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DNA methylation as a mediator of epigenetic regulation in the pathogenesis and precision medicine of osteoarthritis: An updated review

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Abstract: The pathophysiology of osteoarthritis (OA) is multifactorial, with the primary risk factors being obesity, age, environmental variables, and genetic predisposition. The available evidence suggests that genetic diversity does not adequately account for all clinical characteristics and heterogeneity of OA. Genetics has emerged as a nascent and crucial area of research in OA. The epigenetic module presents a potential link between genetic and environmental risk factors and the susceptibility and pathogenesis of OA. As a critical epigenetic alteration, DNA methylation has been shown to have an important role in the etiology of OA and is a viable biomarker for predicting disease progression and medication response, as shown in this research. This review aims to update knowledge in the field of DNA methylation associated with OA to better identify the essential features of OA subtypes and pathological conditions, hence accelerating individualized treatment and precision medicine.

Introduction

It is estimated that 250 million individuals worldwide are living with some kind of osteoarthritis (OA), the most prevalent cause of joint disability, degeneration, and pain (Wilkinson and Zeggini, 2021). The pathogenesis of OA is also uncertain, including stress stimulation, age, genetics, inflammation, obesity, injury, and environmental factors, as well as nuclear and mitochondrial genetics associated with the disease (Molnar *et al.*, 2021). Currently, there are no disease-modifying agents that can be used to slow or reverse OA. OA treatment is limited to drug pain management and arthroplasty (Primorac *et al.*, 2020). The discovery of diagnostic and prognostic biomarkers for OA has lately received significant attention due to its importance and economic impact.

With the rapid development of epigenetics in recent years, large-scale meta-analyses have shown that epigenetics has a role in the pathophysiology of OA (Boer *et al.*, 2021; Styrkarsdottir *et al.*, 2017, 2018; Tachmazidou *et al.*, 2019; Zengini *et al.*, 2018). Numerous genomes have been linked to OA risk by genome-wide association studies and quantitative trait loci analysis (Gari *et al.*, 2016; Tachmazidou *et al.*, 2019). Aberrant epigenetic modifications are associated with a number of pathological conditions and include DNA methylation, expression of non-coding RNAs, and histone modifications that regulate the gene expression at transcriptional and/or post-transcriptional levels. Here, we will briefly highlight the DNA methylation-mediated regulation of gene expression in OA. The methylation of DNA is an influential epigenetic change that regulates the expression of immune-related genes as well as the development of inflammation (Mazzone *et al.*, 2019). DNA methylation modification involves two different processes: the addition and removal of a methyl group at the fifth position of cytosine or the sixth position of adenine in the DNA (Jiang and Guo, 2020), that is, DNA 5-methylcytosine (5 mC) and DNA 6-adenine methylation (6 mA), respectively. DNA 5-methylcytosine generally occurs in cytosine guanine dinucleotide (CpG), the most prevalent DNA methylation modification in eukaryotic genomes (Greenberg and Bourc'his, 2019). These 5 mC methylation marks are created by DNA methyltransferases

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(DNMTs), such as DNMT3B, DNMT3A, and DNMT1. 5 mC methylation can be oxidized and changed into 5-hydroxymethylcytosine (5 hmC) by ten-eleven translocation (TET) enzymes, TET1, TET2, and TET3 (Skvortsova *et al.*, 2017). However, few enzymes are known to be involved in 6 mA methylation in mammals, such as N6 adenine–specific DNA methyltransferase 1 and alkylated DNA repair protein B homolog 1 (Kweon *et al.*, 2019). Using DNA methylation data together with genotypes can identify genetic variants associated with differential methylation levels at CpGs, i.e., methylation qualitative trait loci (mQTLs) (Venkateswaran *et al.*, 2022). The clinical value of DNA methylation changes, which are involved in the pathological process of OA, has been widely recognized (Tachmazidou *et al.*, 2019).

This review mainly summarizes recent advances in understanding the role of DNA methylation in the pathogenesis of OA. We also have accumulated evidence indicating that methylation is a connection between genetic and environmental exposures, and a potential biomarker for subtype identification, treatment, and diagnosis. Finally, we show that epigenetic modules have the potential to be revolutionary biomarkers and precision medicine tools for the later development of personalized therapy and precision medicine.

Genome-wide methylation analysis was carried out to identify epigenetic variants associated with osteoarthritis

The methylation of DNA is a significant epigenetic modification that is referred to both the expression of genes and the splicing of transcriptional information. Identifying aberrant DNA methylation variations throughout the whole genome gives a comprehensive view of the genetic pattern alterations that occur during OA start and course. The Steinberg group established cell-specific maps of molecular QTLs in chondrocytes and synoviocytes derived from the primary joint tissue of OA patients. Combining these maps with Genome-Wide Association Study (GWAS) results, they discovered substantial evidence of the co-localization of five OA markers aldehyde dehydrogenase 1 family member A2 (ALDH1A2) and family with sequence similarity 53 member A in low-grade OA cartilage, solute carrier family 44-member 2 (SLC44A2), NADPH-cytochrome P450 reductase and SMAD Family Member 3 (SMAD3) in high-grade OA cartilage (Steinberg *et al.*, 2021). Alvarez-Garcia *et al.* (2016) compared DNA from knee cartilage from 11 normal and 12 OA donors for the first time using the Illumina Infinium Human Methylation 450 array, the most comprehensive methylation array available. Methylation profiles identified 929 differentially methylated sites united with 500 distinct genes. Finally, it was revealed that normal and knee OA cartilage exhibit significantly different methylation groups. Comprehensive analysis and functional validation revealed significant hypermethylation and downregulation of a subset of six transcription factors T-box transcription factor 4, atonal BHLH transcription factor 8, zinc finger and BTB domain containing 16, MAF basic leucine zipper transcription, nuclear receptor corepressor 2 and zinc fingers and homeoboxes 2 in OA cartilage.

