European Review for Medical and Pharmacological Sciences 2014; 18: 3056-3062

Exploring the osteoarthritis-related genes by gene expression analysis

Z.-T. RAO, S.-Q. WANG, J.-Q. WANG

Department of Orthopaedic Surgery, Tongji Hospital, Tongji University School of Medicine, Shanghai, China

Abstract. – OBJECTIVE: Osteoarthritis (OA) is a most common chronic degenerative joint lesion, which affects both cartilage and bone. A better understanding of the gene expression profiling of OA may help understanding the pathogenesis of OA and finding the therapy targets for OA treatment.

MATERIALS AND METHODS: GSE8077 was downloaded from Gene Expression Omnibus (GEO) including 5 OA rats induced by anterior cruciate ligament transection and partial medial meniscectomy and 5 rats that were performed sham surgery as control. Differentially expressed genes (DEGs) between OA group and control group were identified by t-test with $p < 0.05$ and the coding genes that transcription factors corresponded were screened by TRANSFAC. Then Gene ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis for DEGs and transcription factors were performed. The DEGs and transcription factors were integrated with information from STRING database to construct PPI network.

RESULTS: A total of 119 up-regulated genes, 39 down-regulated genes and 9 transcription factors were identified in OA sample. The GO enrichment analysis showed that 119 up-regulated genes were significantly enriched in blood vessel development and KEGG pathway enrichment showed that genes were involved in circadian rhythm pathway. In the PPI network, Cd44, Mmp13, Timp1 and Igf1 showed higher degrees.

CONCLUSIONS: The screened genes could provide a new and comprehensive view for treatment of OA.

Key Words:
Osteoarthritis, Differentially expressed genes, Microarray, PPI network.

Introduction

Osteoarthritis (OA), also called degenerative joint disease, causes inflammation of one or more joints in the body and is the most common form of arthritis. OA is a serious threat to human health and quality of life, which affects more than 270 million people, making it a leading cause of disability in adults[1]. Pain and stiffness in the joints are the most common symptoms, and joints of patients may become stiffer and harder to move over time[2]. Thus, it is necessary to elucidate the pathogenesis of OA for treatments.

The etiology and pathogenesis of OA have not been elucidated. It has been reported that articular cartilage and cytokines played important roles in occurrence and development of OA[3]. Recent studies indicated that degeneration of articular cartilage was the root cause of OA[4]. Ma et al[5] demonstrated that *B3GNT9*, *MAN2A1*, *ALG8*, *SERP1*, a cluster of genes related with protein glycosylation, were down-regulated in end-stage OA, contributing to the degradation of cartilage. The severity of OA was closely related to the synthesis and secretion of matrix degradation enzymes[6]. Ehrlich and his colleagues confirmed that collagenase of cartilage with OA was significantly increased, indicating that this enzyme was the major factor in disease progression[7]. The Interleukin 1 (*IL1*) is the main driving factor of cartilage matrix degradation, which promotes synthesis and secretion of other degrading enzymes, including collagenase, stromelysin, gelatinase and tissue-type plasminogen activator[8]. The balance of matrix degradation enzymes relies on two enzymes inhibitors, matrix metalloproteinase (*MMP*) and tissue inhibitor of metalloproteinase (*TIMP*), which can limit the activity of neutral metalloprotease and plasminogen activator[9]. Luo et al[10] indicated that RARA (retinoic acid receptor, alpha) which plays an important role in regulation of cytokine and MMP production could be a potential target of therapeutic intervention in OA and rheumatoid arthritis. Additionally, Zhang et al[11] reported that *PPARG* (peroxisome proliferator-activated receptor gamma) may increase the

Corresponding Author: Shu-Qing Wang, MD; e-mail: wshumotion@163.com

expression of inflammatory and catabolic factors in OA, thus inhibition of *PPARG* expression in chondrocytes by pro-inflammatory cytokines may be an important process in OA pathophysiology.

To explore the key genes in the pathogenesis of OA, animal model was used to mimic the gene expression in human OA and the bioinformatics has been applied to analyze the expression profiling of OA samples[12]. In this study, the microarray data in sham control and OA model rats were selected for differential expression analysis. In addition, Gene ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis were performed for differentially expressed genes (DEGs) and transcription factors (TFs) screening. Finally, to further explore the interaction of DEGs and TFs in OA, they were integrated with information from Search Tool for the Retrieval of Interacting Genes (STRING) database to construct protein-protein interaction (PPI) network, aiming to explore their roles in the occurrence and development of OA.

