BIOMOLECULES & THERAPEUTICS

Review
Biomol Ther 27(4), 342-348 (2019)

Molecular Pathophysiology of Ossification of the Posterior Longitudinal Ligament (OPLL)

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Abstract

Ossification of the posterior longitudinal ligament (OPLL) can be defined as an ectopic ossification in the tissues of spinal ligament showing a hyperostotic condition. OPLL is developed mostly in the cervical spine and clinical presentations of OPLL are majorly myelopathy and/or radiculopathy, with serious neurological pathology resulting in paralysis of extremities and disturbances of motility lowering the quality of life. OPLL is known to be an idiopathic and multifactorial disease, which genetic factors and non-genetic factors including diet, obesity, physical strain on the posterior longitudinal ligament, age, and diabetes mellitus, are involved into the pathogenesis. Up to now, surgical management by decompressing the spinal cord is regarded as standard treatment for OPLL, although there might be the risk of development of reprogression of ossification. The molecular pathogenesis and efficient therapeutic strategy, especially pharmacotherapy and/or preventive intervention, of OPLL has not been clearly elucidated and suggested. Therefore, in this review, we tried to give an overview to the present research results on OPLL, in order to shed light on the potential pharmacotherapy based on molecular pathophysiologic aspect of OPLL, especially on the genetic/genomic factors involved into the etiology of OPLL.

Key Words: OPLL, Pathophysiology, Novel therapeutic approach

INTRODUCTION

Ossification of the posterior longitudinal ligament (OPLL) can be defined as an ectopic ossification (calcification) in the tissues of spinal ligament showing a hyperostotic condition (Matsunaga and Sakou, 2012; Kim *et al*., 2018). OPLL is developed mostly in the cervical spine (about 70%), as well as in the thoracic and lumbar spine, predominantly in males (2 times more prevalent than in females) (Saetia *et al*., 2011; Kawaguchi *et al*., 2013). The clinical presentations of OPLL are majorly myelopathy and/or radiculopathy, with serious neurological pathology resulting in paralysis of extremities and disturbances of motility (motor function) lowering the quality of life. These manifestations are due to a reduction of volume of the spinal canal and the compression and injury of spinal cord by hardened ligament after ossification (Koyanagi *et al*., 2003; Chikuda *et al*., 2011; Kim *et al*., 2017). OPLL is known to be an idiopathic and multifactorial disease, which familial inheritance (genetic factors) and non-genetic factors including diet, obesity, physical strain on the posterior longitudinal ligament, age, and diabetes mellitus, are involved into the pathogenesis (Iwasaki *et al*., 2004; Kobashi *et al*., 2004; Stapleton *et al*., 2011; Ikegawa, 2014; Kawaguchi *et al*., 2016). A multitude of research on OPLL has been performed in Japan, since the prevalence of OPLL has been reported to be 2.0-4.0% in Japan, 1.0-3.0% in other Asian countries including Korea and China, and 0.1-1.7% in North America and continental Europe (Mori *et al*., 2014; Yoshimura *et al*., 2014; Fujimori *et al*., 2015). To date, surgical management by decompressing the spinal cord is regarded as standard treatment for OPLL, although there might be the risk of development of reprogression of ossification (Abiola *et al*., 2016; Shin *et al*., 2017; Beom and Seo, 2018; Lee *et al*., 2018). At the same time, the molecular pathogenesis and efficient therapeutic strategy, especially

Open Access **https://doi.org/10.4062/biomolther.2019.043**

Received Mar 8, 2019 **Revised** Mar 31, 2019 **Accepted** Apr 9, 2019
Published Online May 2, 2019

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pharmacotherapy and/or preventive intervention, of OPLL has not been clearly elucidated and suggested. Therefore, in this review, we tried to give an overview to the present research results on OPLL, in order to shed light on the potential pharmacotherapy based on the molecular pathophysiologic aspect of OPLL, especially on the genetic/genomic factors involved into the etiology of OPLL.

