

Identification of differentially expressed genes in hip cartilage with femoral head necrosis, based on genome-wide expression profiles

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Abstract. Necrosis of the femoral head (NFH), a severe orthopedic disease in adults, involves the collapse of the femoral head. The pathophysiological mechanisms underlying NFH are yet to be fully investigated. The aim of the present study was to identify potentially important genes and signaling pathways involved in NFH and investigate their molecular mechanisms. Gene expression profiles of patients with NFH and healthy controls were compared using the Gene Expression Omnibus (GEO) database repository of the National Center of Biotechnology Information. GSE74089 from the GEO database included 4 patients with NFH and 4 healthy individuals. A total of 1,191 differentially expressed genes (DEGs) were identified between the patients with NFH and controls, including 743 upregulated and 448 downregulated DEGs. Then, Gene Ontology and Kyoto Encyclopedia of Genes and Genomes pathway enrichment analysis revealed

that upregulated DEGs were mainly involved in the phosphoinositide 3-kinase/protein kinase B signaling pathway, focal adhesion and extracellular matrix-receptor interactions. Additionally, protein-protein interaction (PPI) analysis identified the most central DEGs as vascular endothelial growth factor A, Jun proto-oncogene, cyclin D1, fibroblast growth factor 2, HECT domain and ankyrin repeat-containing E3 ubiquitin protein ligase 1, protein kinase α , bone morphogenetic protein 2 and prostaglandin-endoperoxide synthase 2. PPI analysis also identified guanine nucleotide-binding protein, $\gamma 13$ as the most commonly downregulated gene based on different centrality. The results of the present study may provide novel insight into the genes and associated pathways involved in NFH, and aid the identification of novel therapeutic targets and biomarkers in the treatment of NFH.

Introduction

Necrosis of the femoral head (NFH) is a common disease of the hip, with a high incidence among elderly patients; the most common clinical symptom is severe pain (1). The pathological characteristics of NFH include reduced blood supply to the hip, collapse of the femoral head and microfracture accumulation without sustained remodeling (2,3). Early clinical symptoms may involve pain, ultimately leading to loss of movement in the hip (4). NFH is frequently treated by total hip arthroplasty in the end-stage of hip arthritis (5); however, the pathogenesis and molecular mechanisms underlying NFH remain unclear.

Numerous studies have focused on the etiology of NFH. Huang *et al* (6) reported that fibroblast growth factor 2 (FGF2) and family with sequence similarity 201 member A were associated with the development of NFH, and that insulin-like growth factor 1, SOX9 and collagen type II $\alpha 1$ may also affect the pathogenesis of NFH. The signal transducer and activator of transcription (STAT)1-caspase 3 pathway upregulated the expression of caspase 3, resulting in apoptosis in NFH (7). Tian *et al* (8) demonstrated that NFH was associated with the Toll-like receptor 4 signaling pathway, which may serve an important role in the pathogenesis of osteonecrosis. MicroRNAs (miRs) are also notable diagnostic markers and therapeutic targets of NFH. In a study by Li *et al* (9), has-miR-195-5p exhibited notable downregulation during the collapse of osteonecrotic femoral heads, suggesting that

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Abbreviations: NFH, necrosis of the femoral head; NCBI, National Center of Biotechnology Information; GEO, gene expression omnibus; DEGs, differentially expressed genes; GO, Gene Ontology; STAT, signal transducer and activator of transcription; PPI, protein-protein interaction; DAVID, Database for Annotation, Visualization and Integrated Discovery; KEGG, Kyoto Encyclopedia of Genes and Genomes; BP, biological process; CC, cellular component; MF, molecular function; STRING, Search Tool for the Retrieval of Interacting Genes/Proteins; VEGFA, vascular endothelial growth factor A; GNG13, guanine nucleotide binding protein, $\gamma 13$; BMP, bone morphogenetic protein; AAV, adeno-associated virus; MAPK, mitogen-activated protein kinase; PAF, platelet activating factor; PAFR, platelet-activating factor receptor

Key words: differentially expressed gene, necrosis of femoral head, gene expression profiles, enrichment analysis, cartilage

the collapse may be associated with the downregulation of miR-195-5p. Wei *et al* (10) revealed that the long non-coding RNA Hox antisense intergenic RNA may inhibit miR-17-5p to regulate osteogenic differentiation and proliferation in the osteonecrosis of the femoral head. Ma *et al* (11) revealed that Runt-related transcription factor 2 and transcription factor Sp7 were downregulated in a rat model of NFH, whereas AJ18 was upregulated.

Microarray analysis using high-throughput platforms is a promising and efficient tool for the investigation of the molecular mechanisms of disease, and the identification of useful biomarkers for the diagnosis and prognosis of disease. Lin and Lin (12) reported 215 differentially expressed genes (DEGs) based on gene expression profiles generated from 3 steroid-induced samples from a rat model of NFH and 3 normal rat samples. Tong *et al* (13) revealed 190 DEGs in a rat model of NFH, 52 of which were upregulated and 138 downregulated. Biological functions identified from enrichment analysis of DEGs included signal transduction, apoptosis, extracellular matrix (ECM), angiogenesis and oxidative stress.

