Eur Spine J (2007) 16:1075–1084
DOI 10.1007/s00586-007-0356-4

REVIEW ARTICLE

Hormones and growth factors in the pathogenesis of spinal ligament ossification

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Received: 23 January 2007 / Accepted: 6 March 2007 / Published online: 11 April 2007

Abstract Ossification of the spinal ligaments (OSL) is a pathologic condition that causes ectopic bone formation and subsequently results in various degrees of neurological deficit, but the etiology of OSL remains almost unknown. Some systemic hormones, such as 1,25-dihydroxyvitamin D, parathyroid hormone (PTH), insulin and leptin, and local growth factors, such as transforming growth factor-β (TGF-β), and bone morphogenetic protein (BMP), have been studied and are thought to be involved in the initiation and development of OSL. This review article summarizes these studies, delineates the possible mechanisms, and puts forward doubts and new questions. The related findings from studies of genes and target cells in the ligament of OSL are also discussed. Although these findings may be helpful in understanding the pathogenesis of OSL, much more research needs to be conducted in order to investigate the nature of OSL.

Keywords Spine · Ligaments · Ossification · Hormones · Growth factors

Introduction

Ossification of the spinal ligaments (OSL), a multifactorial disease in which complex genetic and environmental factors interact [73, 87, 93], is characterized by ossification of various extraspinal ligaments, such as the posterior longitudinal ligament [ossification of the posterior longitudinal ligament (OPLL)], the ligamentum flavum [ossification of ligamentum flavum (OLF)], and the anterolateral spinal ligament [diffuse idiopathic skeletal hyperostosis (DISH)], and it often causes myelopathy, radiculopathy, or both. Although the aetiology of OSL is still poorly understood, some progress has been achieved in basic research during the past decade on the pathogenesis of OSL, including studies of the involvement of some hormones and growth factors (Table 1). This review will focus on the current knowledge and recent developments in the understanding of the pathogenesis of OSL with an emphasis on the roles played by hormones and growth factors.

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Systemic hormones

Some systemic hormones, such as 1, 25-dihydroxyvitamin D, parathyroid hormone (PTH), insulin, and leptin, have been studied extensively in bone and are thought to be crucial to bones. Meanwhile, a high incidence of some metabolic and endocrine diseases related to them, such as hypoparathyroidism, vitamin D-resistant rickets, diabetes mellitus (DM), and obesity, has been detected in patients with OSL. The relationship between the hormones in these metabolic abnormalities and OSL has been documented, but the underlying mechanisms remain uncertain.

Calcium regulating hormones

Calcium regulating hormones maintain the calcium and phosphorus homeostasis and play vital roles in bone metabolism. The abnormalities of calcium regulating hormones, such as hypoparathyroidism and vitamin D-resistant rickets, result in both calcium and phosphorus imbalance and bone disorders.

Table 1 Hormone and growth factors related to OSL

System hormones	
Calcium regulating hormones	1,25-dihydroxyvitamin D, Parathyroid hormone (PTH)
Glucose and fat metabolism	Insulin, leptin
Growth hormones	
Sex hormones	
Vitamin A	
Local growth factors	Transforming growth factor-β1 (TGF-β1), bone morphogenetic protein (BMP-2/4/7), cartilage-derived morphogenetic protein (CDMP)-1, insulin-like growth factor-1 (IGF-1), and connective tissue growth factor (CTGF)

The presence of OSL has been identified in patients with hypoparathyroidism and vitamin D-resistant rickets [1, 69, 81], but the association between them remains unclear. Takuwa et al. [84] observed significantly low-calciuric responses to an oral calcium load in some OSL patients. Seichi et al. [76] found a strong relation between a low calciuric response and the incidence of OSL progression. The calciuric response to an oral calcium load reflects intestinal calcium absorption regulated by 1,25-dihydroxyvitamin D. However, the levels of the serum 25-hydroxyvitamin D and 1,25-dihydroxyvitamin D in these patients were not significantly different from those in controls, so the reduced intestinal activity of vitamin D or the related deficiency of 1,25-dihydroxyvitamin D was conjectured to be related to the development of OSL [89]. The pathology of bone metabolism in vitamin D-resistant rickets is characterized by decreased bone mineral density (BMD) and an increased volume of non-mineralized bone matrix, while patients with OPLL have significantly higher BMD in both the spine and radius [95]. It is difficult to define how 1,25-dihydroxyvitamin D reduces osteoclast or increases osteoblast recruitment and activation and is therefore able to increase the spine mineralization; however, 1,25-dihydroxyvitamin D has a stimulatory effect on the recruitment and differentiation of both osteoblasts and osteoclasts.

