Analysis of CircRNA expression profile of pathological bone formation in ankylosing spondylitis

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	abbreviations
Gene Ontology	GO
Kyoto Encyclopedia of Genes and Genomes	KEGG
Ankylosing spondylitis	AS
Fibroblast growth factor	eFGF
Epidermal augmentum factor	EGF
Platelet derived growth factor	PDGF
Alkaline phosphatase	ALP
Osterix	OSX
Extracellular matrix	ECM
Focal adhesion kinase	FAK

Abstract

Objective: To screen and analyze the function of specific circular RNA involved of pathological bone formation in ankylosing spondylitis.

Methods: From September 2019 to October 2020, Ossification capsule of 3 patients

with ankylosing spondylitis developed hip joint fusion and capsule of 3 patients with femoral neck fracture were enrolled as the experimental group and the control group, respectively. The circular RNA expressions of hip capsule were analyzed by arraystar circRNA chip, bioinformatics analyses including the circRNA/miRNA/mRNA interaction network, Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis.

Results: The results showed that there totally were 25 up-regulated and 39 down-regulated differential circRNAs, among these circRNAs,we screened most up-regulated 10 circRNAs and most down-regulated 13 circRNAs in ankylosing spondylitis (FC⩾2,$P < 0.05$). Further analysis demonstrated that two signal ways were most involved and correslated with the different circRNAs-"Focal Adhesion signal pathway" and "Rap1 signal pathway"

Conclusion: The circular RNAs involved of pathological bone formation in ankylosing spondylitis were significantly different from that of the control group. These differentially expressed circular RNAs may be closely related to the occurrence and development of pathological bone formation in ankylosing spondylitis.

Key words: CircRNA, Ankylosing spondylitis, Gene chip, Pathological bone formation

Introduction

Ankylosing spondylitis (AS) is an immune-mediated chronic inflammatory disease. The pathogenesis of the disease is attributed to the complex interaction of genetic, environmental and immune factors[1]. The main clinical manifestations are sacroiliac arthritis and enthesis, which mainly affect the axial bone, peripheral joints and extra-articular structures can also be involved, and can cause ankylosis and fibrosis of spinal deformities in the late stage, and even cause serious functional damage. Bone remodeling in SpA should be distinguished from classical bone remodeling,which is the continuous renewal of the skeleton by the balanced activity of boneforming

osteoblasts and bone-resorbing osteoclasts, which is orchestrated by the mechanosensitive osteocytes[2], and Lories RJ et.al found processes involved in new bone formation in AS are orchestrated by proliferation, differentiation, maturation, and migration of cells as well as cell death. Osteproliferation in SpA is a complex tissue remodeling process rather than simple proliferation of bone and shares similarities with joint remodeling in osteoarthritis[3], Moreover, control of inflammation reverts the trabecular bone loss[4]but does not seem to affect the progression of ankylosis.11And other studys think the pathogenesis of new bone formation in AS have closely relationship with Inflammatory factors、osteoblast、signal ways and so on. In the late stage, most patients with AS require surgery because of new bone formation and fusion in the axial joint and the large peripheral joint. Therefore, finding the factors that causing the new bone formation of AS will help improving the quality of life of patients with AS, and can also help us early diagnosis、treatment and prognosis

Different from traditional linear RNA (including 5 'and 3' ends),the circular RNA (circRNA) is a single-stranded or double-stranded non-coding RNA with closed ring structure and it is structurally stable and not easy to degrade .In recent years, thousands of circRNAs have been found in eukaryotes with the second generation sequencing, and some circRNAs have been found to be highly expressed in specific cells or tissues[5]. At present, it is believed that the functions of circRNA include regulating transcription, binding protein, miRNA sponge, protein complex and protein induction and stabilization, which are closely related to the occurrence and development of tumor, cardiovascular, diabetes, rheumatoid arthritis and other diseases[6]. Several circRNAs abnormal expressions with high sensitivity and specificity can be used as diagnostic markers, such as hsa-circRNA-103809 and hsa-circRNA-104700 may be involved in the occurrence of colorectal cancer, and can be used as new biomarkers in the diagnosis of colorectal cancer[7]. Some studies have found that circRNA5846 and circRNA1914 in osteoarthritis can promotes osteogenesis-related responses through fibroblast growth factor(eFGF), EGF, PDGF and Wnt signaling pathways[8].so far, to the best of our knowledge, the expression and function of circRNAs involved in pathological bone

formation in ankylosing spondylitis have not been investigated.

Therefore, this study aims to find the circRNA expression profile in ankylosing spondylitis,and predict their interactive miRNA、mRNA as well as analyse GO、KEGG and signal ways. Due to the relatively slow progression of ossification in the spine, sacroiliac and hip joint, ossification problems caused by AS are often ignored in the clinical diagnosis and treatment process, leading to the delay of treatment timing. So these results may provide evidence of pathological bone formation and the potential targets for the development of novel diagnostic and therapeutic strategies in AS.

Materials and Methods

Clinical Specimen Acquisition

The hip joint capsules were obtained from three male patients with ankylosing spondylitis developed hip joint ankylosis and fusion receiving total hip arthroplasty in the third affiliated Hospital of Southern Medical University from September 2019 to October 2020 were enrolled the experimental group(use the Modified New York Criteria as a standard guide to determine diagnosis).Meanwhile, The hip joint capsules were obtained from three male patients with femoral neck fracture were enrolled the control group (excluding Diffuse idiopathic skeletal hyperostosis、osteoarthritis、rheumatoid arthritis、other spondyloarthritis and other potential immune system diseases). This study was approved by the Ethics Committee of our hospital, informed the patients and signed the informed consent form.

Extraction of RNA from Specimens and Detection by CircRNA Chip

Separate 5mg tissue from each specimen(Hip joint capsule),and then extract total RNA using the TRIzol((Life Technologies, Carlsbad, CA,USA). Then the RNA quantification and quality was examined by using the Nanodrop ND-1000 spectrophotometer. RNA integrity and gDNA contamination was tested by denaturing agarose gel electrophoresis. The sample preparation and microarray hybridization were

performed based on the Arraystar's standard protocols provide by KANGCHENG Inc. (Shanghai, China).Firstly, total RNAs of two groups were digested with Rnase R(Epicentre, Inc.) to remove linear RNAs and enrich circular RNAs respectively. Secondly, the enriched circular RNAs were amplified and transcribed into fluorescent cRNA utilizing a random priming method (Arraystar Super RNA Labeling Kit; Arraystar). Thirdly, the labeled cRNAs were hybridized onto the Arraystar Human circRNA Array (8x15K, Arraystar). Finally, after having washed the slides, the arrays were scanned by the Agilent Scanner G2505C.

circRNA/miRNA interaction analysis

TargetScan and miRanda were used to predict that the circRNA with FC ≥ 2 times or more which is the most likely to combine with miRNA, to screen the top five miRNA with the highest matching degree.

circRNA/miRNA/mRNA regulatory networks analysis

The top five circRNAs were selected, with the largest up-and-down difference, and TargetScan and miRanda were used to predict the related target genes of their corresponding miRNA, then take the crossover results, and use Cytoscape3.7.0 to construct a ceRNA network map of osteogenesis-related signal pathways.

