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Original Article

Circular RNA expression profiles in synovial fluid: a promising new class of diagnostic biomarkers for osteoarthritis

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Abstract: Background: Previous studies suggest that circRNAs abnormally function in the progression of osteoarthritis (OA). However, little is known about the diagnostic value of circRNAs in patients with OA. To assess potential applications of circRNAs as diagnostic tools in OA, expression profiles of circRNAs in synovial fluid from OA patients and healthy subjects were obtained. Methods: Microarray analysis was performed to profile the expression of circRNAs in an unbiased manner. CircRNA expression in synovial fluid was identified by real-time quantitative polymerase chain reaction (RT-qPCR). The diagnostic value was evaluated using receiver operating characteristics (ROC) curves and the area under the ROC curves (AUC). Spearman correlation analysis was performed to assess the correlation of circRNAs and clinical parameters. Results: We identified five circRNAs that were significantly elevated in synovial fluid from OA patients compared with those of the healthy controls. Among these five circRNAs, hsa_circ_0104873, hsa_circ_0104595, and hsa_circ_0101251 could effectively separate patients with OA from healthy controls with high AUC (0.683, 0.708 and 0.754, respectively). Furthermore, we found that three circRNAs were positively correlated with the degree of radiographic grading and symptomatic severity of OA patients. Conclusion: This study suggests that increased expression of hsa_circ_0104873, hsa_circ_0104595, and hsa_circ_0101251 in synovial fluid from OA patients may serve as potential biomarkers for OA screening.

Keywords: Osteoarthritis, circular RNA, biomarker, synovial fluid

Introduction

Osteoarthritis (OA) is most frequently found in adults, which is an age-associated disease characterized by degeneration of articular cartilage, menisci, ligaments and subchondral bone, and is accompanied with severe pain, joint stiffness, reduced motion, swelling, and crepitation [1, 2]. At present, the diagnosis of OA is primarily based on plain radiography (X-ray) by measuring the joint-space width (JSW) or magnetic resonance imaging (MRI) by calculating cartilage volume [3, 4]. However, abnormalities of articular cartilage are slowly changing in the progression of OA, and pathologic symptoms are often unrecognized until irremediable joint damage has occurred [5]. Therefore, exploring novel and specific biomarkers for the earlier diagnosis and monitoring disease progression of OA should be emphasized.

The biomarkers currently under evaluation include anabolic and catabolic molecules of bone metabolism and inflammatory cytokines representing diverse biological pathways [4, 6, 7]. For instance, urinary C-terminal telopeptides of type II collagen (μCTX-II), serum cartilage oligomeric matrix protein (COMP), and serum hyaluronan are elevated in patients with hip or knee OA [4, 6]. In addition, a large set of inflammatory cytokines, such as C-reactive protein (CRP), tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), IL-6, and IL-18, can serve as serum markers for OA diagnosis [7]. In recent years, non-coding RNAs, including microRNA (miR) and long non-coding RNA (lncRNA), are regarded as a class of promising blood-based biomarkers for early detection of OA [8-11]. To our knowledge, circular RNA (circRNA) as a class of non-coding RNA has not been reported as the biomarkers of OA screening.

CircRNAs exist ubiquitously in the cytoplasm of eukaryotic cells and are generally formed by back-splicing with high stability, covalently cl-

Table 1. The primers of top 10 up-regulated circRNAs

Gene	Forward primer (5'-3')	Reverse primer (5'-3')	Product length (bp)
hsa_circ_0104873	CCTGCAGCTAAACTTGAGGGA	ACCCTTCTGGATTGCAGCAT	131
hsa_circ_0104595	GCTGAGTTTAAGCGAGAAGTGC	GCACTAGCAATGGCTTCTTCA	146
hsa_circ_0101748	TGGATGATGAAGACTGTGAAAGA	GTAGCACCAAAATGACTGTGTCC	182
hsa_circ_0102559	GTCACAACAGCCACTGAACAA	TGCCATCACGGCATATGTAAG	161
hsa_circ_0103994	CCGTGGCAGTCATCCTTAGT	TGGTTCAGGTCTGTGCTTGAA	131
hsa_circ_0101251	GATGGCTTGTGCTGATGGAAG	AGATGCAAGGAGGAATGTTTCA	121
hsa_circ_0101367	GCAGAAGAAATGGCGGACTTC	GGGGGTCCTATCCAATCATGG	124
hsa_circ_0104762	CTGTGGGAGACTGGTCGAAG	GTAACTTCAGTTGTGGATTGCAG	120
hsa_circ_0102061	GTACCATGAATCCCACAGAGCA	TGGAAGTCAGAGAGATCGGGA	121
hsa_circ_0104700	CTGGACGGGAAGATTGACGA	GGTGTTGGGGTCAATCTGGA	150
β-actin	GTGGCCGAGGACTTTGATTG	CCTGTAACAACGCATCTCATATT	73

osed continuous loop, without 5' to 3' polarity and polyadenylated tail, which give them with a stable structure to counteract RNA exonucleolytic digestion [12, 13]. CircRNAs perform a wide variety of biological functions in eukaryotic cells by acting competing endogenous RNAs (ceRNAs) or miRNA sponges, interacting with RNA binding proteins, modulating the stability of mRNAs, regulating gene transcription and translating proteins [14]. It has been demonstrated that circRNAs are involved in development of OA by regulating chondrocyte proliferation, apoptosis, and extracellular matrix degradation [15-18]. Recently, more and more research has found that circRNAs are stably present in plasma and serum and can serve as novel non-invasive biomarkers in various diseases, including arthritis [19], nervous system diseases [20] and malignancy [21]. Therefore, we aimed to identify the clinical diagnostic significance of circRNAs in OA.