In addition, because metastatic calcification occurs when the calcium×phosphate product exceeds 70, which is often seen in patients with hypoparathyroidism, some authors [89] thought that ligamentous calcification is substantially a form of metastatic or dystrophic calcification, rather than ectopic ossification. However, the pathology of OSL has testified the presence of mature bone in the ossified tissue. Furthermore, it is impossible for the metastatic calcification to provide a scaffold for the initiation of ossification because (1) hypocalcemia, hyperphosphatemia and the decreased level of PTH appear not to influence the incidence of OSL [69] and (2) correcting the serum level calcium and phosphate with supplementation of phosphate and vitamin D could not prevent or reverse the ossification, but rather it aggravated the ossification [5, 22].

The genic studies on vitamin D receptor (VDR), PTH or PTH receptor-1 (PTHR1) in OSL were inconsistent and did not provide meaningful evidence of their association [18, 65, 78]. To date, there has been no report of OSL in a large series of patients with vitamin D-resistant rickets or hypoparathyroidism. Therefore, it is difficult to determine whether there is really an association between vitamin D, PTH and OSL based on the findings of current studies.

Glucose and fat metabolism hormones

The association of obesity, not-insulin-dependent DM (NIDDM) and OPLL has been established and attracted much attention, but the real mechanism remains unknown. Insulin is speculated widely to play a key role in the underlying mechanism, because epidemiological studies [20, 43, 66, 79] have testified that obesity (BMI>=25), impaired glucose tolerance, and NIDDM are the major risk factors for OPLL, and that insulin resistance and hyperinsulinemia often occur in these endocrine abnormalities. Does the exposure of ligament cells to high levels of insulin cause the ossification? Akune et al. [2] proposed this hypothesis based on factors related to the extent of OPLL after finding that but the insulinogenic index (a ratio of the increment of the serum level of insulin to that of glucose), but not the fasting serum insulin level, had a strong positive correlation with the extent of OPLL, and that even the severity of glucose intolerance did not correlate with the extent of OPLL. They thought that the elevated insulin secretion from β-cells might be responsible for the extensive ossification. The patients with higher BMI have more severe hyperinsulinemia, so BMI also has a positive correlation with the extent of OPLL. However, this is not the case in NIDDM. The extent of OPLL in patients with NIDDM, who often have a hyperinsulinemia, is less than that in patients with impaired glucose tolerance, who have a relatively low serum insulin level. In addition, according to their hypothesis, insulin treatment for NIDDM may make the ossification more severe. As poor control of NIDDM was a risk of postoperative reoccurrence of OPLL

Table 2 Relations between DISH and DM, impaired glucose tolerance, and obesity

Authors	Subjects	Results
Julkunen et al. [38]	12,858 persons	Association with obesity and IGT
Coaccioli et al. [8]	130 patients with Types 1, 2 DM and obesity	High incidence of DISH in obese-Type 2 DM patients and obese patients (40%), non-obese-Type 2 DM patients (30%), Type-1 DM patients (26.6%), IGT (22.2%)
Kiss et al. [42]	69 M and 62 F DISH versus 69 M and 62 F with spondylosis over the age of 50 years	Association with obesity, DM
Littlejohn et al. [52]	11 M DISH versus in 8 M age and weight matched controls	Significant hyperinsulinemia in the DISH group response to the glucose challenge
Sencan et al. [77]	133 DM versus 133 non-diabetic controls matched for sex, age, and weight	No statistically significant difference in prevalence of DISH (12 vs. 6.8%)
Vezyroglou et al. [90]	100 DISH versus 100 with various rheumatic disorders (DISH-free) matched for age, sex, BMI	No association with DM
Daragon et al. [9]	50 (>=60 years) DISH versus 50 normal controls, matched for sex, age, weight and height	No association with DM, IGT
Mata et al. [56]	56 DISH versus 31 healthy controls versus 43 lumbar spondylosis	No association with plasma glucose level

IGT impaired glucose tolerance, *M* male, *F* female, and *BMI* body mass index

[7], the hypothesis needs firmer evidence to be supported. Hyperinsulinemia does not seem to be clearly related to more extensive ossification.

