Expression of the deubiquitinase cylindromatosis in articular cartilage and subchondral bone is associated with the severity of knee osteoarthritis

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Abstract. Cylindromatosis (CYLD) is a deubiquitinating enzyme that regulates multiple key signaling pathways involved in the pathophysiology of knee osteoarthritis (KOA). Previous studies have indicated that the expression of CYLD in the articular cartilage of patients with KOA is significantly higher than in healthy controls. However, limited data are available regarding the association between CYLD expression and the severity of KOA. The aim of the present study was to investigate the association between CYLD expression in joint tissues and the severity of KOA. A total of 156 individual tibial plateau samples were obtained between January 2011 and January 2016 from patients that had undergone total knee arthroplasty due to KOA or from healthy controls. The severity of KOA was evaluated using the Kellgren Lawrence (KL) and Mankin scoring systems. Additionally, CYLD expression in the articular cartilage and subchondral bone was analyzed using immunohistochemistry. Compared with the healthy controls, patients with KOA exhibited significantly increased CYLD levels in the articular cartilage (6.53±2.01 vs. 28.69±13.23, P<0.001) and significantly decreased CYLD levels in the subchondral bone (11.46±2.34 vs. 3.50±2.54, P<0.001). Correlation analysis indicated that CYLD expression in the articular cartilage was positively correlated with the KL (r=0.837, P<0.001) and Mankin scores (r=0.925, P<0.001), while its expression in the subchondral bone was negatively correlated with the KL (r=-0.802, P<0.001) and Mankin scores (r=-0.844, P<0.001). The results of the present study demonstrate that CYLD levels in the articular cartilage and subchondral bone are associated with the severity of KOA. Thus, CYLD may be a potential diagnostic and predictive biomarker for KOA and a novel target in its treatment.

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Abbreviations: CYLD, cylindromatosis; KOA, knee osteoarthritis; TP, tibial plateau; KL, Kellgren Lawrence; RANK, receptor activator of nuclear factor-κB; TGF-β1, transforming growth factor β1; TP, tibial plateau

Key words: cylindromatosis, articular cartilage, subchondral bone, knee osteoarthritis, biomarker

Introduction

Knee osteoarthritis (KOA) is a common type of joint disease that affects the whole joint, including the articular cartilage, synovial membrane, meniscus and subchondral bone (1). It is characterized by progressive articular cartilage degradation, subchondral bone sclerosis, osteophyte formation and under-mineralization of the trabecular structure (2,3). The precise underlying mechanism responsible for KOA remains poorly understood but it is widely accepted that biochemical and biomechanical factors serve important roles in the pathogenesis of KOA (4-6).

Over the past two decades, studies investigating KOA have focused on bodily fluid biomarkers, tissue biomarkers and novel drug targets (7-12). Previous studies have demonstrated that the receptor activator of nuclear factor-κB (RANK) signaling pathway, transforming growth factor β1 (TGF-β1) pathway and nuclear factor-κB pathway are all associated with KOA progression (13-15). Additionally, Wnt inhibitory factor-1 (9), hypoxia-inducible factor-1α (10), osteopontin and Wnt5a (12) are associated with the severity of KOA.

Cylindromatosis (CYLD) is a deubiquitinating enzyme that has broad regulative effects on KOA, including its negative regulation of the RANK (16) and TGF-β1 signaling pathways (13,17). Furthermore, CYLD is a crucial negative regulator of osteoclastogenesis (16). Given the roles of the aforementioned signaling pathways in articular cartilage degradation and the subchondral bone remodeling processes, it was hypothesized that the expression of CYLD in the articular

cartilage and subchondral bone may be associated with KOA severity.

It has been reported that levels of CYLD mRNA in the articular cartilage of patients with KOA are two-times higher than those in the articular cartilage of healthy controls (18). However, limited data are available regarding the expression of CYLD in other joint tissues and on the association between CYLD expression in joint tissues and the severity of KOA. Thus, the aim of the present study was to analyze the expression patterns of CYLD in different sections of the knee joint in patients with KOA to evaluate its potential association with the severity of KOA.