In the present study, we compared the expression profile of circRNAs in synovial fluid of OA patients and healthy subjects by microarray analysis and then confirmed our findings in larger independent cohorts. Our findings demonstrated that hsa_circ_0104873, hsa_circ_0104595, and hsa_circ_0101251 are sensitive and specific biomarkers for OA, and these three circRNAs are significantly and positively correlated with radiographic and symptomatic severity of OA.

Material and methods

Patients and specimens

Forty-five patients with OA and twenty-nine healthy controls were enrolled at the 309th Hospital of Chinese People's Liberation Army (Beijing, China) from June 2014 to December 2016. Written informed consent was obtained from all of the participants. The study was approved by the Ethics Committee of the 309 th Hospital of Chinese People's Liberation Army (Beijing, China). OA severity was determined using weight-bearing anteroposterior radiographs of the affected knee. Radiographic severity was evaluated according to the Kellgren and Lawrence grading system [22]. The symptomatic severity of the disease was evaluated according to the Western Ontario McMaster University Osteoarthritis Index (WOMAC), which consists of 3 subscales: pain, stiffness, and physical function [23].

Human circRNA microarray analysis

Total RNA was extracted in synovial fluid from three OA patients and three healthy controls. CircRNA was enriched with Rnase R to digest linear RNA (Epicentre, Madison, WI, USA). RNA was labelled with Arraystar Human circRNA Array V2.0 (8×15 K, Arraystar, Rockville, MD, USA) and was scanned using a Agilent Scanner G2505C (Jamul, CA, USA).

Reverse transcription-quantitative polymerase chain reaction (RT-qPCR)

RNA was extracted using TRIzol (Invitrogen; Thermo Fisher Scientific, Inc., Waltham, MA, USA). Moloney Murine Leukemia Virus reverse transcriptase (Promega Corporation, Madison, WI, USA) and oligo dT 15 primers (Thermo Fisher Scientific, Inc.) were utilized to synthesize cDNA. Divergent primers were designed to ensure amplification of the head-to-tail splicing

Table 2. Clinical parameters of healthy controls and OA patients

Characteristics	Healthy controls (n = 29)	OA patients (n = 45)	KL grade 2 (n = 12)	KL grade 3 (n = 20)	KL grade 4 (n = 13)
Age (years)	63.21 ± 5.92	64.42 ± 4.62	65.75 ± 2.45	63.20 ± 5.63	65.08 ± 4.21
Gender (M/F)	12/17	20/25	4/8	9/11	7/6
BMI (kg/m^2)	25.78 ± 2.59	25.70 ± 2.74	25.72 ± 3.21	25.59 ± 2.81	25.86 ± 2.33

OA, osteoarthritis; KL, Kellgren-Lawrence; M, male; F, female; BMI, body mass index.

