”, “therapies AND osteoarthritis” or “environmental factors AND osteoarthritis” for the introduction, and “epigenetics AND osteoarthritis”, “epigenetic therapies AND osteoarthritis”, “DNA methylation AND Osteoarthritis” “noncoding RNA AND osteoarthritis”, “histone modification AND osteoarthritis” “nutraceuticals AND osteoarthritis”, among others, for the results. For the introduction the webpage Fisterra was also used in order to obtain information about pharmacotherapeutic guidelines for OA.

These searches were accompanied by other filters to limit the age of the article (last ten years) and the type of article (review, meta-analysis and clinical trial). Lastly, in order to assure the quality of the selected articles, information about the journal that published each article has been considered.

To have full access to all information required, the databases were accessed through FAMA, a University of Seville platform that gives students access to paid licenses.

4. Results and discussion

1. Epigenetic implications in OA

Formerly, it was believed that physiopathology of OA has to do with a mechanic issue derived from the use of the joint. Nowadays there is sufficient evidence to say that an important part of the pathogenesis of the disease has to do with aberrant gene expression regulated by defective epigenetic mechanisms (Rice et al., 2020).

The heritability of OA is relatively high, around 40-60%, and it is polygenic. There are several genes associated with OA susceptibility, that have been elucidated by genome-wide association studies (GWAS) which are a tool that allows us to find genetic markers and relate them to a phenotype (a pathology). These OA susceptibility genes, however, have a small effect, and they add up to less than 10% of the inheritance. Hence, the remaining inheritability has to be attributed, at least partly, to non-DNA sequence inheritance, like epigenetics, to reach that 40-60% of heritability (Fathollahi et al., 2019; Rice et al., 2020).

Epigenetic mechanisms are active in somatic cells and they can be passed on to future generations of cells. This action enables cells to respond to variations in their environment by adjusting the array of genes expressed. Epigenetic marks are commonly stable. However, external environmental factors as well as genetic factors (such as SNPs) can trigger epigenetic modifications, and hence gene expression changes, that promote OA initiation or progression. In particular, some of the changes that occur in OA are associated with cellular behaviors that are active during skeletal growth and development, such as the transformation between an articular chondrocyte to a growth plate chondrocyte phenotype (Rice et al., 2020).

Epigenetic regulation is also an essential part of development since early stages of gestation, as it is necessary to accomplish cell differentiation (this is, expressing and suppressing a unique set of genes, from the same genome). However, it is also highly correlated with aging and disease. There is evidence that, with age comes a loss of epigenetic control that ends in a loss of the normal chondrocyte phenotype. This altered

cellular state provokes reactivation of maturative pathways in chondrocytes bringing up in them an OA-associated morphology. (den Hollander and Meulenbelt, 2015). DNA methylation, for example, increases with age, and it is thought to have relation with environmental risk factors and disease risk. In fact, methylation patterns can be used to calculate "epigenetic age" which is related to biological age and age-related diseases (van Meurs et al., 2019; del Real et al., 2018; Rice et al., 2020).

Currently, one of the main problems concerning the relation of epigenetics and OA is that it is not clear whether epigenetic marks precede the pathology altogether and are one of its causes or are a consequence of environmental changes within the affected tissue prior to the clinical onset of the disease. That is, it is unclear if the epigenetic regulation of aberrant gene expression is associated to the etiology of OA, or its progression (den Hollander and Meulenbelt, 2015).

2. Epigenetic mechanisms underlying the aging of cartilage

4.2.1 DNA methylation in OA

The predominantly used DNA methylation study is the genome-wide methylation study which identifies the methylation patterns of a few CpGs per gene throughout the whole genome (Simon and Jeffries, 2018). The main advantages of a genome-wide analysis are that a complete evaluation of global epigenetic modifications within a cell type can be obtained, and the CpGs studied are standardized, so multiple OA data sets can be compared directly (Simon and Jeffries, 2018). Genome-wide methylation studies are able, for this reason, to characterize cartilage and stratify distinct patients (Coutinho de Almeida et al., 2017).

Throughout the genome exist what are called OA susceptibility genes, which code for molecules that play a part in OA pathogenesis. The aberrant expression of these genes for different causes will entail the increase in the possibilities of developing the disease. One of the causes of aberrant expression is the presence, in or around the gene, of an SNP which is a mutation in the genome that is present in a sufficient amount of the population. Just like environmental factors can alter the epigenomic landscape, the genetic sequence itself can also influence epigenetic changes and modulate epigenetically controlled

transcription of genes. So, SNPs present in OA susceptibility genes can modulate DNA methylation. In a study in 2015 for example, 31 genes were identified where changes in methylation patterns are affected by local genetic variation, and 26 genes where gene expression changes were affected by epigenetic patterns and genetic variation. Correlations between SNPs in certain loci associated with OA, and methylation patterns in CpGs have been observed. These loci are called methylation quantitative trait loci (mQTLs) (Coutinho de Almeida et al., 2017; Fathollahi et al., 2019; Den Hollander et al., 2015; den Hollander and Meulenbelt, 2015; Rice et al., 2020).

Approximately 20% of OA risk alleles act as mQTLs. This confirms the vulnerability of chondrocytes to a dysfunctional epigenetic regulation. However, a problem about mQTLs analysis being done is that the arrays cover only about 1.6-3% of the total CpGs in the human epigenome. So the data extracted from them is probably an underestimation (Coutinho de Almeida et al., 2017; Rice et al., 2020).