CURRENT SURGICAL AND PHARMACOLOGICAL MANAGEMENT OF OPLL

Surgical management of OPLL

In the current medical practices, OPLL-induced myelopathy in the cervical spine is managed by anterior decompression or posterior decompression. The anterior decompression means that the operational removal of ossified lesion, via the anterior side of the spine. However, it is technically difficult, since the posterior longitudinal ligament exists in front of the spinal cord. Thus, the posterior approach is carried out to achieve the decompression of spinal cord, although the complications associated with decompression surgery including postoperative re-progression of ossification should be overcome (Zeidman *et al*., 1997; Shin *et al*., 2011, 2017; Beom and Seo, 2018; Lee *et al*., 2018).

Pharmacological and non-surgical management of OPLL

The current non-surgical management of OPLL consists of physical therapy, observation, and administration of oral analgesics (Matsunaga *et al*., 2004; Pham *et al*., 2011). Pain and numbness, the symptoms of OPLL, make the patients ask to resolve them promptly. This kind of neuropathic pain can be managed by pharmacotherapy. Nonsteroidal anti-inflammatory drugs (NSAIDs), opioids, antidepressants, local anesthetics, mecobalamin, and anticonvulsants have been utilized for controlling neuropathic pain (Furukawa, 2008; Tu *et al*., 2015; Liu *et al*., 2017). However, these drugs are used just for symptomatic relief. Therefore, the development of a novel agent for curing and/or preventing the myelopathy due to ossification of spinal ligament based upon targeting the molecular pathophysiology of OPLL is essentially required (Table 1).

MOLECULAR PATHOPHYSIOLOGY OF OPLL

The pathogenesis of OPLL has not been clearly understood. Although both genetic and environmental (non-genetic) factors are reported to be associated with the occurrence of OPLL, this disease shows an intense genetic predisposition (Wang *et al*., 1999; Okamoto *et al*., 2004; Furukawa, 2006; Ikegawa, 2014; Liang *et al*., 2018).

Table 1. The management of OPLL

Surgical management	Anterior decompression; Operational removal of ossified lesion via the anterior side of the spine Posterior decompression; Posterior approach carried out to achieve the decompression of spinal cord
Pharmacological and non-surgical management	Physical therapy (and observation) Administration of oral analgesics