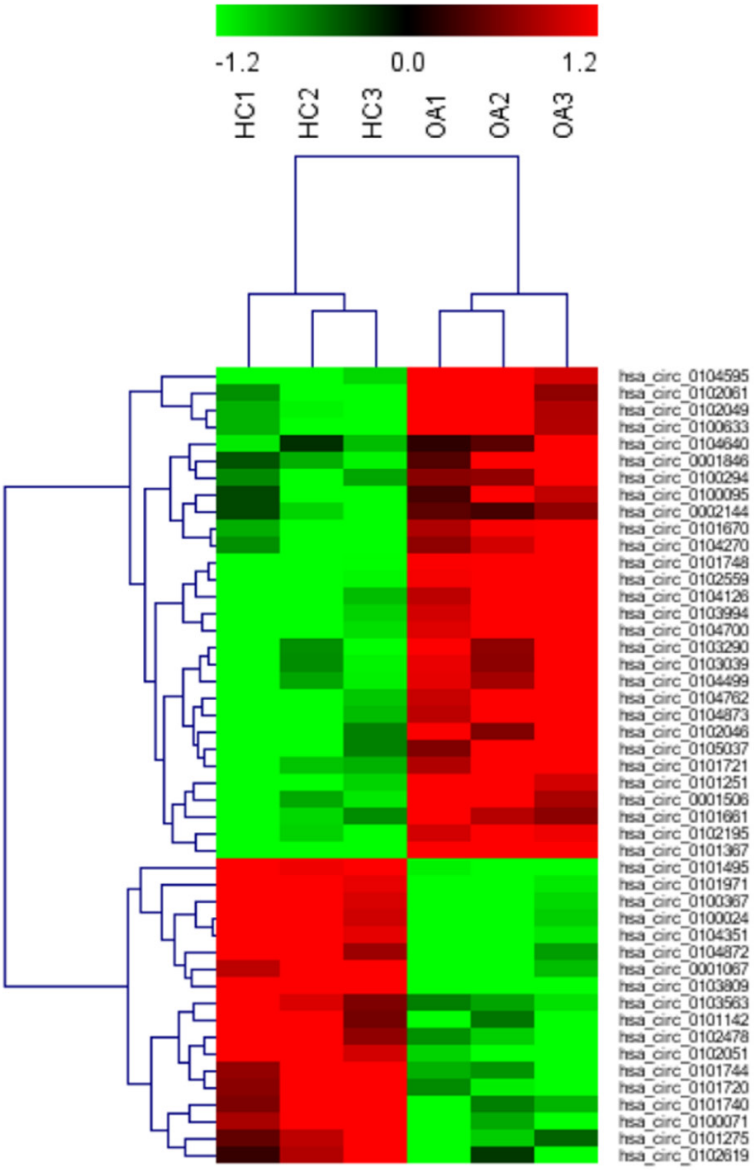

Figure 1. The expression profile of circRNAs in synovial fluid from OA patients and healthy subjects. Microarray and hierarchical cluster analysis were carried out in three synovial fluid samples from OA patients and healthy subjects. Each row represents an individual circRNA and each column represents one sample. The color legend at the top indicates circRNA expression levels, red indicating high expression and green indicating low expression levels.

of circRNA using ABI7300 System (Applied Biosystems, Foster City, CA, USA) with SYBR Select Master Mix (Applied Biosystems), as described previously [24]. Levels of β-actin were utilized to normalize the expression of the circRNAs. The relative expression levels of circRNAs were calculated using the $2^{-\Delta\Delta Ct}$ method [25]. The PCR primers were used in this study as shown in **Table 1**.

Statistical analysis

The data from our study were reported as mean ± standard deviation (SD) for each group. Statistical analysis was performed using IBM SPSS Statistics Version 19.0 (SPSS Inc., Chicago, IL, USA) and GraphPad Prism Version 7.0 (GraphPad Software, Inc., La Jolla, CA, USA). Student's t-test was used to analyze two-group differences. The non-parametric Kruskall-Wallis tests or Chi-square test were used to assess significance in clinical characteristics among the three subgroups or between patients with knee OA and healthy controls.

Spearman's linear regression analysis was used to identify the correlation between circRNAs levels and KL grade or WOMAC scores. Receiver operating characteristic (ROC) curves and the area under the

Table 3. Annotation of top 10 up-regulated circRNAs

circRNA	*p*-Value	Log_2FC	Gene symbol	Spliced length	Position
hsa_circ_0104873	1.17E-04	3.2	IQGAP1	614	chr15:90996006-90999547
hsa_circ_0104595	5.02E-05	3.9	SCAPER	618	chr15:77046148-77059429
hsa_circ_0101748	3.56E-04	3.8	BRMS1L	820	chr14:36299886-36300706
hsa_circ_0102559	6.73E-06	3.7	NUMB	346	chr14:73753817-73759582
hsa_circ_0103994	1.73E-05	3.4	TCF12	511	chr15:57554291-57565460
hsa_circ_0101251	1.89E-07	3.3	RP11-909M7.3	169	chr14:101446913-101448922
hsa_circ_0101367	2.63E-04	3.1	APOPT1	441	chr14:104037959-104053701
hsa_circ_0104762	4.67E-05	3.2	ADAMTSL3	1023	chr15:84566609-84611834
hsa_circ_0102061	5.81E-06	3.2	NIN	711	chr14:51204857-51211083
hsa_circ_0104700	7.14E-04	3.2	HOMER2	1086	chr15:83518440-83561593

