Review

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Genetic, epigenetic, and biomechanical updates on the pathogenesis of ossification of the posterior longitudinal ligament

Ruizhe Wang[a], Minjie Dong[a], Xiaolong Shen[a], Yang Liu[a], Xinwei Wang[a], Wen Yuan[a,*], Chen Xu[a,*], Huajiang Chen[a,*]

Abstract: Ossification of posterior longitudinal ligament (OPLL) is a complex multifactorial spinal disorder characterized by ectopic bone formation within the ligament, leading to progressive spinal canal stenosis and neurological deficits. While its epidemiological and clinical profiles are well-established, the precise molecular pathogenesis remains incompletely understood. This review systematically synthesizes recent advances in understanding the mechanistic underpinnings of OPLL, highlighting the interplay of genetic predisposition, epigenetic regulation, metabolic disorders, and biomechanical stress. Pathological manifestations, anatomical features, and the dual-origin hypothesis of OPLL are elaborated, alongside its phenotypic overlaps with ankylosing spondylitis and the contribution of inflammatory signaling cascades. Susceptibility loci identified via genome-wide association studies and their functional relevance to key regulatory pathways are summarized. Epigenetic regulation, encompassing pre and posttranscriptional modifications, is highlighted with particular attention to the roles of long noncoding RNAs (lncRNAs) and microRNAs (miRNAs). Metabolic mechanisms implicated in OPLL, including diabetes, lipoprotein receptor-related protein 5 signaling, and lipid metabolism dysregulation, are discussed, as is the critical role of biomechanical stress in disease progression. By integrating insights across multiple disciplines, this review establishes a comprehensive pathophysiological framework for OPLL, with the goal of bridging basic science and clinical practice and identifying promising avenues for the development of targeted therapeutic strategies.

Abbreviations: AS = ankylosing spondylitis, BMP = bone morphogenetic proteins, BM-MSCs = bone marrow mesenchymal stem cells, COL6A1 = collagen type VI alpha 1, C-OPLL = cervical OPLL, 1,25$(OH)_2$D = 1,25-dihydroxyvitamin D, ECM = extracellular matrix, FASL = factor-related Apoptosis Ligand, GWAS = genome-wide association studies, HLA = human leukocyte antigen, IL17RC = interleukin 17 receptor C, LDL = low-density lipoprotein, lncRNAs = long noncoding RNAs, LRP5 = lipoprotein receptor-related protein 5, MMPs = matrix metalloproteases, OPLL = ossification of posterior longitudinal ligament, PLL = posterior longitudinal ligament, ROR2 = receptor tyrosine kinase-like orphan receptor 2, RSPO2 = R-spondin 2, sEVs = small extracellular vesicles, SNPs = single-nucleotide polymorphisms, T-OPLL = thoracic OPLL, TIMPs = tissue inhibitor of metalloproteinases, UV = ultraviolet, VDR = vitamin D Receptor, YAP = yes1 associated transcriptional regulator

Keywords: biomechanical stress, epigenetic regulation, genetic regulation, OPLL, pathogenesis

1. Introduction

In 1838, Aston C. Key first reported ossification of the posterior longitudinal ligament (OPLL), which was described in detail by Tsukimoto et al. in 1960.[1] OPLL was defined as a fairly common spinal degenerative disease involving ectopic OPLL, and it is always combined with severe compression of nerve root and spinal cord, contributing to relevant neurological symptoms such as quadriparesis and paraparesis. It predominantly occurs in cervical vertebral (nearly 70%), with the most frequent segment at C5, while the occurrences of thoracic vertebra and lumbar vertebra were both 15%. Besides, its incidence rate among females is less than half that of males. In recent years, numerous studies found that OPLL always occurred after the age of 40 years with an increasing incidence rate nearly 80% for every 10-year increase in age, the average age of onset was 50 years with a younger trend and the peak distribution of incidence at 60–69 years. The overall prevalence of OPLL

RW and MD contributed to this article equally.

[a] Department of Orthopedics, Spine Center, Shanghai Changzheng Hospital, Naval Medical University, Shanghai, China.

* Correspondence: Wen Yuan, Department of Orthopedics, Spine Center, Shanghai Changzheng Hospital, Naval Medical University, 415th Feng Yang Road, Shanghai 200003, China (e-mail: yuanwenspine@smmu.edu.cn); Chen Xu, Department of Orthopedics, Spine Center, Shanghai Changzheng Hospital, Naval Medical University, 415th Feng Yang Road, Shanghai 200003, China (e-mail: chenxu1988@smmu.edu.cn); Huajiang Chen, Department of Orthopedics, Spine Center, Shanghai Changzheng Hospital, Naval Medical University, 415th Feng Yang Road, Shanghai 200003, China (e-mail: spine.chen@163.com).

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is 3.23%–6.1% in China,[2,3] 5.2%–7.7% in Japan,[4] 0.8%–3.0% in other Southeast Asia, 1.3%–3.2% in America, and 1.7%–2.8% in other countries,[5] demonstrating a worldwide distribution. In addition, the prevalence of OPLL in different regions of the spine has also been reported. Liang et al.[3] demonstrated that cervical OPLL most frequently occurred at the C5 segment, thoracic OPLL (T-OPLL) frequently occurred at the cervicothoracic junction segments (T1–T2) and thoracolumbar junctional segments (T11–L1/2), and lumbar OPLL most frequently occurred at the L5–S1 segment, with a similar peak distribution between males and females in each spinal region.

Despite well-established epidemiological and clinical characteristics, the precise molecular pathogenesis of OPLL remains incompletely elucidated, posing a significant obstacle to the development of targeted therapeutic interventions. OPLL is now increasingly recognized as an active, multifactorial process of ectopic ossification driven by a complex interplay of genetic predisposition, epigenetic regulation, metabolic disorders, and biomechanical stress. This evolving understanding underscores the critical importance of a systematic review that synthesizes recent advances across key mechanistic domains. Therefore, this review aims to comprehensively integrate current knowledge on these interconnected aspects—pathological changes, genetic mechanisms, epigenetic regulation, signaling pathways, biomechanical mechanisms, and metabolic mechanisms—to provide a cohesive pathophysiological framework and highlight promising directions for future research and therapeutic development.

2. Method

2.1. Search Strategy

We conducted a literature search in PubMed and Web of Science (1970–2025), with keywords as ("OPLL" OR "ossification of the posterior longitudinal ligament") . We prioritized primary research and reviews that reported genetic, epigenetic, and biomechanical data and handpicked to identify the data and included in the review.