Methylation patterns can vary in anatomically different involved joints and in different stages of the disease (Rice et al., 2020). Consistent findings comparing different studies bring up that the DNA methylome is different when comparing knee and hip tissue, whether they suffer from OA or not (Simon and Jeffries, 2018). A genome-wide methylation study performed on samples of patients with OA showed 550 CpGs with differential methylation, 83 of them in an already known OA susceptibility loci (Fathollahi et al., 2019). The differentially methylated regions (DMRs) related to tissue development are patent in both knee and hip OA cartilage, in comparison with intact cartilage in these joints (Simon and Jeffries, 2018). In samples of hip and knee OA, DMRs for TNF-α, IL-1, IL-6, and a variety of ECM degrading enzymes exist. (Simon and Jeffries, 2018).

Consistent with radiographic findings that noticed subchondral bone lesions prior to cartilage erosion, which raised the question of what tissue was affected first, a study in 2015 identified in subchondral bone at end-stage OA, up to an order of magnitude more DMR CpG sites than in cartilage of the same joint. It was detected that 44% of DMR where in the same genes in both tissues, with very similar methylation patterns (Simon and Jeffries, 2018; Xia et al., 2014). Unfortunately, there are a lot of studies done that

analyze the cartilage, but very few analyzing other tissues in the joint. Moreover, there are lots of studies for late disease stage, but few about joint development. This makes it harder to corroborate the information in respect of other tissues, therefore making it less reliable (Rice et al., 2020).

Target	DNA methylation/gene expression in OA (in comparison with healthy samples)	Function	Reference
MMP-13 promoter	decreased/increased	Metalloproteinase in charge of collagen type II degradation	(Fathollahi et al., 2019; Zhang et al., 2019)
MMP-9 promoter	decreased/increased	Metalloproteinase that degrades the ECM	(den Hollander and Meulenbelt, 2015)
MMP-3 promoter	decreased/increased	Metalloproteinase that degrades the ECM	(den Hollander and Meulenbelt, 2015)
ADAMTS-4 promoter	decreased/increased	ECM degrading enzyme	(Simon and Jeffries, 2018)
iNOS enhancer	decreased/increased	Enzyme that synthesizes NO, which is a pro-inflammatory mediator	(Fathollahi et al., 2019)
GDF-5 promoter (member of TGF-β family)	decreased/increased	Growth factor involved in development, maintenance and repairment of bone, cartilage and other soft tissues	(Fathollahi et al., 2019)
DIO-2 promoter	increased/increased	Enzyme indirectly involved in chondrocyte terminal maturation and bone formation.	(Goldring, 2013; den Hollander and Meulenbelt, 2015)
RUNX2 enhancer	decreased/increased	Transcription factor involved in endochondral ossification and osteoblastic differentiation	(Rice et al., 2020)
RUNX1	decreased/decreased	Transcription factor involved in chondrogenic differentiation and suppression of hypertrophic differentiation. It also promotes ECM formation by transcriptional induction of COL2A1	(Simon and Jeffries, 2018; Yano et al., 2019)
SOX-9 promoter	increased/decreased	Transcription factor involved in chondrogenesis, chondrocyte differentiation and ECM formation	(Fathollahi et al., 2019; Zhang et al., 2019)

BMP-7 (member of TGF-β family)	increased/decreased	Growth factor that regulates chondrocyte function in adult cartilage	(Zhang et al., 2019)
COL9A1 promoter	increased/decreased	Type of collagen in the cartilage	(Fathollahi et al., 2019)
COL11A2 enhancer	increased/decreased	Type of collagen in the cartilage	(Rice et al., 2020)
SUPT3H	decreased/increased	Histone methyltransferase	(Rice et al., 2020)
IL-1β promoter	decreased/increased	Proinflammatory cytokine	(Fathollahi et al., 2019)
IL-6 promoter	decreased/increased	Proinflammatory cytokine	(Raman et al., 2018)
IL-8 promoter	decreased/increased	Proinflammatory cytokine	(Raman et al., 2018)
SOD2 promoter	Increased/decreased	Mitochondrial superoxide dismutase	(Rice et al., 2020)

Table 2. Relevant DNA methylation patterns of OA susceptibility genes in affected cartilage of patients with OA. ***Abbreviations:*** *MMP: metalloproteinase; ECM: extracellular matrix; ADAMTS: A disintegrin and metalloproteinase with thrombospondin motif; iNOS: inducible nitric oxide synthase; NO: nitric oxide; GDF5: growth differentiation factor 5; DIO2: Iodothyronine Deiodinase 2; RUNX: runt-related transcription factor; SOX9: SRY-Box Transcription Factor 9; BMP: Bone Morphogenetic Protein; COL: collagen; SUPT3H: SPT3 homolog, SAGA and STAGA complex component; IL: interleukin; SOD2: superoxide dismutase 2.*

As it can be read in Table 2, in the majority of cases DNA methylation decreases, with the consequently increase in the expression certain genes that mediate OA pathogenesis. This is consistent with data gathered that suggests that the enzyme DNMT3b is downregulated in OA (Rice et al., 2020).