Materials and Methods

Data Resource

The gene expression of GSE8077[13] was downloaded from Gene Expression Omnibus (GEO, http://www.ncbi.nlm.nih.gov/geo/) of NCBI, which was performed on Affymetrix Rat Genome 230 2.0 Array platform. In the expression profile, 5 rats were induced to OA through anterior cruciate ligament transection and partial medial meniscectomy and 5 rats were performed with sham surgery as control. Gene expression profile in the articular chondrocytes of 10 rats was obtained for further analysis.

Identification of DEGs

Initially, the original data were performed for normalization using Affy package in R language[14], and false discovery rate (FDR) correction was performed by Benjamini & Hochberg (BH) method[15]. Further, the microarray probes were annotated according to the information provided by Brain array lab. Finally, the probe signal of each gene was analyzed using Median Polish method[16] to obtain the expression level of each gene. *t*-test was performed for identifying DEGs between OA group and control group[17] with $p < 0.05$. In addition, the fold change of DEGs was required to be not less than 2. TRANSFAC[18] was used to identify the coding genes which TFs corresponding to.

GO Terms and KEGG Pathways Analysis

The DEGs were performed for GO[19] and KEGG pathway enrichment analysis[20] by DAVID[21] with p-value < 0.05 and gene number ≥ 2. The FDR value was required to be 0.01.

Construction of PPI Network

STRING[22] was applied to construct PPI network according to significantly up-regulated DEGs and down-regulated DEGs. The credible protein interaction information from Text Mining, Database or Experiment was extracted for PPI network construction.

Results

DEGs Identification by Microarray Expression Profiling

Basing on microarray expression profiling of OA samples and control samples, the expression difference between these two groups was screened out. The results showed that 119 genes were up-regulated and 39 genes were down-regulated in OA samples. The ratio of the number of up-regulated genes to number of down-regulated genes was about 3.05:1, which indicated that the expression of genes was aberrant in OA samples (Table I). Further analysis showed that a total of nine TFs were selected from 158 DEGs related to OA, in which *Arntl*, *Hey2*, *Lbp*, *Npas2*, *Pbx3*, *Prrx2* and other TFs were up-regulated, whereas *Dbp*, *Id4* and *Nr1d1* were down-regulated in OA group.

Table I. Statistic information of differentially expressed genes. TF: transcription factor.

	Gene Counts	TF Counts	TF genes
Down-regulated genes in OA	39	3	Dbp, Id4, Nr1d1
Up-regulated genes in OA	119	6	Arntl, Hey2, Lbp, Npas2, Pbx3, Prrx2

The expressions of matrix metalloproteinase (*Mmp3*, *Mmp13*), particular cytokines [(*Il1b* (Interleukin-1 beta), *Il10*, *Igf1* (Insulin-like growth factor 1)] and osteoprotegerin [(*Tnfrsf11b* (tumor necrosis factor receptor superfamily member 11b)] between OA samples and control samples were analyzed. The results showed that *Igf1* was significantly up-regulated in OA group, while the expression of *Igf1r* (*Igf1* receptor) did not change significantly between OA and control groups. In addition, the expressions of *Mmp13* and *Timp1* in OA group were both up-regulated. However, the fold change of *Timp1* between OA samples and control samples was 2.04 times while that of *Mmp13* was 2.60 times, indicating that the homeostasis between *Mmp13* and *Timp1* was broken in OA tissue. The expression levels of *Mmp3*, *Tnfrsf11b*, *Il1b* and *Il10* between OA and control groups were basically the same, suggesting that roles of these genes in OA were limited (Figure 1).

Function Enrichment Analysis of DEGs Related to OA

The GO enrichment analysis showed that 119 up-regulated genes were mainly enriched in blood vessel development, cell migration, inflammatory response and other biological processes (Table II). However the significant functional enrichment was not found in down-regulated genes, which indicated that molecular mechanisms of OA were associated with up-regulated genes.