In the present study, DEGs were identified in patients with NFH, and associated pathways were analyzed to identify the underlying molecular mechanisms of this disease. Gene expression profiles in the cartilage of patients with NFH and healthy individuals were acquired from the National Center of Biotechnology Information (NCBI) Gene Expression Omnibus (GEO) database and compared. The GSE74089 microarray dataset was analyzed using R software, Bioconductor packages and the Database for Annotation, Visualization and Integrated Discovery (DAVID) 6.8 online resource. A protein-protein interaction (PPI) network of DEGs was then constructed in order to analyze the putative hub genes of NFH.

Materials and methods

Agilent microarray data processing and gene expression profile mining. The microarray data of GSE74089 (14) in NFH was obtained from the NCBI GEO database (15). GSE74089 contained data from cartilage samples from patients with NFH and healthy controls. The microarray data of GSE74089 were obtained using GPL13497 (Agilent-026652 Whole Human Genome Microarray 4x44K v2; Agilent Technologies, Inc., Santa Clara, CA, USA); the data were based on cartilage samples from 4 patients with NFH and 4 controls.

The pre-processing of gene expression profile data was performed using R software (version 3.4.0; <https://www.r-project.org>) and Bioconductor packages 3.8 (<https://www.bioconductor.org/>) for data analysis. Via the Agilent platform, R software was used to analyze the pre-processing and normalization of Series Matrix Files (.TXT files). The parameters used in the R software included robust multi-array average (for background correction), quantiles (for normalization), perfect match (PM)-only (PM correction) and median polish (as a summary measure).

Identification of DEGs. The Linear Models for Microarray Data 3.8 (LIMMA, <http://www.bioconductor.org/packages/release/bioc/html/limma.html>) package (16) in Bioconductor was employed to evaluate DEGs by comparing

the expression values in the cartilage of patients with NFH and controls. The corresponding P-value of gene symbols following a t-test was defined as the adjusted P-value; log₂ fold change >2 and P<0.01 were considered to be the cut-off criteria for DEGs.

Enrichment analysis of DEGs. DAVID 6.8 was employed for enrichment analysis, in order to investigate DEGs at the molecular and functional level (17,18). DAVID is a gene functional classification tool, which provides typical batch annotation and gene-Gene Ontology (GO) term enrichment analysis to highlight the most relevant GO terms associated with a specific gene list. DAVID was employed for GO function and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analyses of DEGs in NFH (19-21). GO terms included 'cellular component (CC)', 'molecular function (MF)' and 'biological process (BP)'; P<0.05 was set as the cut-off for enrichment analysis.

Analysis of PPI networks. Search Tool for the Retrieval of Interacting Genes/Proteins (STRING; <http://string-db.org>) (22,23) is a database that predicts the PPIs of DEGs. According to the STRING database, PPIs of DEGs with a score (median confidence) of >0.4 were selected, and Cytoscape (Version 3.4.0, available online: <http://www.cytoscape.org/>) was used to analyze the PPI network (24). Hub-proteins are significant nodes of protein interaction within the PPI network (25). To characterize hubs in the PPI network of DEGs, betweenness centrality, degree centrality, Maximum Neighborhood Component (MNC) centrality and stress centrality were evaluated.

Results

Identification of DEGs in NFH. To identify DEGs in the cartilage of NFH and healthy patients, the transcription profile data of GSE74089 were obtained from the NCBI GEO database based on 4 patients with NFH and 4 healthy controls. According to the cut-off criteria, 1,191 DEGs were identified in the cartilage of patients with NFH compared with the controls, including 448 downregulated and 743 upregulated DEGs. DEGs in NFH samples were identified using hierarchical cluster analysis of the data (Fig. 1).

Enrichment analysis of DEGs. GO functional enrichment analysis revealed that the BPs of upregulated DEGs included 'ECM organization', '*in utero* embryonic development', 'collagen catabolic processes', 'collagen fibril organization' and 'angiogenesis' (Table I). CC analysis revealed that upregulated DEGs were primarily enriched in 'proteinaceous ECM', 'collagen trimer' and 'ECM'. The MFs of upregulated DEGs were demonstrated to include 'protein binding', 'platelet-derived growth factor binding', 'ubiquitin-protein transferase activity', 'ECM structural constituent' and 'oxidoreductase activity'. The BPs of downregulated DEGs included 'antigen processing and the presentation of peptide or polysaccharide antigen via major histocompatibility complex (MHC) class II', and 'immunoglobulin (Ig) production associated with Ig-mediated immune response', and 'antigen processing and presentation of exogenous peptide antigen via MHC class II'

Table I. Top 5 terms identified by GO enrichment analysis for the upregulated DEGs in necrosis of the femoral head samples.

