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Original Article

Hylonome

Screening for characteristic genes in osteoarthritis induced by destabilization of the medial meniscus utilizing bioinformatics approach

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Abstract

Objective: To explore the underlying molecular mechanism of the development of OA (osteoarthritis). **Methods:** The expression profile dataset GSE26475 was downloaded from Gene Expression Omnibus database. A total of 21 samples were available, including knee joint samples from OA mice induced by DMM (Destabilization of the medial meniscus) surgery (6h, 3d, 7d), mice undergoing sham (only capsulotomy) surgery (6h, 3d, 7d) and the age matched Naïve mice (normal controls) that were not operated on. The differentially expressed genes (DEGs) were identified and the KEGG pathway enrichment analysis was conducted for all DEGs. **Results:** The number of DEGs between the DMM-induced mice at different times after surgery and normal controls was different that it decreased from 6h to 3d while increased at 7d. The same was true for the change of the number of DEGs between DMM and sham groups. Further analysis revealed that the DEGs between DMM and normal controls were mainly involved in the signaling and inflammation related pathways. Total 16 DEGs between DMM and sham groups at 7d were all involved in the Parkinson's disease, Oxidative phosphorylation and Alzheimer's disease pathways. **Conclusion:** The results presented here may help us to understand the molecular mechanism of OA.

Keywords: Alzheimer's Disease, Bioinformatics, Destabilization of the Medial Meniscus, Differentially Expressed Genes, Osteoarthritis, Pathway Enrichment Analysis

Introduction

As the most common form of arthritis, osteoarthritis (OA) is a major cause of pain and locomotor disability worldwide[1]. The main features of OA are joint space narrowing, osteophyte formation at the joint margins, cartilage destruction, subchondral bone remodeling and synovial inflammation[2]. Previous reports have declared that many factors are implicated in the development of OA, including aging and joint injury[3]. Age affects both the pattern of gene expression in joint tissues and the responses to surgically induced OA[4]. However, the mechanism by which joint injury contribute to the development of OA is still incompletely understood.

To explore the likelihood and mechanism of OA induced by joint injury, several murine surgical models of OA have been studied in the past, including destabilization of the medial meniscus (DMM), transection of the anterior cruciate ligament and removal of the medial meniscus[5]. DMM surgery, which products robust cartilage degration, osteophyte formation and pain, is commonly utilized to induced OA model in recent years[6]. By the DMM-induced OA model, researchers have discovered the alterations in expression of some articular cartilage associated genes, such as aggrecan and aspirin, and some chemotaxis related genes, such as *Ccl21* (Chemokine (C-C motif) ligand 21) and *Cxcr7* (C X C Chemokine Receptor 7)[7]. In addition, IL-8 and its murine equivalent keratinocyte chemoattractant have been found to play important roles in the pathophysiology of OA[8]. Interestingly, plenty of reports have indicated that the Parkinson's disease, Alzheimer's disease, Huntington's disease and OA are all associated with the movement disorder[9]. Some genes, such as *NDUFB-9* (NADH (reduced nicotinamide adenine dinucleotide) dehydrogenase

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	P value <0.01	P value <0.05
(DMM_6hr)-(Naive_0hr)	626	2171
(DMM_3day)-(Naive_0hr)	503	2086
(DMM_7days)-(Naive_0hr)	490	2141
(DMM_6hr)-(Sham_6hr)	368	1398
(DMM_3day)-(Sham)_3day)	217	1382
(DMM_7days)-(Sham_7days)	559	1880

Table 1. The numbers of identified DEGs between DMM (6h) and Naïve samples (0h), as well as DMM and sham (6h, 3d, 7d).

[ubiquinone] 1 beta subcomplex subunit 9), *ACOX-1* (Peroxisomal acyl-coenzyme A oxidase 1) and *PLA2G6* (85 kDa calcium-independent phospholipase A2), have been reported to be differentially expressed in the development of Alzheimer's disease[10]. Although several papers on OA have been published, the underlying molecular mechanism of the development of OA has not been completely explored.