KEGG pathway enrichment analysis showed that *Npas2* and *Artl* (up-regulated genes) in OA group were involved in circadian rhythm pathway.

Construction of PPI Network

To explore the interaction between OA-related genes, DEGs and TFs screened to be related with OA were integrated with information from STRING database to construct PPI network (Table II). Three separate interaction networks were identified (Figure 2). Figure 2A showed that the node degrees of *Cd44*, *Il1b, Mmp13* and *Timp1* were the largest, suggesting that these genes may play central roles in the network. However, the node degree of *Tnfrsf11b* was only three, indicating the role of the gene was limited. Figure 2B showed a network constituted by *Dbp*, *Nr1d1*, *Arntl*, *Hey2*, *Id4* and Figure 2C demonstrated a network consisting of *Mme*, *Anpep*, *Cth*, *Bcat2*, *Tst*, *Eltd1* and *Tnn*.

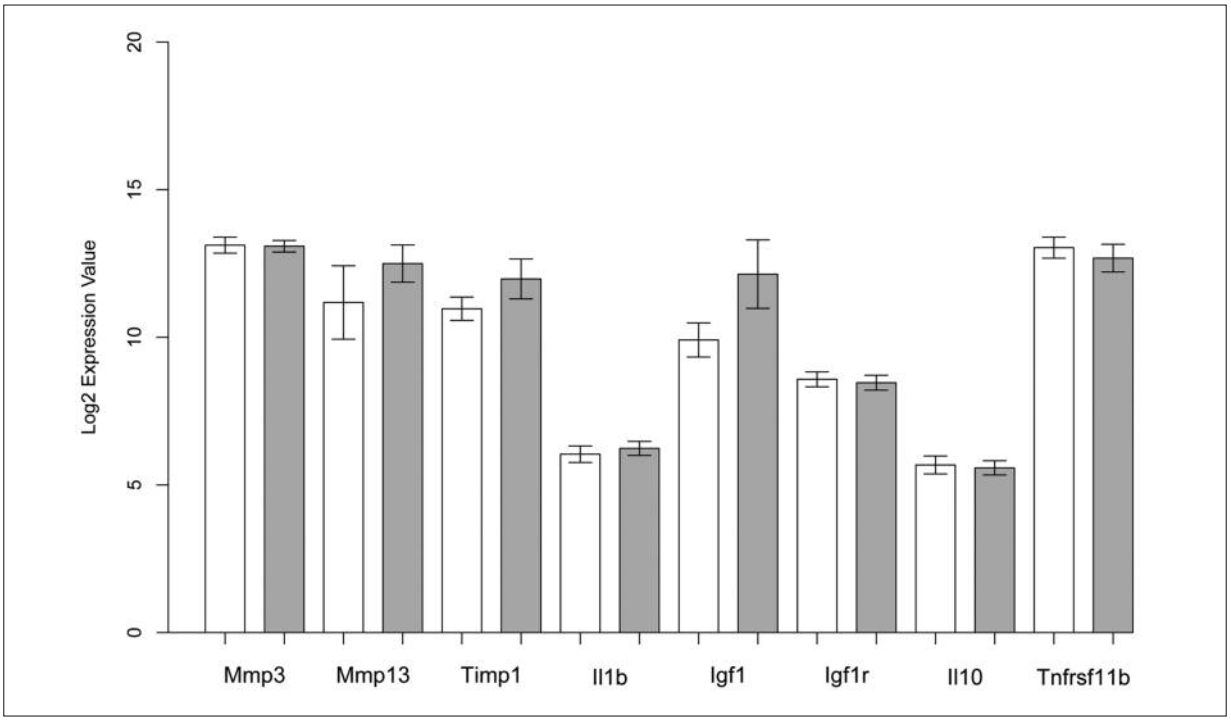

Figure 1. The expression level of osteoarthritis (OA)-related genes based on microarray. The blank columns represent the expression of genes in the sham group. The grey columns represent the expression of genes in the OA group.

Table II. Gene ontology (GO) function enrichment analysis of up-regulated differentially expressed genes in osteoarthritis group.