| Category | Term | Description | Count | P-value | Genes |
|----------|------------|---|-------|------------------------|--|
| BP | GO:0030574 | Collagen catabolic process | 14 | 2.87x10 ⁻⁷ | COL18A1, COL13A1, COL3A1, COL15A1, COL2A1, MMP13, COL5A2, ... |
| | GO:0030198 | Extracellular matrix organization | 24 | 4.46x10 ⁻⁷ | COL18A1, PXDN, COL13A1, LUM, COL3A1, CCDC80, DAG1, ... |
| | GO:0030199 | Collagen fibril organization | 11 | 6.94x10 ⁻⁷ | LUM, TGFBR1, COL3A1, COL1A2, COL2A1, COL1A1, LOX, LOXL2, ... |
| | GO:0001701 | In utero embryonic development | 23 | 7.58x10 ⁻⁷ | SRSF1, MAFF, CCM2, BMP2, ADAM10, TGFBR1, GJA1, UBR3, ... |
| | GO:0001525 | Angiogenesis | 23 | 1.38x10 ⁻⁵ | PRKCA, SLC12A6, COL18A1, PTGS2, COL15A1, RORA, ECM1, THY1, ... |
| CC | GO:0031012 | Extracellular matrix | 37 | 3.37x10 ⁻¹¹ | ASPN, PXDN, LTBP1, LUM, IGFBP7, COL3A1, POSTN, COL2A1, ... |
| | GO:0005578 | Proteinaceous extracellular matrix | 30 | 4.00x10 ⁻⁸ | ASPN, CTHRC1, PXDN, LTBP1, AMTN, MAMDC2, LUM, POSTN, ... |
| | GO:0005581 | Collagen trimer | 17 | 7.43x10 ⁻⁸ | COL18A1, CTHRC1, COL13A1, COL3A1, COL15A1, COL2A1, MMP13, ... |
| | GO:0070062 | Extracellular exosome | 141 | 1.05x10 ⁻⁶ | S100A4, TSPO, RARRES1, FSTL1, AQP1, PNP, OGN, HMCN1, DYSF, ... |
| | GO:0005615 | Extracellular space | 80 | 1.20x10 ⁻⁶ | S100A4, COPA, CTHRC1, PXDN, IGFBP7, FSTL1, POSTN, IL11, OGN, ... |
| MF | GO:0005515 | Protein binding | 361 | 2.83x10 ⁻⁷ | S100A4, XRCC4, LTBP1, PTGS2, SNIP1, LEMD3, PTPN21, FSTL1, ... |
| | GO:0048407 | Platelet-derived growth factor binding | 6 | 1.80x10 ⁻⁵ | COL3A1, COL1A2, COL6A1, COL2A1, COL1A1, COL5A1 |
| | GO:0004842 | Ubiquitin-protein transferase activity | 28 | 2.64x10 ⁻⁵ | KBTBD13, FEM1B, KLHL2, LNX1, ZYG11B, FBXW7, UBE2D2, KLHL24, ... |
| | GO:0005201 | Extracellular matrix structural constituent | 11 | 8.76x10 ⁻⁵ | PXDN, LUM, COL3A1, COL1A2, COL15A1, COL2A1, VCAN, COL1A1, ... |
| | GO:0016641 | Oxidoreductase activity | 4 | 3.79x10 ⁻⁴ | LOXL4, LOXL3, LOX, LOXL2 |

BP, biological process; CC, cellular compartment; DEG, differentially expressed genes; GO, Gene Ontology; MF, molecular function.

(Table II). Additionally, CC enrichment analysis revealed downregulated DEGs to be mainly associated with 'MHC class II protein complex', 'integral component of the luminal side of the endoplasmic reticulum membrane' and 'endocytic vesicle membrane'. The MFs of downregulated DEGs were

demonstrated to include 'MHC class II receptor activity', 'peptide antigen binding', 'MHC class II protein complex binding', 'monooxygenase activity' and 'N,N-dimethylaniline monooxygenase activity'. According to KEGG pathway enrichment analysis, the downregulated DEGs were mainly