3. Pathological changes of OPLL

3.1. Morphological and anatomical characteristics

The posterior longitudinal ligament (PLL) is a dense, collagen-rich connective tissue structure that is composed primarily of type 1 collagen fibers, interspersed with elastic fibers and proteoglycans.[6] It functions as a critical spinal stabilizer by constraining excessive vertebral flexion, resisting anterior disc herniation, and maintaining sagittal balance of the spinal column.[7] In OPLL, this specialized ligament undergoes progressive ectopic ossification, a pathological process characterized by the *de novo* formation of bone tissue within the ligamentous matrix.[5]

The anatomical distribution of OPLL exhibits striking regional predilection, with the cervical spine accounting for approximately 60%–80% of cases, followed by the thoracic (15%–30%) and lumbar (5%–10%) spine.[8] Within the cervical spine, the C5 segment is the most frequently involved, with a predilection for the mid-cervical, which is attributed to the unique biomechanical environment of the cervical spine, including increased range of motion, higher mechanical stress at segmental junctions, and regional variations in ligamentous vascular supply.[9,10]

3.2. Dual-origin mechanisms of OPLL

Numerous histological and translational evidence supports a dual-origin hypothesis for the pathogenesis of OPLL, wherein ectopic bone formation arises from 2 distinct but complementary cellular sources and mechanistic pathways, ligamentous cell transdifferentiation and recruitment of exogenous osteoprogenitor cells (Fig. 1), each regulated by context-dependent microenvironmental cues and signaling cascades.[11–13]

PLL-derived fibroblasts, the primary resident cells of the ligamentous matrix, exhibit inherent plasticity that enables their transdifferentiation into osteoblast-like cells under pathological stimuli (Fig. 1). This phenotypic switch is driven by the convergence of inflammatory mediators, biomechanical stress, and dysregulated signaling pathways, which collectively create a pro-osteogenic microenvironment that overrides the fibroblasts' native fibrous tissue identity.[14] Key signaling cascades orchestrating this transdifferentiation include the Wnt/β-catenin, transforming growth factor-beta/ bone morphogenetic protein (TGF-β/BMP), and mitogen-activated protein kinase (MAPK) pathways.[6] Histopathological studies confirm this process: in early OPLL lesions, PLL fibroblasts adjacent to ossification foci express both fibroblastic markers and osteogenic markers, supporting a gradual phenotypic transition rather than abrupt cell replacement.[15]

The second origin of OPLL involves the recruitment of exogenous osteoprogenitor cells to the PLL, primarily derived from 2 sources: bone marrow-derived mesenchymal stem cells (BM-MSCs) and adjacent vertebral osteophytes (Fig. 1).[15] Circulating BM-MSCs are mobilized from the bone marrow niche and recruited to the PLL lesion site via chemotactic gradients established by pro-inflammatory cytokines and chemokines secreted by infiltrating immune cells and resident fibroblasts.[16–18] Upon homing to the ligament, these BM-MSCs undergo osteogenic differentiation under the regulation of local microenvironmental factors—including bone morphogenetic proteins, TGF-β, and mechanical stress—ultimately contributing to ectopic bone matrix synthesis.[16] The second exogenous source, vertebral osteophytes, represents a contiguous source of osteoprogenitor cells: these bony outgrowths at the vertebral body-disc junction extend into the adjacent PLL tissue through progressive proliferation and matrix invasion. Histological analyses of advanced OPLL specimens reveal direct continuity between vertebral osteophytes and ossified PLL segments, with osteoblasts and osteocytes migrating from the osteophyte into the ligamentous matrix.[19] This invasion

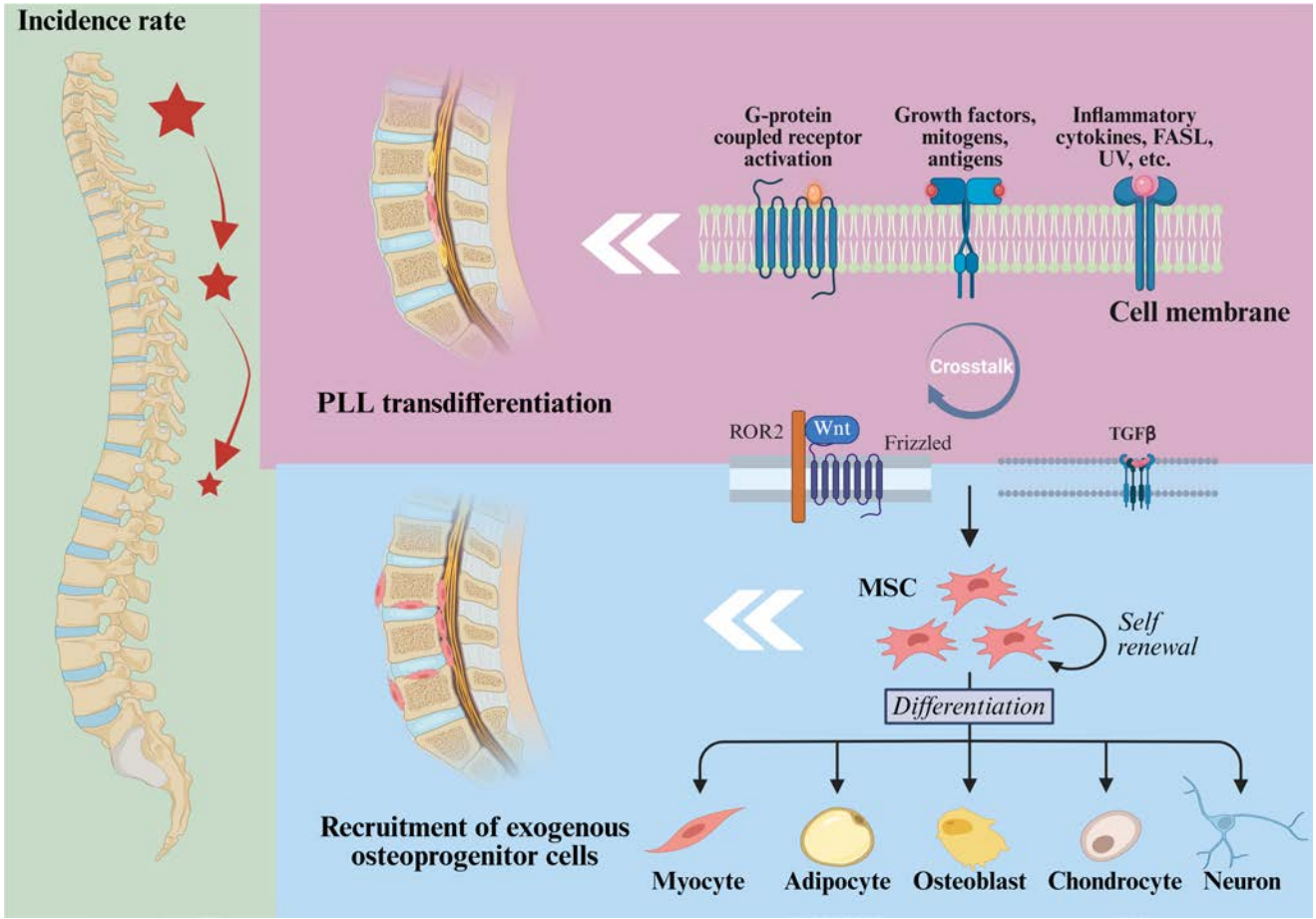

Figure 1. Pathological origin of OPLL. The illustration depicts the dual-origin theory for the development of OPLL according to previous reports. FASL, factor-related apoptosis Ligand; OPLL, ossification of posterior longitudinal ligament; PLL, posterior longitudinal ligament; ROR2, receptor tyrosine kinase-like orphan receptor 2; UV, Ultraviolet.

is facilitated by matrix metalloproteinases (MMPs) and a disintegrin and metalloproteinase with thrombospondin motifs (ADAMTS) enzymes secreted by osteophyte-derived cells, which degrade the extracellular matrix (ECM) of PLL to create a permissive environment for cell migration and bone formation.