Growth differentiation factor 5 (GDF5) and Iodothyronine Deiodinase 2 (DIO2) are two of the most studied loci in regard of DNA methylation in OA. They are considered susceptibility genes for OA when they appear with certain mutations (SNPs) in their risk allele. This mutation has the capacity of promoting changes in the epigenetic landscape, specifically DNA methylation, and therefore gene expression, that make the patient more predisposed to developing the pathology. This event is what is called “disease associated epigenetic control of gene expression” (den Hollander and Meulenbelt, 2015).

SRY-Box Transcription Factor 9 (SOX9) is a key transcription factor in chondrogenesis, and it is considered a typical gene involved in OA pathogenesis. Methylation in SOX9

promoter is increased in patients with OA, which makes the binding of other transcription factors such as cAMP responsive element binding protein (CREB) harder and consequently decreases the expression of SOX9. As a result, SOX9 cannot bind to other genes involved in chondrogenesis and ECM synthesis such as ACAN (aggrecan), COL (collagen)11a1 or COL9a1. All of this lowers the production of AC, and promotes OA progression (Fathollahi et al., 2019; Zhang et al., 2019). Moreover, a hypermethylation of CpG site at the COL9a1 promoter in chondrocytes with OA has been noticed, that downregulates expression because it hinders the binding of SOX9. Although this type of collagen only makes about 1-5% of total cartilage in the joint, and it is not essential for cartilage development, it is still important for the organization and integrity of it, and therefore it is considered a susceptibility gene for OA (Fathollahi et al., 2019).

4.2.2 Post-transcriptional modifications of histones (PTMH) in OA

A number of OA risk loci harbor genes encoding proteins that modify the human epigenome, including the ones that modify histones (Rice et al., 2020). PTMH regulate gene expression by three distinct mechanisms. First, by compacting or loosening the chromatin, second by recruiting transcription factors and cofactors, and third by sending signals to chromatin code readers (Rice et al., 2020).

Acetylation/deacetylation and methylation/demethylation of histones are the main PTMH studied in OA. The N-terminal tails of histones are positively charged, which helps them bind to the DNA (negatively charged) to form nucleosomes. Acetylation neutralizes the charges so the binding with the DNA decreases, chromatin loosens, and transcription is facilitated. Deacetylation has the opposite result. Regarding methylation/demethylation of histones, depending on the methylated residue and the number of methyl groups (mono, di or tri-methylation), the outcome will be different, thus widening the functional diversity of each methylation site (Khan and Haqqi, 2019; Simon and Jeffries, 2018; Wan et al., 2021). Histone methylation mostly occurs in lysine (K) residues at positions 4, 9, 20, 27, 36 and 79 of H3. So, for example, trimethylation in H3K27 or H3K9 is related to transcriptional repression, whereas trimethylation in H3K4, H3K36 or H3K79 is related to transcriptional activation (HE et al., 2020; Khan and Haqqi, 2019).

The principal enzymes involved in PTMH are HATs for acetylation, HDACs for deacetylation, histone methyltransferases (HMTs) for methylation and histone demethylases (HDMTs) for demethylation. HDACs are the most popular among OA studies, and can be sub-classified into two functional groups. The first being the HDACs classes I, II and IV, which are named HDAC1 through HDAC11 and have a classic zinc-dependent active site. And then there is the HDAC class III which require NAD+ cofactor to function, and are called sirtuins (SIRT1 through SIRT7) (Khan and Haqqi, 2019).

PTMH is a type of genetic regulation that has not been deeply studied in OA. However, the expression level, activity and inhibition of enzymes involved in PTMH has. Moreover, it has been difficult to stablish a direct correlation between PTMH and gene expression in OA (Fathollahi et al., 2019). However, there is enough evidence to suggest that PTMH are essential in joint development, homeostasis and degeneration, and that the differential expression of any of the enzymes involved in this process could indirectly alter the chromatin structure and hence gene expression, increasing disease risk (Rice et al., 2020).

Family	Enzyme	Status in OA	Effect	Reference
HDACs	HDAC1 and HDAC2	increased	Negatively affect the expression of COL2A1, ACAN, COMP and COL11A1, thus altering the integrity of AC	(Khan and Haqqi, 2019)
	HDAC3	increased	Affects the SOX9, COL2A1, ACAN and COMP genes and increases chondrocyte hypertrophy	(Wan et al., 2021)
	HDAC4	decreased	Lack of regulation of the expression of RUNX2 and MMPs, promotes catabolic activity in AC	(Khan and Haqqi, 2019)
	HDAC7	increased	By promoting the expression of MMPs, it degrades the ECM	(Khan and Haqqi, 2019)
	SIRT1	Decreased	Decreased expression of COL2A1, COL9A1, ACAN and COMP, and increased expression of MMPs and ADAMTS5. It also affects mitochondrial biogenesis in chondrocytes	(Khan and Haqqi, 2019; Rice et al., 2020)