Differences in DNA methylation patterns may also explain the different phenotypes of OA. Genome-wide association approaches are used in studies of OA patients with distinct characteristics (77,052 cases and 378,169 controls): hip OA, knee OA, knee and/or hip OA, have identified genes enrichment in single-gene forms of skeletal developmental disorders, as well as in biological pathways for collagen formation and extracellular matrix organization (Tachmazidou *et al.*, 2019). Simultaneously, the mechanism of ten possible effector genes, including *fibroblast growth factor 18 (FGF18), cathepsin K (CTSK), transforming growth factor beta 1 (TGFB1), and interleukin 11 (IL11)* supports efficacy evaluation in OA. One research investigated DNA methylation patterns of cartilage from 16 OA hip samples, 62 OA knee samples, and 19 normal hip samples. From a total of 482,421 sites, 12 OA discriminative methylation sites were chosen. These sites are linked to the *Meis homeobox 1 (MEIS1), engrailed homeobox 1 (EN1) gamma-aminobutyric acid type A receptor subunit gamma3 (GABRG3), and retinoid X receptor alpha (RXRA)* genes (Wu *et al.*, 2020). Similarly, Lin *et al.* (2020) quantified CpG methylation in human reinforcers, including methylation profiles of 470,870 CpG probes in 108 samples of hip tissue from hip/knee OA and healthy individuals. The discovery of 8,111 differentially methylated CpGs from enhancer regions shows the importance of DNA methylation changes in both phenotypes of OA. On the side, sex-specific enhancer methylation in hip OA was revealed to be associated with OA phenotype. Subsequently, additional large-scale GWAS studies of hand OA indicate the association between the matrix Gla protein (MGP) variant and increased risk for hand OA, which may increase the burden of hand OA through decreased inhibition of cartilage calcification (den Hollander *et al.*, 2017). Here, we summarize the characteristic genes of aberrant DNA methylation in different clinical phenotypes of osteoarthrosis, as well as the commonly shared genes (Table 1).

Methylation differences may also explain different mechanisms of action in different diseases. The results of genome-wide DNA methylation showed that the overall DNA methylation pattern of rheumatoid arthritis (RA) and OA did differ considerably, but 523 low-methylated regions were exclusive to RA. The presence of different low-methylated regions in synovial cells from RA and OA patients reflects different functional changes and supports their significance as regulatory elements for RA-specific immune function (Ham *et al.*, 2019). Kashin-Beck disease (KBD) is a regional and disabling form of OA that causes severe alterations in the joints and has an ill-defined mechanism (Kang *et al.*, 2022). Patients with OA demonstrated increased methylation in iodothyronine deiodinase-2, glutathione peroxidase 3, and thioredoxin reductase 1 promoter areas compared to persons with those having KBD when MALDI-TOF-MS was utilized. This suggests that distinct epigenetic alterations cause KBD and OA. Furthermore, these modifications cause a disruption in the production of selenoproteins, as well as a regulation of chondrocyte anti-oxidation and anti-apoptosis (Zhang *et al.*, 2021a). Similarly, compared with KBD, differentially methylated genes (DMG) associated with OA are abundant in multiple ion channel pathways, hinting that ion channels are underlying biomarkers of OA. Lastly, the findings

TABLE 1

Summary of the characteristics of abnormal DNA methylation in different clinical phenotypes of osteoarthrosis