Notably, these 2 mechanisms are not mutually exclusive but rather synergistic in promoting OPLL progression. Resident fibroblast transdifferentiation initiates early ectopic ossification, while exogenous osteoprogenitor cell recruitment amplifies and sustains bone formation in advanced lesions.[20] The relative contribution of each pathway may vary by disease stage, anatomical location, and individual genetic background. The fibroblast transdifferentiation predominates in cervical OPLL associated with high mechanical stress, while osteophyte-derived cell invasion is more prominent in T-OPLL linked to degenerative vertebral changes.[14] Elucidating the interplay between these dual origins provides critical insights into OPLL pathogenesis.

3.3. Similarities to ankylosing spondylitis (AS)

OPLL targets PLL, and AS primarily affecting the sacroiliac joints, vertebral endplates, and anterior/posterior spinal ligaments. These two conditions both lead to aberrant ossification of spinal supporting structures.[21] Genetically, emerging genome-wide association studies (GWAS) and candidate gene analyses have implicated human leukocyte antigen B27 (HLA-B27) as a potential susceptibility factor for OPLL, particularly in East Asian populations (Fig. 2).[22,23] Histologically, both conditions exhibit features of chronic inflammation coupled with tissue metaplasia. Immunohistochemical studies confirm overlapping cellular infiltrates in the inflammatory microenvironments of both diseases—cells that secrete pro-osteogenic cytokines and modulate local bone remodeling.[21]

Mechanistically, dysregulated inflammatory cascades represent a central shared pathway. In AS, pro-inflammatory cytokines such as tumor necrosis factor-α (TNF-α), interleukin-6, and interleukin-17A (IL-17A) drive enthesial inflammation and subsequent ossification by activating osteoprogenitor cells and upregulating osteogenic transcription factors (e.g., RUNX2, osterix). Similarly, OPLL ligamentous tissues and adjacent epidural spaces exhibit elevated expression of these cytokines, with *in vitro* studies demonstrating that TNF-α and IL-17A promote osteogenic differentiation of PLL-derived fibroblasts and MSCs via activation of the NF-κB and MAPK pathways.[24,25] Additionally, both diseases share

dysregulation of the TGF-β/BMP signaling axis—a key pathway governing chondrogenesis and endochondral ossification.[25]

3.4. Role of inflammatory responses

Inflammatory responses serve as a central driver in the initiation, progression, and amplification of OPLL, orchestrating a complex interplay between immune cell infiltration, cytokine signaling, ECM remodeling, and osteogenic differentiation.[26]

The infiltrating immune cells secrete a repertoire of pro-inflammatory cytokines, chemokines, and growth factors that converge to modulate multiple facets of OPLL pathogenesis.[27–29] TNF-α exerts pleiotropic effects on OPLL progression. It upregulates the osteogenic transcription in PLL-derived fibroblasts and MSCs, while simultaneously recruiting additional inflammatory cells and MSCs to the lesion site.[27,30] IL-6 activates the Janus kinase-signal transducer and activator of transcription (JAK–STAT) pathway to enhance the osteogenic differentiation potential of MSCs and PLL fibroblasts.[31,32] IL-17A emerges as a critical mediator linking inflammation to osteogenesis in OPLL. It synergizes with TNF-α to amplify RUNX2 expression and alkaline phosphatase (ALP) activity in PLL-derived cells, while also upregulating the chondroid metaplasia and endochondral ossification.[33] Additionally, IL-1β, a pro-inflammatory cytokine associated with acute and chronic inflammation, contributes to OPLL pathogenesis by inducing ECM degradation. This ECM remodeling not only disrupts the structural integrity of the PLL but also releases bioactive fragments that further promote osteogenic differentiation and inflammatory cell recruitment, creating a feed-forward loop that accelerates ossification.[30,34]

4. Genetic mechanisms of OPLL

4.1. Susceptibility loci for OPLL

The heritable predisposition to OPLL has been substantiated through a series of studies, prompting extensive genetic investigations to pinpoint specific risk loci. In 1998, a sib-pair linkage genetic analysis demonstrated that D6S276, on chromosome 6p, was located close to the HLA complex locus.[35] This landmark finding was particularly notable, as the HLA locus is a well-characterized hub for immune regulation and inflammatory disease susceptibility, aligning with emerging evidence linking immune dysregulation and inflammation to OPLL pathogenesis.[6,14] Subsequent candidate gene studies[6,15,28] further supported the involvement of the HLA complex,

