

GENE EXPRESSION PROFILING OF OSTEOARTHRITIS FROM CANINE CHONDROCYTES

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Introduction

Osteoarthritis, also referred to as degenerative joint disease, is a prevalent and debilitating disease in canines. It is often associated with hip dysplasia (1). There is a high degree of similarity between canine and human osteoarthritis making it an excellent animal model of human osteoarthritis. While causative factors remain largely unknown, the disease is characterized by the imbalance of cartilage matrix degradation outweighing cartilage matrix synthesis. Chondrocyte apoptosis and inflammation may also be associated with the disease (2,3).

Gene expression profiling is a powerful tool for determining genes associated with biological processes. Since chondrocytes represent the cellular component of cartilage, we have studied the gene expression profile of osteoarthritic chondrocytes using a combination of differential display and microarray technology.

Methods

Differential Display: RNA was extracted from cartilage that had been snap-frozen in liquid nitrogen and stored at -80°C . Fluorescent differential display was performed using one of three anchored primers in combination with one of 80 arbitrary primers (GenHunter). In all, 240 PCR reactions were carried out. Reactions were separated using PAGE and visualized using a fluorescent scanner (FMBIOII, Hitachi). Bands representing differentially expressed genes were excised, reamplified and run on an agarose gel to verify size. These were subsequently subcloned (PCR-TRAP, GenHunter) and sequenced.

Microarray: Microarray probes were generated by PCR-amplifying clones isolated from differential display. Probes were spotted in duplicate onto poly-L-lysine coated slides using a GMS417 (Affymetrix) arrayer. Osteoarthritic cartilage samples were obtained from the femoral heads of clinically diagnosed canines undergoing total hip replacement (The Ohio State University). RNA was hybridized to the slides using the HC ExpressArray (Digene) kit and visualized using a GMS418 (Affymetrix) scanner. The Image (Biodiscovery) program was used for spot finding and subsequent data analysis was performed using GeneSight (Biodiscovery).

Results

Approximately 1750 clones were isolated using differential display. The qualitative selection of differentially expressed genes included genes only expressed in normal or osteoarthritic samples as well as genes whose expression appeared to be only slightly regulated. A representational polyacrylamide gel image is shown in figure 1. Panel A represents the gel prior to band excision and panel B represents the same gel after band excision. Sizes of clones ranged from 90 b.p. to 1150 b.p., with an average size of 300 b.p. After filtering the sequences for redundant sequences, dimers, E. coli fragments, fragments <100 b.p. etc., approximately 1300 remained.

BLAST analysis enabled the identification of roughly 40% of the clones with an E value less than 1×10^{-10} . Most of this identification was based on human, mouse and rat orthologs due to the limited number of sequenced canine genes. Clones include transcripts such as inducible nitric oxide synthase, COMP, alpha-1 type collagen III, osteonectin and interleukin-8.

The analysis of osteoarthritic cartilage samples by microarray analysis is currently being completed. A representational scatterplot is shown in figure 2. Samples include cartilage from osteoarthritic canines undergoing total hip replacement from various ages (1 year to 8 years of age). Samples are being analyzed to identify specific genes involved in osteoarthritis and their association with the developmental stages of the disease.

Discussion

The use of differential display to isolate gene transcripts has allowed us to develop a microarray chip enriched in transcripts involved in osteoarthritis. The use of this chip to analyze samples from clinically diagnosed canines with osteoarthritis is confirming the role of the isolated differential display clones as well as further characterizing canine osteoarthritis on the molecular level. The identification of the clones shown to be involved in osteoarthritis has been hindered by the inability to find annotated orthologs by BLAST analysis. This will be aided by extending the sequence information of these clones by other means such as 5' RACE or as more canine sequence information becomes available.

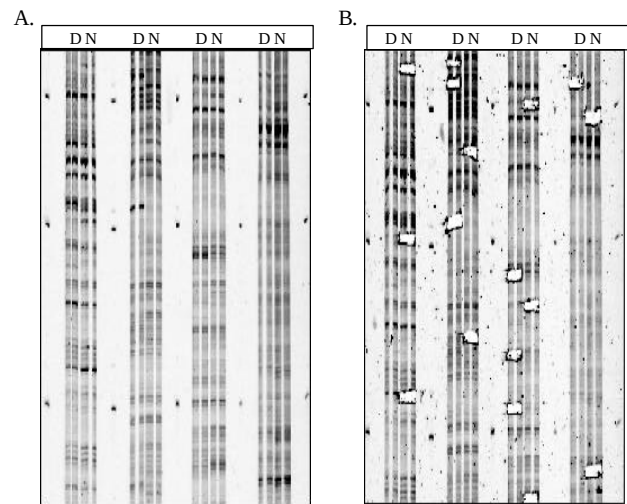


Figure 1. A. Differential display of osteoarthritis vs. normal transcripts loaded in duplicate prior to band excision (D=osteoarthritic, N=normal). B. The same gel after band excision.

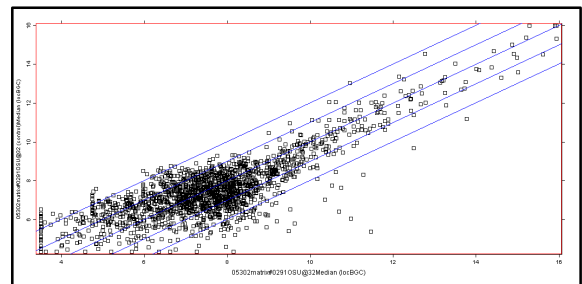


Figure 2. A representational microarray scatterplot of normal vs. osteoarthritic cartilage transcripts.

References

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