Patients and methods

Patients. The protocol of the current study was approved by the Ethics Committees of Shandong Provincial Hospital (Jinan, China), the People's Hospital of Linzi (Linzi, China) and the Central Hospital of Zibo Mining Group (Zibo, China). Human tibial plateau (TP) samples were retrospectively collected from 129 patients with KOA that underwent primary total knee arthroplasty due to KOA and 27 healthy controls who underwent primary amputation due to severe lower-extremity trauma between January 2011 and January 2016. All participants were enrolled from the aforementioned three hospitals. Patients with KOA were diagnosed according to the criteria of the American College of Rheumatology (19). All patients and healthy controls enrolled in the study had signed legally effective informed consent forms. None of the enrolled subjects had a history of bone tumors, conditions affecting bone remodeling, including rheumatoid arthritis, osteoporosis, renal osteopathy or thyroid disease, or use of drugs that affect bone metabolism. The Kellgren Lawrence (KL) score was used to indicate the severity of KOA and this was determined based on knee joint radiographs (20).

Histological analysis. TPs were harvested during surgery, washed with normal saline to remove excess blood, wrapped with gauze and frozen at -70˚C. Samples were removed from storage 48 h prior to use and thawed for 24 h at 4˚C and 24 h at room temperature. For each TP, 9 samples were harvested from the medial, central and lateral regions, respectively, at a depth of 1.0 cm (~0.3x0.3x1.0 cm). A total of 3 samples were harvested from each region. Samples were then fixed in 4% paraformaldehyde at room temperature for 24 h, decalcified in 10% EDTA and dehydrated in graded ethanol. Following dehydration, samples were embedded in paraffin, cut into 5-μm-thick sections, placed on 3-aminopropyltriethoxy-silane coated slides and stored at 4˚C. Hematoxylin and eosin staining and safranin O staining were performed following previously published protocols (21).

Following histological staining, the severity of articular cartilage damage was classified into four categories based on the following modified Mankin system: Grade I (Mankin score, 0-1); grade II (Mankin score, 2-5); grade III (Mankin score, 6-9); and grade IV (Mankin score, ≥10) (22).

Immunohistochemistry. Immunohistochemical staining was performed to assess the expression of CYLD in TP samples using Histostain-SP kits (Invitrogen; Thermo Fisher Scientific, Inc., Waltham, MA, USA) (23). Fixed paraffin-embedded samples were heated at 60˚C for 30 min, deparaffinized in xylene (10 min x 2), rehydrated in alcohol (100% alcohol for 5 min x 2, 95% alcohol for 5 min x 2, and 85, 75 and 50% alcohol for 5 min each), and washed with distilled water and PBS for 5 min each. The samples were then treated successively with 3% H_2O_2 in methanol at room temperature for 10 min and 20% goat serum (both Sigma-Aldrich; Merck KGaA, Darmstadt, Germany) at room temperature for 30 min to block endogenous peroxidase activity and nonspecific antibody binding. Subsequently, sections were incubated with diluted rabbit polyclonal anti-CYLD antibody (1:100; cat. no. ab137524; Abcam, Cambridge, UK) at 37˚C for 2 h, then with goat anti-rabbit immunoglobulin G (1:1,000; cat. no. A0545; Sigma-Aldrich; Merck KGaA) at 37˚C for 30 min. Finally, samples were stained with diaminobenzidine tetrachloride at room temperature for 8 min and counterstained with hematoxylin at room temperature for 1 min. Sections prepared using PBS instead of primary antibody were used as negative controls. All the sections were examined by a blinded independent pathologist using a BX51 microscope (Olympus Corporation, Tokyo, Japan) at a magnification of x100.

CYLD levels were expressed as normalized optical density (OD) values and were determined using a MetaMorph/DPIO/BX41 morphology image analysis system (Olympus Corporation). PBS was used for OD normalization and the experiment was repeated in triplicate. The variation coefficients of CYLD expression in the articular cartilage and subchondral bone were <2%.

Statistical analyses. Data were analyzed using SPSS 17.0 (SPSS, Inc., Chicago, IL, USA). Normally distributed measurement data were expressed as the mean ± standard deviation. Data were compared using one-way analysis of variance with a Tukey's honest significant difference post hoc test or t-tests. Skewed measurement data were expressed as the median and interquartile range and compared using Mann-Whitney U-tests. Numerical data were expressed as percentages and differences between groups were compared using the Pearson's χ^2 test. Associations between CYLD expression in TP samples and the severity of KOA were analyzed using Spearman's correlation analysis. $P<0.05$ was considered to indicate a statistically significant difference.

