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Bioinformatics analysis of gene expression profiles of osteoarthritis

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ABSTRACT

This study aimed to explore the underlying molecular mechanisms of osteoarthritis (OA) by bioinformatics analysis. Synovial tissue samples from five OA and five normal donors (ND) were used to identify the differentially expressed genes (DEGs) by paired *t*-test. Pathway enrichment analysis of DEGs was performed, followed by construction of a protein–protein interaction (PPI) network. A functional enrichment analysis of the modules identified from the PPI network was performed, and the module with the highest enrichment scores was selected for pathway enrichment analysis. A total of 184 DEGs, including 95 up-regulated and 89 down-regulated DEGs, were identified. Up-regulated DEGs were enriched in 6 pathways, such as MAPK signaling and Wnt signaling pathway, while down-regulated DEGs were mainly enriched in glycolysis/gluconeogenesis. In the PPI network, PTTG1 with the highest connectivity degree of 18 was significantly related to nuclear division, mitosis and the cell cycle. Genes in Module A with the highest functional enrichment scores of 9.27 were mainly enriched in the pathways of oocyte meiosis, cell cycle, ubiquitin mediated proteolysis and progesterone-mediated oocyte maturation. The MAPK signaling and Wnt signaling pathways were closely associated with OA. The DEGs, such as PTTG1, MAP2K6, PPP3CC and CSNK1E, may be the potential targets for OA diagnosis and treatment.

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Introduction

Osteoarthritis (OA), as a degenerative disease of articular cartilage, is characterized by an increased tendency for the formation of novel vascular channels (Binks et al., 2013). Characteristic pathological changes of OA include articular cartilage degeneration, angiogenesis, synovial inflammation and osteophyte formation, all of which are related with decreased muscle strength and capsule laxitude (Bonnet and Walsh, 2005; Brandt et al., 2006). Older adults with symptomatic OA undergo significant effects on multiple aspects of health-related quality of life (Salaffi et al., 2005) and OA is an important cause of disability (Peat et al., 2001).

The development and progression of OA have been considered to involve the development of inflammation throughout the disease (Felson, 2006). Magnetic resonance imaging and arthroscopy have demonstrated that progressive OA involves almost all of the articular tissue including proliferation of synovial membranes (Abramson and Attur, 2009; Conaghan et al., 2006). Immunohistochemical studies on patients with early OA have revealed that synovial tissue is characterized by production of pro-inflammatory cytokines, mononuclear cell infiltration and mediators of articular damage (Benito et al., 2005; Smith et al., 1997). The hypothesis that synovial tissue inflammation may be an essential etiological factor for OA is supported by increasing the levels of serum C reactive protein, which has a close association with the progression of OA (Sharif et al., 1997; Sowers et al., 2002). Genetic factors are also critical in the progression of OA. Remst et al. (2013) demonstrated that elevation of transforming growth factor β (TGFβ) in OA could mediate the onset and persistence of synovial fibrosis. In addition, Ghosh et al. (2002) showed that calcium pentosan polysulfate (CaPPS) could inhibit the activity of enzymes, which are responsible for degradation of proteoglycans and collagen, and increase the translation of tissue inhibitor of metalloproteinase-3 (TIMP-3) by synovial fibrosis in OA. However, the specific underlying molecular mechanisms during osteoarthritis associated with synovial tissue development and progression are still poorly understood.

In this study, we downloaded GSE1919 and identified the differentially expressed genes (DEGs) between the OA and normal donors (ND) synovial tissue samples to understand the molecular mechanisms of OA. We also constructed pathway enrichment analysis, protein–protein interaction (PPI) networks and module

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analysis to study and identify the target genes for diagnosis and treatment of OA. Findings of this study might contribute to a better understanding and lead to an improved diagnosis of OA.

Materials and methods

Affymetrix microarray data

The gene expression profile dataset GSE1919 (Ungethuem et al., 2010) was downloaded from the Gene Expression Omnibus (GEO, http://www.ncbi.nlm.nih.gov/geo/) database. The dataset contains 5 OA, 5 rheumatoid arthritis (RA) and 5 normal donors (ND) synovial tissue samples derived from a human study using the Affymetrix Human Genome U95 Array platform. In this paper, the OA and ND samples were analyzed by bioinformatics.

Data preprocessing and analysis of DEGs

The original probe-level data in CEL files were converted into expression measures and quartile data normalized by the robust multiarray average (RMA) in R affy package (Gautier et al., 2004). After this, 32,887 genes were obtained.

The paired *t*-test based on the multtest package (Benjamini and Hochberg, 1995) in R language was used to identify genes that were significantly differentially expressed between OA and ND samples. Multiple testing was corrected by the Benjamini and Hochberg (BH) method (Benjamini and Hochberg, 1995) to obtain the adjusted p-value. And then, log 2-fold change (log FC) was calculated. Only genes with the adjusted p-value < 0.05 and $|\log FC| > 2$ or $|\log FC| < 0.5$ were regarded as DEGs, which were the signature genes of OA.