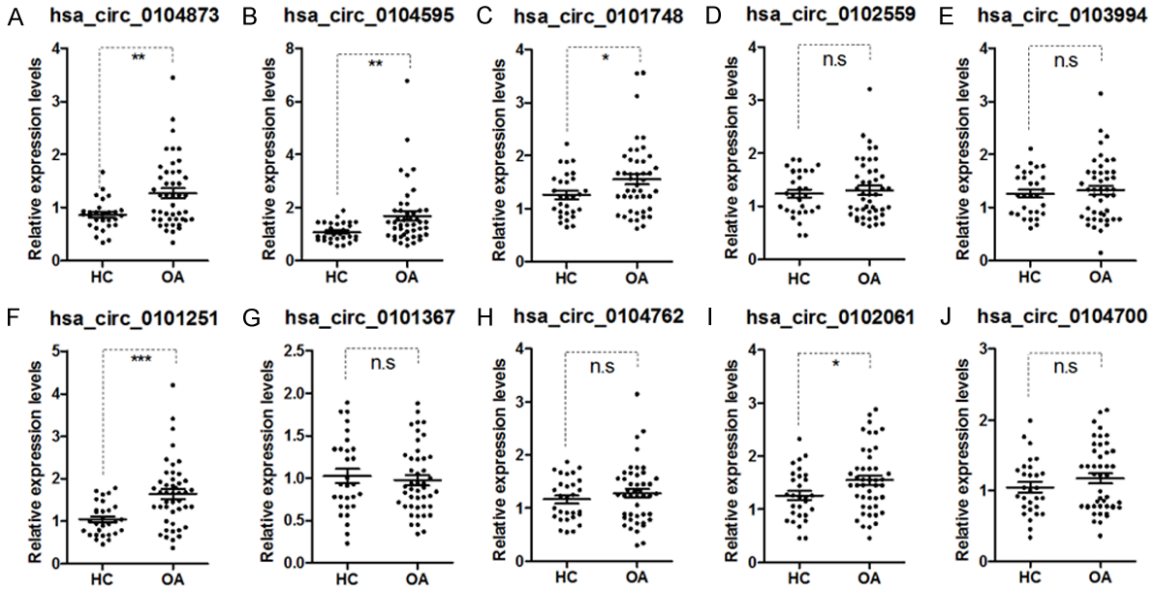

Figure 2. Verification of the top 10 up-regulated circRNAs by qRT-PCR. The expression levels of hsa_circ_0104873 (A), hsa_circ_0104595 (B), hsa_circ_0101748 (C), hsa_circ_0102559 (D), hsa_circ_0103994 (E), hsa_circ_0101251 (F), hsa_circ_0101367 (G), hsa_circ_0104762 (H), hsa_circ_0102061 (I) and hsa_circ_0104700 (J) were measured by qRT-PCR in synovial fluid from 45 OA patients and 29 healthy controls. $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$; n.s, no significant.

ROC curve (AUC) were used to assess the ability of using synovial fluid circRNAs as diagnostic tools for OA. The maximum value of the Youden index was used as a criterion for selecting the optimum cut-off point. *P* values less than 0.05 were considered statistically significant difference.

Results

Clinical characteristics of OA patients and healthy subjects

Demographic features of 45 OA patients and 29 healthy controls are summarized in **Table 2**. The average age, sex ratio, and BMI were not obviously different between OA patients and healthy controls ($P > 0.05$). According to the KL grading system, the OA group was divided into three subgroups, and the demographic features were not significantly different among the three subgroups ($P > 0.05$).

The expression profile of circRNAs in synovial fluid from OA patients and healthy subjects

To investigate differentially expressed circRNAs in synovial fluid, 3 OA patients and 3 healthy subjects were selected. Microarray analysis of the expression profiles of circRNAs in synovial

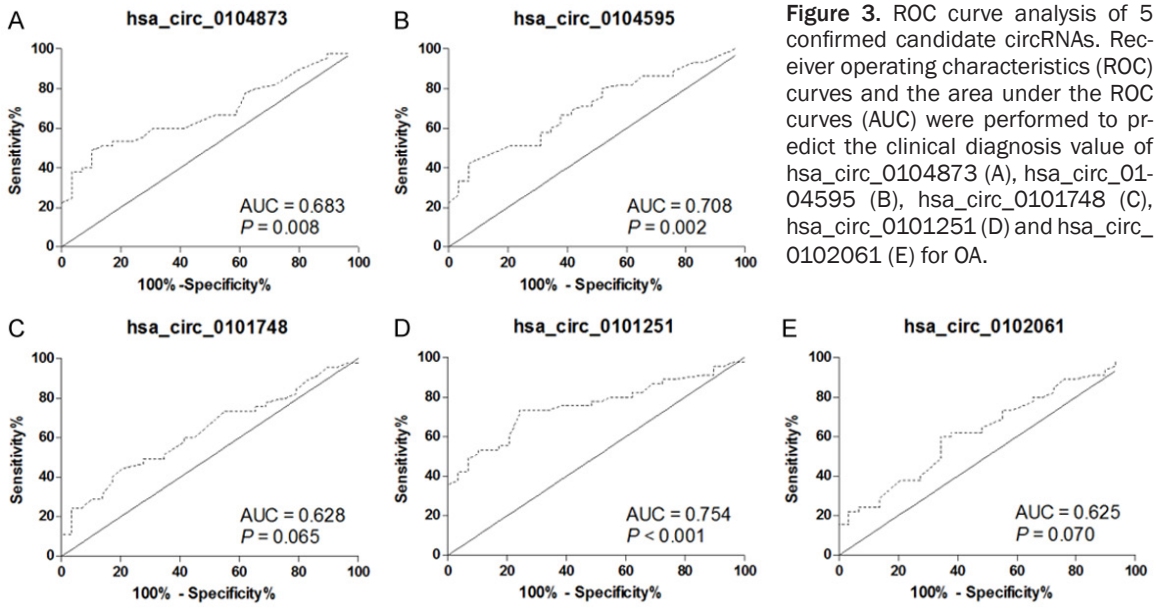

Figure 3. ROC curve analysis of 5 confirmed candidate circRNAs. Receiver operating characteristics (ROC) curves and the area under the ROC curves (AUC) were performed to predict the clinical diagnosis value of hsa_circ_0104873 (A), hsa_circ_0104595 (B), hsa_circ_0101748 (C), hsa_circ_0101251 (D) and hsa_circ_0102061 (E) for OA.