Bioinformatics analysis

The prediction-related target genes were analyzed by gene ontology (Geneoncology,GO) and biological metabolic pathway (KEGG) analysis using DAVID online software, and osteogenesis-related signal pathways were deeply studied.

Statistical Analysis

Data are presented as mean ± standard deviation (SD), SPSS 20.0 (Chicago, US) were used for all statistical analyses,and $P < 0.05$ was considered statistically significant. CircRNA expression profiles in testicular tissue samples of the AS and control group were analyzed by using paired t test.

Results

Quality control

The ratio of OD (260 nm) / OD (280 nm) of total RNA extracted from 6 samples of hip joint capsule was more than 1.8 and less than 2.0, respectively. The ratio of OD (260nm) / OD (230nm) was more than 1.8 respectively. It shows that RNA can be used for further experimental analysis. (Tab. 1)

Results of CircRNA Expression Profile Microarray

There were 25 up-regulated and 39 down-regulated differential circRNAs, among of these circRNAs, 10 circRNAs were up-regulated and 13 circRNAs were down-regulated in ankylosing spondylitis($FC \geqslant 2, P < 0.05$).(Tab.2 and 3)The expression of different circRNAs were analyzed by hierarchical cluster analysis, and the related scatter diagram was drawn. Red represents relatively high expression of circRNA, and green represents lower circRNA. According to these differentially expressed circRNA, ankylosing spondylitis and femoral neck fracture can be distinguished from ankylosing spondylitis at the genetic level.(Fig.1A) .The scatter plot reflects the overall concentrated distribution of circRNA in the hip joint capsule of AS and disease controls (Fig.1B). The volcano map can screen out the differential expression of circRNA. (Fig.1C)

Table.1 the purity and concentration of total RNA in each group were detected by NanoDrop ND-1000.

Sample	OD260/280 Ratio	OD260/230 Ratio	Conc. (ng/μl)	Volume (μl)	Quantity (ng)	QC Purity Pass or Fail
AS1	1.89	1.87	349.53	50	17475.50	Pass
AS3	1.88	2.32	471.00	40	18840.00	Pass
N1	1.96	2.28	748.31	20	14966.20	Pass
N2	1.90	1.97	466.20	30	13986.00	Pass
N3	1.86	1.97	206.69	15	3100.35	Pass
AS4	1.89	2.28	402.07	30	12062.1	Pass

Table 2. 10 differential circRNA up-regulated more than 2-fold in hip joint capsule specimens of ankylosing spondylitis.

CircRNA	p-value	FDR	FC(abs)	Alias	GeneSymbol
has_circRNA_404474	0.023517	0.999649	3.238364		TRIM62
has_circRNA_103454	0.031443	0.999649	2.59865	hsa_circ_0067103	ZNF148
has_circRNA_004496	0.006917	0.999649	2.573734	hsa_circ_0004496	VEGFC
has_circRNA_002649	0.035793	0.999649	2.415517	hsa_circ_0002649	DAB2
has_circRNA_009052	0.000355	0.999649	2.140777	hsa_circ_0009052	MRC2
has_circRNA_104135	0.005898	0.999649	2.104583	hsa_circ_0007874	MTO1
has_circRNA_083369	0.016804	0.999649	2.088818	hsa_circ_0083369	LOC100506990
has_circRNA_103591	0.01473	0.999649	2.063285	hsa_circ_0069029	BC042823
has_circRNA_104812	0.015597	0.999649	2.015876	hsa_circ_0007351	AGTPBP1
has_circRNA_101721	0.035112	0.999649	2.011754	hsa_circ_0038095	ABCC1

Table 3 13 differential circRNA down-regulated more than 2 times in hip joint capsule specimens of ankylosing spondylitis.

circRNA	P-value	FDR	FC (abs)	Alias	GeneSymbol
hsa_circRNA_020273	0.035931	0.999649	3.683941	hsa_circ_0020273	HTRA1
hsa_circRNA_403520	0.045872	0.999649	3.12779		GFPT2
hsa_circRNA_101744	0.018123	0.999649	2.800685	hsa_circ_0005699	C16orf62
hsa_circRNA_104940	0.013161	0.999649	2.706086	hsa_circ_0089153	NUP214
hsa_circRNA_025460	0.003029	0.999649	2.682278	hsa_circ_0025460	YBX3
hsa_circRNA_050648	0.042913	0.999649	2.569779	hsa_circ_0050648	HSPB6
hsa_circRNA_048764	0.025315	0.999649	2.385396	hsa_circ_0048764	RPL36
hsa_circRNA_404655	0.028028	0.999649	2.304858		XLOC_000566
hsa_circRNA_104137	0.009703	0.999649	2.199079	hsa_circ_0076995	EEF1A1
hsa_circRNA_400071	0.000612	0.999649	2.155393	hsa_circ_0092283	MYH9
hsa_circRNA_000735	0.038402	0.999649	2.139321	hsa_circ_0000735	P2RX1
hsa_circRNA_101693	0.035383	0.999649	2.073838	hsa_circ_0007788	NMRAL1
hsa_circRNA_003300	0.023192	0.999649	2.058175	hsa_circ_0003300	LRCH2