	Gene symbol	References
Hip OA specific DMGs	*COMP*, *CHADL*	Styrkarsdottir *et al.* (2017)
	JPH3, *TGFA*, *PLEC*, *MAP2K6*, *RWDD2B*	Zengini *et al.* (2018)
	IL11, *FGF18*, *DIABLO*, *CRHR1*, *MAPT*	Tachmazidou *et al.* (2019)
	SPAG17, *C2orf40*, *RTP4*, *LPP*, *LEPREL1*, *CTC-537E7*.1, *RP11-284G10.1*, *RP11281A20.2*, *RALGPS1*, *TEAD1*, *NACA2*, *TSEN15*, *DGKI*, *RNU6-996P*, *CHST3*, PTPRJ, *TLN2*, *CTD-2623N2.11*, *H1F0*, *COL11A1*, *RP11-95P13.1*, *ITIH1*, *SLC39A8*, *FGF18*, *HFE*, *HLA-DPA1*, *RUNX2*, *BMP5*, *FILIP1*, *HDAC9*, *SMO*, *RP11-274M4.1*, *TNC*, *ASTN2*, *RP11-123K19.1*, *EHBP1L1*, *KDM2A*, *TSKU*, *RP11-993B23.1*, *LRIG3*, *CRADD*, *FAM101A*, *USP8*, *FTO*, *NACA2*, *MAP2K6*, *IL11*	Boer *et al.* (2021)
	SMAD3	Steinberg *et al.* (2021)
	GABRG3, *FAM87B*	Wu *et al.* (2020)
	GRINA	Rice *et al.* (2019)
Hand OA specific DMGs	*AQPEP*, *AC005592.2*, *IRF2BP1*, *C1orf177*, *SLC27A6*, *TEAD1*, *RNF144B*, *C12orf60*, *SNAP47*, *ALDH1A2*	Boer *et al.* (2021)
	MGP	den Hollander *et al.* (2017)
	ALDH1A2	Shepherd *et al.* (2018)
Knee OA specific DMGs	*MOB3B*, *ZNF345*	Zengini *et al.* (2018)
	FGF18	Tachmazidou *et al.* (2019)
	RPL19P11, *WSCD2*, *FABP3P2*, *RP11-35O15.1*, *NMRAL1*, *MN1*, *GDF5*, *SLC44A2*, *SLC27A6*, *DGKI*, *RNU6-996P*, *PTPRJ*, *POLD3*, *SOX5*, *RP1-228P16.4*, *CTD-2623N2.11*, *RP11-95P13.1*, *LTBP1*, *ITIH1*, *SLC39A8*, *HLA-DPA1*, *RUNX2*, *GLIS3*, *TSKU*, *RP11-993B23.1*, *COL27A1*, *CRADD*, *C12orf65*, *USP8*, *FTO*, *WWP2*, *GDF5*	Boer *et al.* (2021)
	ALDH1A2, *TMEM18*	Steinberg *et al.* (2021)
	EN1, *HOXD10*, *MEIS1*, *RXRA*	Wu *et al.* (2020)
Common shared DMGs	*JPH3*, *ZC3H11B*, *UQCC1*, *GDF5*	Zengini *et al.* (2018)
	TGFB1, *GDF5*, *CTSK*, *DPEP1*, *TNFSF15*, *MEIS1*	Tachmazidou *et al.* (2019)
	RNU6-815P, *RNU2-40P*, *RP11-501E14.1*, *FOXP2*, *PARD6G*, *SLC44A2*, *SMAD3*, *MAML2*, *C17orf67*, *RNU2-17P*, *TGFA*, *RAPH1*, *TACC3*, *RNU6-962P*, *SMG6*, *TGFB1*	Boer *et al.* (2021)
	NPC1, *SLC44A2*	Steinberg *et al.* (2021)

Note: DMGs: differentially methylated genes.

demonstrated that a great number of intriguing differentially expressed genes are associated with DMGs. *SPARC-related modular calcium binding 2* and *homeobox D3*, two key genes that govern cartilage and skeletal physiologic and pathologic processes, are examples of differentially methylated regions (DMRs) and DMGs. These genes are also abundant in pathways associated with the skeletal system and limbs (Fan *et al.*, 2021). These findings highlight the importance of examining alterations in DNA methylation across disorders to discover common and disease-specific components of epigenetic dysfunction. In addition, further studies have identified differentially expressed genes and differentially methylated sites for OA via the combined gene expression and bioinformatics analyses. Some intriguing biomarkers were found, such as microtubule-associated protein 1B, fibronectin type III domain containing 1 (FNDC1), anillin, potassium calcium-activated channel subfamily N member 4, sodium channel epithelial 1α subunit, and stanniocalcin 2 (Ball *et al.*, 2022; Yi *et al.*, 2021). The relationship between single nucleotide polymorphism changes and surrounding DMR in the proximal regions of several genes associated with OA risk has been the topic of numerous studies that

have interpreted the correlation plectin (PLEC), glutamate ionotropic receptor NMDA type subunit associated protein 1 (GRINA), ALDH1A2, MGP (Rice *et al*., 2019; Shepherd *et al*., 2018; Shepherd *et al*., 2019). Zhao *et al*. (2017) first found hypomethylation of *TNF receptor-associated factor 1, C-X3-C motif chemokine ligand 1, and connective tissue growth factor* genes in OA chondrocytes. They also observed a consistent relationship between hypomethylation and mRNA expression, indicating that DNA methylation may impact gene and non-coding RNA expression as well as gene transcription and translation. These findings imply that DNA methylation signals might aid in the early detection of OA or risk assessment.

In this review, we summarized a list of the differentially methylated genes of OA that have previously been reported in the scientific literature. We then utilized the GeneMANIA website (Yue *et al*., 2021a) (http://genemania.org) to predict genes functionally comparable to hub genes and built a related PPI network (Ratnakumar *et al*., 2020). The PPI network can forecast the relationships between functionally similar genes and hub genes, including positive regulation of DNA-binding transcription factor activity: positive regulation of DNA-binding transcription factor activity, embryo development culminating in birth or egg hatching, shared protein domains, response to bone morphogenetic proteins, transmembrane receptor protein serine/threonine kinase signaling pathway, ossification, positive regulation of cell adhesion, skeletal system development, co-expression, co-localization, genetic interactions, and so forth (Fig. 1).