Results

Patient characteristics. A total of 156 participants were enrolled in the present study. Baseline features of the patients that may have been associated with the severity of articular cartilage degeneration are listed in Table I. No significant differences were identified in the age, sex or body mass index between healthy controls and patients with KOA ($P>0.05$). KL and Mankin scores of the patients with KOA from three hospitals are listed in Table II. Disease severity did not differ significantly among the patients from the different hospitals ($P>0.05$).

Expression of CYLD in different TP regions. CYLD expression in the TP samples was detected by immunohistochemistry and was determined using normalized OD values. As presented in

Table I. Baseline features of patients.

Parameter	n	Age [years, M (Q_R)]	Sex (n, % female)	BMI (kg/m^2, $\bar{x}$±s)
Control group	27	61.50 (49.00-71.00)	12 (44.4)	25.38±3.55
KOA group	129	63.00 (49.00-71.00)	63 (48.8)	26.89±3.58
$\chi^2/t/z$	NA	-0.203	0.173	1.975
P-value	NA	0.839	0.678	0.048

KOA, knee osteoarthritis; M (Q_R), median (interquartile range); NA, not applicable; BMI, body mass index; $\bar{x}$±s, mean standard deviation.

Table II. Baseline features of patients with knee osteoarthritis from the three different hospitals.

Hospital names	n	Age (years)	Sex (n, % female)	BMI (kg/m^2)	KL scores (n, %)		Mankin scores (n, %)		
					III	IV	II	III	IV
SDH	59	65.00 (47.00-72.00)	30 (50.8)	27.14 (21.93-33.72)	13 (22.03)	46 (77.97)	8 (13.56)	18 (30.51)	33 (55.93)
LZH	42	60.00 (48.00-71.00)	19 (45.2)	25.64 (20.86-32.16)	10 (23.81)	32 (76.19)	7 (16.67)	18 (42.86)	17 (40.47)
ZBH	28	64.00 (49.00-71.00)	14 (50.0)	28.24 (23.03-34.32)	7 (25.00)	21 (75.00)	6 (21.43)	9 (32.14)	13 (46.43)
χ^2/z	NA	-1.310	0.328	-1.642	0.104		3.120		
P-value	NA	0.190	0.849	0.102	0.949		0.538		

All values are presented as the median (interquartile range), unless otherwise specified. NA, not applicable; SDH, Shandong Provincial Hospital; LZH, People's Hospital of Linzi; ZBH, Central Hospital of Zibo Mining Group; M (Q_R), median (interquartile range); BMI, body mass index; KL, Kellgren Lawrence.

Table III. Expression of CYLD in the TP of patients with KOA and healthy controls.

CYLD levels (%)	Articular cartilage	Subchondral bone	*t*	P-value
Control group	6.53±2.01	11.46±2.34	8.295	<0.001
KOA group	28.69±13.23	3.50±2.54	21.235	<0.001
t	8.66	-15.004	NA	NA
P-value	<0.001	<0.001	NA	NA

All values are presented as the mean ± standard deviation. CYLD, cylindromatosis; TP, tibial plateau; KOA, knee osteoarthritis; NA, not applicable.

Table III, CYLD expression in the articular cartilage of patients with KOA was significantly higher than that of the healthy controls (t=8.66, P<0.001). By contrast, CYLD expression in the subchondral bone of patients with KOA was significantly lower than in the healthy controls (t=-15.004, P<0.001).

Representative immunohistochemical staining images and radiographs of the TP samples are presented in Fig. 1. Immunohistochemistry detected CYLD expression in the cell nuclei and cytoplasm; regions with positive CYLD immunostaining were indicated by dark brown granular staining (Fig. 1A and B). Safranin O stained sections revealed that the degree of cartilage destruction was positively associated with the severity of KOA (Fig. 1C). Radiographs of the KOA patients identified clear narrowing of the joint space, which was also positively associated with the severity of KOA (Fig. 1D). As shown in Fig. 1A compared with Fig. 1C and D, elevated CYLD expression in the articular cartilage of patients with KOA was concomitant with the severity of KOA. The opposite pattern of CYLD expression was observed in the subchondral bone of patients with KOA, which was demonstrated in Fig. 1B compared with Fig. 1C and D.

Association between CYLD expression and KL score. KL scores of all patients with KOA were >III, which was in accordance for what is expected in patients requiring total knee replacement. The potential association between CYLD expression in the TP samples and KL score was analyzed by Spearman's correlation analysis. The results are presented in Table IV and indicate that the expression of CYLD in the articular cartilage was positively correlated with the KL

Table IV. Association between CYLD expression in the TP and KL scores.

