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Molecular mechanisms of osteoarthritis using gene microarrays

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ABSTRACT

This study aimed to investigate the molecular mechanisms of osteoarthritis (OA) by microarray analysis. Three gene expression datasets GSE1919, 19664 and 55235 were downloaded from the Gene Expression Omnibus, and data of OA samples and healthy controls were used. After data preprocessing, differential expression analysis between the OA group and controls was performed using LIMMA (Linear Models for Microarray Data) package and genes with $|\log_2 FC \text{ (fold change)}| > 1$ and $P < 0.05$ were screened as DEGs (differentially expressed genes). The screened DEGs were then subject to functional annotation and pathway enrichment analysis using DAVID (Database for Annotation Visualization and Integrated Discovery). Next, gene-set enrichment analysis was performed using Enrichment map Cytoscape plug-in, followed by detecting sub-networks using clusterONE. Finally, risk subpathways were screened using iSubpathwayMiner package. A total of 141 DEGs were screened, including 52 up-regulated ones and 89 down-regulated ones. These DEGs were enriched in 48 GO terms that were mainly related to locomotory behavior, taxis, adhesion, and 11 pathways that were related to cytokine-cytokine receptor interaction, ECM-receptor interaction, focal adhesion, as well as several signaling pathways. The enrichment map enriched gene-sets mainly related to cell death and apoptosis, and extracellular components. The risk pathways up-regulated DEGs were exclusively related to arachidonic acid metabolism and glycosphingolipid biosynthesis, and the top two risk pathways were tyrosine metabolism for the down-regulated ones. From this study we conclude that genes involved in cell death and apoptosis, as well as cell–extracellular matrix interaction, may be essential for OA pathogenesis by altering multiple signaling pathways.

Introduction

OA (osteoarthritis) is typically characterized by deformed joints and can lead to serious consequences such as degradation of articular cartilage with loss of cartilage matrix, osteophyte formation, subchondral sclerosis, meniscal degeneration, bone marrow lesions and synovial proliferation in OA patients (Abramson and Attur, 2009).

Chondrocytes are the only cell typesurviving in articular cartilage. Synovial fibroblasts, a most abundant resident cell type in human synovial tissue, display a significant hyperplasia in chronic arthritis (Izquierdo et al., 2011). Previous studies have suggested that a complex signalling network is involved in chondrogenesis, chondrocyte differentiation and chondrocyte apoptosis (Islam et al., 2001). For example, epidermal growth factor (EGF) has been shown to regulate chondrogenesis negatively by modulating the ERK-1 and p38MAPK signaling pathways (Yoon et al., 2000), and its receptor, EGFR, is a protein tyrosine kinase (PTK) receptor. *SOX 9* is another PTK gene required for chondrocyte differentiation, whose expression is stimulated by fibroblast growth factors (FGFs) via the mitogen-activated protein kinase (MAPK) pathway (Murakami et al., 2000), indicating that MAPK plays an important role in chondrogenesis. Interleukin-1 (IL1) can induce the expression of two matrix metalloproteinase members MMP-13 and MMP-1via the p38/c-JUN N-terminal kinase (JNK) pathway and p38/MEK signaling pathway, respectively (Mengshol et al., 2000). Both MMP-13 and MMP-1 can cleave cartilage matrix collagen, especially MMP-13, which preferentially degrades type II collagen (Knäuper et al., 1996), and aberrant expression of these two genes has been reported previously in OA patients (Tetlow et al., 2001; Attur et al., 2009). Additionally, when the survival signals from extracellular matrix or growth factors are lost, programmed cell death or apoptosis occurs in chondrocytes (Del Carlo and Loeser, 2008). Many studies have demonstrated articular chondrocyte death, mostly in end-stage OA patients, although whether it happens at the early stages in this disease is still unclear.