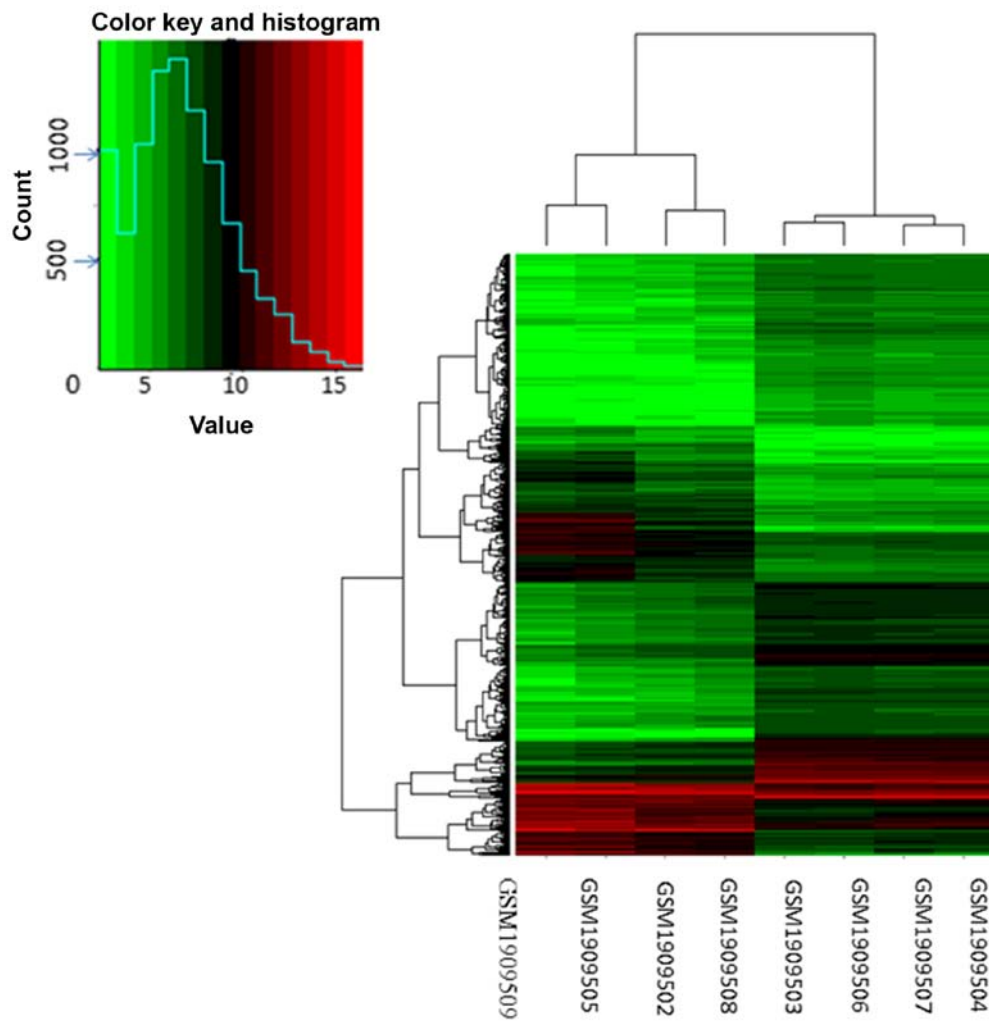


Figure 1. Heat map of DEGs in NFH. Tissue samples are presented as columns; individual genes are presented as rows. Green indicates upregulated genes and red indicates downregulated genes in patients with NFH. The patients in the right four columns (GSM1906503, 4, 6 and 7) were controls; the patients in the left four columns (GSM1909502, 3, 5 and 8) were patients with NFH. DEGs, differentially expressed genes; NFH, necrosis of the femoral head.

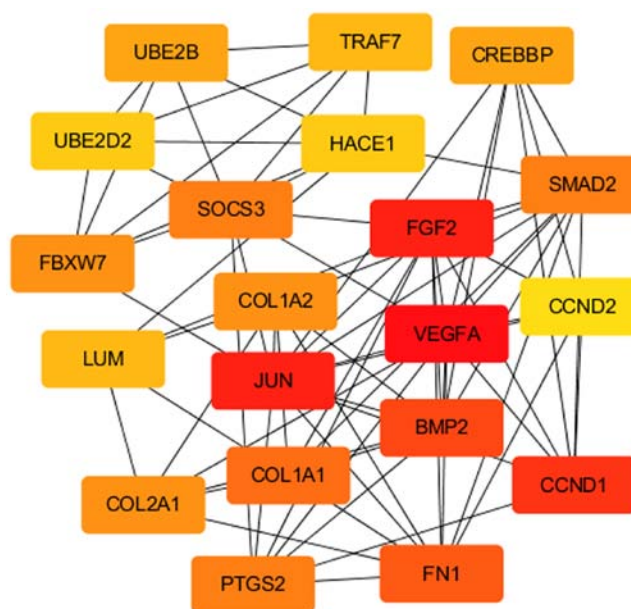


Figure 2. Module identified from the PPI network of the top 20 hub proteins of upregulated DEGs based on MNC centrality. The colors indicate the number of PPIs; red-labeled proteins exhibited an increased number of interactions compared with yellow-labeled proteins. DEGs, differentially expressed genes; MNC, Maximum Neighborhood Component; PPI, protein-protein interaction.

