Genome-wide expression and methylation profiling reveal candidate genes in osteoarthritis

Y. Fan, J. Chen, Y. Yang, J. Lin, Z. Wu

Department of Orthopaedics, Peking Union Medical College Hospital, Peking Union Medical College and Chinese Academy of Medical Sciences, Beijing, P.R. China.

Abstract

Objective

Osteoarthritis (OA) is a common degenerative disease of the synovial joints. Although numerous studies have been performed, the aetiology of OA remains unclear. Evidence suggests that DNA methylation plays important roles in OA.

Methods

Integrated analysis of five gene expression and one methylation profilings in OA was performed to identify differentially expressed genes (DEGs) and differentially methylated genes (DMGs), respectively. Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment were then conducted to reveal the biological functions of DEGs and DMGs. The protein-protein interaction network was finally constructed.

Results

A set of 500 DEGs and 1219 DMGs in OA was found when compared with normal tissues. Function analysis of DEGs and DMGs revealed 3 critical OA-related pathways. A total of 20 DEGs were screened whose expressions showed strongly negative correlations with DNA methylation levels. Among them, 4 up-regulated DEGs (BST2, HDAC4, ITGB2 and VCAM1) may be closely related to the pathogenesis of OA.

Conclusion

The results of integrated analysis explored 3 OA-related pathways (rheumatoid arthritis, osteoclast differentiation and ECM-receptor interaction) and 4 candidate genes of OA (BST2, HDAC4, ITGB2 and VCAM1) that may be therapeutic targets.

Key words

osteoarthritis, mRNA expression, DNA methylation, integrated analysis

*Yu Fan, PhD**
*Jun Chen, PhD**
Yuemei Yang, PhD
Jin Lin, MD
Zhihong Wu, MD

**These authors contributed equally to this study.*
This work was carried out at the Department of Orthopaedics, Peking Union Medical College Hospital.

Please address correspondence to:
Dr Jin Lin,
Department of Orthopaedics,
Peking Union Medical College Hospital,
Peking Union Medical College and Chinese Academy of Medical Sciences,
No. 1 Shuaifuyuan,
Beijing 100730, P.R.China.
E-mail: linjinpumch@sina.com

and to: Zhihong Wu
E-mail: gukebz@126.com

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Introduction

Osteoarthritis (OA) is a common degenerative disease of the synovial joints (1), and is characterised by progressive loss of articular cartilage and subchondral bone changes, leading to joint pain, stiffness, joint deformity and functional limitation (2). It was reported that OA can affect 250 million people worldwide and thus resulting in a great economic burden (3, 4). Although the prevalence of severe OA which requires joint replacement (5) is increasing, there have been no disease-modifying agents approved for the treatment of OA.

The major risk factors for OA include aging, gender, obesity, previous joint injury, malalignments, genetic predisposition and environment (6, 7). Although numerous studies have been performed**,** the aetiology of OA remains unclear. Currently, it is believed that the extracellular matrix (ECM) of cartilage undergoes constant remodelling (8, 9) with the balance between catabolism and anabolism of chondrocyte molecules. Once the chondrocytes suffer a phenotypic change, an imbalance of cartilage homeostasis occurs toward overall matrix degradation, contributing to the occurrence of OA (10).

Ongoing research attempts to define a group of molecular biomarkers, with the greatest potential for future application in clinical practice. A recent review summarised the utility of biomarkers in the pathophysiology of hip OA (11). In total, 33 different biomarkers were demonstrated of associations with hip OA. Sixteen studies and 16 biomarkers were characterised as relevant to diagnosis and disease staging, respectively, in which carboxy terminal peptide of human type II collagen (CTX-II) (12-14) and C-reactive protein (CRP) (15-17) were supported by more evidences. In addition, six biomarkers were associated with hip OA prognosis, in which cartilage oligomeric matrix protein (COMP) may be more important (18-20).

Epigenetics is a common mechanism whereby organisms alter gene expression in response to both external and internal environmental cues. It has been hypothesised that epigenetic alterations in chondrocytes could be a key driver of OA pathogenesis (21, 22). As the most extensively studied epigenetic regulator, DNA methylation plays a key role in the onset and progression of common diseases by providing a link between genetic and environmental risk factors (23). Researches have concerned the DNA methylation in articular cartilage of OA, and a number of CpG sites and genes have been reported to be involved in potential aetiologic mechanisms of OA (24-26). Recently, an integrated study of gene expression and methylation profile of OA was made to investigate the pathogeny of OA at multi-omic levels (27).