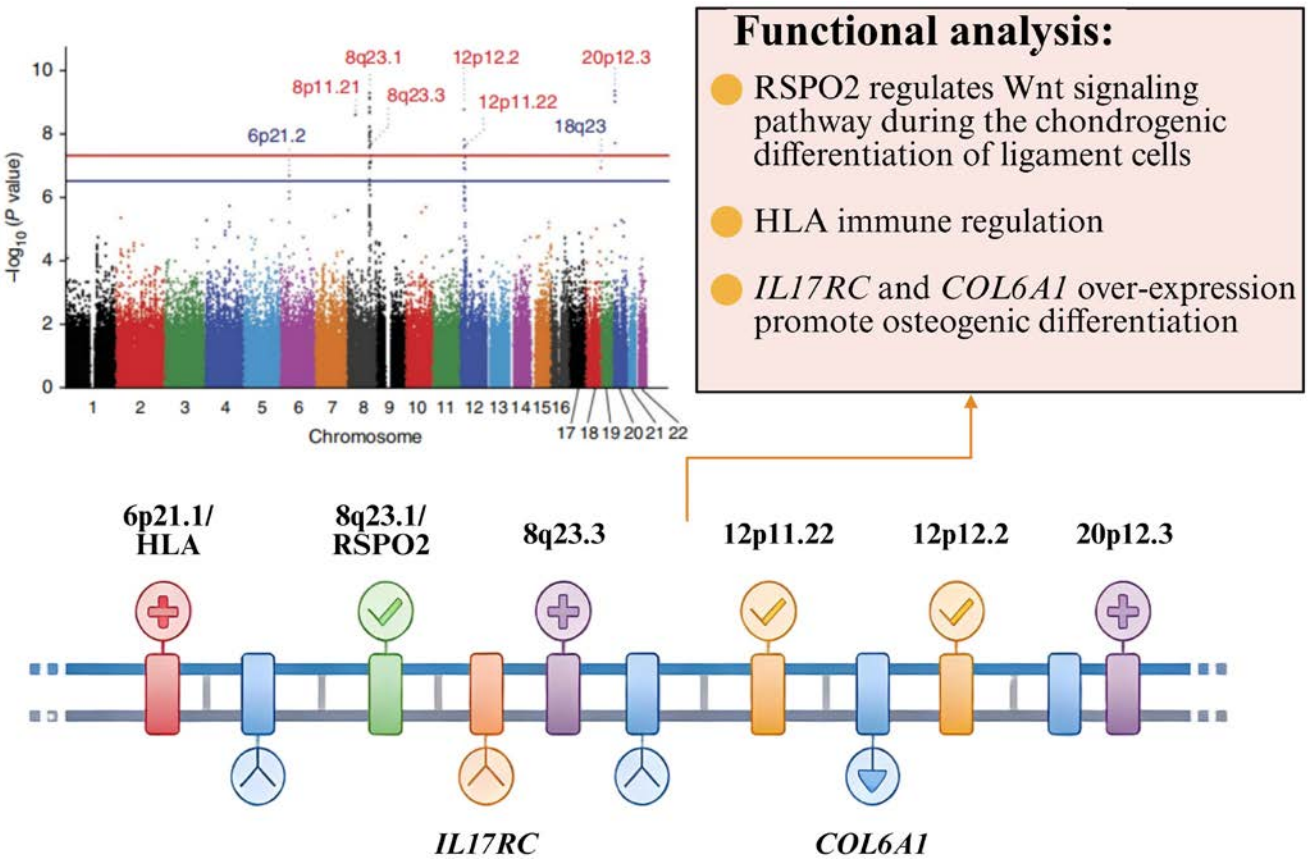

Figure 2. Susceptible loci for OPLL. Important genetic variations unveiled by GWAS showing the susceptible loci of OPLL development, and their pathological functions have been partially revealed. FASL, factor-related apoptosis Ligand; GWAS, genome-wide association studies; HLA, human leukocyte antigen; IL17RC, Interleukin 17 receptor; OPLL, ossification of posterior longitudinal ligament; RSPO2, R-spondin 2.

with associations reported between OPLL and specific HLA alleles in East Asian populations, reinforcing the hypothesis that immune-related genetic variants contribute to disease risk.

A GWAS was performed in nearly 8000 Japanese individuals and followed by a replication investigation with an additional nearly 7000 Japanese individuals (Fig. 2).[36] The 8 potential susceptibility loci for OPLL were explored in the first GWAS: 8p11.21, 8q23.1, 8q23.3, 12p12.2, 12p11.22, 20p12.3, 6p21.2, and 18q23. Combining the subsequent replication GWAS, 6 of 8 potential susceptibility loci were selected as the susceptibility targets: 8q23.1 (rs374810), 8q23.3 (rs13279799), 6p21.1 (rs927485), 12p11.22 (rs1979679), 12p12.2 (rs11045000), and 20p12.3 (rs2423294).[36] The identification of these loci has been instrumental in shifting the research focus towards understanding the functional mechanisms underlying these genetic associations. Notably, these validated loci map to or near genes with functional relevance to pathways critical for OPLL pathogenesis, including cellular signaling, skeletal development, immune regulation, and osteogenic differentiation.

Besides, a whole-genome sequencing study was performed in 30 unrelated northern Han Chinese patients. It indicated that 6 single-nucleotide polymorphisms (SNPs) (rs201153092, rs13051496, rs199772854, rs76999397, rs189013166, and rs151158105) in the *Interleukin 17 receptor (IL17RC)* and *COL6A1* genes were potentially associated with T-OPLL.[37] The 6 SNPs in the *IL17RC* and *COL6A1* genes were further analyzed in 200 northern Chinese individuals (100 patients and 100 control subjects) using the Sequenom system. And revealed 5 SNPs in the *IL17RC* and *COL6A1* genes that represented potentially pathogenic mutations in patients with T-OPLL.[38]

4.2. Functional analysis of susceptibility genes

Among the validated loci, the 8q23.1 region has garnered particular attention. This locus contains the gene encoding R-spondin 2 (RSPO2), a secreted agonist of the canonical Wnt/β-catenin signaling pathway, a critical regulator of bone formation.[39] Functional studies[39,40] have revealed that the expression of RSPO2 is suppressed during the chondrogenic differentiation of ligament cells, a key step in the endochondral ossification process characteristic of OPLL. The risk allele at this locus is associated with reduced expression of RSPO2, potentially leading to a dysregulation of the finely balanced Wnt signaling pathway and tipping the scales towards aberrant ossification.[40] Similarly, the locus on chromosome 6p21.1 is situated within the HLA region, which is pivotal for immune regulation.[36,41] It is hypothesized that certain HLA alleles might predispose individuals to an abnormal immune response against ligament tissue, triggering a cascade of events that culminates in metaplasia and ossification.[42] The other loci (e.g., 12p11.22, 20p12.3) are located in genomic regions containing or near genes involved in cellular signaling and development.

The discovery of these susceptibility loci represents a cornerstone in OPLL research. It not only confirms the strong genetic component of the disease but also opens up entirely new avenues for mechanistic exploration. However, these genetic discoveries alone are insufficient to explain the mechanistic basis of OPLL. A deeper exploration of epigenetic regulation is essential to translate these genetic findings into a comprehensive pathophysiological model.

5. Epigenetic regulation of OPLL

Epigenetic regulation refers to changes in gene expression that do not involve alterations in the DNA sequence, including pretranscriptional regulation and posttranscriptional regulation.[19,20] Epigenetic mechanisms play an important role in the pathogenesis of OPLL by regulating the expression of genes related to bone metabolism, inflammation, and cell differentiation (Fig. 3).

5.1. Pretranscriptional regulation

The pretranscription regulation mainly includes DNA methylation and histone modification.[43] Integrated analysis of RNA-Seq and MBD-Seq reveals that reduced DNA methylation in $Dnmt1^{\Delta Prx1}$ chondrocytes leads to increased expression of genes related to ossification.[43] The $Dnmt3b^{Agc1ER}$ fracture demonstrated defects in chondrogenesis and chondrocyte maturation, as well as a delay in the subsequent processes of angiogenesis, ossification, and bone remodeling.[44] A study employed genome-wide microarray analysis to compare non ossification MSCs (untreated or treated with the DNA methyltransferase inhibitor, 5AdC) with ossification MSCs (untreated or treated with 5AdC). Among them, only 2 genes, *GDNF* and *WNT5A*, exhibited significantly higher expression levels in ossification MSCs compared with non ossification MSCs without 5AdC treatment. Moreover, it was proposed that the osteogenic characteristics of MSCs are promoted by unmethylated *WNT5A* and *GDNF* genes.[45] Histone modifications influence chromatin structure, but direct OPLL evidence is limited. Numerous studies have shown that it regulates endochondral ossification through modifying chromatin structure.[46–48]

5.2. Posttranscriptional regulation

5.2.1. Long noncoding RNAs (lncRNAs). lncRNAs are a class of noncoding RNAs with a length of more than 200 nucleotides.[28] They can regulate gene expression at multiple levels, such as transcriptional regulation, posttranscriptional regulation, and epigenetic regulation, and play an important role in the pathogenesis of OPLL (Fig. 3).[39,49]