Leptin is not only an important factor in fat metabolism, but is also involved in bone formation. The relationship between leptin and OSL is supported by the findings of some studies as follows: (1) significantly higher serum leptin levels were found in females with OSL than those in controls and expression of leptin receptor mRNA in female spinal ligaments was confirmed [80], (2) genic analysis studies indicated a significant association of the leptin receptor (LEPR) gene with the extent of OPLL [18, 25, 83], and (3) as an animal model of OPLL, Zucker fatty rats have an aberration of the leptin receptor gene [68]. However, the association may be indirect and has only been shown in women. First, leptin does not affect alkaline phosphatase activity (ALP) or procollagen type-I carboxyl-terminal peptide (PICP) content of the female spinal ligament cells in vitro [80]. Second, the higher serum leptin level was not found in male patients, and, in the female patients, the serum leptin level positively correlated with the serum insulin level. Third, no significant association was found between the LEPR gene and the prevalence of OPLL [18, 25, 83].

Hyperglycemia and advanced glycation end product (AGE), the typical characteristics of NIDDM, play important roles in the development of NIDDM. The fasting plasma glucose level, the hemoglobin A_{1c} level, and the stage of glucose tolerance have been found not to be associated with the extent of ossification [2], but the extent of OPLL is not equal to the prevalence of OPLL, as no studies have shown an association between the serum glucose level and prevalence of OPLL. Because high glucose is related to arterial calcification in NIDDM and since it can alter the extracellular matrix and cell growth in both renal and skin fibroblasts [6, 30, 46, 49, 50, 96], the association between high glucose and spinal ligament fibroblasts should be extensively researched.

The association between DISH and obesity is similar to that between OPLL and obesity. However, the association between DISH and NIDDM remains controversial. It has been shown that the prevalence of NIDDM or impaired glucose tolerance in patients with DISH is not significantly different from that in controls and that there is no difference in the plasma glucose level between patients with and without DISH, particularly when the influence of weight was excluded [9, 77, 90] (Table 2). Insulin is also thought to be a key factor in the incidence of DISH, but a high incidence of DISH was also found in patients with type I DM due to insulin deficiency. Combined metabolic features of DM + DL (dyslipidemia), DM + HU (hyperuricemia), or DM + DL + HU were shown to be the main risk factors for DISH, so the key factors in the incidence of DISH may be different from those in OPLL. Therefore, there may be some differences in the pathological mechanisms of OPLL and DISH.

Growth hormone

The GH/insulin-like growth factor (IGF-1) paracrine axis improves both bone formation and remodeling. Since

Table 3 Association of serum GH with DISH or OPLL

Authors	Method	Results
Scarpa et al. [74]	54 acromegaly patients versus 54 sex, age and BMI matched healthy controls	Association with DISH, the severity of DISH not related to GH and IGF-I levels.
Ikegawa et al. [27]	26 OPLL versus 19 age matched controls	High GHBP level, normal GH, (IGF)-1 in OPLL
Denko et al. [11]	Patients with DISH versus patients with osteoarthritis (OA) versus normal	Elevated GH, normal IGF-I levels in DISH
Denko et al [10]	Symptomatic DISH groups versus asymptomatic DISH groups	Higher GH and IGF-I in symptomatic groups
Altomonte et al. [3]	Six non-obese patients, with normal glucose olerance, DISH	Significant increased GH in DISH patients after IVITT

GHBP growth hormones-binding protein, *IGF* insulin-like growth factor and *IVITT* an intravenous insulin tolerance test

Julkunen et al. [37] reported the presence of OPLL or DISH in patients with giantism and acromegaly, some clinical observations have focused on the association of growth hormones (GH) with OPLL or DISH (Table 3). The discrepancy in these results may be due to the different controls or different clinical stages in DISH. It is also noted [12, 13] that non-steroid anti-inflammatory drugs and/or corticosteroids could improve DISH symptoms and result in lower serum GH levels, thus suggesting that an elevated GH level might contribute to the progression of clinical symptoms in DISH. Like its effect on the growth plate, GH may stimulate spinal ligament cells directly or indirectly through IGF-I, but a detailed mechanism needs to be researched.