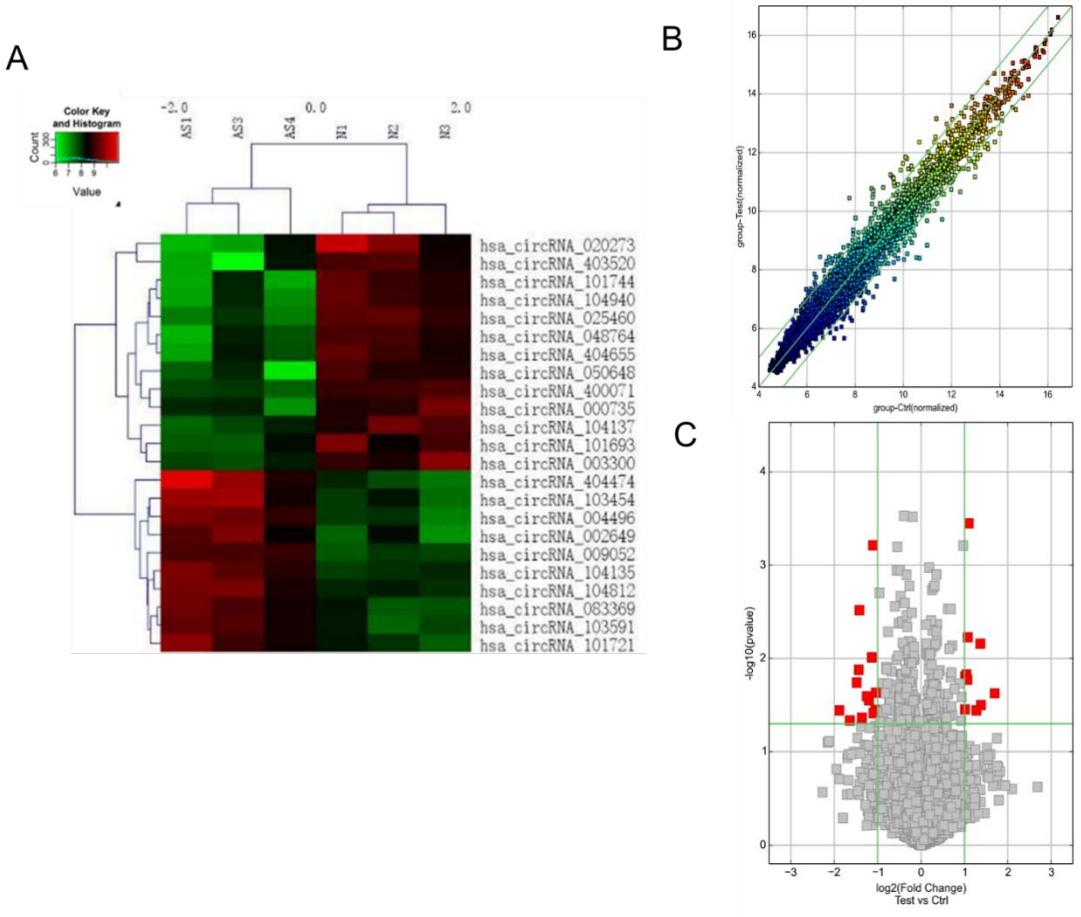
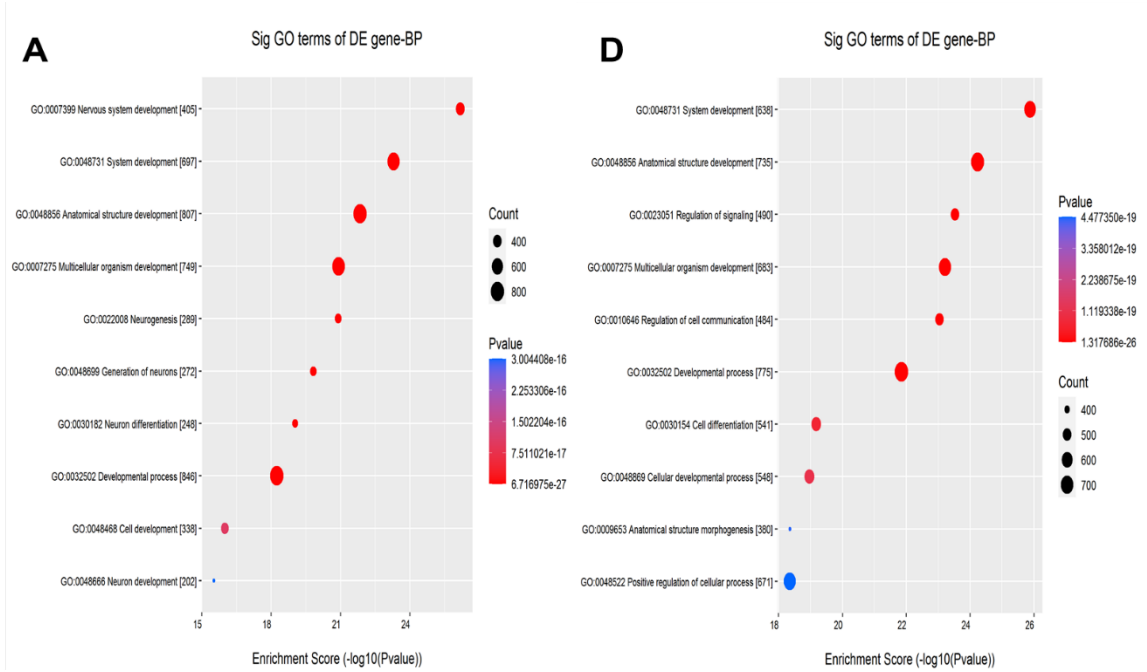


Fig.1 AS differential expression of circRNA between hip joint capsule of femoral neck fracture and hip joint capsule of femoral neck fracture. **A.** Hierarchical cluster analysis.**B.** Scatter plot Note: the data above the green line at the top and below the green line at the bottom represent the difference circRNA (FC ≥2.0 and P < 0.05). **C.**

Volcanic map Note: the red area represents circRNA (FC≥2 P < 0. 05).

Bioinformatics analysis

The target genes involved in the competitive binding of 5 different cirRNA (multiple of difference ranking) were selected for GO biological process enrichment analysis and KEGG pathway analysis. The maximum biological processes of (BP) enrichment include: development, signal regulation, regulation of cellular communication, etc. (Fig 2A). Cellular components (CC): cell connection, projection neurons, synapses, synaptic compartments etc. (Fig.2B). Molecular function (MF): regulates transcriptional activity, binding proteins and enzymes, regulating molecular function etc.(Fig.2C) KEGG pathway is mainly up-regulated: focal adhesion kinase signal pathway, cancer pathway, Rap1 signal pathway, actin cytoskeleton regulation pathway and so on (Fig. 3A). Down-regulation: Wnt signal pathway, inflammatory mediator regulation pathway of TRP channel, FoxO signal pathway, MAPK signal pathway, ERBB signal pathway, Ras signal pathway and so on. (Fig.3B) The“focal adhesion kinas” signal pathway and the “Rap1” signal pathway were the meaningful pathways (Fig.3C and 3D).