Table II. Top 5 terms identified by GO enrichment analysis for the downregulated DEGs in necrosis of the femoral head samples.

| Category | Term | Description | Count | P-value | Genes |
|----------|------------|---|-------|------------------------|---|
| BP | GO:0002504 | Antigen processing and presentation of peptide or polysaccharide antigen via MHC class II | 9 | 8.90x10 ⁻¹¹ | HLA-DQB1, HLA-DRB1, HLA-DRB3, HLA-DRB4, HLA-DRB5, ... |
| | GO:0002381 | Immunoglobulin production involved in immunoglobulin mediated immune response | 5 | 3.28x10 ⁻⁷ | GAPT, HLA-DQB1, HLA-DRB1, HLA-DRB4, HLA-DRB5 |
| | GO:0019882 | Antigen processing and presentation | 9 | 2.62x10 ⁻⁶ | HLA-DQB1, HLA-DRB1, HLA-DRB3, HLA-DRB4, HLA-DRB5, ... |
| | GO:0019886 | Antigen processing and presentation of exogenous peptide antigen via MHC class II | 10 | 1.76x10 ⁻⁵ | HLA-DQB1, HLA-DRB1, HLA-DRB3, SPTBN2, HLA-DRB4, ... |
| | GO:0060333 | Interferon- γ -mediated signaling pathway | 9 | 1.83x10 ⁻⁵ | HLA-DQB1, HLA-DRB1, HLA-DRB3, HLA-DRB4, HLA-DRB5, ... |
| CC | GO:0042613 | MHC class II protein complex | 9 | 1.58x10 ⁻⁹ | HLA-DQB1, HLA-DRB1, HLA-DRB3, HLA-DRB4, HLA-DRB5, ... |
| | GO:0071556 | Integral component of luminal side of endoplasmic reticulum membrane | 8 | 4.17x10 ⁻⁷ | HLA-DQB1, HLA-DRB1, HLA-DRB3, HLA-DRB4, SPPL2B, ... |
| | GO:0030666 | Endocytic vesicle membrane | 9 | 1.49x10 ⁻⁵ | HLA-DQB1, HLA-DRB1, HLA-DRB3, WNT3A, HLA-DRB4, ... |
| | GO:0012507 | ER to Golgi transport vesicle membrane | 8 | 2.56x10 ⁻⁵ | HLA-DQB1, HLA-DRB1, FOLR1, HLA-DRB3, HLA-DRB4, ... |
| | GO:0030658 | Transport vesicle membrane | 7 | 3.89x10 ⁻⁵ | HLA-DQB1, HLA-DRB1, HLA-DRB3, HLA-DRB4, HLA-DRB5, ... |
| MF | GO:0032395 | MHC class II receptor activity | 7 | 7.34x10 ⁻⁸ | HLA-DQB1, HLA-DRB1, HLA-DRB3, HLA-DRB4, HLA-DOA, ... |
| | GO:0042605 | Peptide antigen binding | 7 | 4.63x10 ⁻⁶ | HLA-DQB1, HLA-DRB1, HLA-DRB3, HLA-DRB4, HLA-DRB5, ... |
| | GO:0023026 | MHC class II protein complex binding | 4 | 0.001983 | HLA-DRB1, HLA-DMB, HLA-DOA, HLA-DRA |
| | GO:0004497 | Monoxygenase activity | 6 | 0.00239 | FMO1, FMO2, FMO6P, CYP4F22, CYP2E1, CYP4B1 |
| | GO:0004499 | N,N-dimethylaniline monoxygenase activity | 3 | 0.003718 | FMO1, FMO2, FMO6P |

BP, biological process; CC, cellular compartment; DEG, differentially expressed genes; ER, endoplasmic reticulum; GO, Gene Ontology; MF, molecular function; MHC, major histocompatibility complex.

enriched in pathways involved in *staphylococcus aureus* infection, asthma and graft-versus-host disease. The upregulated DEGs were mainly enriched in pathways involved in focal adhesion, phosphoinositide 3-kinase (PI3K)/protein kinase B (Akt) signaling, pathways in cancer and ECM-receptor interactions (Table III).

PPI network analysis is important for understanding the biological responses of NFH. The STRING online database and Cytoscape software were employed to analyze the identified DEGs. The top 20 upregulated genes were evaluated for MNC centrality, betweenness centrality, stress centrality and degree centrality in the PPI network (Table IV). Based on various centrality, vascular endothelial growth factor A

(VEGFA) was the most notable gene in the PPI network. Jun proto-oncogene (JUN), cyclin D1 (CCND1), FGF2, HECT domain and ankyrin repeat-containing E3 ubiquitin protein ligase 1 (HACE1), protein kinase C α (PRKCA), bone morphogenetic protein (BMP) 2 and prostaglandin-endoperoxide synthase 2 (PTGS2) were within the top 5 genes of at least one of the centrality rankings. The significant network module generated based on MNC centrality is presented in Fig. 2. The top 15 downregulated DEGs were analyzed in the PPI network (Table V). Guanine nucleotide-binding protein, γ 13 (GNG13) was the most common gene based on various centrality. According to the MNC centrality, the significant network module is presented in Fig. 3.