CYLD levels (%)	I-II	III	IV	*r*	P-value
n	27	30	99	NA	NA
Articular cartilage	6.53±2.01	14.22±4.17	33.08±11.83	0.837	<0.001
Subchondral bone	11.46±2.34	6.77±2.22	2.51±1.64	-0.802	<0.001

All values are presented as the mean ± standard deviation. CYLD, cylindromatosis; NA, not applicable; TP, tibial plateau; KL, Kellgren Lawrence.

Table V. Association between CYLD expression in the TP and Mankin scores.

CYLD levels (%)	I	II	III	IV	*r*	P-value
n	27	21	45	63	NA	NA
Articular cartilage	6.53±2.01	14.23±4.66	21.13±5.13	38.91±10.82	0.925	<0.001
Subchondral bone	11.46±2.34	7.81±1.66	3.97±1.41	1.73±1.17	-0.844	<0.001

All values are presented as the mean ± standard deviation. CYLD, cylindromatosis; NA, not applicable; TP, tibial plateau.

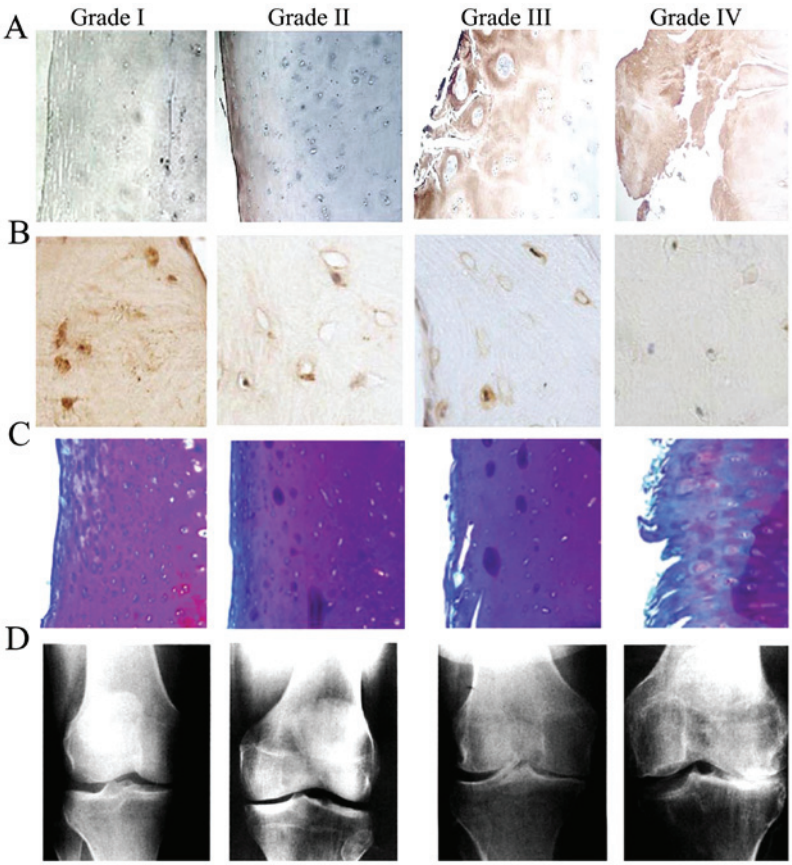

Figure 1. Representative staining images and radiographs of TPs. (A) CYLD immunohistochemical staining in the articular cartilage (magnification x100). (B) CYLD immunohistochemical staining in the subchondral bone (magnification x100). (C) Safranin O staining of the articular cartilage (magnification x100). (D) Radiographs of TPs. Grades I-IV: Severity of knee osteoarthritis graded using (A-C) the modified Mankin system or (D) the Kellgren Lawrence system. TP, tibial plateau; CYLD, cylindromatosis.

score (r=0.837, P<0.001), and that CYLD expression in the subchondral bone was negatively correlated with the KL score (r=-0.802, P<0.001).

Associations between CYLD expression and Mankin score. Articular cartilage sections were classified using modified Mankin scores. As presented in Table V, the articular

cartilage sections classified as grades I, II, III and IV exhibited CYLD expression of 6.53±2.01, 14.23±4.66, 21.13±5.13 and 38.91±10.82%, respectively. CYLD expression in the corresponding graded subchondral bone sections of TP samples were 11.46±2.34, 7.81±1.66, 3.97±1.41 and 1.73±1.17%, respectively.