Term	Count	FDR	Genes
GO:0001568~blood vessel development	14	3.35E-05	EMCN, ANPEP, PRRX2, MMP14, KDR, CDH13, S1PR1, CD44, CXCR4, HMOX1, HEY2, TGFA, COL1A1, ANGPT2
GO:0001525~angiogenesis	10	6.65E-04	CDH13, EMCN, S1PR1, CXCR4, HMOX1, TGFA, ANPEP, MMP14, ANGPT2, KDR
GO:0016477~cell migration	13	0.001054329	CTHRC1, CCL2, TNFRSF12A, MMP14, KDR, CDH13, CD44, CXCR4, FCER1G, HBEGF, TNN, LBP, DCLK1
GO:0006954~inflammatory response	11	0.010128467	CCL2, CD44, C4B, HMOX1, F3, SERPINA1, SERPING1, C1S, C2, LBP, CCL7
GO:0006952~defense response	14	0.01744045	CCL2, C4B, SERPING1, C1S, CD1D1, CD74, CCL7, CD44, HMOX1, F3, FCER1G, SERPINA1, C2, LBP
GO:0048584~positive regulation of response to stimulus	11	0.018232417	CDH13, GIMAP5, S1PR1, C4B, FCER1G, SERPING1, C1S, C2, LBP, CD1D1, KDR

Discussion

OA is one of the most common forms of arthritis, which results into more dependency in walking, stair climbing, and other lower extremity tasks than any other disease, especially in the elderly. In our study, a total of 158 DGEs (including 9 TFs) were identified in OA samples, in which 119 genes were up-regulated and 39 genes were down-regulated. These 119 up-regulated genes were primarily enriched in blood vessel development, cell migration and inflammatory response with GO enrichment, and only *Npas2* and *Artnl* that were up-regulated genes were in-

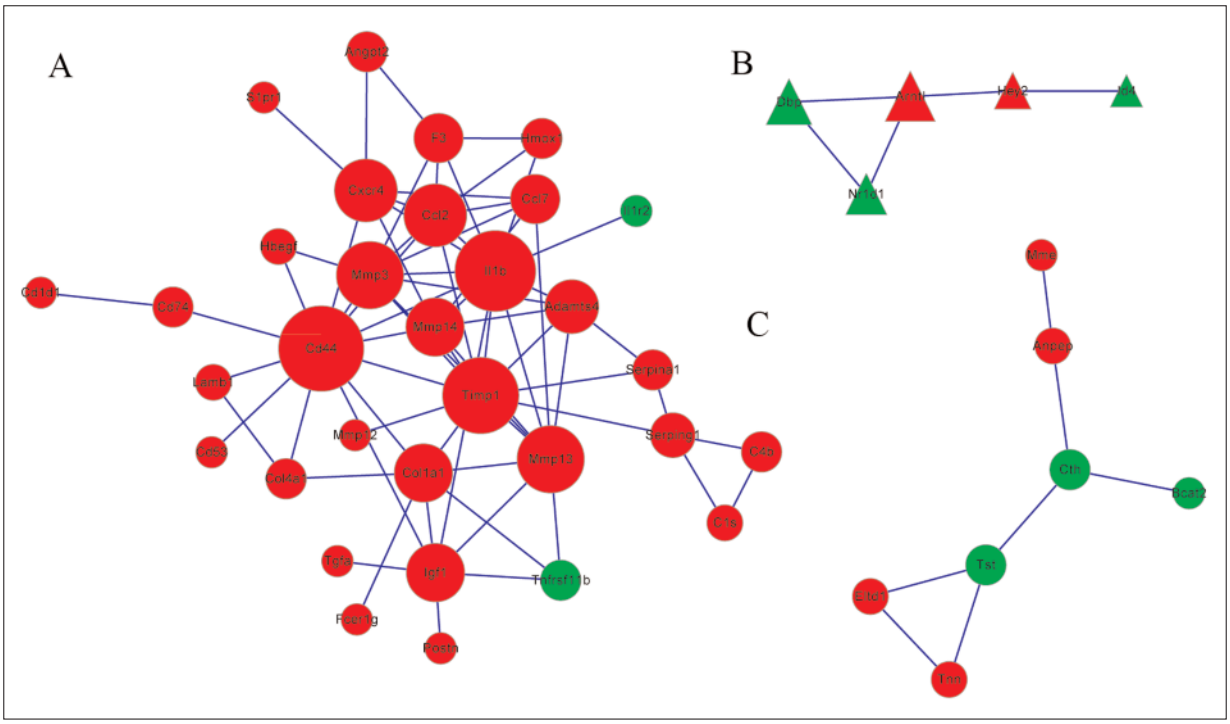

Figure 2. Protein-protein interaction network constituted by differentially expressed genes identified in this study and known osteoarthritis (OA)-related genes in the Text Mining, Database or Experiment databases. Red nodes represent the up-regulated genes in OA group, and green nodes represent the down-regulated genes in OA group.