Genetics of the susceptibility to OPLL

A multitude of study found the associated genetic loci linked to susceptibility to OPLL. Karasugi *et al*. (2013) reported that OPLL-associated loci showing potential linkages at 20p12, 16q24, 7q22, 2p22-2p24, and 1p21 were suggested as a result of a genome-wide linkage study using 214 siblings-pairs of OPLL. Also, 6p21.1, 8q23.1, 8q23.3, 12p11.22, 12p12.2, and 20p12.3 were identified as OPLL-susceptibility loci based on a result of a genome-wide association study (GWAS) (Nakajima *et al*., 2014). GWAS for OPLL provided substantial information on chromosomal positions associated with OPLL, although the causal genes of OPLL and functional genomic positions are required to be connected closely by the efficient target gene association studies. The prevalence of OPLL in cervical spine was 26.15% in the parents and 28.89% in the siblings of probands from 347 families with cervical OPLL, which are higher than those in the general population (Terayama, 1989). The siblings sharing identical human leukocyte antigen (HLA) haplotypes from families of 24 OPLL patients showed the higher prevalence of OPLL and a significant linkage on D6S276 with OPLL (Matsunaga *et al*., 1999). Tanaka *et al*. (2003) suggested that another potential genetic factor of OPLL, collagen 6A1 (COL6A1), exists on 21q (D21S1903). In cartilage and bone, the amount of transforming growth factor-β (TGF-β) is relatively high and there are many target cells for TGF-β. By autocrine and paracrine secretions, TGF-β plays a pivotal role in keeping and proliferating mesenchymal stem cells and progenitors of osteoblasts (Bonewald and Mundy, 1990; Chen *et al*., 2012). Therefore, the TGF-β genes, especially TGF-β1, are considered the major candidates promoting the susceptibility to OPLL, because of its significance in controlling bone metabolism (Bonewald and Dallas, 1994; Kamiya *et al*., 2001; Kawaguchi *et al*., 2003). It is known that the TGF-β superfamily consists of TGF-βs, Activin, bone morphogenetic proteins (BMPs) and Nodal, totally forty or more members. In mammalian development, intracellular signaling of TGF-β/BMPs in concert with MAPK, FGF, Notch, Wnt, and Hedgehog signaling pathways are very important in the formation of bone (Chen *et al*., 2012). There are many reports on single nucleotide polymorphism (SNP) in TGF-β genes and their significance in the prevalence of OPLL. However, to date, the results of the studies are not consistent and more investigations are needed for reaching the conclusion (Kamiya *et al*., 2001; Kawaguchi *et al*., 2003; Horikosi *et al*., 2006; Han *et al*., 2013; Jekarl *et al*., 2013). Collagen is known to play an important role during the development of cartilage and bone. Several genes encoding collagen, COL6A1, COL11A2, and COL17A1, have been reported to be associated with OPLL. Diverse pathological phenotypes of connective tissues might be provoked by aberrant expressions and/or mutations of collagen genes (Tsukahara *et al*., 2005; Kong *et al*., 2007; Kim *et al*., 2014). Similar to the case of TGF-β genes, many studies on SNPs in genes of collagen and their association in the occurrence of OPLL have been performed. Some of them suggested that a specific gene is linked to the prevalence of OPLL, although the relevance of these SNPs of genes encoding collagen in the etiology of OPLL is still not obvious (Koga

et al., 1998; Sakou *et al*., 2000; Maeda *et al*., 2001; Wei *et al*., 2014). Ectonucleotide Pyrophosphatase/Phosphodiesterase 1 (ENPP1) is a type II transmembrane metalloenzyme. It is known to control the calcification of soft tissue and bone, as an inhibitor of calcification by producing inorganic bisphosphate (PPi) (Sun and Mauerhan, 2012). An animal model for human OPLL, the tiptoe walking (twy) mice, shows a spontaneous development of abnormal ossification of the posterior longitudinal spinal ligament (Hosoda *et al*., 1981; Uchida *et al*., 2012). ENPP1 was reported to be the responsible gene for phenotypes of twy mice (Okawa *et al*., 1998). The four SNPs in ENPP1 have been reported to be linked to the development and/or severity of OPLL in humans (Koshizuka *et al*., 2002; Tahara *et al*., 2005; Horikoshi *et al*., 2006). He *et al*. (2013) reported that patients with a specific SNP in ENPP1 showed a good prognosis after surgery for decompression of the spinal cord. BMPs, a group of TGF-β family, are known to induce the differentiation of chondrocytes and osteoblasts in the development of cartilage and bone. Intracellular signaling of Smad provoked by BMP has been known to regulate Msx2, Runx2, Dlx5/6, Osterix and Sox9, the transcriptional factors required for the chondrogenesis and osteoblastogenesis (Marcellini *et al*., 2012; Shi *et al*., 2013; Rahman *et al*., 2015; Sanchez-Duffhues *et al*., 2015). In the tissues of ligament affected by OPLL, receptors for BMPs are expressed in higher levels than in the normal ligament tissues, suggesting that BMP might mediate the occurrence of OPLL (Yonemori *et al*., 1997). The SNPs in genes of BMPs have been studied in relation to the development of OPLL (Wang *et al*., 2018). A specific SNPs of BMP2 was found in the OPLL patients with higher rate than in the normal population (Wang *et al*., 2008; Yan *et al*., 2013; Li *et al*., 2014) and significantly linked to strengthened susceptibility to OPLL (Ren *et al*., 2012). Taken together, it is essentially required to characterize the SNPs in the ligament tissues of OPLL from the big size of samples systematically, since the majority of studies on various genes linked to susceptibility to OPLL have been based upon the small number of sequence variants and small sample sizes. Moreover, the evident functional relationship between the SNPs associated with OPLL and the progression and occurrence of the disease should be clarified through future study (Table 2).