Table 4. Clinical diagnostic significance of circRNAs in OA patients

	AUC	*p*-Value	95% CI		Sensitivity	Specificity	Youden index	Cut-off
			Lower	Upper				
hsa_circ_0104873	0.683	0.008	0.563	0.802	0.489	0.897	0.386	1.225
hsa_circ_0104595	0.708	0.002	0.592	0.824	0.422	0.931	0.353	1.495
hsa_circ_0101748	0.628	0.065	0.501	0.754	0.444	0.793	0.238	1.605
hsa_circ_0101251	0.754	< 0.001	0.645	0.863	0.733	0.759	0.492	1.270
hsa_circ_0102061	0.625	0.070	0.497	0.754	0.600	0.655	0.255	1.445

fluid was performed using the Agilent human circRNA Array (V2.0). Based on the FDR ≤ 0.01 Log_2 (fold change) ≥ 1.5, $P < 0.001$, we found that 47 circRNAs were differentially expressed between OA patients and healthy subjects, among which 29 circRNAs and 18 circRNAs were up-regulated and down-regulated, respectively (**Figure 1**). Among the differentially expressed circRNAs, we selected out top 10 circRNAs, which were significantly up-regulated in synovial fluid from OA patients compared with healthy subjects, and the expression levels and annotation of these circRNAs are summarized in **Table 3**. Gene symbols, splice length, and position according to circBase (http://www.circbase.org) are indicated in the table. Interestingly, all of the top 10 circRNAs came from chromosome 14 (chr14) and chr15. OA-related circRNAs might be predominantly transcribed from chr14 and chr15. Therefore, the distribution status of circRNAs in chromosomes might be related to the progression of OA.

Verification of top 10 up-regulated circRNAs by qRT-PCR

To validate the 10 candidate circRNAs, qRT-PCR was performed in an independent cohort including 45 OA patients and 29 healthy controls. The results demonstrated that the levels of hsa_circ_0104873 (**Figure 2A**), hsa_circ_0104595 (**Figure 2B**), hsa_circ_0101748 (**Figure 2C**), hsa_circ_0101251 (**Figure 2F**) and hsa_circ_0102061 (**Figure 2I**) in synovial fluid from OA patients were significantly higher than those of the healthy controls, while the expression of hsa_circ_0102559 (**Figure 2D**), hsa_circ_0103994 (**Figure 2E**), hsa_circ_0101367 (**Figure 2G**), hsa_circ_0104762 (**Figure 2H**) and hsa_circ_0104700 (**Figure 2J**) did not show any remarkable differences between OA patients and healthy controls.

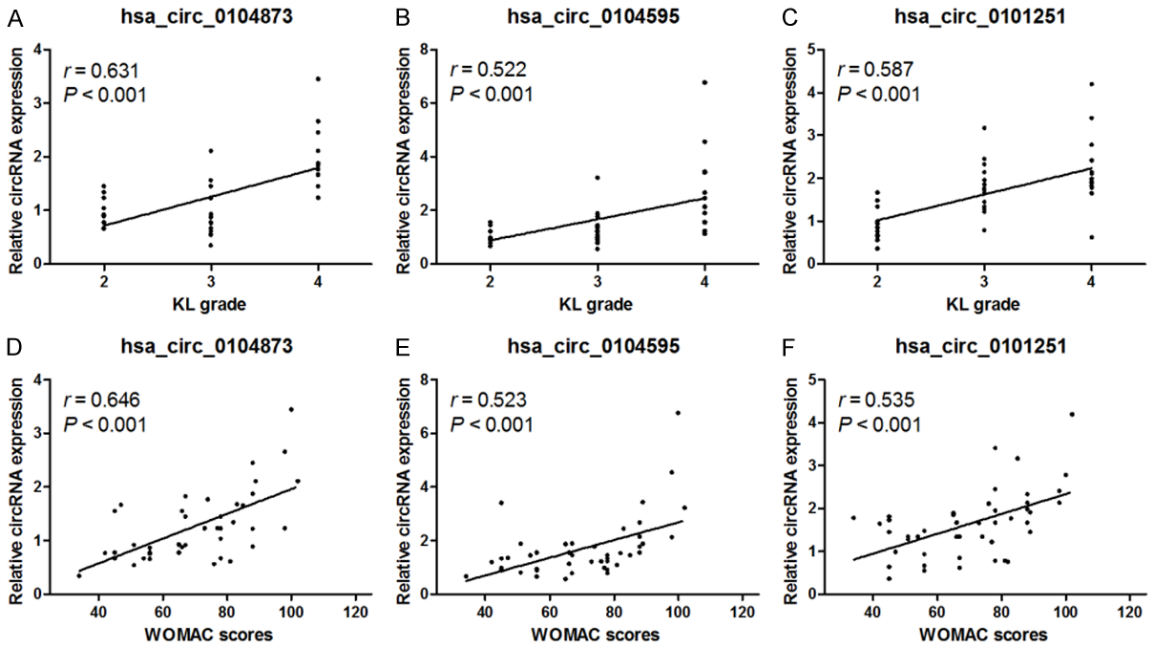

Figure 4. The correlation between circRNA expression levels and KL grade or WOMAC scores. The correlation between hsa_circ_0104873 (A), hsa_circ_0104595 (B) and hsa_circ_0101251 (C) levels of OA patients and disease severity classified according to KL grading system was performed. The correlation between hsa_circ_0104873 (D), hsa_circ_0104595 (E) and hsa_circ_0101251 (F) levels of OA patients and WOMAC scores was performed. KL, Kellgren-Lawrence; WOMAC, Western Ontario McMaster University Osteoarthritis Index.