Table III. Degree of differentially expressed genes in the protein-protein network.

Gene	Degree	Gene	Degree	Gene	Degree
Cd44	13	Adamts4	6	Col4a1	3
Il1b	12	Arntl	5	Cth	3
Timp1	11	Ccl7	5	Hlf	3
Mmp13	9	F3	5	Hmox1	3
Mmp3	9	Dbp	4	Nr1d1	3
Ccl2	8	Npas2	4	Nrp1	3
Cxcr4	8	Serping1	4	Serpina1	3
Col1a1	7	Angpt2	3	Tnfrsf11b	3
Igf1	7	C4b	3	Tst	3
Mmp14	7	Cd74	3	Anpep	2

volved in circadian rhythm pathway with KEGG pathway enrichment, which were consistent with previous reports[23,24]. The PPI network showed that *Cd44*, *Mmp13*, *Timp1* and *Il1b* had higher degrees in the network.

Igf-1 initiates intracellular signaling by binding to its specific receptor, the *Igf-1* receptor[25], which can increase proteoglycan synthesis of chondrocyte to reduce cartilage degradation[26]. In our study, *Igf1* was up-regulated, indicating that *Igf1* in OA tissue might be likely to attempt to repair the injury cartilage. The OA is closely associated with synthesis and secretion of matrix degradation enzymes in chondrocytes, and the dynamic imbalance between *Mmp13* and *Timp1* is an important mechanism for the degradation of cartilage[27]. The *Mmp13* can degrade cartilage collagen, leading to fibrosis of cartilage and disintegration of matrix, however the *Timp1* can limit the activity of neutral metalloproteinase and plasminogen activator. Our results showed that the fold change of *Timp1* between OA samples and control samples was 2.04 times while that of *Mmp13* was 2.60 times, indicating that the homeostasis between *Mmp13* and *Timp1* was broken in OA tissue. The DEGs were enriched in many GO terms, in which the blood vessel development was discovered to be closely related to OA. The blood vessel development is a normal and vital process in growth and development, which required distinct genetic interactions between multiple vascular endothelial growth factor (*VEGF*) receptors in the zebrafish[28]. *VEGF* has been demonstrated to be a major contributor to the generation of articular cartilage matrix[29]. Thus, blood vessel development may be related with OA.

The PPI network analysis showed that the network among *Cd44*, *Mmp13*, *Timp1* and *Il1b* had the largest degree. The *Mmp13* and *Timp1* have been reported to be related with OA in the above, but *Cd44* and *Il1b* were newly discovered in this study. *Cd44* is a receptor for hyaluronic acid and can interact with other ligands, such as osteopontin, collagens, and MMPs[30]. Hence, *Cd44* may affect the occurrence of OA by regulating the expression of genes associated with OA. *Il1b* is involved in the inflammatory response, and is reported to stimulate the release of collagenase from synovial cells[31]. The node degree of *Tnfrsf11b* was only three, but its protein-osteoprotegerin has been reported to play an important role in bone remodeling. Osteoprotegerin encoded by *Tnfrsf11b* is a specific protein which could increase bone mineral density and bone volume by binding to receptor activator of nuclear factor kappa B ligand on osteoblast cells[32]. Our results showed that the expression of *Tnfrsf11b* was down-regulated. But above all, *Tnfrsf11b* may be associated with OA.

Conclusions

Our results confirmed that *Igf1*, *Mmp13*, *Timp1* and *Tnfrsf11b* play important roles in pathogenesis of OA and the up-regulated genes were mainly enriched in blood vessel development. The OA-related network showed that *Cd44*, *Mmp13*, *Timp1* and *Igf1* can be selected as therapeutic targets for OA. The regulatory mechanism was newly discovered to be relevant to the occurrence of OA, which could provide a new and comprehensive view for OA treatment. However, experiments are needed to validate these results in our study.