Epigenetic regulation in the underlying pathogenesis of osteoarthritis

Epigenetic changes regulate gene expression by affecting gene transcription, which in turn causes variations in the amount of the protein encoded by the gene. Epigenetic processes have an effect on somatic cells that are operating normally, including articular chondrocytes. These mechanisms serve to stabilize the phenotype of the somatic cells. Epigenetic modification of chondrocytes can modify their phenotype and function, causing them to overexpress chondrodenatase and proinflammatory mediators. By attracting methyl-binding and chromatin-silencing proteins, CpG methylation promotes transcription factor binding and/or boosts gene expression. Inflammatory signals in OA joints enhance DNA CpG demethylation, and some research has revealed changes in DNA methylation of genes affecting cartilage catabolism (de Andrés *et al*., 2013; Hashimoto *et al*., 2013; Taylor *et al*., 2014) and anabolic metabolism (Imagawa *et al*., 2014; Kim *et al*., 2013) during OA progression. Changes in the methylation pattern of DNA seem to trigger the generation of inflammatory cytokines and matrix-degrading mediators, for example, interleukin (IL)-6 (Yang *et al*., 2017), matrix metalloproteinase 13 (MMP13) (Li *et al*., 2017) and tumor necrosis factor-α (Zhang *et al*., 2021b). These proteins contribute to cartilage degeneration and synovial inflammation in knee OA.

Studies of DNA methylation patterns in relation to OA have focused on candidate genes associated with the pathophysiology of OA. Singh *et al*. (2021) integrated transcription and epigenome data in OA cartilage and confirmed the increased expression of leucine-rich repeat containing 15 (LRCC15) in human OA cartilage samples. Additionally, they demonstrated that changes in DNA methylation might partially drive the differential expression of LRRC15 in OA cartilage. They also highlighted the probable role that LRCC15 plays in OA illness as a component of a functional network involved in chondrocyte phenotypic alterations and stress response coordination. These reactions include hypertrophic changes and extracellular matrix disintegration. The genotype rs11732213 is associated with DNA methylation at nine CpGs, which forms a DMR, which acts as an enhancer and demethylates CpGs that alter the expression of transmembrane protein 129 (TMEM129), which is a target of OA genetic risk at this locus (Brumwell *et al*., 2022). According to other research findings, the expression of C-terminal binding proteins is shown to be mediated by DNA methylation. Overexpressed C-terminal binding proteins form a transcriptional complex with p300 and AP1. The CtBP-p300-AP1 complex transcriptional machinery selectively stimulates the NLR family pyrin domain containing 3 (NLRP3) expression and its downstream signaling in this complex, which eventually leads to an amplification of the inflammatory response and plays a role in OA pathogenesis (Sun *et al*., 2020). Subsequently, based on the cross-omics analysis, other candidate methylation markers that are specific and sensitive and associated with the diagnosis are summarized, such as DNMT3B (Shen *et al*., 2017; Xiong *et al*., 2021; Zhou *et al*., 2019), cartilage oligomeric matrix protein (COMP), ALDH1A2 (Steinberg *et al*., 2018), and PLEC (Rice *et al*., 2019; Sorial *et al*., 2020). By recapitulating the mechanism of these candidate methylation markers, we could link OA genetic susceptibility with a number of cellular pathways or targets, which may provide a scope for the development of simpler OA therapeutics to target this mechanism (Table 2).

DNA methylation as a biomarker in the diagnosis of osteoarthritis

DNA methylation refers to a chemical modification process in which the base on the DNA sequence changes s-adenosine methionine into a methyl group by means of covalent bonding under the action of DNA methyltransferase without changing the genetic expression (Ming *et al*., 2020). DNA methylation is the most prevalent kind of epigenetic control of gene expression in mammalian cells. This alteration occurs naturally and often in eukaryotic cells and is considered normal. Although methylation can be modified in a variety of ways, the bases of the modified sites can be adenine N-6, guanine N-7, cytosine N-4, and cytosine C-5, which are catalyzed by different DNA methylases, but most occur in the gene promoter region CpG island. How DNA methylation patterns are regulated at each site in the genome and in different tissues or cells is a fundamental question that needs to be answered in epigenetics. It is diffusely accepted that dynamic DNA methylation signatures at particular CpG loci can serve as biomarkers of age-related and aging diseases. These biomarkers, which represent essential clinical goals for diagnosis, prognosis, and the prediction of treatment