ROC curve analysis of confirmed 5 candidate circRNAs

To investigate the diagnostic value of 5 candidate circRNAs for OA, receiver operating characteristics (ROC) curves and the area under the ROC curves (AUC) were performed on data from all participants including 45 OA patients and 29 healthy controls. The ROC curves showed that hsa_circ_0104873 (AUC = 0.683, P = 0.008; **Figure 3A** and **Table 4**), hsa_circ_0104595 (AUC = 0.708, P = 0.002; **Figure 3B** and **Table 4**) and hsa_circ_0101251 (AUC = 0.754, P < 0.001; **Figure 3D** and **Table 4**) could separate the patients with OA from the healthy controls. Hsa_circ_0101748 (AUC = 0.628, P = 0.065; **Figure 3C** and **Table 4**) and hsa_circ_0102061 (AUC = 0.625, P = 0.070; **Figure 3E** and **Table 4**) showed lower AUC than three others circRNAs and did not effectively separate OA patients from the healthy controls.

Correlation between circRNAs expression levels and KL grade or WOMAC scores

According to the KL grading system, OA patients were divided into 3 subgroups. The association of hsa_circ_0104873, hsa_circ_0104595 and hsa_circ_0101251 levels in synovial fluid with radiographic severity and WOMAC scores is illustrated in **Figure 4A-C**. Spearman's rank correlation analysis showed that the levels of hsa_circ_0104873 (r = 0.631, P < 0.001), hsa_circ_0104595 (r = 0.522, P < 0.001) and hsa_circ_0101251 (r = 0.587, P < 0.001) in synovial fluid were significantly and positively correlated with radiographic severity in OA patients. Similarly, hsa_circ_0104873 (r = 0.646, P < 0.001; **Figure 4D**), hsa_circ_0104595 (r = 0.523, P < 0.001; **Figure 4E**) and hsa_circ_0101251 (r = 0.535, P < 0.001; **Figure 4F**) levels had a positive correlation with WOMAC scores in OA patients.

Discussion

CircRNAs are widely and abundantly expressed in mammalian cells, and their expression levels are far higher than their linear isomers [26]. Moreover, circRNAs show more stability than other noncoding RNAs, such as miRNAs and lncRNAs [27]. These properties endow circRNAs the potential of being applicable biomarkers for human diseases screening in routine clinical practice. Using circRNA microarray analysis, our study revealed significant differ-

ences of 47 circRRNAs, 29 up-regulated, and 18 down-regulated, with different expression levels in synovial fluid between OA patients and healthy controls. Among top 10 up-regulated circRNAs, microarray profiling and qRT-PCR validation identified hsa_circ_0104873, hsa_circ_0104595, hsa_circ_0101748, hsa_circ_0101251, and hsa_circ_0102061 were increased in the synovial fluid of patients with OA. We conclusively proved that hsa_circ_0104873, hsa_circ_0104595, and hsa_circ_0101251 have high potential as diagnostic biomarkers for OA.

Biomarkers of OA can be roughly classified into four categories: markers of bone, cartilage, synovium metabolism (including synthesis and degradation), and systemic inflammation [1]. Emerging evidence suggests that many of non-coding RNA, including miRNAs and lncRNA, may reflect underlying disease status and disease severity of OA and can serve as potential markers for disease activity [10, 28]. Many of the dysregulated miRNAs, such as miR-9, miR-21, and miR-148a, have been shown to regulate inflammatory pathways and articular cartilage degeneration in the progression of OA [29-31]. Moreover, lncRNA-HOTAIR, GAS5, and PMS2L2 may be new biomarkers for diagnosis or novel therapeutic targets of OA [11, 32]. CircRNAs as a new class of non-coding RNA have a few correlative studies on arthritis [19, 33].

In our study, hsa_circ_0101251 showed the highest diagnostic value for OA among the three candidate biomarkers. Hsa_circ_0101251 is located in chr14:101446913-101448922, the spliced sequence length is 169 ribonucleotides, and its associated-gene symbol is RP11-909M7.3. To the best of our knowledge, hsa_circ_0101251 is quite new, and there is no correlative literature to uncover its functions. In addition, hsa_circ_0104873 and hsa_circ_0104595 originate from IQGAP1 and SCAPER, respectively. IQ motif-containing GTPase-activating protein 1 (IQGAP1) is a highly conserved cytoplasmic scaffold protein, which is involved in multiple signaling pathways in a variety of cell types [34]. Up-regulation of IQGAP1 plays a critical role in regulating cell spreading and actin skeleton formation in the early stages of osteoblast differentiation [35]. IQGAP1 silencing shows a marked decrease in the expression of matrix metalloproteinase 9 (MMP9), which is significantly elevated and may play a role in the development of OA [36]. S-phase cyclin A associated protein in endoplasmic reticulum (SCAPER) localizes primarily to the endoplasmic reticulum (ER) and regulates cell cycle progression by associating with cyclin A/Cdk2 signaling [37, 38]. These results suggest that IQGAP1 and SCAPER are critical regulators of basic cellular physiology and may be involved in chondrocyte dysfunction induced OA. These work and our findings pave new avenues for understanding the role of IQGAP1 and SCAPER and their circRNAs in the pathogenic mechanism of OA.

