

Original Article

The Wnt pathway regulator expression levels and their relationship to bone metabolism in thoracolumbar osteoporotic vertebral compression fracture patients

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Abstract: Objective: To investigate of the Wnt pathway serum regulator expression levels and their relationship with bone metabolism (BM) in thoracolumbar osteoporotic vertebral compression fracture (OVCF) patients. Methods: In this study, 40 healthy controls (group A), 33 osteoporotic patients (group B), and 47 thoracolumbar OVCF patients (group C) were recruited as the study cohort during the same period. The Wnt pathway serum regulator levels, bone density, BM-related inflammatory cytokines, bone formation markers, and bone resorption markers were compared among the three groups, and the correlation between the Wnt pathway serum regulators and BM was analyzed. Results: The β -catenin levels, the BALP, the densities at the femoral neck and lumbar spine, and the PINP, IL-10, OPG and BGP in groups B and C were lower than they were in group A, and the above indices in group C were lower than they were in group B ($P < 0.05$). Groups B and C showed higher CDKK-1, RANKL, TRACP-5b, β -CTX, IL-2, IL-6, MMP-2, MMP-9, Leptin, and TNF- α levels than group A, and the above indicators in group C were higher than they were in group B ($P < 0.05$). A Pearson's correlation analysis showed that the MMP-2, MMP-9, RANKL, β -CTX, and TRACP-5b levels were negatively correlated with β -catenin ($r < 0$, $P < 0.05$) and were positively correlated with DKK-1 ($r > 0$, $P < 0.05$). The BGP, PINP, OPG, and BALP levels were positively correlated with β -catenin ($r > 0$, $P < 0.05$) and were negatively correlated with DKK-1 ($r < 0$, $P < 0.05$). Conclusion: Patients with thoracolumbar OVCF have abnormal Wnt pathway serum regulator expression levels, low bone density, and abnormal BM, and the patients' Wnt/ β -catenin and DKK-1 levels are closely related to BM, so they may be potential targets for the prevention and treatment of metabolic bone diseases.

Keywords: Osteoporosis, osteoporotic vertebral compression fracture, Wnt pathway, bone metabolism

Introduction

Osteoporosis is a metabolic bone disease characterized by the loss of bone matrix, the destruction of the bone microstructure, and a reduction in the number of bone trabecula [1]. Under normal circumstances, bone formation and bone resorption are balanced. Once this balance is disrupted, it easily increases the risk of bone metabolic disease [2]. Thoracolumbar osteoporotic vertebral compression fractures (OVCF) are the most common type of fracture in patients with osteoporosis, and most occur in the elderly who are susceptible to muscle tension, the high forces of falling objects and falls [3]. The common clinical symptoms are limited

thoracolumbar activity, lower extremity dysfunction, and low back pain [4]. In the field of thoracolumbar OVCF prevention and treatment, osteogenesis and osteotomy have become research hotspots, among which the Wnt signaling pathway is the focus of extensive research [5].

The Wnt signaling pathway is highly conserved in many organisms. It regulates osteogenesis and osteoblast activity, and it is involved in cell growth, differentiation, and apoptosis [6]. The Wnt signaling pathway is involved in the dorsal/ventral limb and proximal-distal growth during skeletal development, which promotes the formation of bone, cartilage, fat and muscle, pre-

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venting osteoblasts from differentiating into chondrocytes and inhibiting osteoclast differentiation, and it can affect limbs, joints, and craniofacial development [7]. It has been found that the classical Wnt signaling pathway is closely correlated with cellular osteogenic differentiation and can alter bone mass through multiple pathways, but the relationship between this pathway and bone metabolism (BM) is unclear [8, 9]. Therefore, it is necessary to investigate the correlation between the Wnt pathway serum regulator expression levels and BM, which is important for elucidating the pathogenesis of disease, reconstructing bone tissue, and maintaining bone metabolic balance. This study analyzed the expression levels of the serum Wnt pathway regulators and their relationship with BM in thoracolumbar OVCF patients, aiming to explore new therapeutic options with more evidence-based and targeted properties.