In the present study, we performed integrated analysis for mRNA and DNA methylation profiles of OA, which were from Gene Expression Omnibus (GEO) database. The differentially expressed genes (DEGs) and differentially methylated genes (DMGs) in OA were screened respectively when compared with the normal tissues. Functional enrichment analysis was then performed and potentially OA-related pathways were predicted. Moreover, integrated analysis of DEGs and DMGs were performed and the candidate genes of OA were identified.

Materials and methods

Gene expression and DNA methylation profiles of OA

In order to explore alternations in gene expression and epigenetics, the GEO database (28) was used to obtain mRNA and DNA methylation profiles of OA. "Osteoarthritis" and "homo sapiens" were used as the keywords and the study type was defined as "expression profiling by array" or "methylation profiling by array". The case and control groups were selected by researchers from different countries according to the inclusion exclusion criteria of the database. There were total 63 controls and 111 cases screened into our scope of research, they were from Spain, Australia, Belgium, Germany, USA and United Kingdom.

Screening of DEGs and DMGs

The mRNA expression profiles were initially preprocessed by background

Table I. The eligible mRNA expression and DNA methylation profiles of OA used in the study.
There were five mRNA expression profiles (GSE29746, GSE41038, GSE46750, GSE55235 and GSE64394) and one DNA methylation profile of OA (GSE63695) met the inclusion exclusion criteria, then they were downloaded for further analysis.

	GEO ID	Platform	control: case	Country	Time
mRNA expression profiles	GSE29746	GPL4133 agilent-014850 whole human genome microarray 4x 44K G4112F (feature number version)	11:11	Spain	2011
	GSE41038	GPL6883 illumina human ref-8 v. 3.0 expression beadchip	4:3	Australia	2012
	GSE46750	GPL10558 illumina human HT- 12 v. 4.0 expression beadchip	12:12	Belgium	2013
	GSE55235	GPL96 [HG-U133A] Affymetrix human genome U133A array	10:10	Germany	2014
	GSE64394	GPL6244 [HuGene-1_0-st] Affymetrix human gene 1.0 ST array	5:2	USA	2015
DNA methylation profile	GSE63695	GPL13534 illumina human methylation 450 (beadchip (human methylation450_15017482)	21:73	United Kingdom	2014

GEO: Gene expression omnibus

correction and normalisation. Gene expression level of each gene was calculated by *t*-test with limma package in R, and the differentially methylated levels were compared with City of Hope CpG Island Analysis Pipeline (COHCAP) package. The false discovery rate (FDR) was also obtained using the Benjamini-Hochberg method. All the analyses were performed between normal and OA samples and genes with FDR of less 0.05 were considered as DEGs and DMGs.

Functional annotation of DEGs and DMGs

The database for annotation, visualisation, and integrated discovery (DAVID) (16) bioinformatics resource provides an integrated biological knowledgebase to systematically discover biological roles from large gene and protein lists. In this study, DAVID was used to identify the significantly enriched Gene Ontology (GO) categories (29). Kyoto Encyclopedia of Genes and Genomes (KEGG, http://www.genome.jp/kegg/pathway.html) database was used to perform the pathway enrichment (30).

Integrated analysis of DEGs and DMGs

Based on the datasets of DEGs and DMGs, the overlapping genes between DEGs and DMGs were selected. Correlations between gene expression and methylation status were then analysed for each gene. The DEGs whose expression changes showed a strong negative correlation with DNA methylation levels were obtained, and protein-protein interaction (PPI) analysis was performed according to Biological General Repository for Interaction Datasets (BioGRID) (31, 32) and visualised by Cytoscape software (33).

Table II. GO and KEGG pathway analysis of DEGs in OA.
DEGs enriched in the multiple pathways of OA through GO and KEGG pathway analysis. One of the most relevant pathways was cell cycle (p=2.34E-09, FDR=3.23E-07), followed by DNA replication (p=4.98E-07, FDR=3.43E-05).