Physical stress and OPLL

Interestingly, physical (mechanical) stress to the tissues of ligament, especially a cyclical stretch, has been reported to increase the expression levels of various genes including osteopontin, alkaline phosphatase (ALP), endothelin-1, BMP-2, Type I collagen, Cbfa1 (an osteoblast-specific transcription factor), BMP-4, osteocalcin, BMP receptors, and integrin β1 and induce OPLL development and its progression (Iwasaki *et al*., 2004; Furukawa, 2006; Iwasawa *et al*., 2006). Physical stress also controlled the expression of a specific subtype of purinoceptors in OPLL cells and stimulated the progression of OPLL (Sawada *et al*., 2008). Ohishi *et al*. (2003) reported that the synthesis of prostaglandin in the cells of ligament of OPLL patients increased by physical stress and it stimulated the differentiation of osteoblasts. Overexpression of vimentin, one of the filament proteins, in osteoblasts was reported to inhibit the differentiation of osteoblasts resulting in suppression of mineralization. Physical stress decreased the expression level of vimentin and stimulated the progression of OPLL (Shapiro *et al*., 1995; Zhang *et al*., 2014). Physical stress increased the expression level of connexin 43 (Cx43, gap junction alpha-1 protein), the protein provoking OPLL, via p38 MAPK and ERK signaling pathway in spinal ligament fibroblasts from OPLL patients (Yang *et al*., 2011; Chen *et al*., 2014, 2016).

Table 2. The major genes associated with the susceptibility to OPLL

Ectonucleotide Pyrophosphatase/Phosphodiesterase 1 (ENPP1), Integrin β1
Transforming growth factor-β1 (TGF-β1), Endothelin-1
Bone morphogenetic proteins (BMPs)-2, Bone morphogenetic proteins (BMPs)-4
COL6A1, COL11A2, and COL17A1, Type I collagen
Osteopontin, Alkaline phosphatase (ALP), Osteocalcin
Cbfa1 (an osteoblast-specific transcription factor), BMP receptors

Biomarkers of OPLL

Serum levels of insulin, leptin, and osteocalcin, a bone-formation marker, are positively associated with the occurrence of OPLL (Akune *et al*., 2001; Sugimori *et al*., 2003; Ikeda *et al*., 2011). In the ligaments of OPLL, the levels of nebulin-related anchoring protein and osteoglycin increased, while those of biliverdin reductase B, alpha-1 collagen VI, NAD(P) dependent steroid dehydrogenase-like, and carbonic anhydrase I decreased (Zhang *et al*., 2015). Sclerostin and dickkopf-1 (DKK1) are known to exert a significant activity on the formation of bone and sclerostin levels in serum are higher in the male OPLL patients than those in the normal people (Morvan *et al*., 2006; Modder *et al*., 2011; Szulc *et al*., 2013). It was reported that serum levels of DKK1 were negatively correlated in the patients with OPLL (Kashii *et al*., 2016). Also, the serum levels of osteocalcin and carboxyterminal propeptide of human type 1 procollagen (PICP) are higher in patients with OPLL than in normal people (Matsui *et al*., 1996) (Table 3).