Materials and methods

Clinical data

In this study, 33 osteoporosis patients (group B) and 47 thoracolumbar OVCF patients (group C) were recruited for the study during the period September 2018 to February 2020. In addition, 40 healthy people (group A) were recruited during the same period as a control group. Inclusion criteria: the patients in group B met the criteria in [10] *Diagnostic Guidelines for Primary Osteoporosis* (2017) with a bone density of the lumbar spine < -2.5 SD and confirmed using dual-energy X-ray bone densitometry. The patients in group C were diagnosed through imaging (CT, X-ray, etc.). All the patients voluntarily signed the informed consent form. Exclusion criteria: patients with coagulation abnormalities, bleeding disorders, osteoarticular infections, pathological fractures caused by occupational lesions, bone tumors and inflammation, secondary osteoporosis due to parathyroid dysfunction, etc., spinal stenosis, disc herniation and burst fractures, vertebral fractures, rheumatoid arthritis, vertebral dislocation, ankylosing spondylitis, a combination of severe primary diseases limiting daily activities, the presence of nerve damage symptoms in both the lower extremities or in the saddle region. This study was approved by the Medical Ethics Committee of Zhujiang Hospital, Southern Medical University.

Methods

(1) Serum Wnt pathway regulators. 5 mL of fasting venous blood was collected from all of the patients, and the serum was obtained using centrifugation (3000 r/min, radius 6 cm, 5 min), and their serum β -catenin and DKK-1 levels were determined using enzyme-linked immunosorbent assays (Beijing Biolab Technology Co.).

(2) Bone density. The bone density was measured at the femoral neck and lumbar spine using a DSC-600EVX dual-energy X-ray bone densitometer (Hanfei Medical Instrument Co.).

(3) BM-related inflammatory cytokines, bone formation markers, and bone resorption markers. The interleukin (IL)-2, IL-6, IL-10, tumor necrosis factor- α (TNF- α), transforming growth factor- β 1 (TGF- β 1), leptin, β -CTX, TRACP-5b, type I amino-terminal peptide (PINP), bone alkaline phosphatase (BALP), osteoprotegerin (OPG), bone calcitonin (BGP), matrix metalloproteinase (MMP)-2, and MMP-9 levels were determined using enzyme-linked immunosorbent assays. All the assays were performed in strict accordance with the kit instructions.

Statistical analysis

SPSS 23.0 statistical software was used, with $\bar{x} \pm s$ indicating the measurement data. One-way variance with *post hoc* LSD tests were used for the comparisons among multiple groups. The correlation analyses were performed using Pearson tests. $P < 0.05$ indicated that a difference was significant.

Results

Baseline data

The baseline data, such as age, body mass index, and gender had no significant difference among the three groups ($P > 0.05$). Groups B and C showed no significant differences in terms of comorbidity or the duration of the osteoporosis ($P > 0.05$), so they were comparable (**Table 1**).

Bone density

Group C had the lowest bone density, followed by group B, and group A had the highest bone density ($P < 0.05$), indicating that patients with

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Table 1. Comparison of the baseline data [n/($\chi \pm S$)]

| Baseline data | | Group A (n=40) | Group B (n=33) | Group C (n=47) |
|----------------------------------|--|-------------------|-------------------|-------------------|
| Gender | M/F | 15/25 | 13/20 | 18/29 |
| Age (years) | | 68.26 \pm 5.14 | 67.19 \pm 4.48 | 68.25 \pm 5.29 |
| BMI (kg/m ²) | | 23.61 \pm 3.11 | 24.19 \pm 3.06 | 25.14 \pm 3.18 |
| Duration of osteoporosis (years) | | -- | 5.16 \pm 1.38 | 5.67 \pm 1.43 |
| Comorbidity | Hypertension/hyperlipidemia/diabetes/other | -- | 4/6/3/2 | 6/5/4/3 |
| Fracture site | t8/t9/t10/t11/t12/l1/l2/l3 | -- | -- | 9/7/6/6/5/5/5/4 |

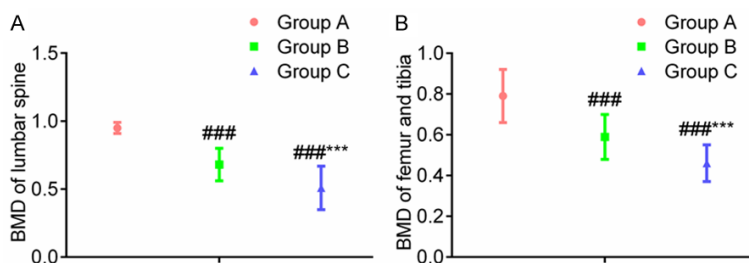


Figure 1. Comparison of the bone density (g/cm²). Note: A. Lumbar spine; B. Femoral tibial. Compared with group A, ### $P < 0.001$; compared with group B, **** $P < 0.001$.

thoracolumbar OVCF have lower bone density (Figure 1).