Spearman's correlation analysis was conducted to assess the association between CYLD expression and Mankin scores. The results indicated that CYLD expression in the articular cartilage and subchondral bone was significantly correlated with the Mankin score (r=0.925 and r=-0.844, all P<0.001). Scatter diagrams were used to plot the correlation between CYLD expression and Mankin score for the TP samples (Fig. 2) and demonstrated that an increased Mankin score is correlated with increased CYLD expression in the articular cartilage and reduced CYLD expression in the subchondral bone.

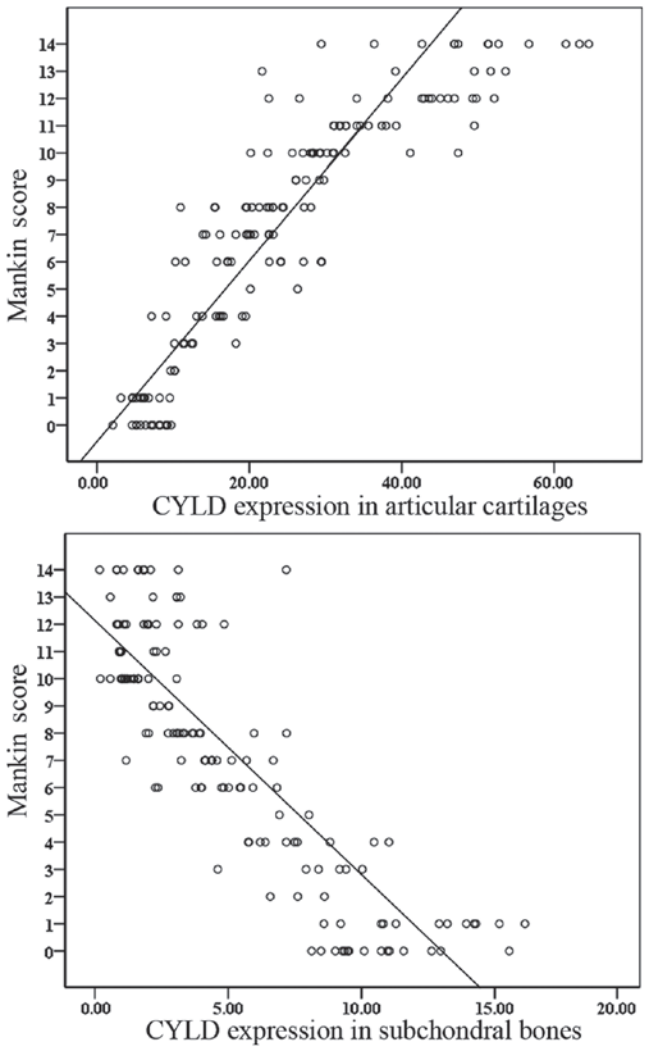

Figure 2. Associations between CYLD expression in the tibial plateau and the Mankin score. Scatter diagrams indicated that CYLD levels in articular cartilage and subchondral bone were correlated with the severity of knee osteoarthritis. CYLD, Cylindromatosis.

Discussion

The present study may represent the first attempt to systematically evaluate CYLD expression in the TP tissue of patients with KOA and determine its association with the severity of KOA. KL and Mankin scores were used to grade the severity of knee OA and the expression of CYLD in the articular cartilage and subchondral bone was determined by immunohistochemistry. Notably, it was determined that CYLD expression was significantly increased in the articular cartilage but significantly reduced in the subchondral bone of patients with KOA. Although there are very few published studies investigating the expression of CYLD in TP tissue, the results of certain reports are partially consistent with those of the current study. Song *et al* (18) reported that CYLD expression in the articular cartilage of patients with KOA was significantly higher than in healthy controls.

Progressive articular cartilage degradation and subchondral bone sclerosis are typical pathological changes that occur in KOA. Despite extensive investigations into the sequence of these pathological changes, a generally accepted mechanism has yet to be established (24-28). However, an increasing number of studies have demonstrated that there is molecular crosstalk between the articular cartilage and subchondral bone (28-31). In the present study, it was identified that the expression of CYLD in the articular cartilage was positively correlated with the KL (r=0.837, P<0.001) and Mankin scores (r=0.925, P<0.001), whereas CYLD expression in the subchondral bone was negatively correlated with KL (r=-0.802, P<0.001) and Mankin scores (r=-0.844, P<0.001). These results indicate that CYLD expression may be a potential biomarker for the diagnosis of KOA, as well for monitoring the severity of KOA. Changes in the expression of CYLD may be an early event involved in the pathological processes of articular cartilage degradation and subchondral bone remodeling abnormalities. Additionally, CYLD may serve a crucial role in the molecular crosstalk that occurs between the articular cartilage and subchondral bone in KOA.