GO ID	GO Term	Count	*p*-value	FDR
GO:0045995	Regulation of embryonic development	2	2.17E-04	1.00E+00
GO:0032677	Regulation of interleukin-8 production	2	2.87E-04	6.90E-01
GO:2001236	Regulation of extrinsic apoptotic signalling pathway	5	3.34E-04	5.36E-01
GO:0032075	Positive regulation of nuclease activity	2	3.45E-04	4.15E-01
GO:1901681	Sulfur compound binding	8	1.10E-04	1.12E-01
GO:0008201	Heparin binding	6	5.22E-04	2.65E-01
hsa04110	Cell cycle	14	2.34E-09	3.23E-07
hsa03030	DNA replication	7	4.98E-07	3.43E-05
hsa05140	Leishmaniasis	5	9.10E-06	1.79E-04
hsa05323	Rheumatoid arthritis	8	2.47E-05	3.10E-04
hsa04380	Osteoclast differentiation	8	0.000426	0.001897
hsa04512	ECM-receptor interaction	6	0.001207	0.003332

GO: gene ontology; KEGG: Kyoto Encyclopedia of Genes and Genomes; DEGs: differentially expressed genes; OA: osteoarthritis; FDR: false discovery rate.

Results

DEGs and DMGs screening

Based on the inclusion criteria, five mRNA expression profiles (GSE29746, GSE41038, GSE46750, GSE55235 and GSE64394) and one DNA methylation profile of OA (GSE63695) were downloaded. There were 42 controls and 38 cases in the mRNA expression profiles, and 21 controls and 73 cases in the DNA methylation profile. The characteristics of each study for integrated analysis were summarised in Table I.

In total, 500 DEGs were screened in the mRNA expression profiles, which include 83 up-regulated and 417 down-regulated genes. The most significantly up- and down-regulated genes were ITGB2 (FDR = 3.24E-04) and HSPA1A (FDR = 2.28E-05), respectively. The hierarchical clustering analysis indicated that the DEGs in OA were distinguished from that in normal tissues (Fig. 1). Additionally, 2725 differentially methylated sites (1636 hyper methylated and 1089 hypo methylated positions) were identified, which were distributed in 1219 DMGs.

Functional enrichment of DEGs and DMGs

The GO enrichment showed that the top three enriched biological process terms for DEGs were regulation of embryonic development, regulation of interleukin-8 (IL-8) production and regu-