Sex hormones

The influence of sex hormones on ligaments has been extensively studied in the injury of the knee anterior cruciate ligament (ACL) of female athletes. Cyclic fluctuations of sex hormones can weaken the ligament's mechanical properties by regulating ligament cell growth and synthesis [48, 61]. For OSL, Okada et al. [67] noted an imbalanced state for sex hormones (higher serum total estrogen level and lower serum 5α-$(OH)_2$ testosterone level) in male patients with OPLL, but not in females. The serum total estrogen level positively correlated with the extent of ossification. In vitro 3, 17 β-estradiol accelerated the production of osteocalcin and proliferation in cells from OPLL patients by a higher affinity estradiol receptor [92]. In a rabbit experiment [60], high-vegetable protein and high-salt foods were found to lead to a sex hormone imbalance affecting the development of chondroblasts and fibroblasts during the attachment of the posterior longitudinal ligament to the vertebral body. In a male rabbit model with instability of cervical vertebrae, the administration of testosterone could cause similar pathological changes in the posterior longitudinal ligament. In addition, tension could attenuate estrogen-stimulated collagen synthesis of fibroblasts from porcine ACL [51], while 1β-estradiol combined with progesterone could result in a dose-dependent reduction in cell proliferation and procollagen synthesis in human ACL fibroblasts in vitro [99]. Based on the above-mentioned facts, the dietetic habit-related change of sex hormone, combined with local mechanical stimulus, seems to affect the ligament cell and extracellular matrix, and then weaken the structure of ligaments. However, a change in sex hormones in males seems to be more closely related to OSL than in females, although changes in sex hormones occur more frequently or markedly in females, such as during menses or menopause.

Vitamin A

Heterotopic ossification, including OPLL and DISH, as found frequently in patients with long-term administration of vitamin A, is generally thought to be a toxic effect of vitamin A therapy [14, 72]. After administration of vitamin A for as long as 6 months, osteophytes or heterotopic ossification could be induced in the tendons or joint capsules of the animal [34]. Retinoic acid can suppress proliferation and stimulates maturation of MC3T3-E1 cells in vitro [36, 62]. Clinically, the levels of serum retinol and retinol binding protein (RBP) are significantly higher in OPLL patients, especially in female patients [44]. Interestingly, genetic analysis [53, 54] revealed that the absence of retinoic acid receptors (RARs) leads to severe deficiencies in cartilage formation at certain anatomical locations while promoting ectopic cartilage formation at other sites. All of these findings suggest that vitamin A may contribute to the development OPLL.

Local growth factors

Local growth factors play important roles in the onset and development of OSL. They may be related to changes in the micro-environment of spinal ligaments. Moreover, the

influence of systemic hormones on OSL may be regulated by the local growth factors. Many growth factors are involved in the osteogenesis, bone growth and chondrogenesis. Transforming growth factor (TGF) superfamily members, especially TGF-β1 and bone morphogenetic protein (BMP), are thought to be of great importance in the pathology of OSL.

BMPs

BMPs were first identified as proteins that were able to induce ectopic osteogenesis and were shown to enhance the osteogenic differentiation of both osteoblast-like and osteoprogenitor cells. They are thought to play important roles in bone formation during development and fracture repair. In addition, they are the proteins which have been researched most extensively in the study of OSL.

Immunohistochemical studies [21, 40, 85, 97] indicated the presence of BMPs in ossified matrix, chondrocytes, and fibroblasts near the ossified areas in surgical specimens of OPLL and OLF. BMPRs (BMP type IA, IB, II receptors) appeared in chondrocytes around the ossified areas and in fibroblasts and chondrocyte-like cells far from the ossified areas. These findings implied that BMPs conduct their function through the whole ossification process of OPLL and their target cells may be fibroblasts and chondrocytes differentiated from fibroblasts in the spinal ligaments. Animal experiments showed that BMP implanted in rat or mice lumbar ligament flavum could induce a local pathological change closely resembling OLF in human, including factors such as chondrocyte proliferation and endochondral ossification in hypertrophied ligamentum flavum [57, 58, 61]. Although the severity of spinal cord compression was much milder than that illustrated in clinical settings, which may be explained by the shorter observation period in experiments, it indicated that BMPs may act as initiators in ligament ossification.