	SIRT3	decreased	It affects SOD2 expression, which disturbs mitochondrial functionality.	(Wan et al., 2021)
	SIRT6	Decreased	Increase of NF-kB dependent inflammatory genes	(Khan and Haqqi, 2019)
HATs	P300/CBP	decreased	It cannot bind with SOX9 in COL2A1 promoter to increase transcription.	(Raman et al., 2018)
HMTs	DOT1L	decreased	It cannot suppress expression (via H3K79 methylation) of Wnt signaling pathway genes, so cartilage homeostasis is lost. Likewise, it cannot promote expression of COL2A1 and ACAN.	(Rice et al., 2020; Wan et al., 2021)
	KMT2F	increased	Methylation of H3K4 in COX2 and iNOS promoters contributes to their increased expression in OA	(Wan et al., 2021)
	KMT2D	increased	Indirectly inhibits expression of SOX9 by methylating H3K4 in SHOX2 gene promoter	(Wan et al., 2021)
HDMTs	LSD1	increased	Increases expression of mPGES-1 by demethylation of H3K9 in its promoter. LSD1 recruitment is induced by IL-1β	(HE et al., 2020; Wan et al., 2021)
	KDM3A (JHDM2A)	decreased	Decreases expression of NFAT1 (which maintains cartilage homeostasis in adult cartilage). This promotes upregulated expression of proinflammatory cytokines and MMPs	(HE et al., 2020; Zhang et al., 2019)
	KDM6B	decreased	It cannot upregulate COL2A1 and ACAN by binding to their promoters, hence, cannot regulate cartilage homeostasis by anabolic mechanisms	(HE et al., 2020)
	KDM4B	decreased	It cannot demethylate histone H3K9 in SOX9 promoter, so SOX9 expression decreases	(HE et al., 2020)

Table 3. Effect of altered levels of relevant histone modification enzymes in OA.

Abbreviations*: COL: collagen; ACAN: aggrecan; COMP: cartilage oligomeric matrix protein; RUNX: runt-related transcription factor; MMP: metalloproteinase; ADAMTS: A disintegrin and metalloproteinase with thrombospondin motif; SOD2: superoxide dismutase-2; NF-kB: nuclear factor kappa B; SOX9: SRY-Box Transcription Factor 9; mPGEs-1: microsomal prostaglandin E synthase-1; DOT1L: disruptor of telomeric silencing 1-like; H3K79: histone 3 lysine 79; H3K4: histone 3 lysine 4; KMT: lysine methyltransferase; COX: cyclooxygenase; iNOS: inducible nitric oxide synthase; SHOX: short stature*

homeobox; LSD1: lysine-specific histone demethylase; H3K9: histone 3 lysine 9; NFAT1: nuclear factor of activated T cells 1; KDM: lysine demethylase

The balance between HATs and HDACs is important in chondrocyte phenotype. Recent studies (Table 3) have demonstrated that uncontrolled activation of HDACs contributes to initiation and progression of OA (Khan and Haqqi, 2019). HDAC inhibition prevents IL-1β induced MMP upregulation in human chondrocytes (Simon and Jeffries, 2018) and multiple KDM (lysine demethylase) have been shown as important regulators of chondrocyte differentiation and pathogenesis of OA (Wan et al., 2021).
Knockout of HDAC3, HDAC4, HDAC5 or HDAC7 impairs endochondral ossification in mice. So, all of them have to be in some way implicated in cartilage development (Rice et al., 2020).

Nuclear factor of activated T cells 1 (NFAT1) is a transcription factor that regulates cytokine expression during the immune response (HE et al., 2020). It is not essential for the development of skeletal tissues (including AC), but it is required to maintain cartilage homeostasis in adults (Zhang et al., 2019). In normal conditions, the expression of this gene starts in adults, and then decreases with age, causing chondrocyte disfunction and increased expression of proinflammatory cytokines and ECM degrading enzymes. Mice with global deletion of the gene start to show articular chondrocyte disfunction and OA-like changes as young adults (Zhang et al., 2019). Transcription repression of NFAT1 is related to increased H3K9me2, whereas transcription activation is related to increased H3K4me2. So, histone modification may play a key role in age-related NFAT1 expression, as seen in Table 3 (Wan et al., 2021).

One of the functions of DOT1L (disruptor of telomeric silencing 1-like), a histone H3K79 methyltransferase, is to block the expression of certain genes within the Wnt signaling pathway (partly through inhibition of SIRT1 activity). In adults, overexpression of Wnt pathway is susceptible to promote progression of OA (He et al., 2020; Rice et al., 2020; Xia et al., 2014). Decreased activity of DOT1L in mice is associated with increased progression of both age-related and post-traumatic OA (He et al., 2020; Rice et al., 2020).
IL-1 decreases mono and di methylation of H3K9 in the promoter of microsomal prostaglandin E synthase-1 (mPGES-1) (which synthesizes PGE2), by recruiting lysine-

specific histone demethylase (LSD1) in OA chondrocytes (Fathollahi et al., 2019). Moreover, IL-1 promotes di and tri-methylation of H3K4 in cyclooxygenase-2 (COX-2) and inducible nitric oxide synthase (iNOS) promoters, which increases their expression (Khan and Haqqi, 2019). COX-2 is also responsible of the synthesis of PGE2. Therefore in these chondrocytes there will be augmented levels of this PG which in turn will increase levels of MMP13 and A disintegrin and metalloproteinase with thrombospondin motif (ADAMTS)-5 induced expression (Fathollahi et al., 2019).

In synovial fibroblasts of OA patients, there is a histone hyperacetylation in the promoter of IL-6, which increases its expression (Fathollahi et al., 2019). Specifically they show increased H3K9, H3K14 and H4K12 acetylation (Raman et al., 2018).