A
Sig GO terms of DE gene-BP
GO:0007399 Nervous system development [405]
GO:0048731 System development [697]
GO:0048856 Anatomical structure development [807]
GO:0007275 Multicellular organism development [749]
GO:0022008 Neurogenesis [289]
GO:0048699 Generation of neurons [272]
GO:0030182 Neuron differentiation [248]
GO:0032502 Developmental process [846]
GO:0048468 Cell development [338]
GO:0048666 Neuron development [202]
15
18
21
24
Enrichment Score (-log10(Pvalue))
Count
400
600
800
Pvalue
3.004408e-16
2.253306e-16
1.502204e-16
7.511021e-17
6.716975e-27
D
Sig GO terms of DE gene-BP
GO:0048731 System development [638]
GO:0048856 Anatomical structure development [735]
GO:0023051 Regulation of signaling [490]
GO:0007275 Multicellular organism development [683]
GO:0010646 Regulation of cell communication [484]
GO:0032502 Developmental process [775]
GO:0030154 Cell differentiation [541]
GO:0048869 Cellular developmental process [548]
GO:0009653 Anatomical structure morphogenesis [380]
GO:0048522 Positive regulation of cellular process [671]
18
20
22
24
26
Enrichment Score (-log10(Pvalue))
Pvalue
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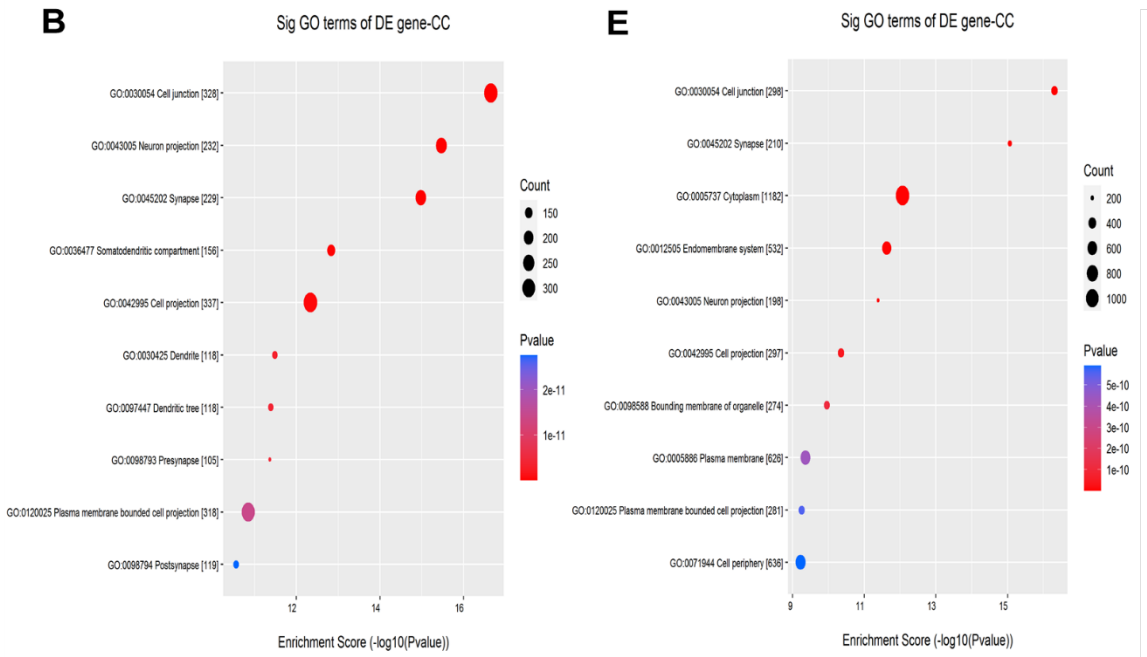
3.358012e-19
2.238675e-19
1.119338e-19
1.317686e-26
Count
400
500
600
700


B
Sig GO terms of DE gene-CC
GO:0030054 Cell junction [328]
GO:0043005 Neuron projection [232]
GO:0045202 Synapse [229]
GO:0036477 Somatodendritic compartment [156]
GO:0042995 Cell projection [337]
GO:0030425 Dendrite [118]
GO:0097447 Dendritic tree [118]
GO:0098793 Presynapse [105]
GO:0120025 Plasma membrane bounded cell projection [318]
GO:0098794 Postsynapse [119]
12
14
16
Enrichment Score (-log10(Pvalue))
Count
150
200
250
300
Pvalue
2e-11
1e-11
E
Sig GO terms of DE gene-CC
GO:0030054 Cell junction [298]
GO:0045202 Synapse [210]
GO:0005737 Cytoplasm [1182]
GO:0012505 Endomembrane system [532]
GO:0043005 Neuron projection [198]
GO:0042995 Cell projection [297]
GO:0098588 Bounding membrane of organelle [274]
GO:0005886 Plasma membrane [626]
GO:0120025 Plasma membrane bounded cell projection [281]
GO:0071944 Cell periphery [636]
9
11
13
15
Enrichment Score (-log10(Pvalue))
Count
200
400
600
800
1000
Pvalue
5e-10
4e-10
3e-10
2e-10
1e-10