Table III. Results of KEGG pathway enrichment analysis of differentially expressed genes.

| Category | Term | Description | Count | P-value | Genes |
|---------------------|----------|----------------------------------|-------|------------------------|---|
| Upregulated genes | hsa04510 | Focal adhesion | 28 | 1.44x10 ⁻⁸ | COL3A1, COL2A1, ITGB8, SOS2, COL6A3, PPP1R12A, COL6A1, PDGFC, ... |
| | hsa04151 | PI3K-Akt signaling pathway | 33 | 2.29x10 ⁻⁶ | PPP2R3A, STK11, COL3A1, COL2A1, GNG12, PKN3, ITGB8, SOS2, ... |
| | hsa04512 | ECM-receptor interaction | 14 | 2.24x10 ⁻⁵ | COL3A1, DAG1, COL2A1, COL5A2, COL5A1, ITGB8, COL6A3, COL1A2, ... |
| | hsa04974 | Protein digestion and absorption | 14 | 2.54x10 ⁻⁵ | COL18A1, COL13A1, SLC16A10, COL3A1, COL15A1, COL2A1, COL5A2, ... |
| | hsa05200 | Pathways in cancer | 31 | 1.96x10 ⁻⁴ | GNAI3, ADCY7, PTGS2, EGLN3, BDKRB1, GNG12, GLI3, MMP1, GLI1, ... |
| Downregulated genes | hsa05150 | Staphylococcus aureus infection | 15 | 3.71x10 ⁻¹⁴ | HLA-DQB1, C3AR1, HLA-DRB1, C4B, HLA-DRB3, HLA-DMB, ITGAM, ... |
| | hsa05310 | Asthma | 9 | 1.12x10 ⁻⁸ | HLA-DQB1, HLA-DRB1, HLA-DRB3, HLA-DRB4, HLA-DRB5, HLA-DMB, ... |
| | hsa05332 | Graft-versus-host disease | 9 | 2.56x10 ⁻⁸ | HLA-DQB1, HLA-DRB1, HLA-DRB3, HLA-DRB4, HLA-DRB5, HLA-DMB, ... |
| | hsa05330 | Allograft rejection | 9 | 6.76x10 ⁻⁸ | HLA-DQB1, HLA-DRB1, HLA-DRB3, HLA-DRB4, HLA-DRB5, HLA-DMB, ... |
| | hsa04940 | Type I diabetes mellitus | 9 | 1.94x10 ⁻⁷ | HLA-DQB1, HLA-DRB1, HLA-DRB3, HLA-DRB4, HLA-DRB5, HLA-DMB, ... |

Akt, protein kinase B; ECM, extracellular matrix; KEGG, Kyoto Encyclopedia of Genes and Genomes; PI3K, phosphoinositide 3-kinase.

Discussion

NFH is a destructive bone disease, mainly induced by disruption of the blood supply and the dysfunction of the coagulation and fibrinolysis systems, which lead to the collapse of the femoral head (26,27). Various molecular and genetic studies have investigated the causes of the disease (28,29); however, the exact pathogenic mechanisms remain unclear. A genome-wide approach was employed to investigate differential gene expression in cartilage samples from patients with NFH and healthy controls. GSE74089 was analyzed to identify potentially important genes in NFH using bioinformatics analysis. The roles and interactions of the identified DEGs in NFH were also determined. A total of 1,191 DEGs were identified in cartilage samples from patients with NFH compared with the control, 448 of which were downregulated and 743 of which were upregulated. These DEGs may serve as important biomarkers with mechanistic relevance to the pathogenesis and progression of NFH.

The DEGs reported in the present study could aid the identification of novel molecules or pathways involved in NFH that may serve as targets in the diagnosis and treatment of the disease, and provide novel insight into its pathogenesis. Upregulated DEGs were involved in the organization of the ECM, collagen catabolism and fibril organization, *in utero* embryonic development and angiogenesis. The genes were mainly enriched in proteinaceous ECM, ECM and collagen trimers for CC enrichment. Liu *et al* (14), the study in which the GSE47089 microarray data were obtained, reported that DEGs in NFH were enriched in growth factors, cytokines, and proteins involved in cell cycle, platelet-derived growth factor binding, the ECM and apoptosis; these DEGs were enriched in collagen, the ECM and extracellular regions and platelet-derived growth factor binding. These findings were consistent with the results of the present study. Upregulated DEGs were mainly involved in PI3K-Akt signaling pathway, focal adhesion and ECM-receptor interactions. Downregulated DEGs were mainly enriched in pathways

Table IV. Evaluation of the top 20 upregulated DEGs of the protein-protein interaction network by MNC centrality, betweenness centrality, stress centrality and degree centrality.