Inflammatory cytokines associated with BM

Group C had the lowest levels of IL-10 and TGF- β 1, group B was the second highest, and group A was the highest ($P < 0.05$). The leptin, IL-2, TNF- α , and IL-6 levels were the highest in group C and the lowest in group A ($P < 0.05$), suggesting that the serum inflammatory factor levels were significantly increased in patients with thoracolumbar OVCF (Figure 2).

The expression of regulators of Wnt pathway

The levels of β -catenin were the lowest in group C and the highest in group A ($P < 0.05$). The DKK-1 levels were the highest in group C and the lowest in group A ($P < 0.05$), indicating that patients with thoracolumbar OVCF showed low expression levels of β -catenin and high expression levels of DKK-1, respectively (Figure 3).

Bone resorption markers

The MMP-2, MMP-9, RANKL, and β -CTX levels were the highest in group C, followed by group B, and the lowest in group A ($P < 0.05$), showing

that the expression levels of various bone resorption markers were elevated in the patients with thoracolumbar OVCF (Figure 4).

Bone formation markers

The PINP, OPG, BALP, and BGP levels were the lowest in group C, followed by group B, and the highest in group A ($P < 0.05$), indicating that the bone formation marker levels

were lower in the patients with thoracolumbar OVCF (Figure 5).

Correlation analysis

A Pearson correlation analysis showed that the MMP-2, MMP-9, RANKL, β -CTX, and TRACP-5b levels were negatively correlated with β -catenin ($r < 0$, $P < 0.05$) and were positively correlated with DKK-1 ($r > 0$, $P < 0.05$). The BGP, PINP, OPG, and BALP levels were positively correlated with β -catenin ($r > 0$, $P < 0.05$) and were negatively correlated with DKK-1 ($r < 0$, $P < 0.05$), indicating that the serum expression levels of the Wnt pathway regulators in patients with thoracolumbar OVCF were closely correlated with the bone metabolic parameters (Table 2).

Discussion

Thoracolumbar OVCF refers to a destruction of the bone microstructure, an increase of brittleness, and a decrease of bone strength caused by osteoporosis, and the occurrence is a complex biological process involving multiple genes and factors, and multiple stages of development [11]. The common treatments include conservative treatment and surgical treatment. Although they are effective in correcting the

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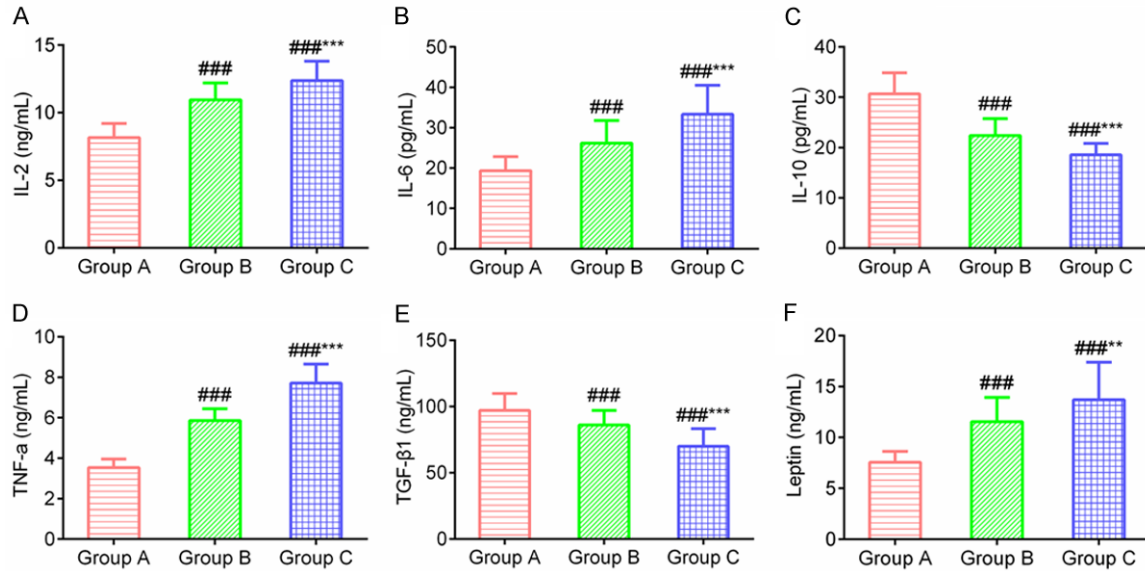