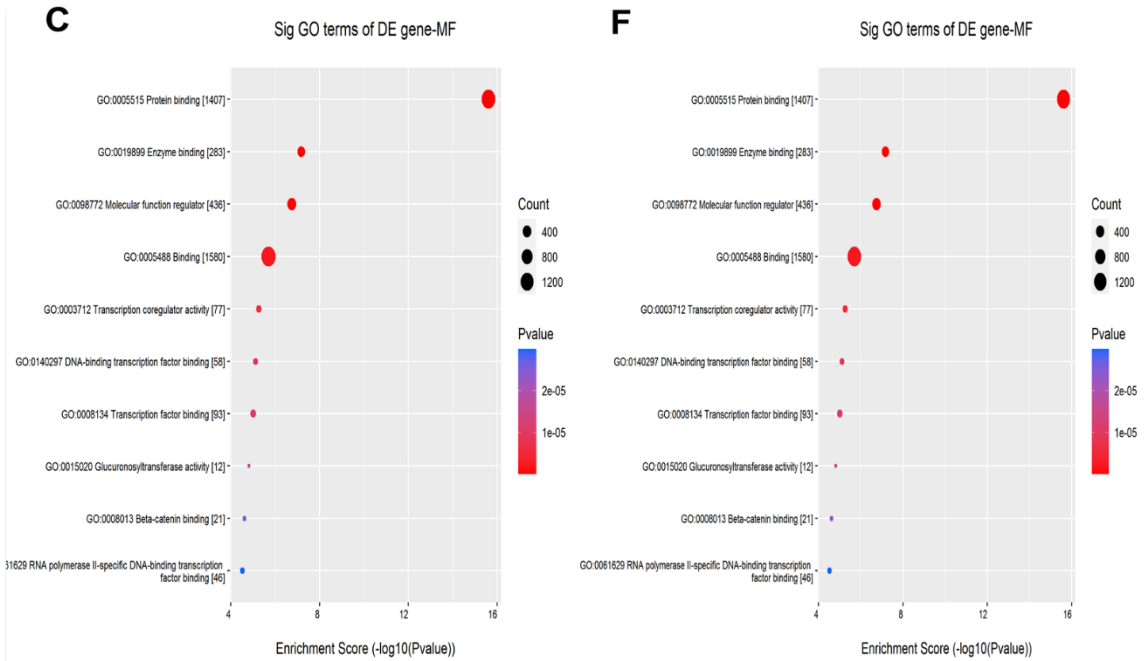

C
Sig GO terms of DE gene-MF
GO:0005515 Protein binding [1407]
GO:0019899 Enzyme binding [283]
GO:0098772 Molecular function regulator [436]
GO:0005488 Binding [1580]
GO:0003712 Transcription coregulator activity [77]
GO:0140297 DNA-binding transcription factor binding [58]
GO:0008134 Transcription factor binding [93]
GO:0015020 Glucuronosyltransferase activity [12]
GO:0008013 Beta-catenin binding [21]
31629 RNA polymerase II-specific DNA-binding transcription factor binding [46]
4
8
12
16
Enrichment Score (-log10(Pvalue))
Count
400
800
1200
Pvalue
2e-05
1e-05
F
Sig GO terms of DE gene-MF
GO:0005515 Protein binding [1407]
GO:0019899 Enzyme binding [283]
GO:0098772 Molecular function regulator [436]
GO:0005488 Binding [1580]
GO:0003712 Transcription coregulator activity [77]
GO:0140297 DNA-binding transcription factor binding [58]
GO:0008134 Transcription factor binding [93]
GO:0015020 Glucuronosyltransferase activity [12]
GO:0008013 Beta-catenin binding [21]
GO:0061629 RNA polymerase II-specific DNA-binding transcription factor binding [46]
4
8
12
16
Enrichment Score (-log10(Pvalue))
Count
400
800
1200
Pvalue
2e-05
1e-05

Figure 2 .GO analysis of the differentially expressed circRNA target genes in the hip joint capsule of AS and femoral neck fracture. **A.**Up-regulate the maximum biological process classification of target genes; **B.** Up-regulate the cellular component classification of target genes; **C.** Up-regulate the molecular functional classification of target genes; **D.** The maximum biological process of down-regulation of target gene; **E.** Cell composition analysis of down-regulated target gene; **F.** Molecular functional analysis of down-regulated target genes.