| Rank | MNC centrality | Betweenness centrality | Stress centrality | Degree centrality |
|------|----------------|------------------------|-------------------|-------------------|
| 1 | VEGFA | VEGFA | VEGFA | VEGFA |
| 2 | JUN | CCND1 | JUN | JUN |
| 3 | FGF2 | JUN | CCND1 | FGF2 |
| 4 | CCND1 | PRKCA | FGF2 | CCND1 |
| 5 | BMP2 | PTGS2 | HACE1 | HACE1 |
| 6 | FN1 | FGF2 | PTGS2 | BMP2 |
| 7 | COL1A1 | HACE1 | PRKCA | FN1 |
| 8 | PTGS2 | CREBBP | SMAD2 | PTGS2 |
| 9 | SMAD2 | SMAD2 | FN1 | COL1A1 |
| 10 | SOCS3 | FN1 | BMP2 | SMAD2 |
| 11 | COL2A1 | BMP2 | CREBBP | CREBBP |
| 12 | FBXW7 | CD55 | LUM | SOCS3 |
| 13 | COL1A2 | SOCS3 | SOCS3 | COL2A1 |
| 14 | CREBBP | TJP1 | JAK2 | FBXW7 |
| 15 | UBE2B | LUM | CDK9 | PRKCA |
| 16 | LUM | JAK2 | NT5E | UBE2B |
| 17 | TRAF7 | NOTCH3 | TGFBR1 | LUM |
| 18 | HACE1 | TGFBR1 | NOTCH3 | UBE2N |
| 19 | UBE2D2 | NT5E | GNAI3 | COL1A2 |
| 20 | GNAI3 | CDK9 | CD55 | GNAI3 |

DEG, differentially expressed genes; MNC, Maximum Neighborhood Component.

Table V. Evaluation of the top 15 downregulated DEGs of the protein-protein interaction network by MNC centrality, betweenness centrality, stress centrality and degree centrality.

| Rank | MNC centrality | Betweenness centrality | Stress centrality | Degree centrality |
|------|----------------|------------------------|-------------------|-------------------|
| 1 | GNG13 | GNG13 | PTAFR | GNG13 |
| 2 | GNG8 | PTAFR | GNG13 | GNG8 |
| 3 | SAA1 | CTSH | CTSH | SAA1 |
| 4 | CXCR1 | SFTPBP | HLA-DRA | PTAFR |
| 5 | OPRL1 | HLA-DRA | HLA-DRB5 | HLA-DRA |
| 6 | C3AR1 | HLA-DRB5 | HLA-DRB1 | HLA-DRB5 |
| 7 | CORT | HLA-DRB1 | SFTPBP | HLA-DRB1 |
| 8 | PPYR1 | TYROBP | GNG8 | CXCR1 |
| 9 | HTR1B | GNG8 | SAA1 | TYROBP |
| 10 | PTAFR | C1QB | SPTBN2 | OPRL1 |
| 11 | XCR1 | SPTBN2 | TYROBP | C3AR1 |
| 12 | GHSR | SAA1 | XCR1 | CORT |
| 13 | LTB4R2 | ITGAM | C1QB | PPYR1 |
| 14 | HLA-DRA | XCR1 | ITGAM | HTR1B |
| 15 | HLA-DRB5 | CD163 | CD163 | XCR1 |

DEG, differentially expressed genes; MNC, Maximum Neighborhood Component.

involved in *staphylococcus aureus* infection, asthma and graft-versus-host disease. Focal adhesion kinase (FAK) is a non-receptor tyrosine kinase associated with a number of

different signaling proteins (30). Zhang *et al* (31) reported that CXC chemokine ligand 13 (CXCL13)/CXC chemokine receptor 5 (CXCR5)/FAK signaling is involved in the

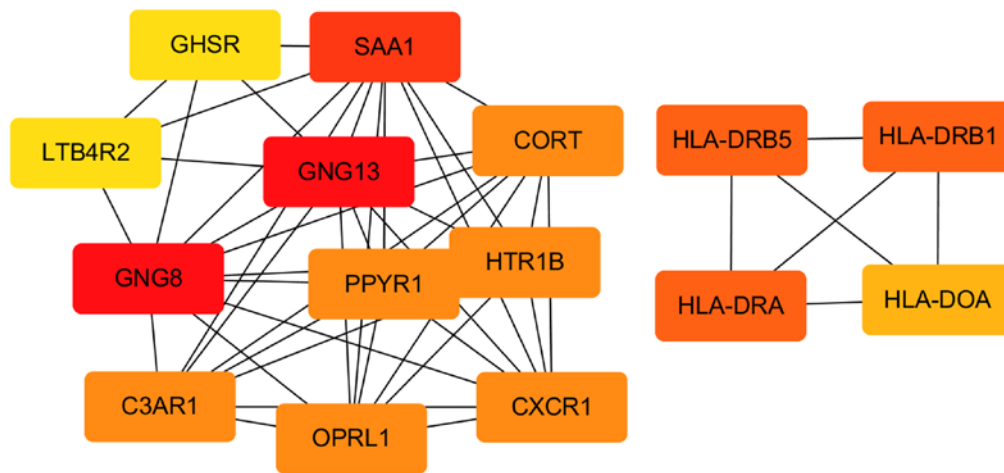


Figure 3. Module identified from the PPI network of the top 15 hub proteins of downregulated DEGs based on MNC centrality. The colors indicate the number of PPIs; red-labeled proteins exhibited an increased number of interactions compared with yellow-labeled proteins. DEGs, differentially expressed genes; MNC, Maximum Neighborhood Component; PPI, protein-protein interaction.