Genic analysis studies provided inconsistent findings with regard to the association between BMPs and the incidence of OPLL [18, 25, 62], thus suggesting that BMPs might be only an irreplaceable local factor in ossification process in OSL, rather than the plague spot in OSL. That is, the factor which induces production of BMPs from ligament cells is of primary importance to the development of OSL. So, what induces the production of BMPs in ligament cells? Tanno et al. [86] found that mechanic stress significantly up-regulated the mRNA level of BMPs and BMPRs as well as the production of BMPs in OPLL cells. Although no change was demonstrated in non-OPLL cells, this study indicated that BMPs and BMPRs may be the bridges linking local and external factors. In addition, the immunohistochemical evaluations [23] of the spinal posterior longitudinal ligament and intervertebral disc of the tiptoe walking mouse, a mouse model for OPLL, showed a close relationship, pathologically, between disc degeneration and OPLL. The authors hypothesized that the spinal ligament transforms itself to cartilage or bone in order to repair degeneration of the disc in the tiptoe walking mouse [23], but disc tissue may provide an autocrine signal such that some cytokines repair the disc during the degeneration. Most recently, significantly higher mRNA levels of endogenous BMP-2 and BMP-7 were shown in both the annulus fibrous and nucleus pulpous from degenerated rabbit discs [61]. So, do the exogenous BMPs from discs stimulate nearby spinal ligaments to undergo abnormal ossification? Is OSL essentially a part of the repair process for disc degeneration?

Although BMP-2, BMP-4, and BMP-7 have been shown to induce bone in vivo and to trigger the differentiation of mesenchymal stem cells in vitro, their individual functional roles in OSL pathology are largely unclear. BMP-2 promotes osteogenic differentiation, the matrix production and proliferation of spinal ligament cells both in the OPLL and in some non-OPLL cell lines, but stimulating effects on the OPLL cells are more significant [26, 47]. Treatment with BMP-2 or adenovirus-mediated BMP-2 cDNA gene transfer, results in up-regulation of the expression of osteogenic phenotypes and bone nodule formation in human ligamentum flavum cells [59]. Therefore, BMP-2 may act as both an initiator and a promoter of the ossification. BMP-4 can induce ossification of the ligamentum flavum in mice. It is located in a manner similar to BMP-2 in immunohistochemical studies, and mechanical stress could stimulate its expression in OPLL cells in vitro [21, 40, 88, 97]. The presence of share stress responsive elements was found in the promoter region of mouse BMP-4/2 [16, 17], so both BMP-2 and BMP 4 may be related to mechanical stimuli. BMP-7 was found in hypertrophic chondrocytes during long bone development [91], but it was mainly located in chondrocytes near the ossified zone in OSL. As BMP-7 stimulates the in vitro expression of cartilage markers in fibroblasts from intrarticular ligaments [4], it may play a role in the cartilage ossification of OSL.

TGF-β1

Like BMPs, TGF-β1 is a multifunctional growth factor involved in cell proliferation and differentiation, and extracellular matrix protein synthesis. In OSL, TGF-β1 is located in the ossified matrix, chondrocytes adjacent to ossified ligaments, and in undifferentiated pericytes in unossified fibrous tissues, but not in fibroblasts, while its receptors are confirmed to the ligament cells in OPLL. These receptors are thought to function in cartilage ossification and the vessel invading process [29, 40]. TGF-β1 also seems to be responsible for ligament tissue hypertrophy. In vitro, it