Figure 2. Comparison of the bone metabolism-related inflammatory cytokines. Note: A. IL-2; B. IL-6; C. IL-10; D. TNF- α ; E. TGF- β 1; F. Leptin. Compared to group A, #### $P < 0.001$; compared to group B, ** $P < 0.01$, *** $P < 0.001$.

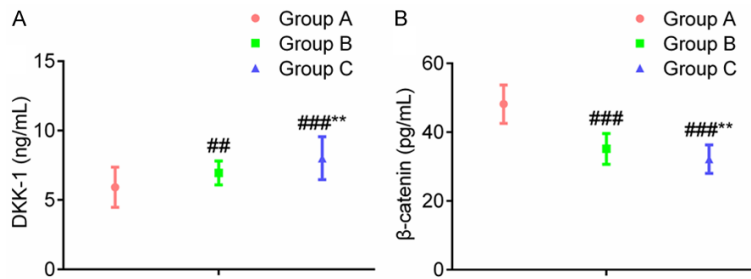


Figure 3. Comparison of the Wnt pathway regulator expression levels. Note: A. DKK-1; B. β -catenin. Compared to group A, ## $P < 0.01$, ### $P < 0.001$; compared to group B, ** $P < 0.01$, *** $P < 0.001$.

loss of height and promoting the bone healing process, the treatment efficacy is unsatisfactory. New, anti-osteoporotic molecular targets have attracted great clinical attention [12]. At present, studies on the signaling molecules, and the expression and regulatory mechanisms of the Wnt signaling pathway have made progress, among which the Wnt/ β -catenin signaling pathway is the most critical. Studies have focused the role of Wnt/ β -catenin in the differentiation process of osteoblasts and bone formation [13-15].

Cytoplasmic β -catenin can form complexes with intracellular proteins such as GSK-3 β and Axin, which are degraded by the proteasome under ubiquitination modification and phosphorylation, resulting in lower levels of β -

catenin in the cytoplasm [16]. Under stimulation, Axin binds to the cytoplasmic tails of LRP5 and LRP6 and can inhibit GSK-3 β function, causing β -catenin to accumulate in large amounts in the cells and gradually migrate into the nuclei, and, in combination with TCF/LEF transcription factors, activate the intracellular transcription process and regulate the transcription levels of many target genes,

such as the core-binding factors and proto-oncogenes [17, 18]. In a normal organism, both conditions co-exist and maintain a dynamic equilibrium. DKK-1 is a secreted Wnt inhibitor with obvious characteristics, and it can form a trimer with kremen1, kremen2, LRP5, LRP6, and undergoes a rapid endocytosis, decreasing LRP5, LRP6 on the cell membrane and inhibiting Wnt signaling to the cell. Moreover, it competitively inhibits the LRP5, LRP6 receptors and Wnt ligands [19]. Evidence has shown that blocking the DKK-1 function plays a role in preventing systemic bone loss and promoting bone formation [20]. MacDonald et al. [21] found that the bone mass in mice with reduced DKK-1 expressions is relatively increased, indicating that DKK-1 overexpression can inhibit the Wnt signaling pathway, resulting in reduced