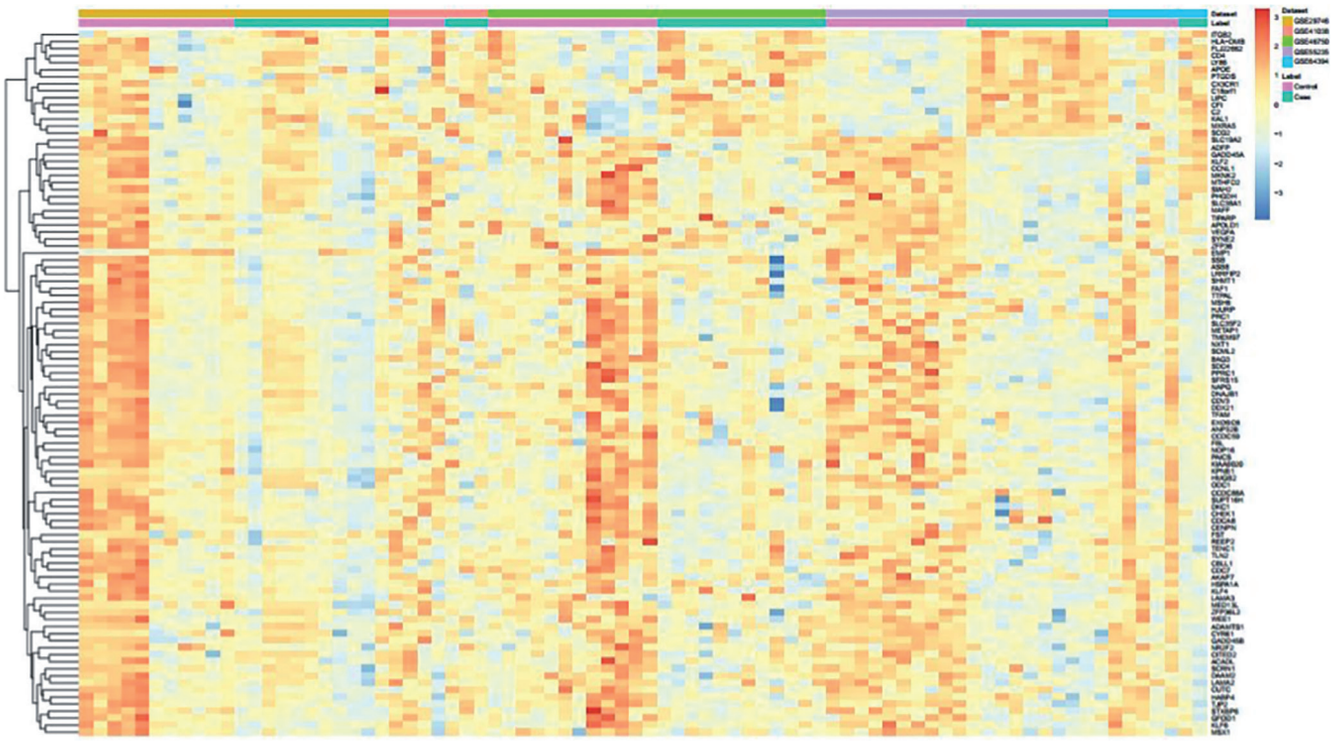

Fig. 1. Hierarchical clustering of top 100 DEGs among five mRNA expression datasets of OA.

lation of extrinsic apoptotic signalling pathway. The enriched molecular function terms for DEGs were sulfur compound binding and heparin binding. For DMGs, they were mainly enriched in the biological process of negative regulation of transforming growth factor beta receptor signalling pathway and negative regulation of cellular response to transforming growth factor beta stimulus, and mostly involved in the molecular function of tumour necrosis actor-activated receptor activity and death receptor activity.

Table III. GO and KEGG pathway analysis of DMGs in OA.
GO and KEGG were used to analyse DMGs involved in pathogenesis of OA. Among them, focal adhesion in the first place (p=9.45E-08, FDR=3.26E-05). Other signalling pathway included negative regulation of transforming growth factor beta receptor signalling pathway, organelle part, death receptor activity, MAPK signalling pathway, ECM-receptor interaction, Osteoclast differentiation, rheumatoid arthritis, etc.

ID	Term	Count	p-value	FDR
GO:0030512	Negative regulation of transforming growth factor Beta receptor signalling pathway	2	9.50E-04	1.00E+00
GO:1903845	Negative regulation of cellular response to transforming growth factor beta stimulus	2	9.50E-04	1.00E+00
GO:0044446	Intracellular organelle part	167	5.91E-04	4.11E-01
GO:0044422	Organelle part	171	7.14E-04	2.48E-01
GO:0005031	Tumour necrosis factor-activated receptor activity	2	9.92E-05	1.40E-01
GO:0005035	Death receptor activity	2	9.92E-05	7.01E-02
hsa04510	Focal adhesion	24	9.45E-08	3.26E-05
hsa04010	MAPK signalling pathway	26	1.59E-06	1.83E-04
hsa05200	Pathways in cancer	30	1.12E-06	1.94E-04
hsa04512	ECM-receptor interaction	12	3.07E-05	0.00106
hsa04380	Osteoclast differentiation	13	0.00042	0.004461
hsa05323	Rheumatoid arthritis	7	0.02600	0.044639

GO: gene ontology; KEGG: Kyoto Encyclopedia of Genes and Genomes; DEGs: differentially expressed genes; FDR: false discovery rate.