The present study was aimed to identify the characteristic genes which were differentially expressed in the DMM induced OA mice, so as to reveal the possible pathogenesis of OA. The samples in different time courses of induction were compared and the differentially expressed genes (DEGs) were screened out. Additionally, the enriched pathways, in which the DEGs were involved, were explored. Our findings may provide a novel insight into the mechanism of OA and the resulted candidate genes may be used as the therapeutic targets for OA.

Materials and methods

Knee joints expression profile data

The gene expression profile dataset GSE26475[11] based on the Affymetrix Mouse Gene 1.0 st v1 platform was downloaded from the NCBI (National Center for Biotechnology Information) GEO database (Gene Expression Omnibus, http://www.ncbi.nlm.nih.gov/geo/). Surgical model of murine OA was induced by DMM. Gene expression in whole knee joints at 3 early time points post DMM surgery, 6h, 3d and 7d, was examined. The age matched Naïve mice that were not operated on and the mice which underwent sham surgery (only capsulotomy) were used as controls. The sham surgery had the same time points post-surgery as DMM samples (6h, 3d and 7d). There were 3 biological replicates per condition (total 21 samples).

Data preprocessing

The derived original data were subjected to background correction and quantile normalization[12]. When two or more probes mapped to the same Entrez Gene identifier (Entrez Gene ID), the mean expression value of all these probes was calculated as the final expression value of this gene[13].

Differentially expressed genes (DEGs) analysis

The classical t-test[14] was applied to identify genes that were differentially expressed among the 9 samples from DMM-induced mice, 9 samples from sham mice and 3 samples from Naïve mice. Only the genes with *P* values <0.05 were screened out as DEGs.

Pathway enrichment analysis

The Kyoto Encyclopedia of Genes and Genomes (KEGG) stands for a database consisting of known genes and their respective biochemical functionalities, linking genomic information with higher order functional information[15]. DAVID (a Database for Annotation, Visualization, and Integrated Discovery) provides a set of data-mining tools that systematically combine functionally descriptive data with intuitive graphical displays[16].

The KEGG pathway enrichment analysis was performed for the identified DEGs using the DAVID database. The pathways between DMM (6h) and Naïve samples, as well as DMM and sham samples (6h, 3d, 7d) were compared and only the ones with *P* values less than 0.05 were selected as the pathways significantly associated with OA. Besides, the *P* values were adjusted by Bonferroni method[17].

Results

DEGs analysis

Finally, the expression data of 19310 genes were available for all the 21 samples, which were then organized in a matrix, where the rows represented genes and the columns represented samples. Differential expression analysis, based on the matrix, revealed that the number of DEGs between samples derived from DMM and Naïve mice was more than that between DMM and sham groups (Table 1). Meanwhile, the number of DEGs with *P* values less than 0.05 was decreased with the time extension from 6h to 3d (DMM vs. Naïve: from 2171 to 2086, DMM vs. Sham: from 1398 to 1382, respectively); however, the number was increased at 7d (2141, 1880, respectively). In addition, the change trend of the number of DEGs with *P* values <0.01 was similar to that of DEGs with *P* values <0.05.

The enriched pathways between DMM and Naïve samples

Total 11 enriched pathways with *P* values less than 0.05 were identified between DMM (6 h) and Naïve samples (Table 2). Half of the pathways were signaling molecules related pathways, such as ECM (Extracellular Cell Matrix)-receptor inter-

Term	P value	Bonferroni
mmu04512:ECM-receptor interaction	2.95E-05	0.004469
mmu04510:Focal adhesion	1.97E-04	0.029477
mmu04610:Complement and coagulation cascades	0.004713	0.51229
mmu04060:Cytokine-cytokine receptor interaction	0.005501	0.567628
mmu04621:NOD-like receptor signaling pathway	0.005905	0.593497
mmu04640:Hematopoietic cell lineage	0.009296	0.758199
mmu05200:Pathways in cancer	0.009831	0.777255
mmu04062:Chemokine signaling pathway	0.011955	0.839292
mmu00240:Pyrimidine metabolism	0.019762	0.951871
mmu05211:Renal cell carcinoma	0.03655	0.996516
mmu04010:MAPK signaling pathway	0.047517	0.999389

Table 2. The enriched pathways between DMM (6h) and Naïve samples with *P* value <0.05.