Conflict of Interest

The Authors declare that there are no conflicts of interest.

References

1) Vos T, Flaxman AD, Naghavi M, Lozano R, Michaud C, Ezzati M, Shibuya K, Salomon JA, Abdalla S, Aboyans V. Years lived with disability (YLDs) for 1160 sequelae of 289 diseases and injuries 1990-2010: a systematic analysis for the Global Burden of Disease Study 2010. Lancet 2013; 380: 2163-2196.
2) Conditions NCCfC. Osteoarthritis: national clinical guideline for care and management in adults. Royal College of Physicians, 2008.
3) Zhang W, Nuki G, Moskowitz R, Abramson S, Altman R, Arden N, Bierma-Zeinstra S, Brandt K, Croft P, Doherty M. OARSI recommendations for the management of hip and knee osteoarthritis: part III: Changes in evidence following systematic cumulative update of research published through January 2009. Osteoarthritis Cartilage 2010; 18: 476-499.
4) Sandell LJ, Aigner T. Articular cartilage and changes in arthritis. An introduction: cell biology of osteoarthritis. Arthritis Res 2001; 3: 107-113.
5) Ma C, Lv Q, Cao Y, Wang Q, Zhou X, Ye B, Yi C. Genes relevant with osteoarthritis by comparison gene expression profiles of synovial membrane of osteoarthritis patients at different stages. Eur Rev Med Pharmacol Sci 2014; 18: 431-439.
6) Buckwalter J, Mankin H. Articular cartilage: degeneration and osteoarthritis, repair, regeneration, and transplantation. Instr Course Lect 1997; 47: 487-504.
7) Altman R, Brandt K, Hochberg M, Moskowitz R, Bellamy N, Bloch DA, Buckwalter J, Dougados M, Ehrlich G, Lequesne M, Lohmander S, Murphy WA Jr, Rosario-Jansen T, Schwartz B, Trippel S. Design and conduct of clinical trials in patients with osteoarthritis: recommendations from a task force of the Osteoarthritis Research Society. Results from a workshop. Osteoarthritis Cartilage 1996; 4: 217-243.
8) Frisbie D, Ghivizzani S, Robbins P, Evans C, McIlwraith C. Treatment of experimental equine osteoarthritis by in vivo delivery of the equine interleukin-1 receptor antagonist gene. Gene Ther 2002; 9: 12-20.
9) Kossakowska AE, Edwards DR, Lee SS, Urbanski LS, Stabbler AL, Zhang C-L, Phillips BW, Zhang Y, Urbanski SJ. Altered balance between matrix metalloproteinases and their inhibitors in experimental biliary fibrosis. Am J Pathol 1998; 153: 1895-1902.
10) Luo F, Yuan F, Peng Z, Zhou W, Fang L, Cai J. Regulation different network analysis of rheumatoid arthritis (RA) and osteoarthritis (OA). Eur Rev Med Pharmacol Sci 2013; 17: 2504-2511.
11) Zhang B, Xie Q, Quan Y, Pan X. Expression profiling based on graph-clustering approach to determine osteoarthritis related pathway. Eur Rev Med Pharmacol Sci 2013; 17: 2097-2102.
12) Appleton C, Pitelka V, Henry J, Beier F. Global analyses of gene expression in early experimental osteoarthritis. Arthritis Rheum 2007; 56: 1854-1868.
13) Duval E, Bigot N, Hervieu M, Kou I, Leclercq S, Galéra P, Boumediene K, Baugé C. Asporin expression is highly regulated in human chondrocytes. Mol Med 2011; 17: 816-823.
14) Gautier L, Cope L, Bolstad BM, Irizarry RA. Affy-analysis of Affymetrix GeneChip data at the probe level. Bioinformatics 2004; 20: 307-315.
15) Benjamini Y, Hochberg Y. Controlling the false discovery rate: a practical and powerful approach to multiple testing. J R Stat Soc Series B Stat Methodol 1995; 289-300.
16) Xie W, Chen Y, Zhou G, Wang L, Zhang C, Zhang J, Xiao J, Zhu T, Zhang Q. Single feature polymorphisms between two rice cultivars detected using a median polish method. Theor Appl Genet 2009; 119: 151-164.
17) Baldi P, Long AD. A Bayesian framework for the analysis of microarray expression data: regularized t-test and statistical inferences of gene changes. Bioinformatics 2001; 17: 509-519.
18) Matys V, Fricke E, Geffers R, Gössling E, Haubrock M, Hehl R, Hornischer K, Karas D, Kel AE, Kel-Margoulis OV. TRANSFAC®: transcriptional regulation, from patterns to profiles. Nucleic Acids Res 2003; 31: 374-378.
19) Ashburner M, Ball CA, Blake JA, Botstein D, Butler H, Cherry JM, Davis AP, Dolinski K, Dwight SS, Eppig JT. Gene Ontology: tool for the unification of biology. Nat Genet 2000; 25: 25-29.
20) Nakaya A, Katayama T, Itoh M, Hiranuka K, Kawashima S, Moriya Y, Okuda S, Tanaka M, Tokimatsu T, Yamanishi Y. Kegg OC: a large-scale automatic construction of taxonomy-based ortholog clusters. Nucleic Acids Res 2013; 41: D353-D357.
21) Da Wei Huang BTS, Lempicki RA. Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. Nat Protoc 2008; 4: 44-57.
22) Franceschini A, Szklarczyk D, Frankild S, Kuhn M, Simonovic M, Roth A, Lin J, Minguez P, Bork P, von Mering C. STRING v9. 1: protein-protein interaction networks, with increased coverage and integration. Nucleic Acids Res 2013; 41: D808-D815.
23) Kouri V-P, Olkkonen J, Kaivosoja E, Ainola M, Juhila J, Hovatta I, Konttinen YT, Mandelin J. Circadian timekeeping is disturbed in rheumatoid arthritis at molecular level. PloS one 2013; 8: e54049.
24) Zhang Q, Cheng B, Yang C, Ge H. Interaction relationships of osteoarthritis and rheumatoid arthritis related genes. Eur Rev Med Pharmacol Sci 2014; 18: 179-184.
25) Pollak M. The insulin and insulin-like growth factor receptor family in neoplasia: an update. Nat Rev Cancer 2012; 12: 159-169.
26) Key TJ, Appleby PN, Reeves GK, Roddam AW, Helzlsouer K, Alberg A, Rollison D, Overvad K, Kaaks R,