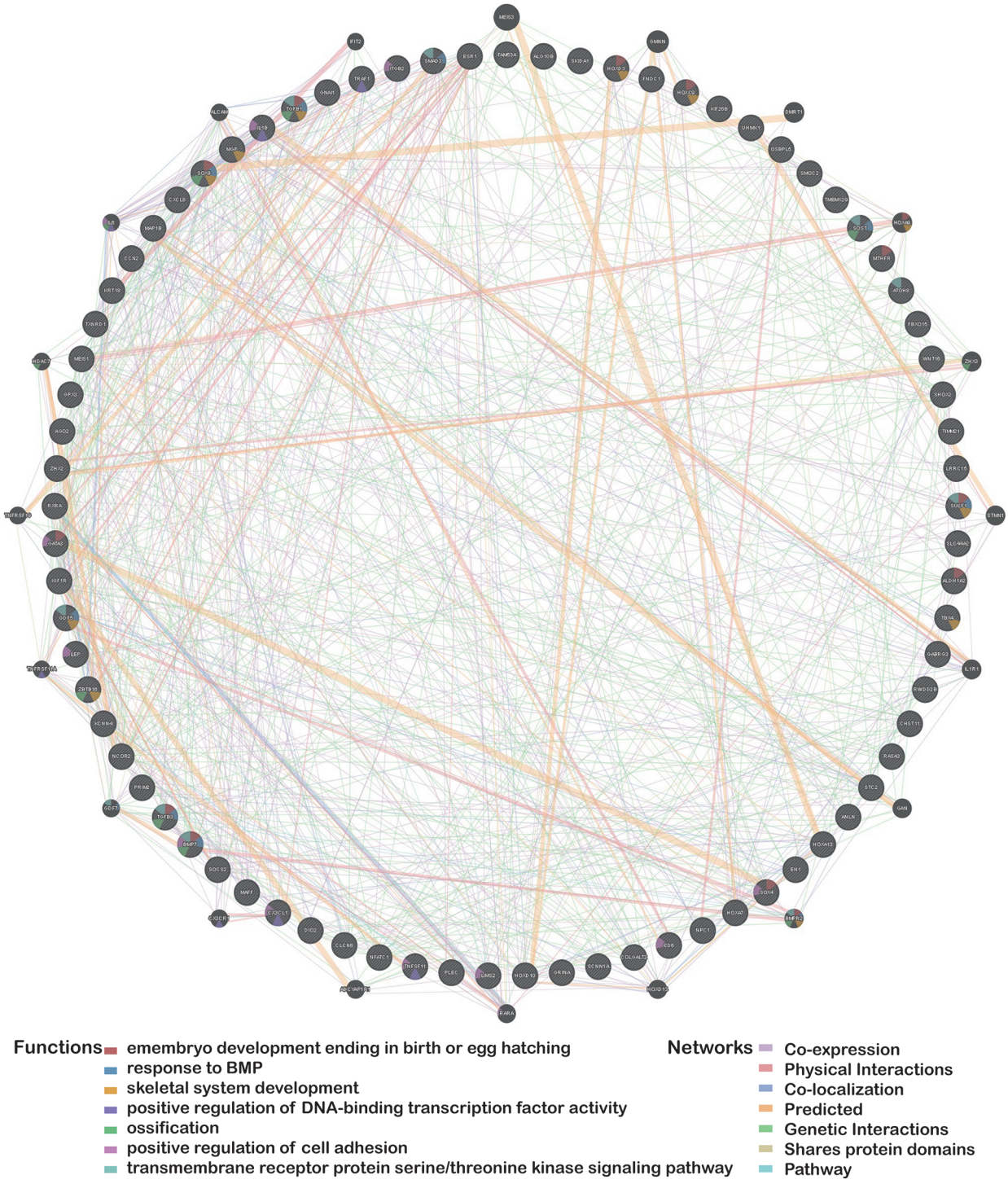

FIGURE 1. Protein-protein interaction network of hub genes.

response, are found at specific loci (Salameh *et al*., 2020). The epigenetic regulation mechanism of whole blood, cartilage, and synovium in OA patients is described in Table 3.

Whole blood

Monitoring changes in methylation might be a useful technique for predicting the progression of a disease and determining how well a patient responds to therapy. Recent research has investigated the methylation status of readily accessible tissues, such as blood and synovial fluid, to ascertain whether these biomarkers indicate synovial disease.

Patterns of DNA methylation in the blood may have a lot of promise as non-invasive biomarkers as well. Dunn *et al.* (2019) concluded peripheral blood epigenetic pattern of OA for the first time. DNA from peripheral blood mononuclear cells (PBMCs) was analyzed at the time of visit in 58 future radiographic progressions (joint space narrowing at 24 months, sustained at 48 months) compared to that of 58 non-progressors. The collected data was utilized to create a prediction approach based on DNA methylation to distinguish between two groups. Methylation in PBMCs might be beneficial as indicators of OA development, and

TABLE 2

Summary of candidate methylation markers in osteoarthritis

Gene symbol	Function	Methylation status
COMP (Steinberg *et al.*, 2018; Styrkarsdottir *et al.*, 2017)	A crucial gene presented in the interterritorial matrix is involved in collagen fibrillogenesis.	Hypermethylated
PCYOX1 (Zengini *et al.*, 2018)	Rs3771501 located was involved in catalysis the of degradation of prenylated proteins.	Hypermethylated
Bach1 (Zengini *et al.*, 2018)	Rs6516886 located was associated with meniscal degeneration in osteoarthritis.	Hypermethylated
FNDC1 (Yi *et al.*, 2021)	With a putative role in inhibiting the synthesis of cartilage matrix and stimulating the release of proinflammatory cytokines and MMP.	Hypermethylated
PLEC (Rice *et al.*, 2019; Sorial *et al.*, 2020)	A cytoskeletal protein that maintains tissue integrity.	Hypermethylated
GRINA (Rice *et al.*, 2019)	A gene encoding ionotropic glutamate receptor TMBIM3.	Hypermethylated
ALDH1A2 (Shepherd *et al.*, 2018; Steinberg *et al.*, 2018)	A crucial gene involved in post-natal bone health and bone remodeling.	Hypermethylated
Lrrc15 (Singh *et al.*, 2021)	A crucial gene involved in matrix remodeling and cartilage catabolism in OA via NF-κB and JNK/AP1 pathways.	Hypermethylated
TMEM129 (Brumwell *et al.*, 2022)	Rs11732213 located was involved in protein degradation within the endoplasmic reticulum in osteoarthritis.	Hypomethylated
CtBPs (Sun *et al.*, 2020)	One gene forms a transcription complex with p300 and AP1 to activate NLRP3 expression and its downstream signaling pathways.	Hypermethylated
DNMT3B (Shen *et al.*, 2017; Xiong *et al.*, 2021; Zhou *et al.*, 2019)	With a putative role in facilitating articular cartilage homeostasis and slowing the onset of osteoarthritis caused by trauma or chemicals in OA.	Hypomethylated