4.2.3 ncRNAs in OA

ncRNAs are a type of RNA that do not translate to a protein, but instead are part of the wide network of epigenetic regulation. They are categorized by their length, in small ncRNA (<200 nucleotides) and long ncRNA (>200 nucleotides). They have various functions, and are involved in OA pathogenesis (Razmara et al., 2019).

miRNAs are negative regulators of gene expression, mainly at a posttranscriptional level, that are localized in intergenic zones, introns of protein-coding genes and, to a lesser extent, in exons. They modulate gene expression through a variety of mechanisms such as binding to complementary sites of mRNA for its degradation by a protein complex called RNA-induced silencing complex (RISC), binding and destabilization of mRNA without cleavage and reduction of the efficiency of ribosomal translation. Each miRNA can have multiple target mRNAs. They can be taken into consideration as pathogenic contributors, therapeutic targets or circulating biomarkers of disease (Razmara et al., 2019; Rice et al., 2020; Simon and Jeffries, 2018; Swingler et al., 2019; Zhang et al., 2019)

ncRNAs themselves need epigenetic mechanisms for their regulation. For example, some miRNAs located in introns share the epigenetic regulations with their host genes, because of the epigenetic modifications within the promoter, that affect the expression of both. (Coutinho de Almeida et al., 2017). Therefore, not only can miRNAs modulate the

expression of genes, but also genes encoding miRNAs can suffer epigenetic regulation that modulates the expression of the miRNA itself (Yu et al., 2020).

It is important to analyze (Table 4) how variations in the regulation of miRNAs, that maintain normal chondrocyte phenotype or mediate inflammatory pathways, influence OA pathogenesis (Goldring and Marcu, 2012).

miRNA	Status in OA	Effect	Reference
miR-140	decreased	It cannot carry out its effects on multiple targets, which leads to chondrocyte hypertrophy, loss of cartilage homeostasis and upregulation of inflammatory genes.	(Razmara et al., 2019; Simon and Jeffries, 2018; Swingler et al., 2019)
miR-146	decreased	It cannot target TNF-α nor SMAD4 and it cannot repress gene expression induced by IL-1	(Goldring and Marcu, 2012; Razmara et al., 2019)
miR-21	increased	It targets GDF5 during regulation of chondrogenesis. It increases destruction of cartilage.	(Razmara et al., 2019; Swingler et al., 2019)
miR-22	increased	targets BMP-7 and PPARα which results in increased levels of IL-1β and MMP13	(Razmara et al., 2019; Xie et al., 2020)
miR-27a	decreased	It cannot decrease MMP13, IGFBP-5 and ADAMTS expression in chondrocytes. It cannot regulate chondrocyte hypertrophy by targeting PPARγ2.	(Fathollahi et al., 2019; Goldring and Marcu, 2012; Razmara et al., 2019)
miR-145	increased	Attenuates chondrocyte differentiation, and reduces expression of COL2A1 and ACAN by blocking SOX9	(Fathollahi et al., 2019; Razmara et al., 2019)
miR-455	increased	It modulates TGF-β signaling pathway through inhibition of SMAD2 and SMAD3 which indirectly entails cartilage degradation. It can also regulate chondrogenic differentiation by influencing DNA methylation.	(Fathollahi et al., 2019; Razmara et al., 2019; Rice et al., 2020)
miR-98	increased	It blocks Bcl-2 activity, therefore promoting chondrocyte apoptosis.	(Xie et al., 2020)
miR-16	increased	Through blocking of SMAD7 it decreases levels of MEG3 which is inversely correlated with VEGF levels and angiogenesis. It can also target SMAD3 to promote ECM degradation	(Razmara et al., 2019; Xie et al., 2020)
miR-34a	increased	Prevents proliferation and promotes apoptosis of chondrocytes through targeting of SIRT1 protein	(Fathollahi et al., 2019)

miR-181b	increased	It inhibits PTEN (tumor suppressor gene) in chondrocytes. So, it prevents their proliferation and promote their apoptosis.	(Fathollahi et al., 2019)
miR-101	decreased	It cannot regulate cartilage homeostasis via integrin-α1, through the blocking of DNMT3B	(Coutinho de Almeida et al., 2017; Yu et al., 2020)
miR-125b	decreased	It cannot block ADAMTS4 activity	(Coutinho de Almeida et al., 2017)
miR-320	decreased	It cannot block MMP13 activity	(Coutinho de Almeida et al., 2017)
miR-449a	increased	Inhibits expression of COL2A1, while enhancing MMP13 expression through SIRT1	(Coutinho de Almeida et al., 2017)
miR-29	increased	Regulates TGF-β, NF-kB and Wnt signaling pathways. It also targets and represses COL2A1 and COL1A2 expression.	(Fathollahi et al., 2019)
miR-210	Decreased in AC/increased in synovial fibroblasts	its effects on chondrocyte proliferation and ECM deposition are diminished and chondrocyte apoptosis is increased. In synovial tissue, upregulation of this miRNA induces angiogenesis via activation of VEGF.	(Fathollahi et al., 2019)

Table 4. Effect of altered levels of relevant miRNAs in OA. ***Abbreviations:*** *TNF-α: tumor necrosis factor alpha; IL: interleukin; GDF5: growth differentiation factor 5; BMP: bone morphogenetic protein; PPAR: peroxisome proliferator activated receptor; MMP: metalloproteinase; IGFBP: insulin-like growth factor binding protein; ADAMTS: A disintegrin and metalloproteinase with thrombospondin motif; COL: collagen; ACAN: aggrecan; SOX9: SRY-Box Transcription Factor 9; TGF-β: transforming growth factor beta; Bcl-2: B-cell lymphoma 2; MEG3: maternally expressed gene 3; VEGF: vascular endothelial growth factor; ECM: extracellular matrix; PTEN: SIRT: sirtuin; DNMT: DNA methyltransferase NF-kB: nuclear factor kappa B*