Several signaling pathways have been implicated in the molecular crosstalk between articular cartilage and subchondral bone, including the TGF-β and Wnt signaling pathways (28-31). CYLD may negatively regulate these signaling pathways during the pathological processes of KOA (16,17). This may explain why CYLD expression is increased in the articular cartilage but decreased in the subchondral bone of patients with KOA and may explain its correlation with the severity of KOA.

TGF-β also exhibits inverse expression trends in KOA; its expression is decreased in the articular cartilage and increased in the subchondral bone (29,32,33). Furthermore, inhibition of TGF-β expression in the articular cartilage or upregulation of TGF-β expression in the subchondral bone aggravates the degeneration of articular cartilage (32). CYLD negatively regulates TGF-β expression by deubiquitinating protein kinase B (17). Elevated Wnt signaling may also induce bone sclerosis (34) and this may be associated with the reduced deubiquitinating activity of CYLD (35). Additionally, decreased CYLD expression in the subchondral bone may induce subchondral bone remodeling abnormalities via negative regulation of the RANK signaling pathway (16). Collectively, the aforementioned findings support the hypothesis that CYLD exhibits regulatory activity during the processes of articular cartilage degradation and subchondral

bone remodeling in KOA. However it remains unknown whether the articular cartilage and subchondral bone influence each other via CYLD expression. Further studies are required to elucidate the precise mechanisms of CYLD in KOA, particularly regarding its potential effects on osteoblasts, osteoclasts and chondrocytes.

The sample size of the present study was larger than that of previous studies (9,12), and in the present study, TP samples were collected from subjects admitted to three different public hospitals, including one 2A hospital, one 3B hospital and one 3A hospital. Hospitals in China are classified into 9 grades according to the size of the hospital, medical technology, medical equipment, management and medical quality; they are as follows: 1A, 1B, 1C, 2A, 2B, 2C, 3A, 3B and 3C. Hospitals grades as 1A, 1B and 1C are township hospitals, which provide preventive care and minimal health care. Hospitals grades as 2A, 2B, 2C, 3A, 3B and 3C are affiliated with large and medium-sized cities, and responsible for providing specialist health services. Most Chinese patients with mild and moderate-severe diseases tend to choose large hospitals, including 2A, 3A, 3B and 3C hospitals, for specialist treatment (36). This means that samples included in the present study were more likely to be representative of all patients with KOA. Nevertheless, the present study still had a number of limitations. The current study was retrospective; thus, the collection of blood or synovial fluid samples from patients was not possible. Given that biomarkers included in the bodily fluid are more favorable for diagnosis (37), further studies are required to investigate the associations between CYLD levels in bodily fluids and the severity of KOA, which may assist the early diagnosis and estimations of prognosis in patients. Furthermore, KL and Mankin scores are artificial classification systems used for grading the severity of KOA. The KL score is considered to be imprecise and indefinite (37,38) and neither of these classification systems fully reflect the severity of subchondral bone remodeling abnormalities. Therefore, more detailed studies are necessary to investigate the associations between CYLD levels in TP tissues, and the activities of osteoblasts, osteoclasts and chondrocytes.

In conclusion, despite these limitations, the present study demonstrated that CYLD levels in the articular cartilage and subchondral bone of patients with KOA were associated with the severity of KOA. Thus, CYLD may be a potential diagnostic and predictive biomarker for KOA.