Note: COMP: cartilage oligomeric matrix protein; PCYOX1: prenylcysteine oxidase 1; Bach1: BTB domain and CNC homolog 1; FNDC1: fibronectin type III domain containing 1; PLEC: plectin; GRINA: glutamate ionotropic receptor NMDA type subunit associated protein 1; ALDH1A2: aldehyde dehydrogenase 1 family member A2; Lrcc15: leucine-rich repeat containing 15; TMEM129: transmembrane protein 129; CtBPs: C-terminal binding proteins; DNMT3B: DNA methyltransferase 3 beta.

bigger patient cohorts should be studied further (Dunn *et al.*, 2019). In a different study examining DNA methylation at more than 850K locations, the researchers discovered 28,549 CpG sites that were methylated in the same way in bone and blood. This research aimed to determine whether or not blood can be deemed a viable bone-surrogate (Ebrahimi *et al.*, 2021). Numerous key genes involved in bone metabolism are represented, such as *EN1, estrogen receptor 1 (ESR1), TNF superfamily member 11 (RANKL), and Wnt family member 16 (Wnt16)*, and major bone-regulating pathways, such as Wnt signaling and estrogen response, are enriched among similarly methylated sites, further substantiating the feasibility of our approach. In general, our findings imply that bone methylation groups and trapping sites linked with bone modulation might be reflected in peripheral blood.

In a similar manner, as a target of the rs6516886 OA association signal, the RWD domain containing 2B (RWDD2B) is accompanied by differential methylation of Cg20242 and additional CpGs in its vicinity as intermediaries in the regulation of the gene. RWDD2B is derived from peripheral blood, cartilage, and synovium and codes for a poorly understood protein, and additional analysis of genetic risk targets for OA will provide fresh insights into this complex disease (Parker *et al.*, 2021). Teerawattanapong *et al.* (2019) measured line-1 methylation levels in 104 patients with knee OA and 96 healthy controls, and the results showed that long interspersed nuclear element-1 (LINE-1) methylation in white blood cells was linked to radiographic severity of knee osteoarthritis and increased risk. Patients suffering from knee OA were found to have higher than normal amounts of 8-hydroxy-2′-deoxyguanosine (8-OHdG) in their synovial fluid, suggesting that their elevated levels can be potentially used as biomarkers for the severity of knee OA and possible involvement in its pathology.

In general, these results suggest peripheral blood can reflect bone methylation and explore sites associated with bone regulation.

Cartilage tissue

Epigenetic changes in cartilage are strongly associated with OA. As part of methylation analysis, genes related to matrix metalloproteinases and leptin genes *(IL1β, SOD2, NOS2, SOX9, GDF5*), linked to obesity and inflammation, showed significant changes (Koike *et al.*, 2018). In a study (Chen *et al.*, 2019), the expression of the long non-coding RNAs (LncRNA) XIST and tissue inhibitors of metalloproteinases 3 (TIMP-3) were analyzed, and chondrocytes were separated and identified from OA cartilage tissues and normal cartilage tissues. Functional investigations involving the recruitment of DNMT3A, DNMT3B, and DNMT1 to the TIMP-3 promoter have shown that the LncRNA XIST enhances the methylation of the TIMP-3 promoter. This, in turn, stimulates collagen breakdown in the chondrocytes of OA patients after tibial plateau fracture. These two