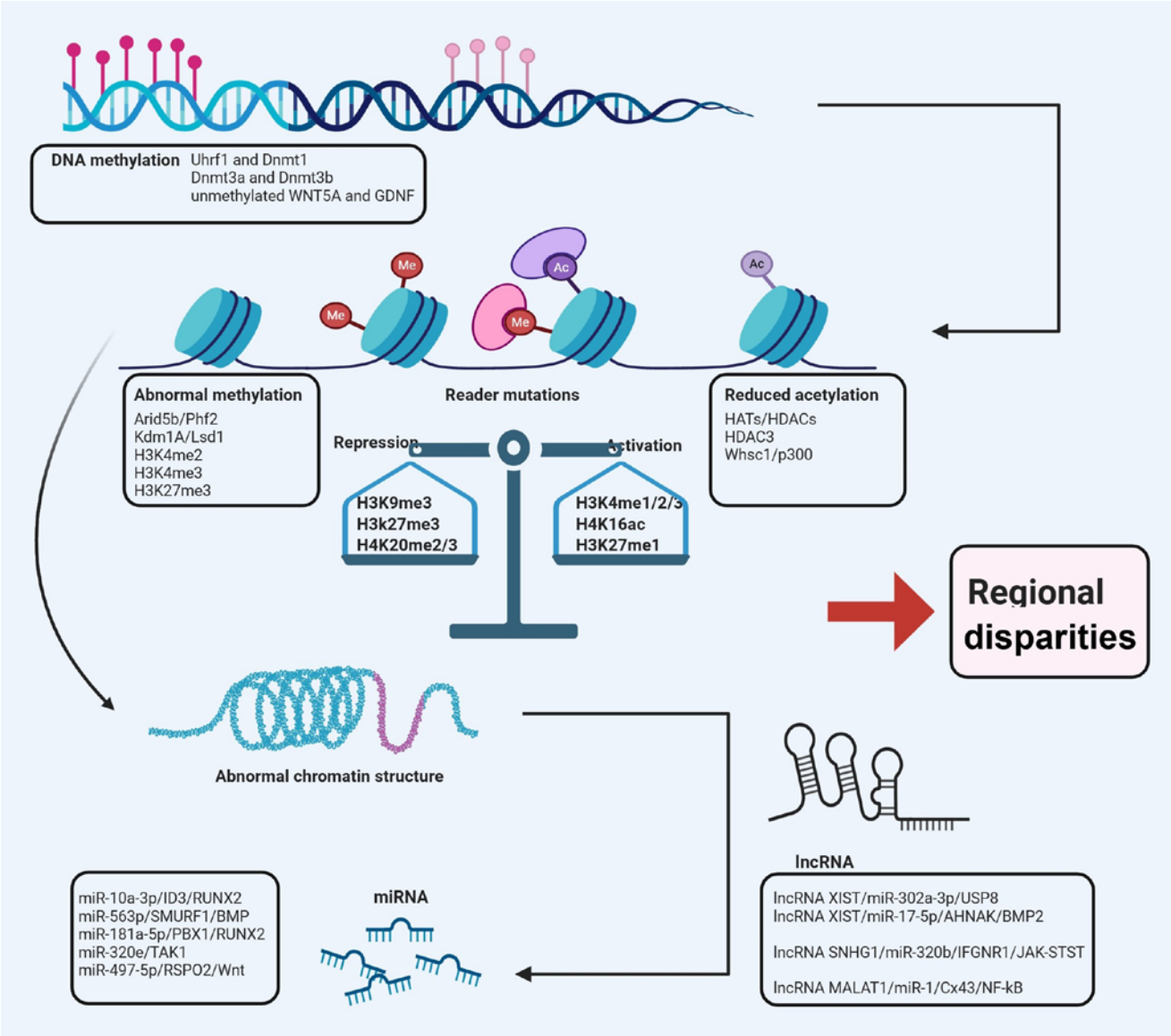

Figure 3. Epigenetic regulation of OPLL. Epigenetic regulations take a critical part in the development of OPLL, and many targets were discovered previously, including DNA methylation, histone modification, lncRNAs, and miRNAs. They may have a critical influence in causing the regional disparities of OPLL. OPLL, ossification of posterior longitudinal ligament; miRNAs, microRNAs; lncRNAs, long noncoding RNAs.

lncRNA XIST can regulate OPLL progression through multiple pathways. A recent study[50] has shown that miR-302a-3p, as the downstream target of lncRNA XIST, significantly decreased osteogenic differentiation of primary ligament fibroblasts. The study indicated that lncRNA XIST promotes OPLL progression via the miR-302a-3p/USP8 pathway. However, in another study, Liao et al.[51] found that mechanical stress increased the lncRNA XIST expression and decreased the level of miR-17-5p, which is identified as the downstream of lncRNA XIST based on the bioinformatic analysis. An additional mechanism study found that overexpression of miR-17-5p significantly repressed the levels of BMP2 and RUNX2, which revealed that miR-17-5p ameliorates the mineral deposition and osteogenic differentiation via the targets AHNAK/BMP2 signaling pathway under mechanistic stress.

lncRNA SNHG1 can promote the osteogenic differentiation of OPLL-derived ligament fibroblastic cells by sponging miR-320b.[52] Further mechanism study has revealed that lncRNA SNHG1-induced JAK–STAT signaling pathway activation is partially ameliorated by miR-320b. Therefore, miR-320b, which is sponged by SNHG1 in ligament fibroblastic cells, can truly suppress IFGNR1 and inhibit the JAK–STAT signaling pathway, thereby alleviating osteogenic differentiation and mineral deposition.

lncRNA MALAT1 is upregulated in the PLL cells of OPLL patients.[53] miR-1 showed a significant increase after siMALAT1 treatment. The results of the dual-luciferase-reporter assay indicated that Cx43 is the target of miR-1 in OPLL cells. Consequently, authors demonstrated that the MALAT1–miR-1–Cx43 axis regulates the OPLL progress via the NF-κB signaling pathway.[53]

5.2.2. MicroRNAs (miRNAs). Numerous studies indicated that miRNAs play vital roles in regulating ossification of various tissues.[54–56] Besides, miRNAs are also considered as the upstream of ossification-related genes in OPLL, which helps to explore the specific regulatory mechanism (Fig. 3).

Jiang et al.[28] predicted vital miRNAs according to the different expression of circRNAs and lncRNAs based on high-throughput sequencing of peripheral blood mononuclear cells of OPLL patients. In our previous study,[57] we first performed high-throughput sequencing and bioinformation to provide an integrative analysis of the whole transcriptome and its regulatory miRNA networks in OPLL, and the majority of 218 differentially expressed miRNAs were found to be OPLL-specific and strongly correlated to ossification, which also generated a related OPLL-specific miRNA signature for the first time. Xu et al.[58] showed 16 identification of hub genes and hub miRNAs, and presented a potential miRNA-mRNA regulatory network adequately based on our submitted database (Gene Expression Omnibus [GEO] dataset GSE69787).

For specific miRNAs, we demonstrated that miR-10a-3p promotes the ossification level of ligament cells by targeting ID3, thus increasing the binding of RUNX2 to ossification-related genes both *in vitro* and *in vivo*.[12] Besides, we also found that miR-10a-5p, miR-563, and miR-210-3p showed high accuracy and significance in identifying OPLL from other groups individually, which indicates that these miRNAs may play vital roles in diagnosing OPLL.[59] We found that miR-563 indeed induced OPLL through the downstream target SMURF1.[60] SMURF1 not only negatively mediates the BMP signaling pathway by mediates ubiquitination and degradation of SMADs,[61] but also induces RUNX2 degeneration in osteoblast differentiation.[62] Accordingly, we also demonstrated that miR-181a-5p promotes the ossification level of ligament cells by targeting PBX1, thus increasing the binding of RUNX2 to ossification-related genes by improving histone deacetylase and reducing histone methylation at the promoter region both *in vitro* and *in vivo*, which provides a basal theory for therapeutic application of the miR-181a-5p antagomir in OPLL treatment.[11] In a recent study,[13] we first manipulated small extracellular vesicles (sEVs) secretion inhibitors into OPLL model mice and found that sEVs secretion inhibition significantly repressed heterotopic OPLL compared with control groups, which indicates that OPLL-derived sEVs may play vital roles in OPLL progression. According to the further mechanism study, we demonstrated that miR-320e of OPLL-sEVs promotes ossification via inhibiting the downstream target TAK1 *in vivo* and *in vitro*.