Term	Genes
mmu04512:ECM-receptor interaction	COL1A1, COL1A2, COL4A1, COL4A2, COL11A1, COL11A2, ITGA3, ITGA5, ITGA7, LAMA2, VAMP1
mmu04060:Cytokine-cytokine receptor interaction	CXCL1, CXCL2, CXCL5, CXCL13, CCL2, CCL6, CCL9, CCL12, CCL7, PDGFD, FZD2, 111R1, RELN, DAG1, IL7R, IL13RA1, TNFRSF12A, INHBE, 114RA
mmu04640:Hematopoietic cell lineage	IL7R, IL1B, 111R1, CD14, H2EA, ITGA5, ITGA3, FCGR1, 114RA

Table 3. The differentially expressed genes enriched in the signaling molecules related pathways and inflammation related pathways identified between DMM (6h) and Naïve samples.

Term	P value	Bonferroni
mmu04060:Cytokine-cytokine receptor interaction	0.025153	0.964464
mmu05020:Prion diseases	0.032955	0.987597
mmu00120:Primary bile acid biosynthesis	0.035835	0.991609

Table 4. The enriched pathways between DMM and sham samples (6h) with *P* value <0.05.

Term	P value	Bonferroni
mmu05010:Alzheimer's disease	0.00185	0.163126
mmu04060:Cytokine-cytokine receptor interaction	0.00320	0.265114
mmu03320:PPAR signaling pathway	0.01688	0.805019
mmu04640:Hematopoietic cell lineage	0.02069	0.865719
mmu00980:Metabolism of xenobiotics by cytochrome P450	0.04940	0.992299

Table 5. The enriched pathways between DMM and sham samples (3d) with *P* value <0.05

action (*P* value=2.95E-05) and cytokine-cytokine receptor interaction (*P* value=0.005501), and inflammation related pathways (Hematopoietic cell lineage with *P* value= 0.009296). Several collagen genes, including *COL1A1* (Collagen, type I, alpha 1), *COL1A2*, *COL4A1*, *COL4A2*, *COL11A1* and *COL11A2* were observed to be involved in the ECM-receptor interaction pathway. Other genes such as *CXCL1* (Chemokine (C-X-C motif) ligand 1), *CXCL2*, *CXCL5*, *CXCL13*, *CCL2*, *CCL6*, *CCL9*, and *CCL12* were enriched in cytokine-cytokine receptor interaction pathway. Furthermore, *IL7R*, *ITGA5* (Integrin alpha-5) and *ITGA3* were discovered in Hematopoietic cell lineage pathway (Table 3).

Term	P value	Bonferroni
mmu05012:Parkinson's disease	1.36E-05	0.001751
mmu00190:Oxidative phosphorylation	4.20E-05	0.005398
mmu05010:Alzheimer's disease	1.90E-04	0.024277
mmu05016:Huntington's disease	2.04E-04	0.025921
mmu03010:Ribosome	0.001011	0.122377
mmu05322:Systemic lupus erythematosus	0.003069	0.327299
mmu00020:Citrate cycle (TCA cycle)	0.004056	0.407993
mmu00860:Porphyrin and chlorophyll metabolism	0.019619	0.922381
mmu00010:Glycolysis / Gluconeogenesis	0.030991	0.982769

Table 6. The enriched pathways between DMM and sham samples (7d) with *P* value <0.05

Pathways identified between DMM and Sham groups

There were 3, 5 and 9 enriched pathways between DMM and Sham samples at 6h, 3d and 7d post-surgery, respectively (Table 4, 5 and 6). Additionally, the number of enriched pathways was increased with time; therefore, the differences between DMM and Sham samples were most significant at 7 days.