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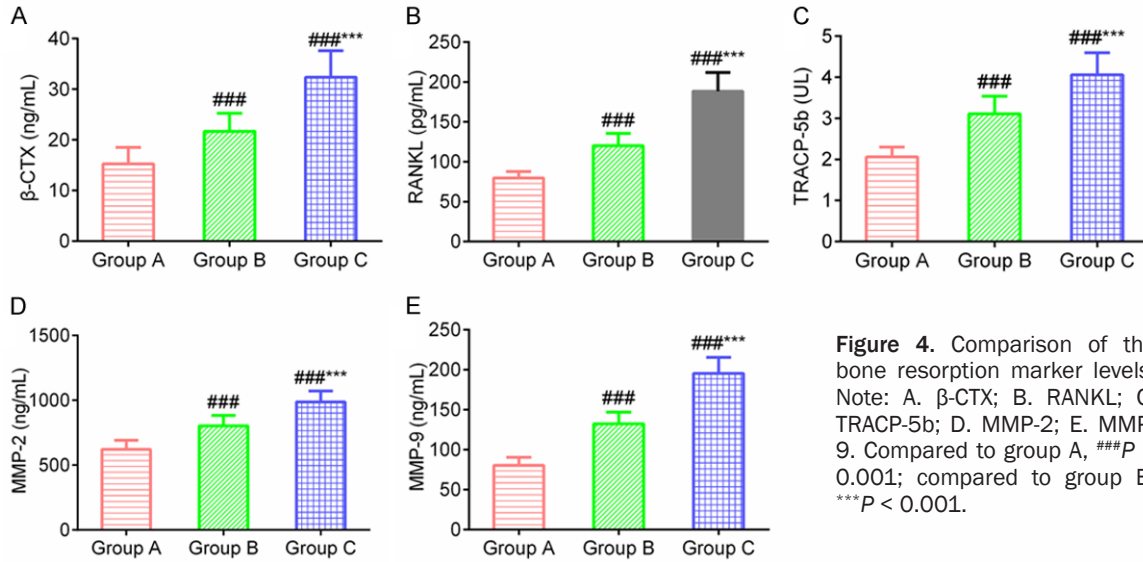


Figure 4. Comparison of the bone resorption marker levels. Note: A. β -CTX; B. RANKL; C. TRACP-5b; D. MMP-2; E. MMP-9. Compared to group A, ### P < 0.001; compared to group B, *** P < 0.001.

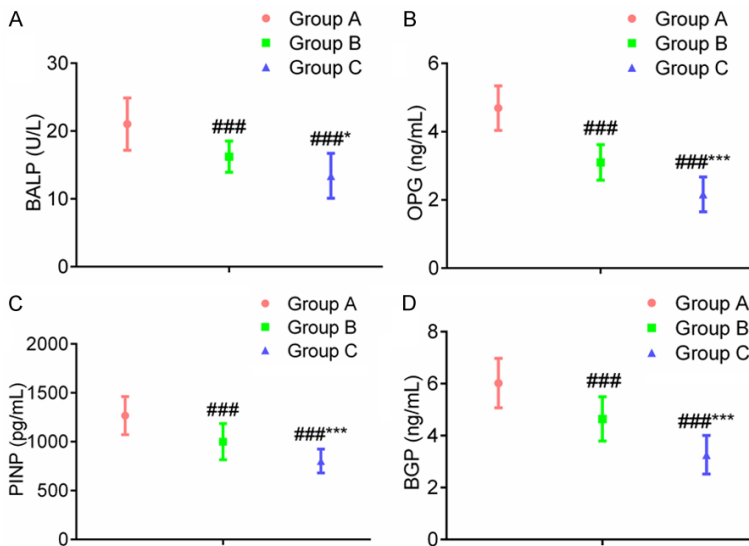


Figure 5. Comparison of the bone formation marker levels. Note: A. BALP; B. OPG; C. PINP; D. BGP. Compared to group A, ### P < 0.001; compared to group B, * P < 0.05, *** P < 0.001.

bone density and decreased bone formation. In the present study, DKK-1 levels: group C > group B > group A and β -catenin levels: group C < group B < group A. It can be seen that the DKK-1 levels were highly expressed, and the β -catenin levels were lower in patients with thoracolumbar OVCF. Ding [22] found that the combined quantification of β -catenin and DKK-1 has an important reference value for the bone metabolic status of knee osteoarthritis patients who also have osteoporosis, and the Wnt/ β -catenin signaling pathway and its

related regulatory factors may be involved in the occurrence and development of this disease, a finding basically consistent with this study.