Trichopoulos D. Insulin-like growth factor 1 (IGF1), IGF binding protein 3 (IGFBP3), and breast cancer risk: pooled individual data analysis of 17 prospective studies. Lancet Oncol 2010; 11: 530-542.

27) Culhaci N, Metin K, Copcu E, Dikicioglu E. Elevated expression of MMP-13 and TIMP-1 in head and neck squamous cell carcinomas may reflect increased tumor invasiveness. BMC Cancer 2004; 4: 42.

28) Carmeliet P, Ferreira V, Breier G, Pollefeyt S, Kieckens L, Gertsenstein M, Fahrig M, Vandenhoeck A, Harpal K, Eberhardt C. Abnormal blood vessel development and lethality in embryos lacking a single VEGF allele. Nature 1996; 380: 435-439.

29) Ferrara N, Gerber H-P, LeCouter J. The biology of VEGF and its receptors. Nat Med 2003; 9: 669-676.

30) Wang C-T, Lin Y-T, Chiang B-L, Lin Y-H, Hou S-M. High molecular weight hyaluronic acid down-regulates the gene expression of osteoarthritis-associated cytokines and enzymes in fibroblast-like synoviocytes from patients with early osteoarthritis. Osteoarthritis Cartilage 2006; 14: 1237-1247.

31) Baune BT, Dannlowski U, Domschke K, Janssen DG, Jordan MA, Ohrmann P, Bauer J, Biros E, Arolt V, Kugel H. The interleukin 1 beta (IL1β) gene is associated with failure to achieve remission and impaired emotion processing in major depression. Biol Psychiatr 2010; 67: 543-549.

32) Lacey D, Timms E, Tan H-L, Kelley M, Dunstan C, Burgess T, Elliott R, Colombero A, Elliott G, Scully S. Osteoprotegerin ligand is a cytokine that regulates osteoclast differentiation and activation. Cell 1998; 93: 165-176.