In Table 6, the top four remarkable pathways were Parkinson's disease (*P* value=1.36E-05), Oxidative phosphorylation (*P* value=4.20E-05), Alzheimer's disease (*P* value=1.90E-04) and Huntington's disease (*P* value=2.04E-04). A total of 16 genes, such as *COX7A1* (cytochrome c oxidase subunit VIIa polypeptide 1), *SDHA* (Succinate dehydrogenase complex, subunit A), *SDHD, NDUFB2* (NADH dehydrogenase [ubiquinone] 1 beta subcomplex subunit 2), *NDUFB3, NDUFB6, NDUFB8, NDUFB9, NDUFA2* and *NDUFV1*, were the common DEGs involved in all the top four pathways (Table 7).

Gene ID	Symbol
100043111	Gm4237
66043	Atp5d
66218	NDUFB9
22273	Uqcrc1
638710	LOC638710
12859	Gm11273
11950	Gm12231
67264	Ndufb8
66925	SDHD
66495	NDUFB3
17995	Ndufv1
230075	NDUFB6
68198	NDUFB2
17991	Ndufa2
66945	SDHA
12865	COX7A1

Table 7. The 16 common differentially expressed genes involved in the top 4 enriched pathways between DMM and sham samples (7d).

Discussion

Although many papers on the development of OA have been published, the underlying molecular mechanisms are still unclear. The use of an animal model opens up the possibility of studying on the early stage of disease progression. In the present study, the expression profiles of the whole knee joints from mice induced by DMM and sham surgery were compared at different time courses, including 6h, 3d and 7d. Additionally, the DEGs between the DMM induced mice and the Naïve mice which were not operated on were identified. The numbers of DEGs in these groups were different at the indicated time points. Between the DMM (6h) and Naïve groups, the DEGs, such as *COL1A1, CXCL1, CC12* and *IL7R*, were closely associated with the signaling molecules related pathways and inflammation related pathways. In addition, the differences in the expression of genes between DMM and sham samples were most significant at 7d. The identified 16 DEGs, including *COX7A1, SDHA, SDHD* and *NDUFB2*, were discovered contributed to the development of Parkinson's disease, Alzheimer's disease and Huntington's disease.

Firstly, the present study showed that the number of DEGs between DMM and Naïve samples was more than that of DMM and sham groups. This finding indicated that surgeries may lead to the occurrence of some events, such as infection, and consequently lead to the expression alterations of some genes. Meanwhile, the number of DEGs was found decreased from 6h to 3d but was increased at 7 days after surgery. These data suggested that there were dynamic changes during the development of OA[11]. Further analysis revealed that the enriched pathways between DMM and normal controls were signaling molecules and inflammation related pathways, including ECM-receptor interaction, cytokine-cytokine receptor interaction and Hematopoietic cell lineage. OA is a group of mechanical abnormalities involving the degradation of joints including articular cartilage and subchondral bone. Articular cartilage, including chondrocytes and the ECM produced by them, plays a critical role in the degeneration and deform of joints[18]. Both integrin and nonintegrin ECM receptors (such as CD44) are expressed in articular cartilage and provide chondrocytes the means to 'sense' changes through interactions with their prin-

cipal ligands in the ECM environment, which are responsible for maintaining the homeostasis in articular cartilage[19]. The degeneration of ECM can be controlled by inflammatory mediators, such as cytokines, released by cartilage, bone and synovium. Such inflammatory cytokines are among the critical mediators of the disturbed processes implicated in OA pathophysiology[20]. In addition, osteoclasts derive from bone marrow hematopoietic cells, play vital roles in the pathogenesis of OA. Therefore, these three pathways, which screened out in our study, are associated with the development of OA[21].