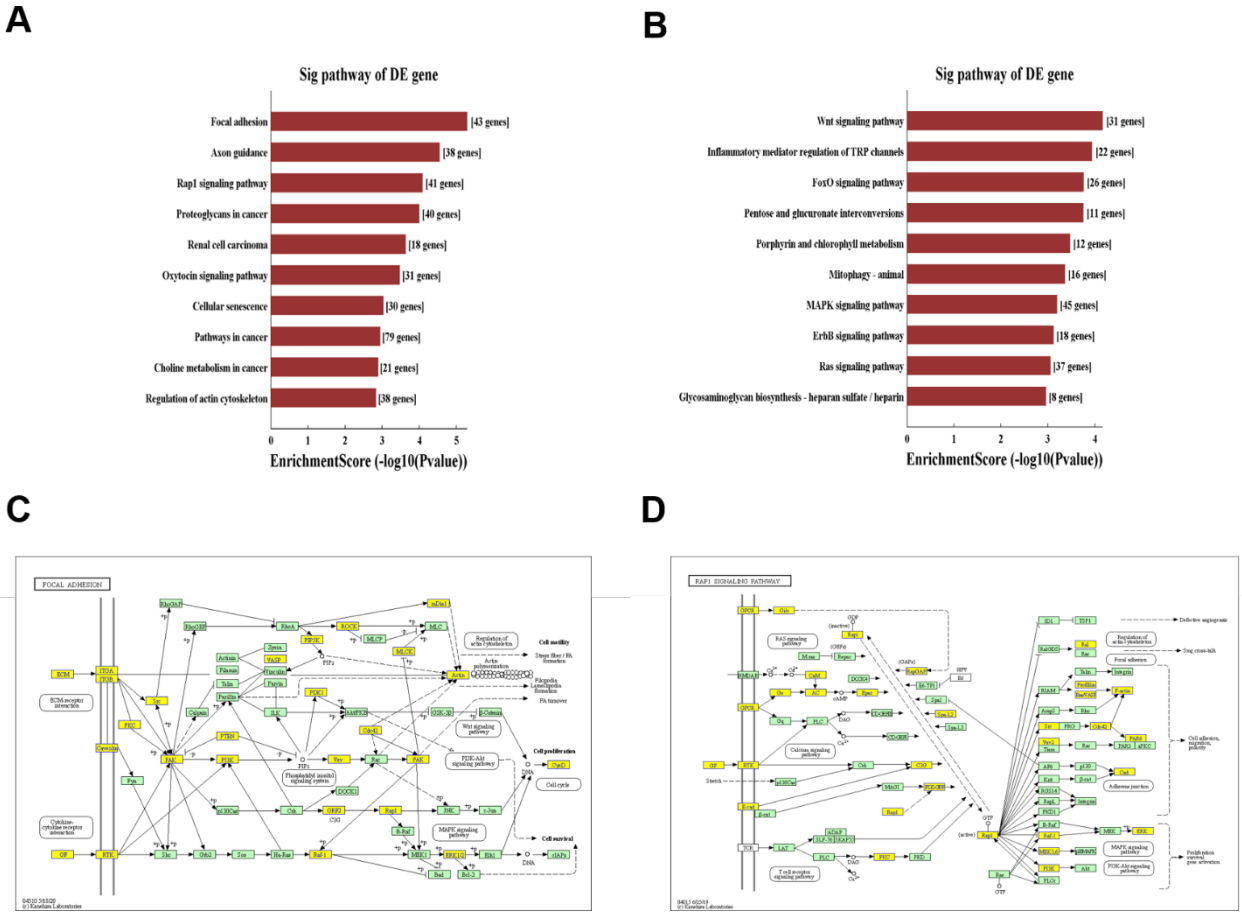


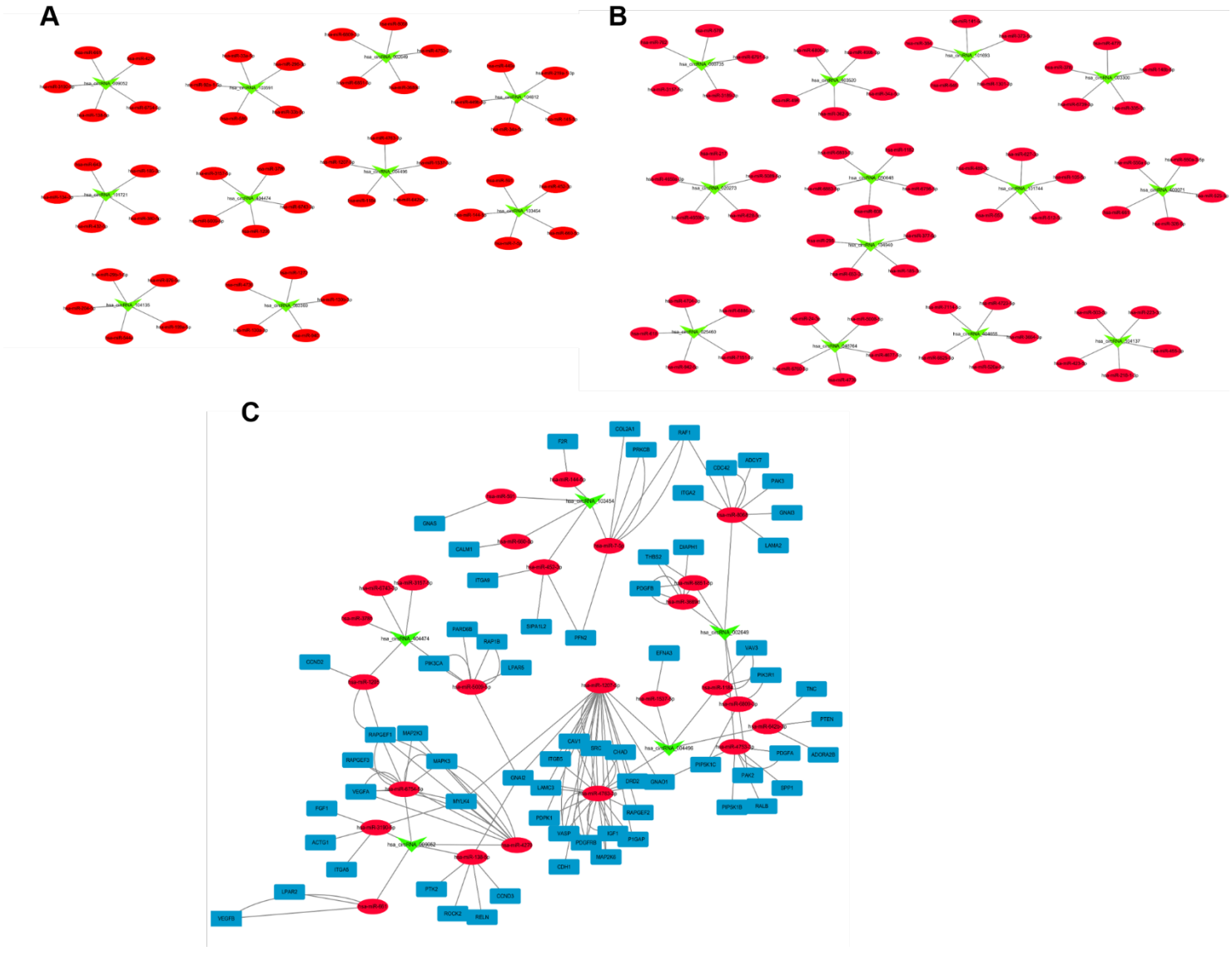
Figure 3 KEGG analysis of differentially expressed circRNA target genes between AS and femoral neck fracture. **A.** Pathway analysis of up-regulated target genes; **B.** Pathway analysis of down-regulated target genes;**C.** focal adhesion kinase signal pathway diagram; **D.**Rap1 signal pathway diagram.(download from KEGG. https://www.genome.jp/kegg/)

Prediction of miRNA

As a sponge of miRNA, circRNA has a large number of miRNA binding sites in the molecular structure. To fully understand the underlying mechanisms of circRNA and pathological bone formation , based on differentially expressed circRNA data, we used a database to predict target miRNAs interacting with circRNA, and Cytoscape was used to construct a circRNA-targeted miRNA gene network map. For a particular miRNA, circRNA has many targets, and the network map illustrates the top five predicted miRNA targets that differentially express circRNA. (Fig. 4A and 4B)

Prediction of the CircRNA/microRNA/mRNA Interaction Network

The ceRNA network is then constructed by using Cytoscape 3.7.0 to the genes



related to focal adhesion kinase signal pathway and Rap1 signal pathway.(Fig. 4C)

Fig.4 miRNA-circRNA network(A and B):Prediction of miRNA sites of differential circRNA adsorption;(A.up-regulation B. down-regulation) C. ceRNA network diagram of FAK and Rap1 signal pathway. CircRNA: green V ; MiRNA: red oval;Gene: blue square. line represents the interaction between circRNAs and miRNAs or miRNAs and mRNA.

Discussion

Ankylosing spondylitis is a rheumatic immune disease, and its pathogenesis is related to the interaction between genes, molecules and signal pathways. Any single gene protein or signal pathway is difficult to explain this complex syndrome. The current diagnostic methods of ankylosing spondylitis are not specific and delayed, moreover the effect of drug treatment is not good, so there is an urgent need for a sensitive and specific diagnostic index to deepen our understanding of the occurrence, development and pathological bone fromation of AS,and it also can provide potential targets for the development of novel diagnostic and therapeutic strategies against AS.