5.3. Epigenetic regulation and regional disparities

Epigenetic modifications are being increasingly acknowledged as crucial mediators that connect environmental, metabolic, and lifestyle factors to regional disparities in the prevalence of OPLL.[11,63] In contrast to genetic variants, which remain constant within populations, epigenetic marks are highly sensitive to external signals.[64] These context-dependent modifications have the potential to regulate the expression of genes controlling osteogenic differentiation, inflammation, and ECM remodeling, thus contributing to the remarkable regional variations in the incidence and prevalence of OPLL observed worldwide (Fig. 3).[65]

OPLL demonstrates a well-documented ethnic and geographic bias, with a significantly higher prevalence in East Asian populations than in Western populations.[6,26,36] This disparity cannot be comprehensively accounted for by genetic factors alone. Instead, mounting evidence indicates that region-specific environmental and lifestyle factors prompt epigenetic divergence, influencing the penetrance of genetic risk variants and ultimately affecting disease susceptibility.[66–68]

Mechanistically, these regional environmental cues converge to modify key epigenetic pathways implicated in OPLL. DNA methylation of the promoters of osteogenic and inflammatory genes can either enhance or inhibit their expression.[69] For instance, hypomethylation of the RUNX2 and BMP4 promoters in East Asian individuals may promote ligamentous ossification by increasing their transcriptional activity.[70–72] Histone modifications, such as H3K4 activation and H3K27 repression, further precisely regulate gene expression in response to environmental stimuli, with regional disparities in histone modifier enzyme activity contributing to differential OPLL susceptibility.[73]

6. Biomechanical mechanisms of OPLL

Mechanical stress has been considered an incredible factor in the development and progression of OPLL. The spinal column endures diverse mechanical forces, including tensile stress, compressive stress, shear stress, and torsional stress, throughout daily activities. Abnormal mechanical stress can instigate a series of biological responses within ligament cells, resulting in ectopic ossification. The mechanical stress significantly increased the Cx43 expression, ER stress, and GLI1 expression (Fig. 4).

6.1. Upregulation of Cx43 expression

Cx43, a crucial constituent of gap junctions, assumes a significant role in cell-to-cell communication. Mechanical stress can notably upregulate the expression of Cx43 in ligament cells. Cx43 is capable of transducing mechanical signals into intracellular biochemical signals, facilitating the osteoblastic differentiation of ligament cells. Subsequent to mechanical stimulation, Cx43 initiates the downstream NF-κB signaling and inflammatory response, which can be reversed by Cx43 siRNA or an NF-κB (p65) inhibitor.[74,75] Moreover, higher levels of Cx43 activate the ERK1/2, p38 MAPK, and JNK pathways, yet the knockdown of Cx43 protein expression

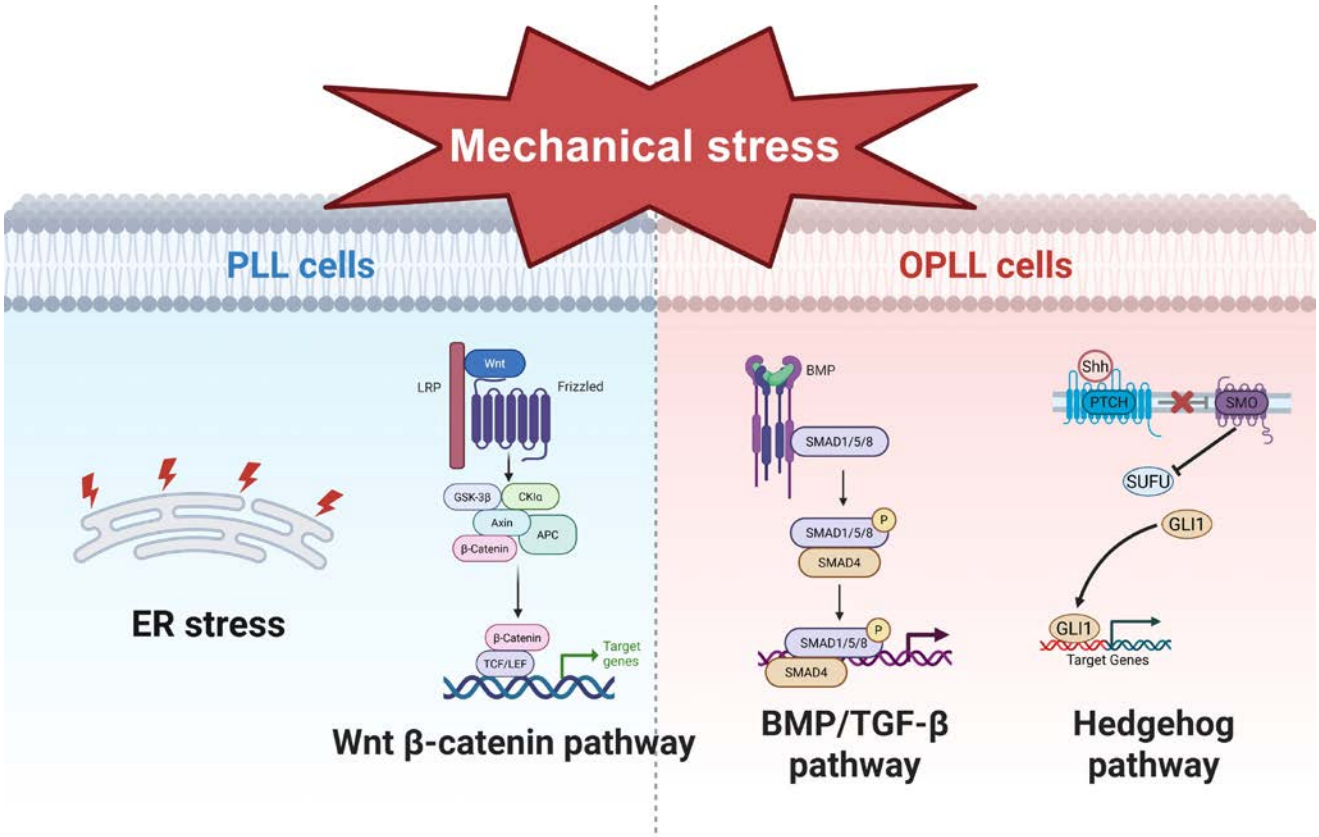

Figure 4. Mechanical stress takes part in the development of OPLL. The illustration depicts the mechanical signaling pathways that influence the development of OPLL according to previous reports. OPLL, ossification of the posterior longitudinal ligament; PLL, posterior longitudinal ligament.

inhibits the expressions of osteocalcin (OCN), ALP, and type I collagen(COL I) by blocking the ERK1/2 and p38 MAPK pathways rather than the JNK pathway.[76]

6.2. Induction of ER stress

Another study demonstrated that the ER stress was increased in non ossified areas of the ligament tissues of OPLL patients but not expressed in the ligaments of non-OPLL patients. The mechanical stress also improved ER stress, which influences the MAPK signaling pathway to promote osteoblastic differentiation.[77] Yuan et al.[78] indicating that after ER stress induction, USP9X activated Cx43 ubiquitination, and decreased ossification of primary ligament fibroblasts and nuclear translocation of NF-κB p65 by regulating Cx43 expression. So, the study suggested that the ER stress–NF-κB–USP9X–Cx43 signaling pathway plays an important role in OPLL progression.