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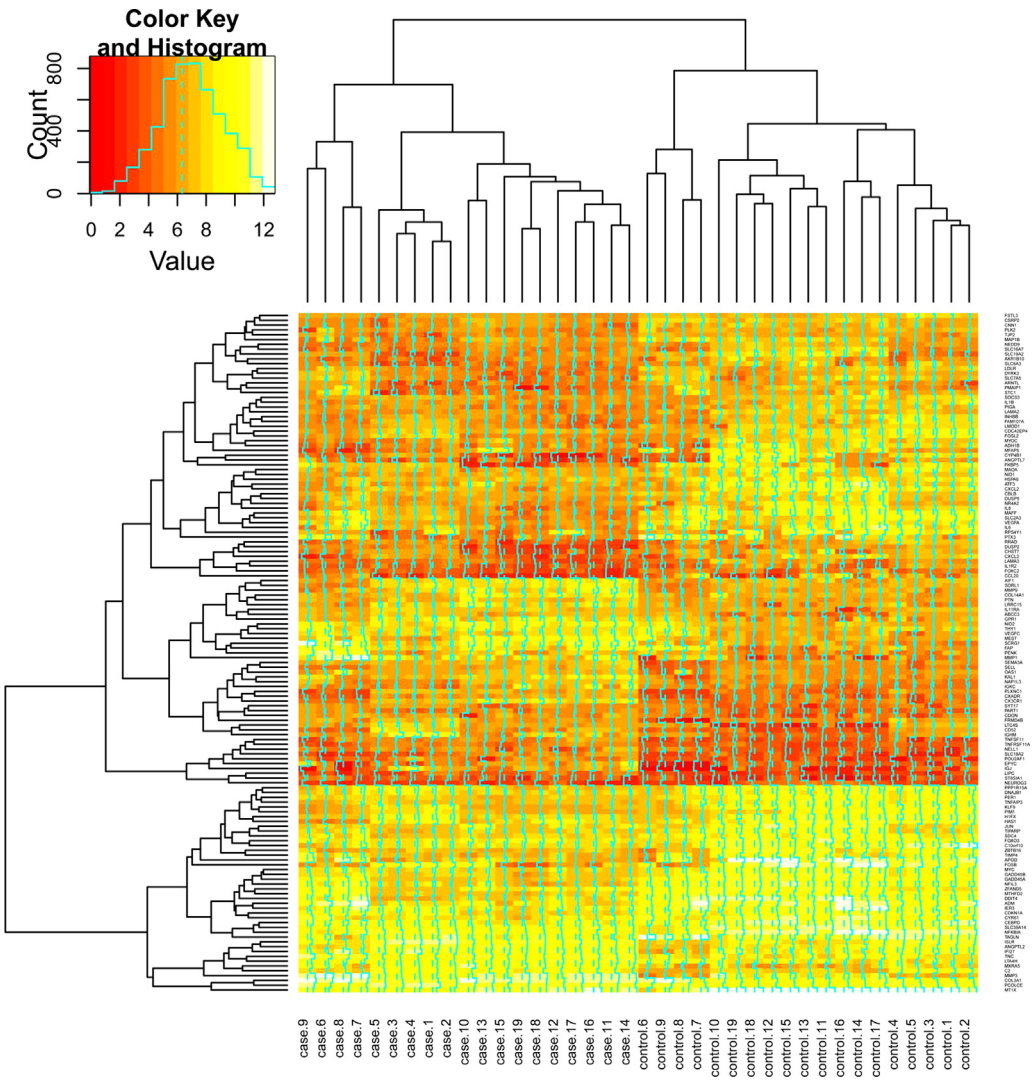

Fig. 1. *Cluster analysis based on 141 significantly differentially expressed genes.* Each row, a single gene; each column, a tissue sample. A darker yellow indicates a higher expression level, and a darker red indicates a lower expression level; color in-between indicates expression level between the highest and the lowest levels. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

In the present study, we used three different microarray datasets and attempted to make an in-depth analysis of OA pathogenesis with reference to previous studies.

Data and methods

Microarray data

Three gene microarray datasets GSE1919 (Ungethuem et al., 2010), GSE19664 (Bernstein et al., 2010) and GSE55235 (Woetzel et al., 2014) were downloaded from GEO (Gene Expression Omnibus, http://www.ncbi.nlm.nih.gov/geo/) database. For the first dataset (GSE1919), only mRNA expression data of 5 patients with OA and 5 healthy controls were used in the present study; the platform is Agilent-014850 Whole Human Genome Microarray 4x44K G4112F. Gene expression data of the 5 OA patients and 5 healthy controls in the second dataset (GSE19664) were used in our study; the annotation platform is GPL9828 [HG-U133_Plus_2] Affymetrix Human Genome U133 Plus 2.0 Array. For the third dataset, only mRNA expression data of the 10 OA patients and 10 healthy controls were used.

Microarray data preprocessing

After annotation of probes from different microarray data and comparison, 7740 common genes were obtained. First, batch error was removed using the SVA (Surrogate variable analysis) package of R/Bioconductor (Pirooznia et al., 2013). Then, normalization between arrays was performed using preprocess Core package of R/Bioconductor (Bolstad and Bolstad, 2013).

Screening of DEGs (differentially expressed genes)

After data preprocessing, the differential expression analysis between the OA group and the control group was performed using Limma (Linear Models for Microarray Data) package of R/Bioconductor (Smyth, 2005). Genes with $|\log_2 \text{FC}$ (fold change)$| > 1$ and $P \leq 0.05$ were screened as DEGs (differentially