During BM, the bone resorption mediated by osteoclasts and the bone formation mediated by osteoblasts are closely related to the occurrence and progression of osteoporosis, and alterations in the cellular activity of osteoclasts and osteoporosis lead to the destruction of bone microstructure and bone loss. OPG, secreted by osteoblasts, can block the maturation and differentiation of osteoclasts and further accelerate the process of bone syn-

thesis. As a regulatory molecule in the synthesis of type I collagen, the PINP level can reflect the activity of collagen synthesis and play a key role in maintaining the integrity of bone microstructure. BGP and BALP can inhibit the formation of abnormal hydroxyapatite crystals and maintain a normal mineralization rate [23]. In this study, the BALP, OPG, PINP, and BGP levels were the lowest in group C, followed by group B and were the highest in group A, proving that during the progression of thoracolumbar OVCF, the bone formation mediated by

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Table 2. The correlations between the serum Wnt pathway regulator expression levels and the bone metabolism indicators *r* (*P*)

| Expression levels | DKK-1 | β -catenin |
|-------------------|----------------|------------------|
| β -CTX | 0.721 (0.000) | -0.599 (0.038) |
| RANKL | 0.623 (0.011) | -0.633 (0.007) |
| TRACP-5b | 0.555 (0.036) | -0.709 (0.001) |
| MMP-2 | 0.619 (0.012) | -0.685 (0.006) |
| MMP-9 | 0.702 (0.003) | -0.716 (0.000) |
| BALP | -0.456 (0.039) | 0.569 (0.035) |
| OPG | -0.568 (0.035) | 0.711 (0.000) |
| PINP | -0.699 (0.005) | 0.685 (0.005) |
| BGP | -0.736 (0.000) | 0.413 (0.041) |

osteoblasts is diminished and the differentiation and maturation are impeded. A further analysis of the correlation between the serum Wnt pathway regulators and bone resorption showed that BALP, OPG, PINP, and BGP are strongly correlated with the DKK-1 and β -catenin levels, suggesting that decreased β -catenin levels and increased DKK-1 secretions can hinder the differentiation and maturation of osteoblasts as well as their mediated bone formation. β -CTX is a byproduct of collagen hydrolysis in the bone matrix, and MMP-2 and MMP-9 are important catalytic enzymes of type I collagen hydrolysis, leading to bone loss and the destruction of bone microstructure through collagen loss and hydrolysis in the bone matrix [24]. In this study, the β -CTX, RANKL, TRACP-5b, MMP-2, and MMP-9 levels were the highest in group C, followed by group B and the lowest in group A, and the levels of the above indicators were closely related to DKK-1 and β -catenin, suggesting that during the progression of osteoporosis to thoracolumbar OVCF, the cleavage products of collagen increased, the protease activity increased, and the synthesis products decreased, *i.e.*, increased osteoclast activity results in enhanced osteoclast-mediated bone resorption, while increased secretions of β -catenin and decreased secretions of DKK-1 promote increased protease activity, enhance osteoclast-mediated bone resorption and further degrade collagen in the bone matrix. The reason may be that the Wnt/ β -catenin signaling pathway plays a key role during osteoclast formation and osteoclast apoptosis. Activation of the Wnt signaling pathway not only inhibits osteoclastogenesis, but it also stimulates osteogenesis and increases bone mass [25]. In

addition, β -catenin binding to corresponding molecules in this pathway (e.g. the TCF protein) can positively regulate OPG expression and negatively affect osteoclasts. As an inhibitor of the Wnt signaling pathway, DKK-1 promotes the low expression of the downstream signaling molecules after phosphorylation, increases the rate of osteoblast apoptosis, and inhibits the binding of the signaling molecules to kremen1, kremen2, LRP5, LRP6, thus hindering the complex formation, BM, and decreasing osteoclastogenesis [26].

In conclusion, patients with thoracolumbar OVCF have abnormal serum Wnt pathway expressions, low bone density, and abnormal BM. The Wnt/ β -catenin and DKK-1 levels are closely related to BM, and can be used as targets for the prevention and treatment of bone metabolic diseases. At present, there are many studies on the relationship between the Wnt pathway and osteoporosis, but there are few studies on thoracolumbar OVCF, and the relationship between this pathway and bone metabolism in patients with osteoporotic fractures is still unclear. Therefore, we chose to research this topic in order to provide a target for the prevention and treatment of bone metabolic diseases. However, there are still some deficiencies in the current study, such as the correlations between serum the Wnt pathway expression and the bone density and the correlation between serum the Wnt pathway expression and the inflammatory cytokines have not been analyzed, so this will be addressed in future prospective studies.

Disclosure of conflict of interest

None.

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