Different methodologies, complexity of pathways regulated by miRNAs, disease stage, severity of inflammation and type of the samples used for these studies are factors to be taken into consideration when interpreting this data, as some of it may contradict other authors (Fathollahi et al., 2019).

miR-140 is a miRNA vastly studied in OA. It is located in intron 16 of WWP2 gene, which plays a role in protein ubiquitination and is a direct target of transcription factor SOX9. Therefore, expression of SOX9, WWP2 and miR-140 are correlated, and when SOX9 acts on WWP2, levels of miR-140 also increase. miR-140 targets the expression

of several genes both directly like HDAC4, ADAMTS5 and SMAD3 and indirectly such as RUNX2, MMP13 and insulin-like growth factor binding protein (IGFBP)-5. Because of this activity, miR-140 is implicated in chondrogenesis, chondrocyte differentiation and cartilage homeostasis, and inhibits chondrocyte hypertrophy and the upregulation of inflammatory genes. Overexpression of miR-140 in chondrocytes is protective against induced OA in mice, and mice lacking miR-140, WWP2 or both, develop OA-like disease (Goldring and Marcu, 2012; Razmara et al., 2019; Rice et al., 2020; Simon and Jeffries, 2018; Swingler et al., 2019).

lncRNAs can fold into specific conformations to bind DNA, RNA and proteins, that enable them to form broad regulatory networks (Sun et al., 2019). They have three main functions; they modify mRNA and protein stabilities, they are also involved in chromatin modification and they act as "sponges" for miRNAs (that is, they bind to them to block their activity). When a lncRNA acts as a sponge, it is named competing endogenous RNA (ceRNA) (Coutinho de Almeida et al., 2017). The interaction between lncRNAs and miRNAs plays an important role in the development of OA (Xie et al., 2020).

lncRNAs are particularly tissue and cell type specific. This data along with the facts that they have a poor conservation between species and that there are a lot of them for which a function is unknown, make the study of lncRNAs for a specific disease quite challenging (Coutinho de Almeida et al., 2017; Rice et al., 2020).

lncRNA	Status in OA	Outcome	Reference
HOTTIP	Increased	Increases the level of cartilage destruction by downregulating HOXA13 and its downstream target, integrin-α1	(Sun et al., 2019)
HOTAIR	increased	Upregulation of ADAMTS5. Contributes to OA progression via Wnt pathway	(Razmara et al., 2019; Sun et al., 2019)
GAS5	increased	Stimulates chondrocyte apoptosis and increases expression of MMPs (2, 3, 9 and 13) and ADAMTS4. It can also block miR-21 activity.	(Sun et al., 2019)
PVT1	increased	Promotes chondrocyte apoptosis. It binds to and blocks miR-488 (which is	(Razmara et al., 2019; Sun et al., 2019)

		involved in chondrogenic differentiation)	
UFC1	decreased	There is no blocking of miR-34a and therefore it cannot facilitate chondrocyte proliferation nor inhibit chondrocyte apoptosis.	(Sun et al., 2019)
lncRNA-MSR	increased	Decreases expression of COL2A1 and ACAN and increases expression of MMP13 and ADAMTS5	(Sun et al., 2019)
lncRNA-CIR	increased	Increases expression of MMP13 and ADAMTS and decreases expression of COL2A1 and COL1A1	(Coutinho de Almeida et al., 2017)
Nespas	increased	Decreases COL2A1 expression and increases expression of MMP2 and MMP13	(Sun et al., 2019)

Table 5. Effect of altered levels of relevant lncRNAs in OA. ***Abbreviations:*** *HOTTIP: HOXA distal transcript antisense RNA; HOXA13: homeobox A13; HOTAIR: HOX transcript antisense RNA; ADAMTS: A disintegrin and metalloproteinase with thrombospondin motif; GAS5: Growth Arrest Specific 5; MMP: metalloproteinase; PVT1: plasmacytoma variant translocation 1; UFC1: ubiquitin-fold modifier conjugating enzyme 1; MSR: mechanical stress related; CIR: cartilage injury-related; COL: collagen; ACAN: aggrecan*

Results from some studies (Table 5) suggest a correlation between increased levels of certain lncRNAs and perturbed AC. lncRNAs have been demonstrated to be crucial regulators for maintenance or degradation of ECM (Sun et al., 2019).

3. Alternative therapies for OA

4.3.1 Epigenetic therapies for OA

Epigenetic changes are tissue and cell specific and nowadays there are not selective drugs that can change the epigenetic landscape in OA. There are a few limitations for the development of possible epigenetic pharmacotherapies. The main one being that epigenetic regulators can act broadly upon multiple cell types with the subsequent increase in toxicity. Moreover, although they may have short-term tolerance, the long-term effects of their systemic use are not only unknown, but also unpredictable. A solution for this problem is to consider a short-term and/or local treatment, for a more targeted pharmacological action, to act on the microenvironment of the affected tissue specifically (Park et al., 2012; van Wijnen Andre J, 2016).