6.3. Activation of the Hedgehog pathway

The cyclic tensile strain induces abnormal activation of the Hedgehog pathway to facilitate OPLL.[79] Meanwhile, a study indicated that the upstream Hedgehog pathway interacts with the downstream BMP pathway to influence the pathogenesis of OPLL.[80] The Hedgehog-GLI1 pathway can regulate the osteogenic differentiation of human cervical PLL cells by regulating the BMP signaling pathway.

6.4. Biomechanical environment of predilection sites

OPLL exhibits a predisposition towards specific spinal segments, including the cervical C5 segment, the cervicothoracic junction, and the thoracolumbar junction.[81] The cervicothoracic and thoracolumbar junctions serve as transition zones between different spinal regions, where there are substantial disparities in mobility and mechanical properties.[82] This leads to concentrated mechanical stress, which may also account for the high incidence of OPLL in these areas. The biomechanical mechanisms of OPLL are driven by abnormal mechanical stimuli that disrupt the microenvironment and cellular homeostasis of PLL. Chronic cyclic tensile strain, compressive loading, and shear stress activate mechanosensitive signaling pathways. These factors induce osteogenic differentiation, promote inflammatory cytokine secretion, and alter ECM remodeling, ultimately facilitating ectopic ossification.

7. Metabolic mechanisms of OPLL

Metabolic disorders, including diabetes, abnormal lipid metabolism, and dysregulation of bone metabolism-related molecules, are intricately associated with the pathogenesis of OPLL.[20] These metabolic factors can influence the equilibrium between bone formation and resorption, along with the function of ligament cells and MSCs, consequently facilitating the progression of OPLL (Fig. 5).

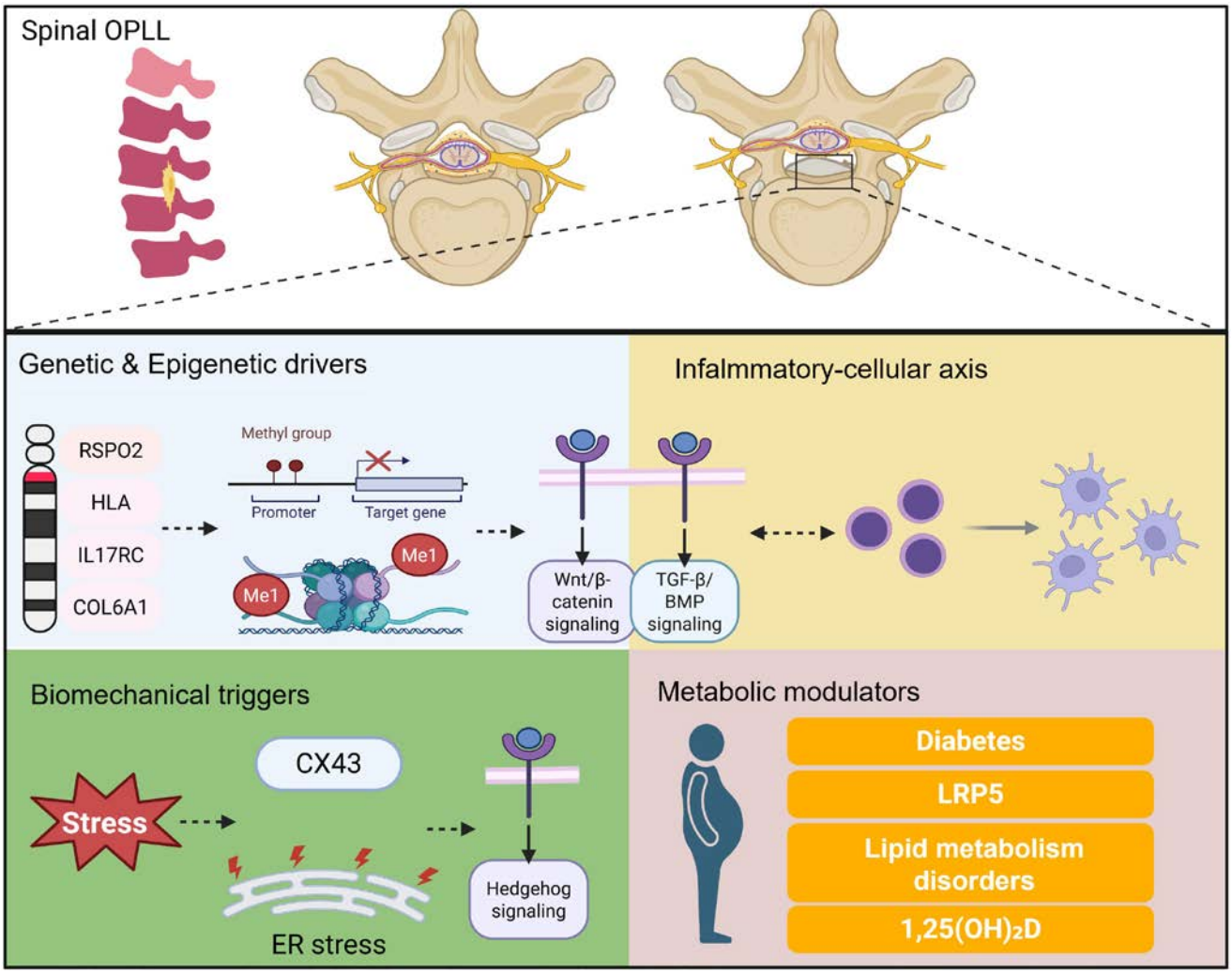

Figure 5. Summary of pathological factors that affect the development of OPLL. The illustration summarizes the genetic, epigenetic, metabolic, and mechanical signaling pathways that influence the development of OPLL according to previous reports. OPLL, ossification of posterior longitudinal ligament; LRPS, lipoprotein receptor-related protein 5; RSPO2, R-spondin 2.