OPLL and mesenchymal stem cells (MSCs)

MSCs are defined as multipotent progenitor cells differentiating to diverse types of cells, including chondrocytes and osteoblasts. MSCs derived from spinal ligaments may differentiate to chondrogenic, adipogenic, or osteogenic cells and play a pivotal role in abnormal ossification process (Asari *et al*., 2012; Medici and Olsen, 2012; Nelson *et al*., 2012; Nomura *et al*., 2013). The transcription factors including Runx2, Sox9, Osterix, and Msx2 have been reported to control chondrogenesis and osteogenesis from MSCs (Nishimura *et al*., 2012). Also, the expression levels of parathyroid-related peptide hormone (PTHrP), Sox9, and Indian hedgehog (Ihh) in

Table 3. The Biomarkers of OPLL

Positively associated biomarkers	Insulin, Leptin, Osteocalcin, Osteoglycin, Nebulin-related anchoring protein, Sclerostin
Negatively associated biomarkers	Biliverdin reductase B, Dickkopf-1 (DKK1), Carbonic anhydrase I, Alpha-1 collagen VI, NAD(P) dependent steroid dehydrogenase-like

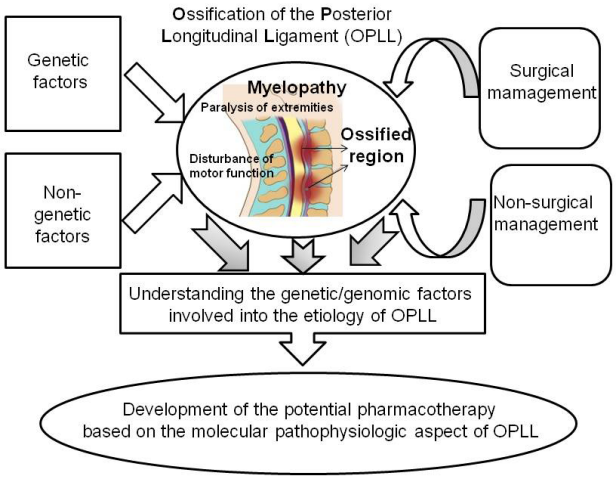

Fig. 1. Overview of etiology, management, and strategy for the development of potential pharmacotherapy.

the tissues of OPLL were reported to be increased than in the normal ligament tissues (Sugita *et al*., 2013). Therefore, through future investigations, it is urgently needed to elucidate the exact role of aberrant intracellular signaling in MSCs derived from spinal ligament, during the process of development and progression of OPLL.

OPLL and dietary factors

Up to now, there are few reports on the relationship between dietary habits and the development of OPLL. Wang *et al*. (1999) reported that, in Taiwan, a high-salt diet through intaking much amount of pickled foods and low intake of meat daily are positively associated with the occurrence of OPLL. Okamoto and his colleagues also reported that, in Japan, frequent intake of chicken and soy foods and frequent consumption of pickles were associated negatively and positively with the development of OPLL, respectively (Okamoto *et al*., 2004). The exact relationship between diets and the occurrence of OPLL is unclear because of the limited results of existing investigational studies. More and more extensive investigations are urgently needed for elucidating the relationship between dietary habits and the risk of development of OPLL.

CONCLUSION AND FUTURE DIRECTION FOR OPLL RESEARCH

In spite of a multitude of investigations, the exact causal genes for the development of OPLL are still not elucidated, although diverse genes showed the evidence of being involved into the occurrence and progression of OPLL. In addition to the current surgical management of OPLL, cutting-edge strategy for prevention and management using pharmacological means is essentially needed and has not been suggested until now. Therefore, novel drugs and/or drug targets should be developed based on the in-depth additional examination of genetic factors involved into the etiology of OPLL. Specifically, novel agents that can possibly regulate the intracellular signaling of TGF-β/BMPs and/or the genes encoding collagen, which is known to play an important role during the development of bone and to be associated with OPLL, including COL6A1, COL11A2, and COL17A1, should be developed. Also, the environmental factors, especially the exact relationship between diets and the occurrence of OPLL, should be specified through future investigational studies (Fig. 1).

CONFLICT OF INTEREST

The authors have no conflict of interest to declare.

ACKNOWLEDGMENTS

This research was supported by NRF-2014R1A6A1029617 and NRF-2017R1C1B1005126, Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education.

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