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Research paper

Comparative analysis of gene expression profiles of hip articular cartilage between non-traumatic necrosis and osteoarthritis

Wenyu Wang [a], Yang Liu [b], Jingcan Hao [a], Shuyu Zheng [c], Yan Wen [a], Xiao Xiao [a], Awen He [a], Qianrui Fan [a], Feng Zhang [a,*], Ruiyu Liu [d,**]

[a] Key Laboratory of Traece Elements and Endemic Diseases of National Health and Family Planning Commission, School of Public Health, Health Science Center, Xi'an Jiaotong University, PR China
[b] Department of Rheumatology, Xi'an Fifth Hospital, PR China
[c] Department of Radiation Oncology, First Affiliated Hospital, Health Science Center, Xi'an Jiaotong University, PR China
[d] Department of Orthopedics, Second Affiliated Hospital of Health Science Center of Xi'an Jiaotong University, Xi'an, Shaanxi, PR China

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ABSTRACT

Hip cartilage destruction is consistently observed in the non-traumatic osteonecrosis of femoral head (NOFH) and accelerates its bone necrosis. The molecular mechanism underlying the cartilage damage of NOFH remains elusive. In this study, we conducted a systematically comparative study of gene expression profiles between NOFH and osteoarthritis (OA). Hip articular cartilage specimens were collected from 12 NOFH patients and 12 controls with traumatic femoral neck fracture for microarray ($n = 4$) and quantitative real-time PCR validation experiments ($n = 8$). Gene expression profiling of articular cartilage was performed using Agilent Human 4×44 K Microarray chip. The accuracy of microarray experiment was further validated by qRT-PCR. Gene expression results of OA hip cartilage were derived from previously published study. Significance Analysis of Microarrays (SAM) software was applied for identifying differently expressed genes. Gene ontology (GO) and pathway enrichment analysis were conducted by Gene Set Enrichment Analysis software and DAVID tool, respectively. Totally, 27 differently expressed genes were identified for NOFH. Comparing the gene expression profiles of NOFH cartilage and OA cartilage detected 8 common differently expressed genes, including COL5A1, OGN, ANGPTL4, CRIP1, NFIL3, METRNL, ID2 and STEAP1. GO comparative analysis identified 10 common significant GO terms, mainly implicated in apoptosis and development process. Pathway comparative analysis observed that ECM-receptor interaction pathway and focal adhesion pathway were enriched in the differently expressed genes of both NOFH and hip OA. In conclusion, we identified a set of differently expressed genes, GO and pathways for NOFH articular destruction, some of which were also involved in the hip OA. Our study results may help to reveal the pathogenetic similarities and differences of cartilage damage of NOFH and hip OA.

1. Introduction

Non-traumatic osteonecrosis of femoral head (NOFH) is a disabling disease, which mainly affects young adults (Malizos et al., 2007). NOFH leads to the collapse of femoral head, secondary osteoarthritis and hip dysfunction (Ito et al., 2003). The unknown pathogenesis of NOFH impedes the development of effective precautions and treatments, leaving the majority of NOFH patients to total hip replacement.

Osteoarthritis (OA) is the most common degenerative joint disorder. It is estimated that one in four people may develop symptomatic hip osteoarthritis in his or her lifetime (Murphy et al., 2010). Hip OA is another major reason for total hip replacement. Both NOFH and OA clinically manifest as serious pain and restricted movement of hip joints. In the early stage of NOFH without significant radiographic changes, it is often difficult to distinguish NOFH from OA.

NOFH is primarily characterized by progressive avascular bone necrosis and joint degeneration of the hip (Ikeuchi et al., 2015). The newly generated bone at the necrotic region of femoral head fails to recover structural integrity and finally leads to the collapse of femoral head (Seamon et al., 2012). Most of previous studies of NOFH focused on the damage of bone and bone marrow. Recently, some studies have demonstrated the important role of hip cartilage destruction in the deterioration of NOFH (Magnussen et al., 2005b; Li et al., 2015a). The

Abbreviations: NOFH, non-traumatic osteonecrosis of femoral head; OA, osteoarthritis; qRT-PCR, quantitative real-time PCR; SAM, Significance Analysis of Microarrays; GO, gene ontology; MMP, metalloproteinase.

* Correspondence to: F. Zhang, School of Public Health, Health Science Center, Xi'an Jiaotong University, Xi'an, PR China.
** Corresponding author.
E-mail addresses: fzhxjtu@mail.xjtu.edu.cn (F. Zhang), liuryu@126.com (R. Liu).

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damage of hip cartilage is a common phenomenon in NOFH, which in turn destabilizes the structure and exacerbates the collapse of femoral head (Seo et al., 2015). Moreover, previous studies observed altered cartilage lesion-related biomarkers in the early stage of NOFH without significant bone destruction (Jingushi et al., 2000; Li et al., 2015a). The altered expression levels of biomarkers appeared to be correlated with the progression of NOFH (Jingushi et al., 2000; Li et al., 2015a). Effective interventions against hip articular cartilage destruction have the potential to delay NOFH deterioration and relieve the dysfunction of hip.

Articular cartilage degeneration is one of the major pathological changes of OA. Extensive studies of OA articular cartilage degeneration have been conducted, but few for NOFH. As a common pathological change, clarifying the similarities and differences of the mechanism of articular cartilage lesions between NOFH and OA may help to utilize OA study results to study the molecular pathogenesis of NOFH cartilage degeneration. To the best of our knowledge, no study has been conducted to compare the genome-wide gene expression profiles of hip articular cartilage between NOFH and OA by now.