To date, limited studies have been conducted on the interaction between circRNA and miRNA in AS. However, some studies have shown that circRNA is related to the inflammation and the expression of osteoblasts and osteoclasts in rheumatic immune system diseases,such as osteoarthritis and osteoporosis [9]. Has-circ-0005105 competitively binds with miR-26a in chondrocytes and up-regulate the target gene NMPT related to bone formation [10]. CircRNA-0048211 / miRNA-3-5p / BMP2 axia plays a role in the progression of postmenopausal osteoporosis. Its overexpression can significantly up-regulate osteogenesis-related genes in bone marrow mesenchymal stem cells and enhance ALP activity and mineralization ability [11]. CircRNA-33287 may increase the expression of Runx3, Runx2, OSX and ALP through competitive binding with MIR-214-3p [12]. Therefore, we speculated that the difference in circRNAs may be related to the initiation and progression of inflammation in ankylosing spondylitis and local bone destruction and fibrosis formation.

In this study, the microarray data revealed that there were 25 circRNAs up-regulated

and 39 circRNAs down-regulated, among these circRNAs ,there were 10 circRNAs were up-regulated and 13 circRNAs were down-regulated($FC \geqslant 2, P < 0.05$). Further systemic bioinformatics analyses including the circRNA/miRNA/mRNA interaction network, GO and KEGG pathway analysis were used to predict the functions of differentially expressed circRNAs suggesting a potential important role of circRNAs in regulating bone formation. The results showed that these differentially expressed circRNAs have not been reported in ankylosing spondylitis, but the research of miRNA in ankylosing spondylitis and orthopedic related diseases is relatively mature. We found that has-miR-34a, has-miR-503-5p and has-miR-138-5p were associated with mechanical reactive osteogenesis, and we found a competitive combination of circRNA in our circRNA-miRNAmRNA network diagram[13-15], so we inferred that differentially up and down regulated circRNAs were associated with pathological osteogenesis and inflammation.

The GO analysis showed that the differentially expressed circRNAs were mainly related to the development of cell tissue and the regulation of molecular function. According to the pathway analysis of differentially up-regulated circRNAs, we found that it was mainly related to focal adhesion kinase signal pathway and Rap1 pathway, furthermore, we found that integrin and mechanical stimulation promoted osteogenesis-related response may mainly through these two pathways.

Integrins are type I heterodimeric transmembrane protein receptor composed of α and β subunits, which not only mediates the signal from the outside to the inside, but also the signal from the inside to the outside. therefore, integrin is essential for a wide range of cellular functions, including leukocyte homing and activation, cell response to mechanical stress, apoptosis and tumor growth and metastasis [16]. Integrin-mediated cell adhesion to the ECM mediates the formation of mechanosensitive structures,and forces allosterically modulate the functions of mechanosensitive proteins within adhesion structures to induce biochemical signalling cascades, which trigger both rapid changes in cellular mechanics by affecting cytoskeletal dynamics and longterm changes in cell proliferation and differentiation by modulating gene expression. [17]. And ECM

contains integrin-binding Arg-GlyAsp (RGD) sequences, it can affect bone metabolism by changing the response signals of resident cells and the specific cellular responses produced by these signals [18-19].

KEGG pathway analysis showed that FAK signal pathway was highly expressed in hip ossification tissue of patients with ankylosing spondylitis. Focal adhesions are specialized sites within the cell where clustered integrin receptors interact with the extracellular matrix on the outside of cells and with the actin cytoskeleton on the inside,and they also act as scaffolds for many signaling pathways triggered by integrin engagement or mechanical force exerted on cells [20]. In further studies by ShinH et al, as expansion continued at a higher passage,they found that the gene and protein expression levels of focal adhesion complex and small RhoGTPases were up-regulated and chondrocytes lost their inherent differentiation characteristics and transformed into fibroblast-like cells, so inhibition of focal adhesion kinase (FAK) could induce the loss of fibroblast properties and type II collagen in differentiated chondrocytes,so he speculated that FAK and Src-mediated activation of focal adhesion complexes can cause chondrogenic dedifferentiation through phenotypic changes or gene/protein regulation. [21]. In the study of PironeDM et al, it was found that FAK may mediate the proliferation signal initiated by adhesion. FAK seems to transduce not only high adhesion signals, to stimulate proliferation ,but also low adhesion signals, to inhibit growth. This dual role highlights the role of FAK as a central control point for growth regulation and emphasizes its key role in integrating multiple adhesion, mechanical and biochemical functions of focal adhesion [22]. Therefore, we speculated that the adhesion signal pathway is closely related to the late osteogenesis of ankylosing spondylitis.

Rap1 is a telomere-associated protein that not only maintains telomere length and structural integrity, but also regulates the transcription of genes related to insulin secretion, peroxidase and metabolism by binding to non-telomere sites [21,23-25]. Zou W et al proposed that RAP1 can activate integrin aggregation, cell adhesion to bone matrix, related cytoskeleton modification and signal transduction, and transmembrane transduction to promote bone formation. Through experiments, Osteoclast specific

deletion of Rap1(CtsK-Rap1), which promotes talin/β integrin recognition, yields similar osteopetrotic mice[26]. Therefore, we infered that integrin was a potential effect factor of Rap1 in regulating bone resorption.

Through gene prediction, we found that ACTG1 is highly expressed in the Focal adhesion signaling pathway and the Rap1 signaling pathway. The actin γ1 encoded by ACTG1 is a cytoplasmic actin that exists in all cell types and involved in the movement of many types of cells and the maintenance of the cytoskeleton. ACTG1 expression is upregulated and participates in the interaction of ECM-receptors and plays an active role in the apoptosis of human chondrocyte [27-29]. The experiments of Shimohira T et al also confirmed that YAG laser ablation of bone can regulate the expression of BCAR1 and ACTG1, which is the main regulator of mechanical transduction in bone tissue, which is helpful to bone healing [30]. By enriching and analyzing the gene expression profiles of chondrocytes and fibroblasts in osteoarthritis, LiC et al inferred that chondrocytes might up-regulate the expression of COL6A3 and ACTG1 to complete fibroblasts transformation through the focal adhesion pathway [31]. Through KEGG and GO analysis of rheumatoid arthritis gene, Liu J et al found that ACTG1 is related to immune-related cellular processes [32]. Therefore, we speculated that ACTG1 may regulate bone formation in the late stage of ankylosing spondylitis through Focal adhesion signal pathway and Rap1 signal pathway.