As shown in Table II and III, the cell cycle was the most significantly enriched pathway in KEGG analysis for DEGs, while for DMGs it was focal adhesion. Moreover, rheumatoid arthritis, osteoclast differentiation and ECM-receptor interaction were observed in both DEGs and DMGs. In the pathway of rheumatoid arthritis, eight DEGs including matrix metalloproteinase-3 (MMP3), Integrin Beta 2 (ITGB2), transforming growth factor beta-3 (TGFB3), HLA class II histocompatibility antigen, DP (W2) beta chain (HLA-DPB1), jun-activation-domain binding protein-1 (JUN), human stromal cell-derived factor-1 (CXCL12), vascular endothelial growth factor A (VEGFA) and HLA class II histocompatibility antigen, DM beta chain (HLA-DMB) were enriched, and seven DEGs including ITGB2, CD86, IL18, TGFB1, CCL5, Cathepsin K (CTSK) and tumour necrosis factor ligand superfamily member 11 (TNFSF11) were obtained (Fig. 2).

Methylation of DEGs

There were 20 overlapping genes, whose DNA methylation level showed a strong negative correlation with

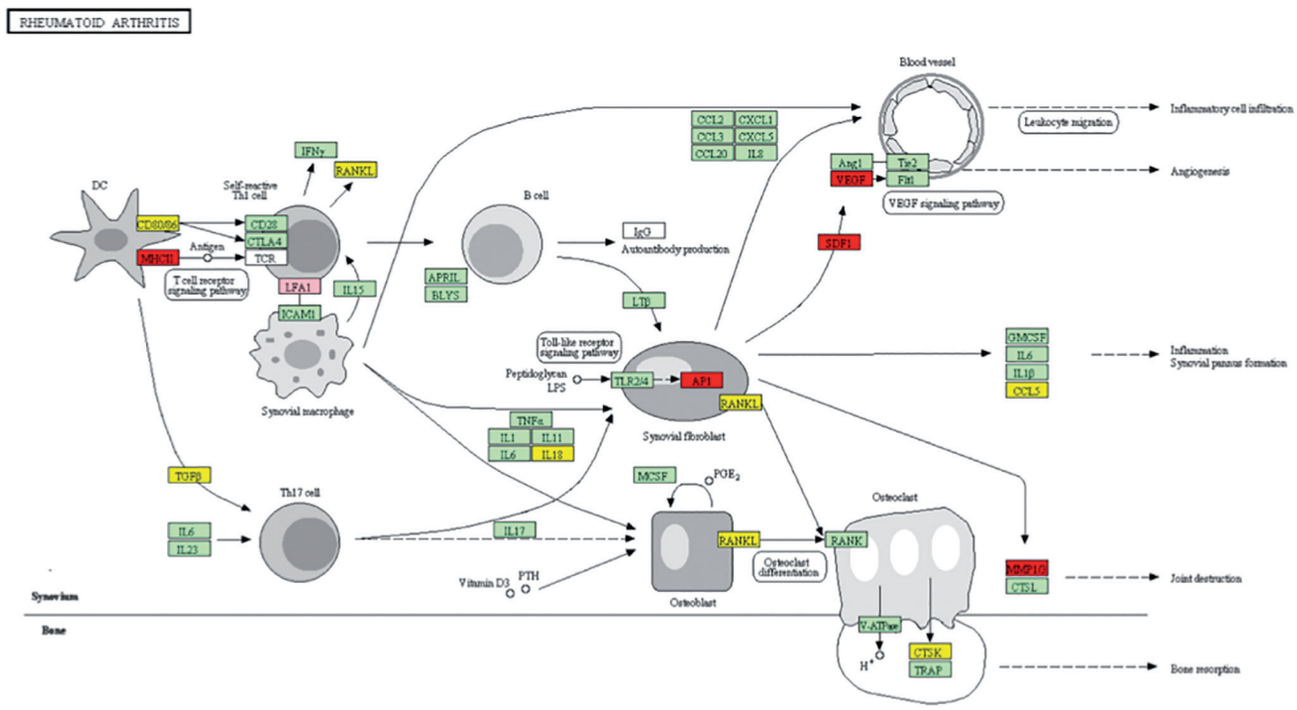

Fig. 2. DEGs and DMGs enriched in the pathway of rheumatoid arthritis of homo sapiens. Red indicates DEGs; Yellow indicates DMGs; Pink indicates the gene was both differentially expressed and differentially methylated. LFA1 is alias of ITGB2. The pathway map was obtained from http://www.genome.jp /kegg/tool/map_pathway2.html.