In this study, we first conducted a genome-wide gene expression profiling of NOFH articular cartilage, and compared it with the gene expression profile of hip OA articular cartilage. The differently expressed genes of NOFH vs. Control and OA vs. Control were then subjected to functional enrichment analysis considering gene ontology (GO) and biological pathways.

2. Materials and methods

2.1. Ethics statement

This study was approved by the Institutional Review Board of Xi'an Jiaotong University. Informed-consent documents were obtained from all study subjects.

2.2. NOFH articular cartilage specimens

Hip articular cartilage specimens were collected from 24 subjects undergoing total hip replacement surgery at the Second Affiliated Hospital of Xi'an Jiaotong University. All participants were Chinese Han. NOFH cartilage specimens were collected from the anterosuperior regions of femoral heads of 12 patients with non-traumatic Ficat classification grade III NOFH (Ficat, 1985). Health control cartilage specimens were collected from the anterosuperior regions of femoral heads of 12 OA-free subjects with traumatic femoral neck fracture. All cartilage specimens were harvested within 2 h of joint replacement surgery. Only the articular cartilage with intact gross appearance and being less than histological grade 2 was used in this study (Mankin et al., 1971; Carlson et al., 2002). All study subjects underwent careful clinical examination and radiography of hips. Clinical data of each subject was recorded by doctor-administered questionnaire, including self-reported ethnicity, lifestyle characteristics, health status, family and medical histories. Based on the radiologic imaging of hip and hematoxylin and eosin staining of hip articular cartilage, the subjects with rheumatoid arthritis or other hip disorders were excluded. The collected cartilage specimens were rapidly dissected and frozen in liquid nitrogen, and stored at −80 °C for subsequent RNA extraction. Specific for this study, 4 and 8 NOFH-control pairs, matched with age and sex, were used for microarray and quantitative real-time polymerase chain reaction (qRT-PCR), respectively (Table 1).

Table 1
Characteristics of study subjects.

	NOFH[a]		Control	
	Sex	Age (years)	Sex	Age (years)
Microarray	Male	41	Male	42
	Male	42	Male	45
	Male	42	Male	42
	Male	42	Male	54
qRT-PCR	Male	43	Male	57
	Female	47	Female	47
	Male	47	Male	61
	Male	47	Male	64
	Female	48	Female	60
	Male	51	Male	53
	Female	54	Female	61
	Female	57	Female	63

[a] Denotes non-traumatic osteonecrosis of femoral head.

2.3. RNA preparation

Frozen cartilage specimens were first rapidly ground in liquid nitrogen using freezer mill. Total RNA was isolated from cartilage specimens using the Agilent Total RNA Isolation Mini kit (Agilent Technologies, Santa Clara, CA, USA) following manufacturer's recommendation. The quality and concentration of isolated total RNA was evaluated by 1% agarose gel electrophoresis and Agilent ND-1000 (Agilent Technologies), respectively.

2.4. Microarray hybridization

The isolated total RNA of 4 NOFH articular cartilage and 4 healthy articular cartilage was translated into cRNA and labeled with Cy3 using the Agilent Quick Amp Labeling kit (Agilent Technologies). According to the Agilent One-Color Microarray experimental protocol (Agilent Technology), the labeled cRNA was purified using RNeasy Mini kit (Qiagen, MD, USA). Agilent ND-1000 (Agilent Technology) was used to determine the concentration and specific activity of labeled cRNA. 1 μg of labeled cRNA was hybridized to the Agilent Human 4 × 44 K Microarray (v2, Agilent Technologies). Hybridization signals were recorded by Agilent microarray scanner (G2505C), and analyzed by Feature Extraction v11.0 and Agilent GeneSpringGX v12.1 software (Agilent Technologies). The quality of fluorescent spots was evaluated, and the fluorescent spots failing to pass the quality control procedures were removed. All the raw data underwent Linear and LOWESS normalization.

2.5. Data analysis

The Significance Analysis of Microarrays (SAM) software (version 4.01, http://statweb.stanford.edu/~tibs/SAM/) was applied for microarray data analysis (Tusher et al., 2001). The differently expressed genes were identified at fold changes >3.0 and false discovery rate (FDR) <0.01. The FDR values were calculated using the permutation-based analysis algorithm of SAM (Tusher et al., 2001). GO functional enrichment analysis of gene expression profile of NOFH was conducted by Gene Set Enrichment Analysis (GSEA, http://www.broadinstitute.org/gsea/index.jsp) software (Subramanian et al., 2005). GSEA is capable of integrating the information of disease-related genes and known functional relationships of multiple genes. It can help to identify disease relevant gene sets with known biological function. The gene ontology collection 3.0 containing 1454 GO terms was downloaded from the Molecular Signature Database (http://www.broadinstitute.org/gsea/msigdb/index.jsp) (Subramanian et al., 2005), and used by GSEA for GO enrichment analysis. Significant GO terms were derived from entire gene expression profile data of NOFH at GSEA P values ≤0.05. For biological pathway enrichment analysis, the differently expressed genes were also input into the functional annotation tool DAVID 6.7 (http://david.abcc.ncifcrf.gov/home.jsp) (Huang da et al., 2009). An enrichment P value was calculated by DAVID for each pathway term. The significant pathways were identified at DAVID P values < 0.05.

2.6. Quantitative real-time PCR validation

Quantitative real-time PCR (qRT-PCR) was conducted to validate the accuracy of microarray experiment using an independent sample of 8