Diabetes mellitus, especially type 2 diabetes, is a risk factor for OPLL. Studies have shown that the prevalence of OPLL is significantly higher in diabetic patients than in nonpatients.[20] The possible mechanisms include insulin resistance and hyperglycemia.[83–85] Insulin resistance can lead to impaired insulin signaling, which may affect the balance of bone metabolism, promoting ectopic ossification.[83]

Low-density lipoprotein receptor-related protein 5 (LRP5) is a key molecule involved in bone metabolism and the Wnt signaling pathway. In OPLL, LRP5 may play a role by regulating the Wnt/β-catenin signaling pathway.[86] The activation of the Wnt/β-catenin pathway mediated by LRP5 can promote the osteogenic differentiation of ligament cells and MSCs, leading to ectopic ossification.[87,88] Additionally, LRP5 may also be involved in the regulation of lipid metabolism, and its abnormal expression may affect lipid homeostasis, thereby indirectly participating in the pathogenesis of OPLL.[87]

Abnormal lipid metabolism is also closely related to OPLL. Studies have shown that the levels of lipids such as cholesterol, triglycerides, and low-density lipoprotein (LDL) are abnormally increased in OPLL patients.[89–92] The LDL can be oxidized to form oxidized LDL, which can activate macrophages to secrete inflammatory cytokines, promoting the inflammatory response in the ligament and facilitating ectopic ossification.[91,92] Lipids can affect the synthesis and degradation of the ECM of the ligament. The abnormal lipid accumulation leads to the degradation of collagen fibers in the ligament, reducing the mechanical strength of the ligament and making it more susceptible to mechanical stress-induced damage and ossification.

1,25-dihydroxyvitamin D [1,25$(OH)_2$D] serves as a crucial modulator of OPLL through its pleiotropic impacts on bone metabolism, inflammatory signaling, and mesenchymal cell fate determination. 1,25$(OH)_2$D directly promotes the osteogenic differentiation of PLL-derived fibroblasts and MSCs by upregulating the expression of core osteogenic transcription factors and osteogenic markers.[93] Treating PLL fibroblasts with 1,25$(OH)_2$D increases mineralized nodule formation and ALP activity, and 1,25$(OH)_2$D regulates inflammatory responses in the PLL microenvironment by controlling the production of pro-inflammatory

cytokines and chemokines.[94] Excessive $1,25(OH)_2D$ stimulates PLL fibroblasts and infiltrating macrophages to secrete TNF-α, IL-6, and CCL2. These, in turn, recruit additional inflammatory cells and MSCs to the lesion site and amplify osteogenic signaling through paracrine mechanisms.[95] Notably, $1,25(OH)_2D$-induced cytokine production is partially mediated by NF-κB activation, establishing a feed-forward loop between inflammation and osteogenesis. $1,25(OH)_2D$ modifies ECM remodeling by upregulating the expression of matrix metalloproteinases and downregulating tissue inhibitors of metalloproteinases (TIMPs) in PLL tissues.[96] This imbalance facilitates ECM degradation, releasing bioactive fragments that further enhance osteogenic differentiation and vitamin D receptor (VDR) activation, thereby aggravating ligamentous ossification.

The metabolic mechanisms underpinning OPLL encompass dysregulated pathways that are pivotal to bone metabolism, glucose homeostasis, lipid metabolism, and mineral regulation. Crucial mediators consist of abnormal $1,25(OH)_2D$-VDR signaling, dyslipidemia, and diabetes-associated hyperglycemia. Genetic variants in metabolic regulators further render individuals predisposed by augmenting pro-osteogenic metabolic signals, whereas the cross-talk among metabolic, inflammatory, and osteogenic pathways expedites ligamentous ossification.

8. Discussion

The mechanistic underpinnings of OPLL present a multifaceted interplay of genetic susceptibility, molecular regulation, and biomechanical factors (Fig. 5). This review has delineated the current understanding of these core drivers, spanning from genetic loci and miRNA-mediated epigenetic control to pivotal signaling pathways and their clinical implications through advanced classification systems. It becomes increasingly evident that OPLL represents an active, pathologically driven process of ectopic ossification, rather than a passive degenerative condition.

A primary cornerstone of OPLL pathogenesis lies in its genetic predisposition. GWAS have robustly identified specific susceptibility loci, notably on chromosomes 6p, 8q, and 12p, underscoring a significant heritable component.[36] These findings suggest that inherent genetic polymorphisms can prime ligament cells towards a heightened osteogenic response. Pretranscriptional regulation via DNA methylation and histone modifications is integral to OPLL pathogenesis, acting as a bridge between genetic susceptibility and environmental factors. DNA hypomethylation tends to activate osteogenic genes, while histone alterations fine-tune chromatin dynamics. While direct evidence of histone modifications in OPLL is limited, insights from endochondral ossification highlight their potential roles. Controlled by histone acetyltransferases and deacetylases, this reversible modification affects ossification pathways via modifying chromatin structure. Concurrently, the role of miRNAs has unveiled a sophisticated layer of regulatory control. The emerging concept that these miRNAs are selectively packaged into sEVs for intercellular communication introduces a novel paracrine mechanism for propagating the ossification signal within the ligamentous tissue microenvironment. This insight not only advances our pathophysiological model but also highlights the potential of these miRNAs as viable diagnostic biomarkers and therapeutic targets.

Central to the ossification process is the dysregulation of core signaling pathways. The Wnt/β-catenin pathway, TGF-β/BMP pathway, and mechanotransduction pathways are not isolated cascades but form a highly interconnected network. Mechanical stress, for instance, can activate yes1 associated transcriptional regulator (YAP1), which subsequently cross-talks with and potentiates the Wnt/β-catenin pathway.[2,38,97,98] Likewise, BMP signaling, a principal driver of osteogenesis, interacts with other pathways. The convergence of these signals on master transcription factors, particularly RUNX2, appears to act as a final common pathway inducing the osteogenic differentiation of ligament fibroblasts. This network perspective clarifies why mono-therapeutic strategies targeting a single molecule often show limited efficacy, given the inherent redundancy and compensatory mechanisms within biological systems. Consequently, future therapeutic paradigms may need to embrace multitarget approaches or focus on pivotal nodes where these pathways integrate.

The instrumental role of chronic mechanical stress provides a critical link to clinical observations. Evidence demonstrating that mechanical stretch upregulates Cx43, induces ER stress, and activates Hedgehog signaling offers a mechanistic explanation for the predilection of OPLL to develop at biomechanically dynamic spinal segments.[74,76,80] The mechanosensitive capacity of ligament cells effectively transduces physical force into pro-ossification biochemical signals, bridging external biomechanics with intrinsic cellular responses. This understanding opens avenues for potential preventive strategies aimed at modulating biomechanical loads in at-risk individuals.

9. Conclusion

In summary, OPLL is a disease characterized by the convergence of genetic predisposition, epigenetic regulation, dysregulated cellular signaling, and biomechanical stress. However, current research has limitations: histone modification's role is inferred from broader studies, and mechanistic insights often rely on bulk analyses. Future work should leverage single-cell sequencing to resolve cellular heterogeneity and multiomics integration to map pathway interactions. A holistic understanding of these intertwined mechanisms is fundamental for pioneering novel diagnostic, preventive, and therapeutic strategies. This review synthesizes the current state of knowledge and aims to provide a foundation for future research directed at improving outcomes for patients with this complex condition.

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Ethical statement

Not applicable.

Conflict of Interest

Huajiang Chen, Associate Editor-in-Chief of *Spine Research*, was not involved in the peer-review processor in any editorial decisions regarding this manuscript. The peer-review process was handled independently by other qualified editors to minimize potential bias. The other authors have no conflicts of interest to disclose.

Data availability statement

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Author contributions

R.W., M.D., W.Y., C.X., and H.C. drafted the manuscript. X.S., Y.L., and X.W. edited the languages. W.Y., C.X., and H.C. supervised and critically revised the manuscript.

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