One of the most studied epigenetic therapies is the inhibition of HDACs with HDAC inhibitors (iHDAC), however of all the clinical trials ongoing or completed with iHDACs, none of them are related to OA. Nevertheless, in *in vitro* studies, some iHDACs have been shown to confer protection and prevent ECM degradation in OA adult chondrocytes. For example, Trichostatin A (TSA), a microbial metabolite isolated from *Streptomyces hygroscopicus*, can inhibit all HDACs in general, and prevents IL-1β induced expression of MMPs in OA chondrocytes. Another example is Vorinostat, an iHDAC similar to TSA in its mechanism of action (Khan and Haqqi, 2019; Park et al., 2012).

SRT1720 is a potent activator of SIRT1 that can decrease synovial inflammation and progression of OA when administered in mice with induced OA. However, it is important to take into consideration that these type of studies cannot confirm if the consequence of this drug is due to increased acetylation of histones (primary function of SIRT1), or whether the effects are non-epigenetic (Grandi and Bhutani, 2020).

Because there is not enough information yet about the use of synthetic epigenetic drugs to modulate, and potentially treat OA, it is necessary to look at other areas that can possibly improve the disease at least partially and temporarily, until effective molecules appear. It has been proven that IL-1β levels in OA are significantly higher, and that OA patients exhibit increased levels of oxidative stress, so the primary target is to boost antioxidant and anti-inflammatory activities in affected tissue (Leong et al., 2013; Sukhikh et al., 2021).

4.3.2 Potential of nutritional therapy and nutraceuticals for changing the epigenetic marks in OA

Nowadays, the majority of chronic illnesses are considered multifactorial and take into consideration epigenetic changes as one of the possible factors. Other factor is nutrition. There are several studies indicating a correlation between nutrition and disease susceptibility, and there is evidence suggesting a direct relation between epigenetic mechanisms and nutritional factors or what could be called as “nutritional epigenetics” (Park et al., 2012).

As certain foods play an important role in regulating epigenetic modifications, it seems reasonable to confirm that bioactive compounds in the diet may modulate initiation and progression of certain diseases. (Arora et al., 2020; Cione et al., 2020). These dietary bioactive compounds are not necessarily essential for life, but they can have a major impact on health, among other effects by both directly and indirectly modifying epigenetic marks (Park et al., 2012).

Nutrition may modulate levels of certain macromolecules within the organism, and these likewise have an influence in inflammation and oxidative stress. Exposure to high glucose, for example, caused a reduction in H3K9 methylation leading to an increased expression of transcription factor NF-kB, and these epigenetic marks were sustained following return to normoglycemic conditions. Moreover, cells exposed to oxidized LDL cholesterol reduced HDAC1 and HDAC2 leading to increased activating histone marks at the IL-8 promoter. Another example, deficiency of B-vitamins, methionine and/or choline can significantly alter DNA methylation by modification of the levels of S-adenosyl methionine (SAM) and S-adenosyl homocysteine (SAH) (methyl group donors) (Park et al., 2012).

Resveratrol is a polyphenolic compound produced by a wide variety of plants and fruits, especially grape. It can be toxic for a variety of tumor cells, but it has been shown to be beneficial for the growth of certain types of cells such as endothelial cells and chondrocytes, and has properties which can be favorable for certain age-related diseases (Arora et al., 2020; Jin et al., 2018)

Resveratrol decreases levels of IL-6, TNF-α and IL-1β. This in consequence, suppresses NF-kB pathway in chondrocytes, with the following reduction of pro-inflammatory products, inhibits IL-1β induced iNOS production, and blocks apoptosis via caspase-3 (Ansari et al., 2020; Jin et al., 2018; Leong et al., 2013; Sukhikh et al., 2021).

Resveratrol was found to decrease apoptosis by increasing SIRT1 activity in chondrocytes (Ansari et al., 2020; Grandi and Bhutani, 2020). The regulation of SIRT1 activity by different polyphenols is a promising strategy against chronic inflammation, which is an important factor in many age-related diseases. (Park et al., 2012).

It has also been observed that Resveratrol increases miR-146 expression in chondrocytes, which is beneficial for OA. It was also noted that this molecule can downregulate expression of miR-21 and miR-181b in cancerous cells, and although it cannot be said that it will do the same in chondrocytes, if it does, it could promote the argument of its relevance in this pathology (Cione et al., 2020; Jin et al., 2018).

Epigallocatechin-3-gallate (EGCG) is a bioactive polyphenol present in green tea that has good potential for OA prevention and treatment. It has innate antioxidant activity, up to 25 times more than vitamin C or E, as it can rise levels of catalase, superoxide dismutase and glutathione peroxidase (Leong et al., 2013; Sukhikh et al., 2021).

It also has anti-inflammatory activity in chondrocytes as it can suppress IL-1β induced expression of pro-inflammatory mediators such as COX-2, NF-kB, PGE2, IL-6, IL-8 and TNF-α. There is evidence as well that EGCG can inhibit expression of ADAMTS-1, ADAMTS-4, ADAMTS-5, MMP-1 and MMP-3 (Ansari et al., 2020; Sukhikh et al., 2021).

It has been observed that EGCG upregulates miR-199a expression, which targets COX-2, and it also upregulates miR-140 in OA chondrocytes stimulated with IL-1β. Therefore, it can be concluded that at least part of EGCG mechanism of action toward OA is through regulation of epigenetic mechanisms, specifically microRNAs (Cione et al., 2020; Rasheed et al., 2016).