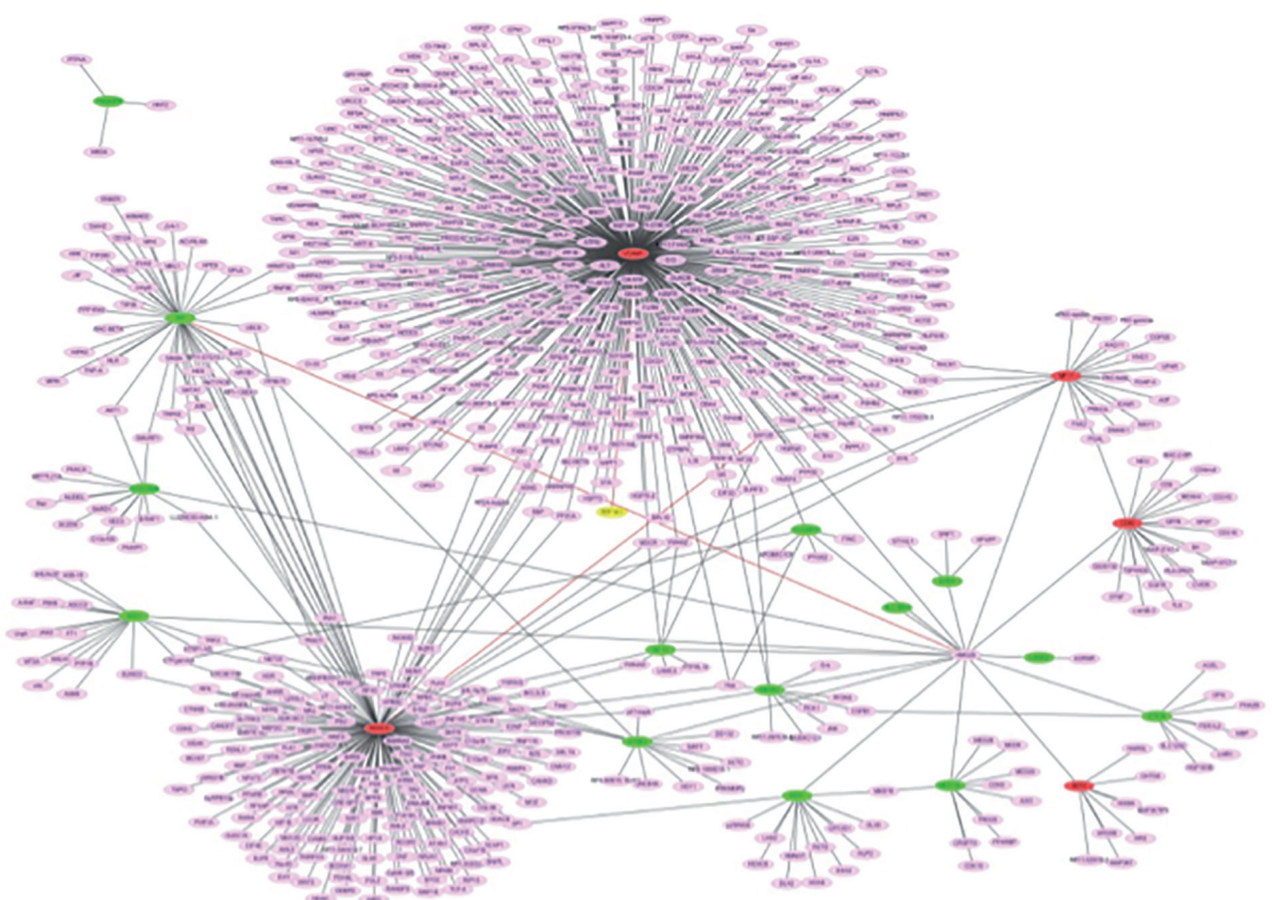

Fig. 3. PPI network for the 20 DEGs whose expression change showed negative correlation with DNA methylation level. Red indicates up-regulated DEGs; Green indicates down-regulated DEGs.

Table IV. DEGs whose expression showed negative correlations with DNA methylation levels. There were 20 overlapping genes between 500 DEGs and 1219 DMGs, including 5 up-regulated and 15 down-regulated. The overlapping genes methylation level showed a strong negative correlation with gene expression changes.