promotes matrix synthesis and inhibits proliferation of ligament cells from patients with OPLL [29, 32]. It is also related to both the hypertrophy of the ligamentum flavum in lumber spinal stenosis and hypertrophy of the posterior longitudinal ligament [71]. An immunohistochemical study of the skin in patients with OPLL found an increase of decorin, a component of the extracellular matrix, in the epidermis of the nuchal skin, indicating an abnormality in the extracellular matrix. Decorin antagonistically regulates the action of TGF-β, so an abnormality in the regulatory system of TGF-β may be involved in the aggradation of the extracellular matrix [28, 35]. The findings of studies regarding whether TGF-β up-regulates ALP activity in OSL cells are inconsistent [29, 48]. TGF-β may improve osteogenic differentiation indirectly because it up-regulates the expression of connective tissue growth factor (CTGF) in both OPLL and non-OPLL cells. CTGF stimulated ALP expression in spinal ligament cells from patients with OPLL, but not in patients without OPLL [94]. In addition, genic analysis demonstrated a significant association between a TGF-β1 polymorphism (C allele) and the area of the ossified lesion, but such a relationship was not observed between the polymorphism and the occurrence of OPLL [41]. Like BMPs, the inducer of TGF-β production and its mechanism of action are not known.

Other growth factors

IGF-1 plays a major role during skeletal growth by regulating cellular activities of growth-plate chondrocytes. The elevated expression of IGF-1 was present in chondrocytes adjacent to ossified ligaments. In vitro, IGF-1 could enhance osteogenic differentiation in OPLL cells and improve proliferation and matrix synthesis in both OPLL and non-OPLL cells [19]. Cartilage-derived morphogenetic protein (CDMP)-1, another member of the TGF-β superfamily, is also located at the site of ossification of the ligamentum flavum (OLF) [63]. Both CDMP-1 mRNA and protein are specifically localized in spindle-shaped cells and chondrocytes in OLF tissues. Based on the previously reported promoting action of CDMP-1 in chondrogenesis, CDMP-1 may be involved in regulating the chondrogenesis process of OLF. However, few studies have been conducted to reproduce these findings, so their precise roles in OSL remain unclear.

Target cells in spinal ligaments

Ligaments connect one bone to another and act to constrain and guide joint motion. They respond to exercise or immobilization by altering their tensile strength and can be repaired after injury. They respond to both mechanical force and growth factors by altering cell proliferation or matrix synthesis [4, 55, 75], so the ligament cells are not silent or blunt, but are active and sensitive.

The identification of target cells in the spinal ligaments will be helpful to comprehensively understand the nature of OSL. Four kinds of cells were depicted in the immunohistochemical study of OSL: (1) chondrocyte, also called fibrocartilaginous cell, in the ossification foci or in the fibrocartilaginous and hyaline cartilaginous tissue near the ossification foci; (2) chondrocyte-like cell, also called a round cell and (3) fibroblast-like cell, also called a spindle cell, near the cartilaginous tissue; (4) fibroblast far from the ossification foci. According to their distribution, a chondrocyte is thought to derive from a fibroblast, and both chondrocyte-like cells and fibroblast-like cells are thought to be a cell types produced during the differentiation of fibroblasts into chondrocytes or osteoblasts [70]. However, in pathological and cell culture studies, there are perhaps four originations of the target cells in the ligaments. (1) One origination source is fibrocartilage cells in the enthesis, the inserting portion of the ligament into the vertebrae. These fibrocartilage cells are susceptible to mechanical stress, which accelerates with aging and degenerative changes, and they form ossified tissue at the enthesis, known as the syndesmophytes. In in vivo animal experiments, the osteogenic positive cells appear in or near the enthesis by pathological examination [23]. However, OSL is a more extensive lesion in ectopic bone formation where proliferating fibroblasts and fibrocartilage cells are distributed more extensively and are not limited to the enthesis. (2) Another origination source is undifferentiated mesenchymal cells in the ligament. In vitro studies [32, 33] demonstrated that the OSL cells were mainly undifferentiated mesenchymal cells distinct from the non-OSL cells—fibroblasts. These cells exhibited an osteoblastic phenotype; they synthesized osteocalcin upon vitamin D3 priming, exhibited high ALP activity and PTH induced an elevation of cAMP levels [33, 34]. But the characteristics are not exclusive to cells in OSL, since they are also seen in ligamentum flavum cells in lumbar spine stenosis [82]. (3) A third origination source is general fibroblasts in the ligament. Actually, all genes expressed in fibroblasts are also expressed in osteoblasts, including two osteoblast-specific transcripts: one encoding Cbfa1/RunX2, a transcription factor, and the other encoding osteocalcin, a secreted molecule that inhibits osteoblast function [15]. Cbfa1/RunX2 and osteocalcin transcriptional activities may be suppressed by a molecular defense protein, Msx2, which colocalizes with Cbfa1/RunX2. So if some factors, such as BMPs, override the molecular defense, or if the molecular de-