Curcumin, a bioactive polyphenol, is the major component of turmeric, and a commonly used spice. It can have regulatory activity for HDACs and HATs, in some cases inhibitory and in other cases stimulatory. Whichever the case, Curcumin has been shown to be able to suppress the expression of IL-1β and its induced activation of NF-kB, decreasing levels of IL-6, IL-8, COX-2, NO and MMPs like MMP-3 and MMP-9, and altogether alleviate cartilage degeneration in rat OA models (Ansari et al., 2020; Leong et al., 2013; Park et al., 2012).

Quercetin is a flavonoid found in many fruits and vegetables. It can reduce expression of TNF-α and IL-1α, and it has a strong antioxidant power, being able to decrease levels of ROS by increasing expression of glutathione and glutathione peroxidase. These characteristics make it a molecule known for improving mitochondrial dysfunction, and decreasing cellular senescence (Ansari et al., 2020; Sukhikh et al., 2021). The effect on the mitochondria also seems to be mediated by increased levels of SIRT1 which upregulates the expression of mitochondrial biogenesis genes (D'Adamo et al., 2020).

Baicalin is a bioactive flavonoid that is not present in food but can be extracted from the plant *Scutellaria baicalensis*. It has anti-inflammatory activity. It can inhibit apoptosis and decrease IL-1β induced production of cytokines such as IL-6, IL-8 and TNF-α. It has the capacity of downregulating miR-126 which consequently blocks the activation of NF-kB signaling pathway. Other bioactive polyphenol, in this case a flavone, found in this plant is Wogonin. Purified Wogonin suppresses the IL-1β induced expression of IL-6, COX-2, iNOS and MMPs (Ansari et al., 2020). Flavonoids are of especial interest for therapeutic strategies to reduce NSAIDs doses in inflammatory pathologies (D'Adamo et al., 2020; Yang et al., 2018).

There is not enough literature yet about the study of certain nutrients and the epigenetic consequences of their usual consumption, at least in OA, but there is certainly evidence that suggest their relevance as possible diet-based therapy approaches for the disease. It has been observed, for example, that extra virgin olive oil has anti-inflammatory effects and helps with diverse chronic inflammatory illnesses. Oleocanthal and Oleuropein are polyphenols present in olive oil that have been shown to inhibit pro-inflammatory molecules such as TNF-α, iNOS and a variety of ILs, and furthermore, they demonstrated inhibitory activity towards MMPs and ADAMTS expression in chondrocytes. Vitamin C is a potent antioxidant compound that is also associated with reduced risk of cartilage loss and OA progression (Ansari et al., 2020; D'Adamo et al., 2020; Leong et al., 2013).

Omega-3 fatty acids are known to have anti-inflammatory activity, whereas omega-6 and saturated fatty acids are associated with a proinflammatory environment. Omega-3 fatty acids such as alpha-linoleic (ALA), eicosapentaenoic (EPA) and docosahexaenoic (DHA) all demonstrated to have anticatabolic activity against matrix degradation in OA as they

have the capability to modulate gene expression by decreasing levels of mRNA of proteins like ADAMTS-4, ADAMTS-5, MMP-3, MMP-13, COX-2, IL-1α, IL-1β and TNF-α. Moreover, it has been observed that intraarticular injection of EPA protected chondrocytes from oxidative-stress induced apoptosis in a mouse OA model (Bortoluzzi et al., 2018; D'Adamo et al., 2020).

Prodelphinidin is a condensed tannin found in pomegranate fruit that has anti-inflammatory properties. It can inhibit IL-1β induced activation of NF-kB and expression of MMP-1 MMP-3, MMP-13 and COX-2 in human chondrocytes (Ansari et al., 2020; Leong et al., 2013; Sukhikh et al., 2021).

Ginger extracts contain gingerols. These bioactive compounds can inhibit COX-2 and 5-1-lipoxygenase, hence decreasing production of PG and leukotrienes. Ginger extract is also effective for downregulating TNF-α expression in human synoviocytes, by modulating NF-kB signaling pathway (Leong et al., 2013; Sukhikh et al., 2021).

5 Conclusions

1. Part of the etiopathogenesis of OA comes from an altered gene expression derived from deficient epigenetic control. The epigenetic landscape found in OA tissues differed considerably from the one in healthy samples. However, it cannot be elucidated whether these changes precede the onset of the disease or are a consequence of it.

2. SNP mutations in the genome can modify epigenetic marks, being therefore a cause of epigenetic alteration and making subjects with this SNP more susceptible to develop the disease

3. Genome wide methylation studies help identify methylation patterns of OA susceptibility loci in diverse tissues. It has been observed that an overall decrease in DNA methylation is patent in OA samples compared to healthy ones.

4. The study of PTMH is limited to the interpretation of the levels of enzymes involved in this epigenetic mechanism, and although it is hard to stablish a correlation between PTMH and gene expression, it was found than in OA almost every HDAC level is altered, so they have to be implicated in its pathogenesis.

5. There are several limitations in the study of ncRNAs, as most of their functions are unknown, however, they can act as pathogenic contributors, circulating biomarkers, and therapeutic targets. miR-140 is the most studied miRNA in OA, and it has substantial implications in its pathogenesis.

6. The limitations in the development of effective and non-toxic epigenetic pharmacotherapies, make it a necessity to look for alternatives that can potentially improve OA physiopathology.

7. In line with the fact that diet is one of the environmental factors with the capacity of modifying epigenetics, bioactive nutritional compounds found in the diet such as Resveratrol or EGCG have been shown to improve the epigenetic landscape in OA, therefore being beneficial for the disease.

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