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References

1. Heidari B: Knee osteoarthritis prevalence, risk factors, pathogenesis and features: Part I. Caspian J Intern Med 2: 205-212, 2011.
2. Man GS and Mologhianu G: Osteoarthritis pathogenesis-a complex process that involves the entire joint. J Med Life 7: 37-41, 2014.
3. Hayami T: Osteoarthritis of the knee joint as a cause of musculoskeletal ambulation disability symptom complex (MADS). Clin Calcium 18: 1574-1580, 2008 (In Japanese).
4. Guilak F: Biomechanical factors in osteoarthritis. Best Pract Res Clin Rheumatol 25: 815-823, 2011.
5. Powell A, Teichtahl AJ, Wluka AE and Cicuttini FM: Obesity: A preventable risk factor for large joint osteoarthritis which may act through biomechanical factors. Br J Sports Med 39: 4-5, 2005.
6. Fukui N, Ikeda Y, Ohnuki T, Tanaka N, Hikita A, Mitomi H, Mori T, Juji T, Katsuragawa Y, Yamamoto S, *et al*: Regional differences in chondrocyte metabolism in osteoarthritis: A detailed analysis by laser capture microdissection. Arthritis Rheum 58: 154-163, 2008.
7. Hunter DJ, Nevitt M, Losina E and Kraus V: Biomarkers for osteoarthritis: Current position and steps towards further validation. Best Pract Res Clin Rheumatol 28: 61-71, 2014.
8. Attur M, Krasnokutsky-Samuels S, Samuels J and Abramson SB: Prognostic biomarkers in osteoarthritis. Curr Opin Rheumatol 25: 136-144, 2013.
9. Gao SG, Zeng C, Liu JJ, Tian J, Cheng C, Zhang FJ, Xiong YL, Pan D, Xiao YB and Lei GH: Association between Wnt inhibitory factor-1 expression levels in articular cartilage and the disease severity of patients with osteoarthritis of the knee. Exp Ther Med 11: 1405-1409, 2016.
10. Qing L, Lei P, Liu H, Xie J, Wang L, Wen T and Hu Y: Expression of hypoxia-inducible factor-1α in synovial fluid and articular cartilage is associated with disease severity in knee osteoarthritis. Exp Ther Med 13: 63-68, 2017.
11. Al-Jarallah KF, Shehab D, Al-Awadhi A, Nahar I, Haider MZ and Moussa MA: Are 25(OH)D levels related to the severity of knee osteoarthritis and function? Med Princ Pract 21: 74-78, 2012.
12. Li Y, Xiao W, Sun M, Deng Z, Zeng C, Li H, Yang T, Li L, Luo W and Lei G: The expression of osteopontin and Wnt5a in articular cartilage of patients with knee osteoarthritis and its correlation with disease severity. Biomed Res Int 2016: 9561058, 2016.
13. Fang J, Xu L, Li Y and Zhao Z: Roles of TGF-beta 1 signaling in the development of osteoarthritis. Histol Histopathol 31: 1161-1167, 2016.
14. Rigoglou S and Papavassiliou AG: The NF-κB signalling pathway in osteoarthritis. Int J Biochem Cell Biol 45: 2580-2584, 2013.
15. Zeng GQ, Chen AB, Li W, Song JH and Gao CY: High MMP-1, MMP-2, and MMP-9 protein levels in osteoarthritis. Genet Mol Res 14: 14811-14822, 2015.
16. Jin W, Chang M, Paul EM, Babu G, Lee AJ, Reiley W, Wright A, Zhang M, You J and Sun SC: Deubiquitinating enzyme CYLD negatively regulates RANK signaling and osteoclastogenesis in mice. J Clin Invest 118: 1858-1866, 2008.
17. Lim JH, Jono H, Komatsu K, Woo CH, Lee J, Miyata M, Matsuno T, Xu X, Huang Y, Zhang W, *et al*: CYLD negatively regulates transforming growth factor-β-signalling via deubiquitinating Akt. Nat Commun 3: 771, 2012.
18. Song J, Jin EH, Kim D, Kim KY, Chun CH and Jin EJ: MicroRNA-222 regulates MMP-13 via targeting HDAC-4 during osteoarthritis pathogenesis. BBA Clin 3: 79-89, 2014.
19. Aletaha D, Neogi T, Silman AJ, Funovits J, Felson DT, Bingham CO III, Birnbaum NS, Burmester GR, Bykerk VP, Cohen MD, *et al*: 2010 rheumatoid arthritis classification criteria: An American College of Rheumatology/European League Against Rheumatism collaborative initiative. Arthritis Rheum 62: 2569-2581, 2010.
20. Edwards MH, Parsons C, Bruyère O, Petit Dop F, Chapurlat R, Roemer FW, Guermazi A, Zaim S, Genant H, Reginster JY, *et al*: High kellgren-lawrence grade and bone marrow lesions predict worsening rates of radiographic joint space narrowing; the SEKOIA study. J Rheumatol 43: 657-665, 2016.
21. Jaiprakash A, Prasadam I, Feng JQ, Liu Y, Crawford R and Xiao Y: Phenotypic characterization of osteoarthritic osteocytes from the sclerotic zones: A possible pathological role in subchondral bone sclerosis. Int J Biol Sci 8: 406-417, 2012.
22. Wei F, Zhou J, Wei X, Zhang J, Fleming BC, Terek R, Pei M, Chen Q, Liu T and Wei L: Activation of Indian hedgehog promotes chondrocyte hypertrophy and upregulation of MMP-13 in human osteoarthritic cartilage. Osteoarthritis Cartilage 20: 755-763, 2012.
23. Welte S, Urbanik T, Elßner C, Kautz N, Koehler BC, Waldburger N, Bermejo JL, Pinna F, Weiss KH, Schemmer P, *et al*: Nuclear expression of the deubiquitinase CYLD is associated with improved survival in human hepatocellular carcinoma. PLoS One 9: e110591, 2014.
24. Burr DB and Gallant MA: Bone remodelling in osteoarthritis. Nat Rev Rheumatol 8: 665-673, 2012.
25. Anderson-MacKenzie JM, Quasnichka HL, Starr RL, Lewis EJ, Billingham ME and Bailey AJ: Fundamental subchondral bone changes in spontaneous knee osteoarthritis. Int J Biochem Cell Biol 37: 224-236, 2005.