Collagen genes have been suggested to mediate the susceptibility to OA[22] and breakdown of cartilage collagen seems to be closely related to the pathogenesis of OA. In the osteoarthritic articular cartilage, increased damage of type II collagen was detected[23,24]. With regard to collagen, several genes including *COL1A1, COL1A2, COL4A1, COL4A2, COL11A1* and *COL11A2* were found to be differentially expressed in the DMM samples, suggesting that these genes may be implicated in the development of OA. Furthermore, these genes may have the potential to be the target of novel drugs for OA. Chemokines, namely by their chemotactic activity, are a family of small heparin-binding cytokines. So far, 4 subfamilies of chemokines, named C, C-C, C-X-C, and C-X3-C chemokines, have been identified. A growing number of reports have illustrated the involvement of chemokines in cartilage abnormalities including OA. In current study, *CXCL1, CXCL2, CXCL5, CXCL13, CCL2* and *CCL6* displayed differential expression in the knee joints of DMM-induced mice. Indeed, an increased production of CXCL1, CCL2 and CCL5 has been observed in OA[25,26]. Integrins are a family of transmembrane receptors that mediate the attachment between a cell and its surroundings, such as other cells or the ECM. As discussed above, integrin ECM-receptors are responsible for the homeostasis in articular cartilage. Abnormal expression of integrin alters the cell/ECM signaling and modifies the chondrocyte synthesis in the development of OA[27]. Integrin α5 (ITGA5) and ITGA3, identified to be differentially expressed in OA mice in present study, are closely associated with the development of OA[28]. Based on the above findings and knowledge, researchers and doctors may develop new therapeutic methods and discover novel drugs for OA. In brief, our findings may not only evolve the present therapies but also stimulate the progress of new treatments, so as to treat the OA more efficiently.

Since the number of DEGs was increased at 7d after surgery, the enriched pathways at 7d were studied carefully. Total 16 common DEGs, including *COX7A1, SDHA, SDHD, NDUFB2, NDUFB3, NDUFB6, NDUFB8, NDUFB9, NDUFA2* and *NDUFV1* were all involved in the Parkinson's disease, Oxidative phosphorylation and Alzheimer's disease pathways. COX7A1 is a subunit of cytochrome c oxidase. SDHA and SDHD belong to the succinate dehydrogenase (SDH) complex. *NDUFB2, NDUFB3, NDUFB6, NDUFB8, NDUFB9, NDUFA2* and *NDUFV1* encode proteins which are subunits of NADH dehydrogenase. NADH dehydrogenase (complex I), succinate dehydrogenase (complex II), cytochrome c reductase (complex III) cytochrome c oxidase (complex IV) are protein complexes of the mitochondrial electron transfer chain that contribute to mitochondrial functions, including respiratory activity and ATP synthase. Mitochondrial dysfunction has been found in human OA chondrocytes and analyses of mitochondrial electron transport chain in these cells revealed a decreased activity of Complexes I, II and III when compared to normal chondrocytes[29,30]. Thus, these common DEGs may take effects in the pathogenesis of OA. Meanwhile, our result indicated that all the identified 16 DEGs may induce OA by disrupting the Oxidative phosphorylation pathway, which agrees with the previous studies[31].

In conclusion, our present study utilized the bioinformatics approach to identify the DEGs between DMM-induced mice and normal controls as well as between DMM and sham mice at different time points. Based on the DEGs screened out in these samples, researchers may understand the molecular mechanism profoundly. Moreover, the DEGs may have the potential to be the drug targets for OA. In brief, our study may assist the researchers and medical personnel to understand the molecular mechanism of OA and screen for new drug targets to treat the disease more efficiently. However, due to the small number of samples and the limitation of the approach used in this study, further studies are still needed.

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