Table 4 Gene research on hormones and growth factors in patients with OSL

Authors	Subjects	Results
Shiigi et al. [78]	27 OSL versus 97 controls	Significant association in VDR gene polymorphism (B allele) (2% vs. 12%)
Ogata et al. [65]	120 OPLL (77M/43F) versus 306 controls (166M/140F)	ESR polymorphism associated with the both incidence and severity of female OPLL, no relation in VDR, PTH polymorphism
Takuya et al. [64]	134 OPLL versus158 controls	Significant associations in RXRβ
Kamiya et al. [39]	46 OPLL versus 273 controls	Significant association in TGF-β1 polymorphism (T8693 $\rightarrow$ C)
Kiss et al. [42]	369 OPLL versus 258 controls	Significant association in TGF-β1 polymorphism (C allele) with the area of the ossified lesion not the occurrence of OPLL
Koga et al. [45]	18 OPLL versus51 controls	No association in BMP-2 gene
Tahara et al. [83]	172 OPLL versus 93 age-matched controls	Association in LEPR gene with extensive of OPLL, not incidence of OPLL
Furushima et al. [18]	126 OPLL sib-pairs versus 250 unrelated OPLL controls and 200 non-OPLL controls	No evidence of linkage in VDR, LEPR, ESR2, BMP-2, BMP-7, TGF-β1, CTGF gene, weak evidence in PTHR1, TGF-β3, IGF-1 gene, suggestive evidence of linkage in BMP-4.
Horikoshi et al. [25]	711 OPLL (489M/222F) versus 896 controls (508M/388F)	No evidence of linkage in LEPR, ESR2, BMP-4, TGF-β1, PTHR1, IGF-1. Strong evidence of linkage in TGF-β3.

Results showed the association in gene with the occurrence of OSL

M male, *F* female, *VDR* vitamin D receptor, *RXR*β retinoic X receptor β, *LEPR* leptin receptor, *ESR* estrogen receptor, and *PTHR* PTH receptor

fense fails, fibroblasts would express osteoblast-specific transcripts [98]. (4) Another origination source is periosteum cells. A pathological study showed that there was not a clear delineation between the fibrous layer of the periosteum and the deep layer of the posterior longitudinal ligament [24]. The cambium layer is located between the body and the fibrous layer of the periosteum, so it can be speculated that active cells with osteogenic potentials in the cambium layer may ''enter'' the deep layer of the posterior longitudinal ligament or paracrine cytokines induce the cell in the posterior longitudinal ligament. Periosteum cells form bone by membranous ossification and sometimes the ossification model is also found in OPLL specimens, but no research has been made to verify this hypothesis.

Genic research on growth factors and hormones in patients with OSL

Several genetic studies (Table 4) on the relationship between hormones and growth factors and OSL in Japan have led to conflicting conclusions. OSL is not a single-gene disease, but may result from the interaction of multiple relevant genes. There may also be subsets of OSL. However, the limitation of gene studies is that they only identify a correlation rather than a causality between particular genetic polymorphism(s) and OSL [31]. Therefore, more investigations based on large-scale populations and other races should be conducted in the future. In addition, studies related to genetic mutations of these hormones or growth factors in OSL may be necessary.

Summary

OSL are multigenic, multifactor diseases. This may the reason why research on the basic pathology of OSL are not converging and are not profound enough to explain and allow for understanding of the nature of the diseases. Many explanations for the onset or development of OSL are in the stage of hypotheses and lack firm and sufficient evidence for support. More epidemiological studies should be performed to reaffirm the risk factors for the onset or progression of OSL. Identification of a precise mechanism for the factors in the progression of OSL may be helpful for clinical treatments. Further studies should be focused on elucidating the interactions between multiple factors.

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