Conclusion

This study is the first to explore the role of circRNAS in the initiation and progression of in ankylosing spondylitis. It is found that circRNAs are specifically expressed in the ossified hip joint capsule tissue of ankylosing spondylitis,which provides a new direction for us to understand the etiology, diagnosis, treatment and prognosis of ankylosing spondylitis at the gene level.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

JW,YZ designed the study and analyzed the data; JW drafted the manuscript,and YZ revision of the manuscript.HC,JW processed the samples,DX,HZ,CZ collected the samples.

Ethics approval and consent to participate

The present study was approved by the Ethics Committee of the third Affiliated Hospital of Southern Medical University. Written informed consent was obtained from all patients.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

[1] Mohammadi H, Hemmatzadeh M, Babaie F, et al. MicroRNA implications in the etiopathogenesis of ankylosing spondylitis. J Cell Physiol 2018;233:5564-5573.

[2] Del F A, Teti A, Rucci N. Bone cells and the mechanisms of bone remodelling. Front Biosci (Elite Ed) 2012;4:2302-2321.

[3] Lories RJ, Luyten FP. Bone morphogenetic protein signaling in joint homeostasis and disease. Cytokine Growth Factor Rev 2005;16:287-298.

[4] Carter S, Lories RJ. Osteoporosis: a paradox in ankylosing spondylitis. Curr Osteoporos Rep, 2011,9:112-115.

[5] Starke S, Jost I, Rossbach O, et al. Exon circularization requires canonical splice signals. Cell Rep 2015;10:103-111.

[6] Han YN, Xia SQ, Zhang YY, et al. Circular RNAs: A novel type of biomarker and genetic tools in cancer. Oncotarget 2017;8:64551-64563.

[7] Li F, Zhang L, Li W, et al. Circular RNA ITCH has inhibitory effect on ESCC by suppressing the Wnt/beta-catenin pathway. Oncotarget 2015;6:6001-6013.

[8] Li F, Zhang L, Li W, et al. Circular RNA ITCH has inhibitory effect on ESCC by suppressing the Wnt/beta-catenin pathway. Oncotarget 2015;6:6001-6013.

[9] Qian D Y, Yan GB, Bai B, et al. Differential circRNA expression profiles during the BMP2-induced osteogenic differentiation of MC3T3-E1 cells. Biomed Pharmacother 2017;90:492-499.

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

[11] Qiao L, Li CG, Liu D. CircRNA_0048211 protects postmenopausal osteoporosis through targeting miRNA-93-5p to regulate BMP2.Eur Rev Med Pharmacol Sci 2020;24:3459-3466.

[12] Peng W, Zhu S, Chen J, et al. Hsa_circRNA_33287 promotes the osteogenic differentiation of maxillary sinus membrane stem cells via miR-214-3p/Runx3.Biomed Pharmacother 2019;109:1709-1717.

[13] Chen L, Holmstrom K, Qiu W, et al. MicroRNA-34a inhibits osteoblast differentiation and in vivo bone formation of human stromal stem cells. Stem Cells 2014;32:902-912.

[14] Liu L, Liu M, Li R, et al. MicroRNA-503-5p inhibits stretch-induced osteogenic differentiation and bone formation. Cell Biol Int 2017;41:112-123.

[15] Chen Z, Zhao F, Liang C, et al. Silencing of miR-138-5p sensitizes bone anabolic action to mechanical stimuli. Theranostics 2020;10:12263-12278.

[16] Hynes RO.Integrins:bidirectional,allosteric signaling machines.Cell 2002;110:673687.

[17] Sun Z, Costell M, Fassler R. Integrin activation by talin, kindlin and mechanical forces. Nat Cell Biol 2019;21:25-31.

[18] Wrighton PJ, Klim JR, Hernandez BA, et al. Signals from the surface modulate differentiation of human pluripotent stem cells through glycosaminoglycans and integrins. Proc Natl Acad Sci USA 2014;111:18126-18131.

[19] Zimmerman D, Jin F, Leboy P, et al. Impaired bone formation in transgenic mice resulting from altered integrin function in osteoblasts. Dev Biol 2000;220:2-15.

[20] Burridge K. Focal adhesions: a personal perspective on a half century of progress. FEBS J 2017;284:3355-3361.

[21] Shin H, Lee MN, Choung JS, et al. Focal Adhesion Assembly Induces Phenotypic Changes and Dedifferentiation in Chondrocytes. J Cell Physiol 2016;231:1822-1831.

[22] Pirone DM, Liu WF, Ruiz SA, et al. An inhibitory role for FAK in regulating proliferation: a link between limited adhesion and RhoA-ROCK signaling. J Cell Biol 2006;174:277-288.

[23] Canto C, Auwerx J. PGC-1alpha, SIRT1 and AMPK, an energy sensing network that controls energy expenditure. Curr Opin Lipidol 2009;20:98-105.

[24] Martinez P, Gomez-Lopez G, Garcia F, et al. RAP1 protects from obesity through its extratelomeric role regulating gene expression. Cell Rep 2013;3:2059-2074.

[25] Yeung F, Ramirez CM, Mateos-Gomez PA, et al. Nontelomeric role for Rap1 in regulating metabolism and protecting against obesity. Cell Rep 2013;3:1847-1856.

[26] Zou W, Izawa T, Zhu T, et al. Talin1 and Rap1 are critical for osteoclast function. Mol Cell Biol 2013;33:830-844.

[27] Bunnell T M, Burbach B J, Shimizu Y, et al. beta-Actin specifically controls cell growth, migration, and the G-actin pool. Mol Biol Cell 2011;22:4047-4058.

[28] Shum MS, Pasquier E, Po'Uha ST, et al. gamma-Actin regulates cell migration and modulates the ROCK signaling pathway. FASEB J 2011;25:4423-4433.

[29] Tsolis KC, Bei ES, Papathanasiou I, et al. Comparative proteomic analysis of hypertrophic chondrocytes in osteoarthritis. Clin Proteomics 2015;12:12.

[30] Shimohira T, Katagiri S, Ohsugi Y, et al. Comprehensive and Sequential Gene Expression Analysis of Bone Healing Process Following Er:YAG Laser Ablation. Photobiomodul Photomed Laser Surg 2021;39:100-112.

[31] Li C, Luo J, Xu X, et al. Single cell sequencing revealed the underlying pathogenesis of the development of

osteoarthritis. Gene, 2020,Oct 5;757:144939.

[32] Liu J, Chen N. A9 mRNAs-based diagnostic signature for rheumatoid arthritis by integrating bioinformatic analysis and machine-learning. J Orthop Surg Res 2021;16:44.