TABLE 3

Summary of DNA methylation studies in osteoarthritis

Tissue	Sample	Technique	Analysis target	Analysis outcome	Reference
Whole blood	58 OA radiographic progressors, 58 OA radiographic nonprogressors	Illumina Infinium HumanMethylation 450 k/850 k arrays	Genome-wide analysis	DNA methylation-based models of PBMCs are predictive of OA radiographic progression.	Dunn *et al.* (2019)
	12 women with OA	Epigenome-wide cross-tissue correlation study	Genome-wide analysis	EN1, ESR1, Wnt16, and RANKL are all methylated in bone tissue and blood.	Ebrahimi *et al.* (2021)
	348 patients who had primary hip or knee OA	Pyrosequencing assays	DNA methylation	The association signal rs6516886 is a multi-tissue mQTL involving cg20220242 and acting on RWDD2B.	Parker *et al.* (2021)
	104 participants diagnosed with primary knee OA, 96 healthy controls	Bisulfite sequencing PCR	LINE-1 methylation	LINE-1 hypomethylation in OA patients.	Teerawattanapong *et al.* (2019)
Cartilage tissue	78 OA patients, 19 healthy subjects	Illumina's Infinium HumanMethylation450 BeadChip	Genome-Wide DNA methylation study	16,816 differentially methylated CpGs, of which 8111 were from enhancers.	Lin *et al.* (2020)
	247 patients with knee, hip, or hand OA	Pyrosequencing assays	*ALDH1A2* locus	*ALDH1A2* locus increases the risk of hand OA, with the effect dependent on rs12915901.	Shepherd *et al.* (2018)
	15 OA patients, 7 healthy subjects	Methylation-specific PCR	Promoter region of the *TIMP-3* gene	Increased methylation level in *TIMP-3* promotor region.	Chen *et al.* (2019)
	60 OA patients, 60 healthy subjects	Methylation-specific PCR	*MiR-34a* gene	Increased methylation level in the *miR-34a* gene.	Zhang *et al.* (2020b)
	137 OA patients	Pyrosequencing assays	COLGALT2 expression	OA risk site rs11583641 regulates COLGALT2 expression.	Kehayova *et al.* (2021)
	306 individuals with hip osteoarthritis or femoral neck fracture	Pyrosequencing assays	*TGFB1* gene	OA risk SNP rs75621460 impacts DNA methylation and regulates*TGFB1 in vitro*.	Rice *et al.* (2021)
	12 patients with hip osteoarthritis	Illumina HumanMethylation 450 arrays	Genome-wide DNA methylation profiling	There were 7,316 methylated CpG sites in the subchondral bone underneath deteriorated cartilage and 1,397 differentially methylated CpG sites in overlaying damaged cartilage, 126 of which were shared.	Jeffries *et al.* (2016)
Synovium	20 OA patients, 15 healthy subjects	Bisulfite sequencing PCR	CpG island in promoter region of the *IL-6* gene	*IL-6* hypomethylation in OA patients.	Li *et al.* (2017)
	202 patients undergoing knee or hip arthroplasty for primary OA	Illumina NextSeq platform	*PLEC*	The gene encoding *PLEC* increase the risk of OA, with the effect dependent on rs11780978.	Sorial *et al.* (2020)
	20 OA patients, 15 healthy subjects	Bisulfite sequencing PCR	*MiR-140* and *miR-146a* genes	Hypermethylation of *miR-140* and *miR-146a* genes.	Papathanasiou *et al.* (2019)
	62 OA patients, 60 healthy subjects	Methylation-specific PCR	*MiR-130a* gene	Increase in methylation of the *miR-130a* gene.	Zhang *et al.* (2020a)

Note: *ALDH1A2: aldehyde dehydrogenase 1 family member A2*; COLGALT2: collagen beta (1-O)galactosyltransferase 2; CpG: cytosine guanine dinucleotide; DNA: deoxyribonucleic acid; EN1: engrailed homeobox 1; ESR1:estrogen receptor 1; *IL-6: interleukin-6*; LINE-1: long interspersed nuclear element-1; mQTL: methylation qualitative trait loci; MiR: MicroRNA; OA: osteoarthritis; PBMCs: peripheral blood mononuclear cells; PCR: Polymerase Chain Reaction; *PLEC: plectin*; RANKL: TNF superfamily member 11; RWDD2B: RWD domain containing 2B; SNP: single nucleotide polymorphism; *TGFB1: transforming growth factor-beta 1; TIMP-3: tissue inhibitors of metalloproteinases 3*; Wnt16: Wnt family member 16.

molecules can potentially be attractive therapeutic targets for people suffering from OA. Certain RNA-seq tests have revealed that the short nucleolar RNA host gene 9 is repressed in OA and inhibits chondrocytes from committing apoptosis by lowering the activity of miR-34a through methylation (Zhang *et al*., 2020b).

In addition, epigenetics and the genetics of alleles linked with an increased risk of OA have direct and functional interactions. Methylation quantitative trait loci, or mQTLs, are genetic polymorphisms that correspond with levels of methylation at a CpG site. There is also a possibility that up to 25% of OA at-risk loci could be mQTLs34. Kehayova *et al.* (2021) built functional analyzes of OA risk loci to demonstrate causal relationships between single genetic risk on nearby genes, DNA methylation, and the expression of target genes. Research utilizing cartilage samples from patients and a chondrocyte cell culture demonstrate that the OA-affected allele rs11583641 is associated with an increase in collagen beta (1-O) galactosyltransferase 2 (COLGALT2) expression. In particular, the researchers found a correlation between rs11583641 and a closed methylation site of a COLGALT2 enhancer in OA the cartilage of patients. In addition, other researchers found that the risk mutation rs75621460 impacts transforming growth factor-beta 1 (TGFB1) expression in distinct tissues. Genotyping articular cartilage samples from 319 individuals with OA revealed that the risk allele "A" of rs75621460 is related to higher methane levels at surrounding methylation sites. These locations were linked to TGFB1 expression and took on opposing effects in OA patients' synovium and cartilage (Rice *et al*., 2021).

To investigate the role of enhancers in OA, 16,816 differentially methylated CpGs were identified, of which approximately half (8,111) were from enhancers, forecasting major DNA methylation changes in the enhancer region for both types of OA (Lin *et al*., 2020). The majority of the differentially methylated CpG sites in the subchondral bone underlying eroded cartilage were hypomethylated, according to a study that investigated the DNA methylation patterns of patients with hip OA. The researchers discovered 7,316 differentially methylated CpG sites in the subchondral bone underneath deteriorated cartilage, the vast majority of which (75%) were hypomethylated, and 1,397 sites in covering damaged cartilage, 126 of which were shared. Some original differentially methylated genes involved in the pathogenesis of OA were found, such as hypomethylated genes *argonaute RISC catalytic component 2, transforming growth factor beta 1, nuclear factor of activated T cells 1, insulin-like growth factor 1 receptor and hypermethylated genes NR2F1 antisense RNA 1, homeobox A7, CD6 molecule*, etc. (Jeffries *et al*., 2016). It seems that the ALDH1A2 locus raises the risk of hand OA via lower ALDH1A2 expression in the joint tissues; the impact relies on rs12915901. These results point to a mechanism that might now potentially be addressed to control the risk of OA (Shepherd *et al*., 2018). These studies uncovered new mQTLs and shed insight into the complex relationship between gene expression in cartilage, the genetic predisposition to OA, and DNA methylation.