In our study, ROC analyses showed that hsa_circ_0104873, hsa_circ_0104595, and hsa_circ_0101251 might be used as novel biomarkers for OA with high degrees of specificity. To further confirm these results, Spearman's rank correlation was used to determine the correlation between the levels of circRNAs in synovial fluid and radiographic grading or symptomatic severity of knee OA. Our results illustrate a pronounced positive correlation of three circRNAs with the degree of radiographic grading and symptomatic severity in OA patients. Therefore, we have reason to believe that hsa_circ_0104873, hsa_circ_0104595, and hsa_circ_0101251 can be used as a new alternative biomarker set to reflect the disease severity of OA. However, the underlying pathogenic mechanisms of OA associated with the present circRNAs needs to be further investigated. Currently, some heuristic results are described by some researchers. For example, Liu et al. described that circRNA-CER function can be a competing endogenous RNA (ceRNA) to enhance MMP-13 expression by sponging miR-136 in human cartilage degradation [15]. Wu et al. demonstrated that hsa_circ_0005105 upregulates NAMPT expression and promotes chondrocyte extracellular matrix degradation by sponging miR-26a [18]. Therefore, abnormally expressed circRNAs probably play important roles in the pathological process of OA.

In summary, hsa_circ_0104873, hsa_circ_0104595, and hsa_circ_0101251 were significantly up-regulated in synovial fluid and were positively correlated with the degree of radiographic grading and symptomatic severity of OA patients. These three circRNAs might be used

as diagnostic biomarkers for OA patients in routine clinical practice.

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Disclosure of conflict of interest

None.

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References

[1] Liu Y, Hou R, Yin R and Yin W. Correlation of bone morphogenetic protein-2 levels in serum and synovial fluid with disease severity of knee osteoarthritis. Med Sci Monit 2015; 21: 363-370.

[2] Bijlsma JW, Berenbaum F and Lafeber FP. Osteoarthritis: an update with relevance for clinical practice. Lancet 2011; 377: 2115-2126.

[3] Zhao XY, Yang ZB, Zhang ZJ, Zhang ZQ, Kang Y, Huang GX, Wang SW, Huang H and Liao WM. CCL3 serves as a potential plasma biomarker in knee degeneration (osteoarthritis). Osteoarthritis Cartilage 2015; 23: 1405-1411.

[4] Attur M, Krasnokutsky-Samuels S, Samuels J and Abramson SB. Prognostic biomarkers in osteoarthritis. Curr Opin Rheumatol 2013; 25: 136-144.

[5] Lotz M, Martel-Pelletier J, Christiansen C, Brandi ML, Bruyere O, Chapurlat R, Collette J, Cooper C, Giacovelli G, Kanis JA, Karsdal MA, Kraus V, Lems WF, Meulenbelt I, Pelletier JP, Raynauld JP, Reiter-Niesert S, Rizzoli R, Sandell LJ, Van Spil WE and Reginster JY. Value of biomarkers in osteoarthritis: current status and perspectives. Ann Rheum Dis 2013; 72: 1756-1763.

[6] Dorleijn DM, Luijsterburg PA, Bay-Jensen AC, Siebuhr AS, Karsdal MA, Rozendaal RM, Bos PK and Bierma-Zeinstra SM. Association between biochemical cartilage markers and clinical symptoms in patients with hip osteoarthritis: cohort study with 2-year follow-up. Osteoarthritis Cartilage 2015; 23: 57-62.

[7] Daghestani HN and Kraus VB. Inflammatory biomarkers in osteoarthritis. Osteoarthritis Cartilage 2015; 23: 1890-1896.

[8] Murata K, Yoshitomi H, Tanida S, Ishikawa M, Nishitani K, Ito H and Nakamura T. Plasma and synovial fluid microRNAs as potential biomarkers of rheumatoid arthritis and osteoarthritis. Arthritis Res Ther 2010; 12: R86.

[9] Kriegsmann M, Randau TM, Gravius S, Lisenko K, Altmann C, Arens N and Kriegsmann J. Expression of miR-146a, miR-155, and miR-223 in formalin-fixed paraffin-embedded synovial tissues of patients with rheumatoid arthritis and osteoarthritis. Virchows Arch 2016; 469: 93-100.

[10] Nugent M. MicroRNAs: exploring new horizons in osteoarthritis. Osteoarthritis Cartilage 2016; 24: 573-580.

[11] Fu M, Huang G, Zhang Z, Liu J, Zhang Z, Huang Z, Yu B and Meng F. Expression profile of long noncoding RNAs in cartilage from knee osteoarthritis patients. Osteoarthritis Cartilage 2015; 23: 423-432.

[12] Vicens Q and Westhof E. Biogenesis of circular RNAs. Cell 2014; 159: 13-14.

[13] Hentze MW and Preiss T. Circular RNAs: splicing's enigma variations. EMBO J 2013; 32: 923-925.

[14] Kulcheski FR, Christoff AP and Margis R. Circular RNAs are miRNA sponges and can be used as a new class of biomarker. J Biotechnol 2016; 238: 42-51.

[15] Liu Q, Zhang X, Hu X, Dai L, Fu X, Zhang J and Ao Y. Circular RNA related to the chondrocyte ECM regulates MMP13 expression by functioning as a MiR-136 'Sponge' in human cartilage degradation. Sci Rep 2016; 6: 22572.

[16] Liu Q, Zhang X, Hu X, Yuan L, Cheng J, Jiang Y and Ao Y. Emerging roles of circRNA related to the mechanical stress in Human cartilage degradation of osteoarthritis. Mol Ther Nucleic Acids 2017; 7: 223-230.