differentiation and trafficking of bone marrow stromal cells (BMSCs) in NFH; CXCL13/CXCR5 signaling was proposed to induce the phosphorylation of FAK via the mitogen-activated protein kinase (MAPK) pathway (32). The PI3K/Akt pathway is associated with various fundamental cellular processes, including survival, proliferation, growth and differentiation (33). Xue *et al.* (34) reported that Salidroside alleviated the dexamethasone-induced apoptosis of osteoblasts by downregulating caspase-3 and activating the PI3K/Akt signaling pathway in osteoblasts. In addition, the osteogenic differentiation-inducing and bone regenerative properties of graphene-incorporated poly(lactic-co-glycolic acid) were mediated via the activation of the PI3K/Akt/glycogen synthase kinase β / β -catenin signaling pathway (35).

PPI networks of DEGs were constructed using STRING and Cytoscape to investigate the associations between important proteins identified by GO enrichment and pathway analyses (36). The upregulated DEGs included VEGFA, JUN, CCND1, FGF2, HACE1, PRKCA, BMP2 and PTGS2. In the early stages of a rabbit model of NFH, BMP and VEGF were co-expressed using an adeno-associated virus (AAV) vector; the AAV-VEGF/BMP vector increased the bone repair capacity of the femoral head by inducing angiogenesis and improving bone quality (37). VEGF-expressing transgenic autologous BMSCs improved bone reconstruction and blood vessel regeneration in a canine model of NFH (38). Adenovirus-mediated expression of BMP2 and basic FGF in BMSCs in combination with a demineralized bone matrix improved bone formation in a dog model of NFH (39). CCND1 is an important regulatory factor of the cell cycle and is a frequently used biomarker for the diagnosis and prognosis of human primary tumors (40). Phosphorylated CCND1 is associated with the development of osteosarcoma, which may occur via MAPK-induced expression of CCND1, and the continuous proliferation of tumor cells (41).

Downregulated DEGs identified in the present study included GNG13, platelet-activating factor receptor (PAFR), GNG8, serum amyloid A1 and cathepsin H. PPI analysis identified GNG13 as a central downregulated DEG. GNG13 is a divergent member of the GNG subunit γ family and

contains a C-terminal NPW tripeptide (42). It is a component of the gustducin G-protein heterotrimer involved in bitter and sweet taste reception in taste bud cells (42). PAF is a potent phospholipid regulator of inflammation; PAFR is expressed on plasma and nuclear membranes in various cell types, and binds to PAF and oxidized phospholipids (43). Activation of PAFR in macrophages induces an anti-inflammatory phenotype (44). PAF activates PAFR, which may serve an important role in the malignant development of esophageal squamous cell carcinoma by stimulating PI3K/AKT activation, and promoting disease progression and metastasis via the initiation of a forward feedback loop between PAFR and STAT3 (45).

There were certain limitations to the present study. Gene expression data were obtained from only a single dataset containing 4 patients with NFH and 4 controls. The use of additional datasets with increased sample sizes in future studies would increase the accuracy and reliability of identified DEGs. Additionally, the potential role of DEGs and PPIs identified in the hip cartilage of patients with NFH require further investigation *in vivo* and *in vitro*; for example, the effects of silencing or upregulating DEGs in cellular or *in vivo* models could be determined. Furthermore, the altered expression of genes and proteins identified by microarray analysis should be investigated via reverse transcription-quantitative polymerase chain reaction and histological analyses of samples from patients with NFH. These experiments may validate the diagnostic and prognostic potential of identified DEGs for the disease.

In conclusion, following integrated bioinformatical analysis of the gene expression profiles of cartilage from patients with NFH and controls, 1,191 DEGs were identified in necrotic samples, 743 of which were upregulated and 448 were downregulated. DEG enrichment analysis identified molecules and pathways, which may provide novel insight into the pathogenesis of NFH. PPI network analysis identified VEGFA, JUN, CCND1, FGF2, HACE1, PRKCA, BMP2 and PTGS2, and GNG13 as central upregulated and downregulated DEGs, respectively. These DEGs and signaling pathways may serve as biomarkers and targets in the treatment of NFH; however, further investigation is required.

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Availability of data and materials

The datasets used and/or analyzed during the present study are available from the corresponding author on reasonable request.

Authors' contributions

WCL made substantial contributions towards the conception and design of the study, and experiments. DLB and YX performed the major bioinformatics analysis of the gene database. RJX and WBH performed the analysis of DEGs. WCL drafted the manuscript, aggregated the figures and discussed the results. RJX contributed to the revision of the manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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