Synovium

The accumulation of inflammatory cells and the expression of inflammatory mediators in synovial tissue are also crucial factors in the pathogenesis of OA. Activated fibroblast-like synoviocytes (FLS) in the OA synovium produce, among other things, cytokines, growth factors, MMPs, and TIMPs to drive catabolic pathways in chondrocytes and contribute to macrophage activation (Haubruck *et al*., 2021).

Yang *et al.* (2017) isolated IL-6 overexpressing FLS from OA patients to examine epigenetic alterations in the IL-6 promoter region. OA patients had a pattern of hypomethylation and histone hyperacetylation, as well as decreased binding of methyl CpG binding protein 2, histone deacetylase 1, and DNMT3a, suggesting the role of epigenetic alterations in the IL-6 promoter at the synovial level in the pathogenesis of OA. Recently, small non-coding RNAs, for example, have been found in FLS, while DNA hypermethylation can silence the transcription of non-coding RNAs. Studies revealed that hypermethylation of *miR-140* and *miR-146a* regulation region-specific CpG sites is associated with down-regulation of *miR-140-5p* and *miR-146a* in FLS and OA chondrocyte, separately. In addition, hypomethylation of the *miR-146a* gene leads to a decrease in the binding affinity of the nuclear factor-κB transcription factor, confirming the inflammatory role of *miR-146a* in OA, which is also a current strategy for treating OA (Papathanasiou *et al*., 2019). In another study (Zhang *et al*., 2020a), analysis of chondrocyte proliferation revealed that overexpression of the *miR-130a* gene, a promoter of chondrocyte proliferation, resulted in increased proliferation rates in chondrocytes from patients with OA. PLEC CpGs were analyzed for mQTLs in cartilage, fat pads, synovium, and peripheral blood of patients with OA, and imbalance in allelic expression in synovium was observed in the same direction as the cartilage, and methylation and PLEC expression were correlated. However, depletion of PLEC and *Plec* affects a wide range of pathways reported to play critical roles in cartilage biology and OA (Sorial *et al*., 2020).

With all things above, a series of analyses were performed to further identify DNA methylation sites among cartilage, synovium, and blood, which, as expected, had distinct methylation patterns. These differential DNA methylation genes from different tissues might be involved in the development and progression of OA, providing potential therapeutic targets for OA. Interestingly, some identical methylated genes, such as RWDD2B (Parker *et al*., 2021) and PLEC (Sorial *et al*., 2020), were found.

DNA Methylation-mediated epigenetic modules are promising targets for osteoarthritis

Epigenetic modification is dynamic, and its reversibility facilitates the development of new drugs. Smeriglio *et al.* (2020) revealed that cytosine hydroxymethylation regulated the activation of Wnt signaling, metalloproteinases, and mTOR through TET1. Inhibition of TET1 activity lowers the production of matrix metalloproteinases MMP3 and MMP13, as well as several inflammatory factors, which have anti-arthritic effects. By targeting myeloid cell leukemia-1 and modulating the downstream phosphoinositide-3-kinase/protein kinase B pathway, *miR-34a* silencing by DNMT3B may substantially decrease chondrocyte ECM breakdown and inflammatory response in OA (Xiong *et al*., 2021). This study demonstrated that miR-34a knockdown may result in

the development of a new OA therapy (Xiong *et al.*, 2021). The current investigation revealed that cryptotanshinone might downregulate the expression of *miR-574-5p* by altering its methylation, hence enhancing YY1-associated factor 2 (YAF2) expression and influencing chondrocyte apoptosis (Yue *et al.*, 2021b). A study by Wang *et al.* (2018) found that Chinese medicine compound Qi-Fang-Xi-Bi-Granules (QFXBGs) promoted methylation of CCAAT enhancer-binding protein alpha 2 (C/ebpα-2) CpG island at ten bases from the promoter, which facilitates cartilage growth and regulates C/ebp expression while reducing chondrocyte apoptosis, restoring cartilage and subchondral bone.

In short, medicines that ameliorate abnormal methylation and target methylation are expected to be used in OA, and genes involved in the epigenetic regulation of OA can be used as new targets.

Current challenges and opportunities in osteoarthritis epigenetics research

OA possesses a unique methylation profile, and DNA methylation profiling has the potential to be a beneficial diagnostic tool, allowing not only the identification of OA but also the identification of potential therapeutic targets for some of the several differential methylation pathways involved in OA-specific pathways. Methylation alterations have been implicated in the development, diagnosis, therapy, and prognosis of OA, according to epigenetic research. Differential methylation genes discovered may be used as biomarkers to predict OA development and disease severity, as well as possible treatment targets for OA. As a drug target, epigenetic modification can provide a new pharmacological direction for the development of new drugs, which supplies a direction for the precise treatment of OA. While DNA methylation studies have led to significant advances in OA, some serious questions remain in current research. More studies are needed to detect the function and targets of differential methylation and to shed light on the pathogenesis and drug discovery of OA.

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