Gene	Fold Change	FDR	CpG ID	CpG Island	Δβ	FDR
Up-regulation/Hypomethylated						
BST2	1.7593	2.36E-02	cg20092122	False	-0.2006	4.53E-06
CD82	1.3854	3.46E-02	cg21108085	True	-0.1070	7.77E-03
HDAC4	1.1307	4.64E-02	cg19449565	False	-0.2381	8.08E-06
			cg20298668	False	-0.1506	1.89E-04
			cg17356718	False	-0.1962	3.63E-07
			cg05171197	False	-0.1643	3.12E-06
			cg27587095	True	-0.1628	4.65E-07
ITGB2	2.7625	3.24E-04	cg22699620	True	-0.1075	9.09E-08
VCAM1	2.0423	1.97E-02	cg27544046	False	-0.1410	3.78E-07
			cg04743650	False	-0.1338	7.36E-04
Down-regulation/Hypermethylated						
ASS1	-1.2936	4.40E-02	cg14010829	True	0.1762	1.19E-05
CCDC88A	-1.4455	8.43E-03	cg13359689	False	0.1707	4.48E-10
CLSTN2	-1.7200	3.95E-02	cg01995815	False	0.1470	1.35E-10
CUGBP2	-1.5753	2.85E-02	cg06352464	False	0.1805	5.17E-04
			cg12756527	False	0.1950	1.13E-08
EHD4	-1.4212	3.90E-02	cg04614155	True	0.2061	1.36E-13
KIF1C	-1.4296	1.74E-02	cg03877174	False	0.1489	2.75E-07
LAMA2	-2.2373	6.03E-05	cg23621912	False	0.1243	8.28E-08
MED13L	-1.3012	1.27E-03	cg20777829	False	0.1065	7.21E-04
MKNK2	-1.8872	7.75E-04	cg00434573	True	0.1392	4.87E-11
MSX1	-1.7505	6.19E-03	cg21538208	True	0.1461	1.20E-08
SKI	-1.4721	1.67E-02	cg04619852	True	0.1106	1.25E-06
SLC38A1	-1.5089	8.08E-03	cg17090968	True	0.1110	3.08E-06
			cg26018685	True	0.1373	3.64E-09
STK39	-1.4034	1.46E-02	cg13431028	False	0.1154	1.35E-03
			cg03351487	False	0.1556	8.80E-07
			cg13345122	False	0.1555	3.27E-04
			cg11775828	True	0.1645	1.97E-08
SYNE2	-1.7820	1.76E-03	cg19987354	False	0.1362	4.68E-05
TNFAIP8	-1.3253	4.84E-02	cg07086380	True	0.1318	1.64E-05

β: absolute difference in methylation value between sample groups (β_{case}-$\beta_{control}$); False (CpG island): CpG site is not in CpG island; True (CpG island): CpG site is in CpG island.

gene expression changes, between 500 DEGs and 1219 DMGs (Table IV). In the 20 genes, 5 were up-regulated, including BST2, CD82, histone deacetylase 4 (HDAC4), ITGB2 and vascular cell adhesion molecule 1 (VCAM1) and 15 were down-regulated including argininosuccinate synthase 1(ASS1), human girdin (CCDC88A), calsyntenin 2 (CLSTN2), elav-like family member 2(CUGBP2), Eps15 homology domain-containing protein 4 (EHD4), kinesin-like protein (KIF1C), laminin alpha 2 (LAMA2), mediator complex subunit 13-Like (MED13L), MAP kinase-interacting serine/threonine-protein kinase 2 (MKNK2), Msh homeobox 1(MSX1), Ski_oncogene(SKI), sodium-coupled neutral amino acid transporter 1 (SLC38A1), STE20/SPS1-related proline-alanine-rich protein kinase (STK39), nuclear envelope 2 (SYNE2) and tumour necrosis factor α inducible protein 8 (TNFAIP8). PPI network analysis showed that it contained 755 nodes and 831 edges. The significant hub proteins contained VCAM1 (degree=438), HDAC4 (degree=179) and SKI (degree=43) (Fig. 3).