Pathway enrichment analysis of DEGs

DAVID (The Database for Annotation, Visualization and Integrated Discovery), as a comprehensive set of functional annotation tools, is used for systematic and integrative analysis of large gene lists (Huang et al., 2008). The Kyoto Encyclopedia of Genes and Genomes (KEGG) (Kanehisa, 2002) pathway enrichment analysis for the identified DEGs was performed by DAVID Fisher test with the thresholds of p value <0.05 and enrichment gene count >2 (Fisher, 1948).

Protein–protein interactions (PPI) analysis and network construction

STRING (Search Tool for the Retrieval of Interacting Genes) is an online database which has been designed as a global perspective to evaluate PPI information (Franceschini et al., 2013). In this paper, the STRING online tool was applied to analyze the PPI of DEGs and only those experimentally validated interactions with a confidence score >0.99 were selected as significant. PPI networks were then constructed using the Cytoscape software (Smoot et al., 2011) based on the PPI relationships. From a previous study on biological networks, most of the PPI networks obeyed the scale-free attribution (Junker et al., 2006). So connectivity degree was analyzed by statistics in the network and the important nodes which participated in PPI relations in the networks were obtained, namely hub proteins (He and Zhang, 2006; Lamb et al., 2006).

Network modeling analysis

Molecular Complex Detection (MCODE) (Bader and Hogue, 2003) was used for the Cytoscape platform to enable discovery of dense clique-like structures within a network. As a result of the complicacy for nodes and edges of the PPI network, the enrichment analysis was conducted through MCODE. The parameters to disclose the enriched functional modules were Degree Cutoff = 2, Node Score Cutoff = 0.2, K-Core = 2 and Max Depth = 100. After that, the gene ontology (GO) functional enrichment analysis of the module genes was performed and the module which had the highest enrichment score was selected for pathway enrichment analysis.

Results

Identification of DEGs between OA and ND samples

A total of 184 DEGs with the cut-off criteria of the adjusted p-value < 0.05 and $|\log FC| > 2$ or $|\log FC| < 0.5$ were selected, including 95 (52%) up-regulated and 89 (48%) down-regulated DEGs.

KEGG pathway enrichment analysis

The significantly enriched pathways for the up-regulated and down-regulated DEGs are shown in Fig. 1. The up-regulated DEGs were enriched in 6 pathways such as MAPK (JUND, PPP3CC, GADD45A and MAP2K6) and Wnt signaling pathway (PPP3CC and CSNK1E). The down-regulated DEGs were enriched in 5 pathways such as glycolysis/gluconeogenesis (AKR1A1 and ALDH3B1) and the MAPK signaling pathway (MAPK13and MAPKAPK3).

PPI network construction

A total of 261 PPI relationships were obtained with the combined score >0.99. The PPI networks were then constructed using Cytoscape (Fig. 2). The genes PTTG1 (pituitary tumor-transforming gene 1, degree = 18), UBC (human ubiquitin C gene, degree = 15), CDC16 (cell division cycle 16, degree = 14), CDC20 (cell-division-cycle 20, degree = 14) and CDC27 (cell-division-cycle 27, degree = 14) were selected as hub nodes with the top five high connectivity degree (Table 1).

Module analysis

A total of 4 significant modules were selected with the parameter of MCODE score ≥4. Module A (MCODE score = 11.818), Module B (MCODE score = 7.429), Module C (MCODE score = 4) and Module D (MCODE score = 4.5) were shown in Fig. 3. Module A had 12 nodes and 65 edges involving one down-regulated gene (PTTG1); Module B with 8 nodes and 26 edges involved one down-regulated gene (FANCC); Module C with 10 nodes and 18 edges involved both the up-regulated (VDR) and down-regulated gene (CDKN1A); Module D with 5 nodes and 9 edges did not involve any DEGs.

The GO functional enrichment scores of Module A, B and C were 9.27, 3.6 and 2.89 respectively. Module D with small number of genes could not be used for enrichment analysis (Table 2). Module A was enriched in 7 functions such as nuclear division, mitosis and cell cycle; Module B was enriched in 4 functions

Table 1
The statistical results of connectivity degrees of the genes in the PPI network.

Gene	Degree	Gene	Degree
PTTG1	18	ANAPC16	12
UBC	15	ANAPC5	12
CDC16	14	ANAPC7	12
CDC20	14	ESR1	12
CDC27	14	ANAPC13	11
ANAPC1	13	ANAPC4	11
CDC23	13	EP300	11
CDC23	13	CREBBP	10
TP53	13	JUN	10

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