[17] Li BF, Zhang Y, Xiao J, Wang F, Li M, Guo XZ, Xie HB, Xia H and Chen B. Hsa_circ_0045714 regulates chondrocyte proliferation, apoptosis and extracellular matrix synthesis by promoting the expression of miR-193b target gene IGF1R. Hum Cell 2017; 30: 311-318.

[18] Wu Y, Zhang Y, Zhang Y and Wang JJ. CircRNA hsa_circ_0005105 upregulates NAMPT expression and promotes chondrocyte extracellular matrix degradation by sponging miR-26a. Cell Biol Int 2017; 41: 1283-1289.

[19] Ouyang Q, Wu J, Jiang Z, Zhao J, Wang R, Lou A, Zhu D, Shi GP and Yang M. Microarray expression profile of circular RNAs in peripheral blood mononuclear cells from rheumatoid arthritis patients. Cell Physiol Biochem 2017; 42: 651-659.

[20] Lu D and Xu AD. Mini review: circular RNAs as potential clinical biomarkers for disorders in the central nervous system. Front Genet 2016; 7: 53.
[21] Meng S, Zhou H, Feng Z, Xu Z, Tang Y, Li P and Wu M. CircRNA: functions and properties of a novel potential biomarker for cancer. Mol Cancer 2017; 16: 94.
[22] Kellgren JH and Lawrence JS. Radiological assessment of osteo-arthrosis. Ann Rheum Dis 1957; 16: 494-502.
[23] Bellamy N, Buchanan WW, Goldsmith CH, Campbell J and Stitt LW. Validation study of WOMAC: a health status instrument for measuring clinically important patient relevant outcomes to antirheumatic drug therapy in patients with osteoarthritis of the hip or knee. J Rheumatol 1988; 15: 1833-1840.
[24] Xia W, Qiu M, Chen R, Wang S, Leng X, Wang J, Xu Y, Hu J, Dong G, Xu PL and Yin R. Circular RNA has_circ_0067934 is upregulated in esophageal squamous cell carcinoma and promoted proliferation. Sci Rep 2016; 6: 35576.
[25] Livak KJ and Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. Methods 2001; 25: 402-408.
[26] Jeck WR, Sorrentino JA, Wang K, Slevin MK, Burd CE, Liu J, Marzluff WF and Sharpless NE. Circular RNAs are abundant, conserved, and associated with ALU repeats. RNA 2013; 19: 141-157.
[27] Liang D and Wilusz JE. Short intronic repeat sequences facilitate circular RNA production. Genes Dev 2014; 28: 2233-2247.
[28] Kong R, Gao J, Si Y and Zhao D. Combination of circulating miR-19b-3p, miR-122-5p and miR-486-5p expressions correlates with risk and disease severity of knee osteoarthritis. Am J Transl Res 2017; 9: 2852-2864.
[29] Jones SW, Watkins G, Le Good N, Roberts S, Murphy CL, Brockbank SM, Needham MR, Read SJ and Newham P. The identification of differentially expressed microRNA in osteoarthritic tissue that modulate the production of TNF-alpha and MMP13. Osteoarthritis Cartilage 2009; 17: 464-472.
[30] Zhang Y, Jia J, Yang S, Liu X, Ye S and Tian H. MicroRNA-21 controls the development of osteoarthritis by targeting GDF-5 in chondrocytes. Exp Mol Med 2014; 46: e79.
[31] Vonk LA, Kragten AH, Dhert WJ, Saris DB and Creemers LB. Overexpression of hsa-miR-148a promotes cartilage production and inhibits cartilage degradation by osteoarthritic chondrocytes. Osteoarthritis Cartilage 2014; 22: 145-153.
[32] Xing D, Liang JQ, Li Y, Lu J, Jia HB, Xu LY and Ma XL. Identification of long noncoding RNA associated with osteoarthritis in humans. Orthop Surg 2014; 6: 288-293.
[33] Zheng F, Yu X, Huang J and Dai Y. Circular RNA expression profiles of peripheral blood mononuclear cells in rheumatoid arthritis patients, based on microarray chip technology. Mol Med Rep 2017; 16: 8029-8036.
[34] Abel AM, Schuldt KM, Rajasekaran K, Hwang D, Riese MJ, Rao S, Thakar MS and Malarkannan S. IQGAP1: insights into the function of a molecular puppeteer. Mol Immunol 2015; 65: 336-349.
[35] Hong D, Chen HX, Yu HQ, Liang Y, Wang C, Lian QQ, Deng HT and Ge RS. Morphological and proteomic analysis of early stage of osteoblast differentiation in osteoblastic progenitor cells. Exp Cell Res 2010; 316: 2291-2300.
[36] Adams SB, Setton LA, Bell RD, Easley ME, Huebner JL, Stabler T, Kraus VB, Leimer EM, Olson SA and Nettles DL. Inflammatory cytokines and matrix metalloproteinases in the synovial fluid after intra-articular ankle fracture. Foot Ankle Int 2015; 36: 1264-1271.
[37] Tsang WY, Wang L, Chen Z, Sanchez I and Dynlacht BD. SCAPER, a novel cyclin A-interacting protein that regulates cell cycle progression. J Cell Biol 2007; 178: 621-633.
[38] Tsang WY and Dynlacht BD. Double identity of SCAPER: a substrate and regulator of cyclin A/Cdk2. Cell Cycle 2008; 7: 702-705.