Discussion

This study was designed to explore the gene expression and epigenetic alternations in OA. In total, 500 DEGs (83 up-regulated and 417 down-regulated) and 1219 DMGs were identified in OA compared with normal tissues. The functional enrichment of DEGs and DMGs revealed three potentially OA-related pathways, including rheumatoid arthritis, osteoclast differentiation and ECM-receptor interaction.

The pathway of rheumatoid arthritis is involved in the chronic autoimmune joint disease where persistent inflammation affects bone remodelling, leading to progressive bone destruction. The pathway of osteoclast differentiation is related to bone resorption. The pathway of ECM-receptor interaction serves an important role in tissue and organ morphogenesis and involves in the maintenance of cell and tissue structure and function. Studies have reported constant remodelling of ECM in cartilage for maintaining the balance between anabolic and catabolic factors, and the phenotypic change of chondrocytes could lead to imbalance of cartilage homeostasis toward overall matrix degradation in OA (8). Our study suggested that these three pathways may play important roles in the pathogenesis of OA.

Integrated analysis of DEGs and DMGs revealed that there were 20 DEGs, whose expression showed strong negative correlations with DNA methylation levels, including 5 up-regulated genes and 15 down-regulated genes. In which several genes were closely related to the pathogenesis of OA, such as BST2, HDAC4, ITGB2, VCAM1 and MSX1.

ITGB2, also known as CD18 or LFA1, plays a crucial role in the immune system (34). A previous study reported that CD11a/CD18 is shed from leukocytes in inflammatory models (35). A recent study reported that the shedding of human CD11/CD18 complexes was involved in the synovial inflammation of rheumatoid arthritis and spondyloarthritis. In addition, soluble adhesion molecules CD11/CD18 were considered as factors balancing chronic inflammation in arthritis and likely as the contributors to disease activity (36). In the present study, we found that ITGB2 was differentially expressed as well as methylated which significantly enriched in the pathway of rheumatoid arthritis. These results may suggest to us the possible critical roles of ITGB2 in OA.

BST2 is a human membrane protein, which can be expressed in bone marrow stromal cell lines and synovial cell lines. Ishikawa and collegues cloned a cDNA encoding BST2 from a rheu-

matoid arthritis-derived synovial cell line and suggested that BST2 may be involved in pre-B-cell growth (37).In this study, we found that BST2 was up-regulated in OA compared with normal tissues, implying that BST2 may possibly participate in the immune reaction process of OA.

As a key regulator of chondrocyte hypertrophy during skeletogenesis of mice, up-regulated HDAC4 was also observed in human OA cartilage, and it is believed that inhibition of HDAC4 might be of benefit to prevent cartilage destruction *in vitro* (38). Our study confirmed up-regulated HDAC4 in OA and provided additional evidence for its association with OA pathogenesis, indicating that HDAC4 might be a powerful pharmaceutical target for the therapeutic interventions of OA.

It has been reported that VCAM1 is involved in osteoclasts. Microarray gene expression profiling revealed differentially expressed VCAM1 between females and males in OA bone, which may partially explain the sex disparity observed in OA (39). In addition, it was proposed that co-expression of Runx1, VCAM1 and lubricant in OA cartilage participates in a cooperative mechanism for a compensatory anabolic function (40). We found that VCAM1 was up-regulated in OA compared with normal tissues, suggesting its role in the bone remodelling of OA.

Additionally, the homeobox gene MSX1 encodes a transcription factor that is highly expressed during embryogenesis and postnatal development in bone (41). A previous study of genome-wide expression profiling in OA showed that MSX1 displays significantly higher expression in OA cartilage than that in normal donor cartilage, which is involved in bone formation (42). However, in the current study MSX1 was found to be down-regulated in OA compared with normal tissues. Further study should be performed to confirm its expression levels and roles in OA.

In conclusion, integrated analysis of mRNA expression profiling and DNA methylation profiling in OA revealed a set of 500 DEGs and 1219 DMGs. Functional analysis explored several key pathways which may be of great importance in the pathogenesis of OA, such as rheumatoid arthritis, osteoclast differentiation and ECM-receptor interaction. In addition, four candidate genes were identified in OA (BST2, HDAC4, ITGB2 and VCAM1), which were all up-regulated and hypomethylated. These results could help to elucidate the pathogenesis of OA and move the field towards novel therapeutic strategies.

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