26. Hayami T, Pickarski M, Zhuo Y, Wesolowski GA, Rodan GA and Duong LT: Characterization of articular cartilage and subchondral bone changes in the rat anterior cruciate ligament transection and meniscectomized models of osteoarthritis. Bone 38: 234-243, 2006.
27. Day JS, Ding M, van der Linden JC, Hvid I, Sumner DR and Weinans H: A decreased subchondral trabecular bone tissue elastic modulus is associated with pre-arthritic cartilage damage. J Orthop Res 19: 914-918, 2001.
28. Zhang LZ, Zheng HA, Jiang Y, Tu YH, Jiang PH and Yang AL: Mechanical and biologic link between cartilage and subchondral bone in osteoarthritis. Arthritis Care Res (Hoboken) 64: 960-967, 2012.
29. Sharma AR, Jagga S, Lee SS and Nam JS: Interplay between cartilage and subchondral bone contributing to pathogenesis of osteoarthritis. Int J Mol Sci 14: 19805-19830, 2013.
30. Yuan XL, Meng HY, Wang YC, Peng J, Guo QY, Wang AY and Lu SB: Bone-cartilage interface crosstalk in osteoarthritis: Potential pathways and future therapeutic strategies. Osteoarthritis Cartilage 22: 1077-1089, 2014.
31. Findlay DM and Kuliwaba JS: Bone-cartilage crosstalk: A conversation for understanding osteoarthritis. Bone Res 4: 16028, 2016.
32. Shen J, Li S and Chen D: TGF-β signaling and the development of osteoarthritis. Bone Res 2: pii: 14002, 2014.
33. Van der Kraan PM: Age-related alterations in TGF beta signaling as a causal factor of cartilage degeneration in osteoarthritis. Biomed Mater Eng 24 (1 Suppl): S75-S80, 2014.
34. Jenkins ZA, van Kogelenberg M, Morgan T, Jeffs A, Fukuzawa R, Pearl E, Thaller C, Hing AV, Porteous ME, Garcia-Miñaur S, *et al*: Germline mutations in WTX cause a sclerosing skeletal dysplasia but do not predispose to tumorigenesis. Nat Genet 41: 95-100, 2009.
35. Tauriello DV, Haegebarth A, Kuper I, Edelmann MJ, Henraat M, Canninga-van Dijk MR, Kessler BM, Clevers H and Maurice MM: Loss of the tumor suppressor CYLD enhances Wnt/beta-catenin signaling through K63-linked ubiquitination of Dvl. Mol Cell 37: 607-619, 2010.
36. Li X, Huang J and Zhang H: An analysis and of hospital preparedness capacity for public health emergency in four regions of China: Beijing, Shandong Guangxi and Hainan. BMC Public Health 8: 319, 2008.
37. Guermazi A, Hayashi D, Roemer F, Felson DT, Wang K, Lynch J, Amin S, Torner J, Lewis CE and Nevitt MC: Severe radiographic knee osteoarthritis-does Kellgren and Lawrence grade 4 represent end stage disease?-the MOST study. Osteoarthritis Cartilage 23: 1499-1505, 2015.
38. Schiphof D, Boers M and Bierma-Zeinstra SM: Differences in descriptions of Kellgren and Lawrence grades of knee osteoarthritis. Ann Rheum Dis 67: 1034-1036, 2008.