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## The Varus Knee Reveals Differential Expression Patterns of miRNAs in Spared vs. Non-spared Compartments

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# THE VARUS KNEE REVEALS DIFFERENTIAL EXPRESSION PATTERNS OF MIRNAS IN SPARED VS. NON-SPARED COMPARTMENTS

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## Introduction

MicroRNAs (miRNAs) function by repressing cellular protein levels to provide a sophisticated level of gene regulation that coordinates a broad spectrum of biological processes. MiRNA inhibition of mRNA translation has emerged as an important regulator of chondrogenic and osteogenic development, osteoblast, osteoclast and chondrocyte cell growth and differentiation, and tissue homeostasis in the adult skeleton. MiRNAs control many layers of regulation in adult tissues connected to both normal biological and pathologic cellular activities. The study of miRNAs in skeletal disorders is in its infancy. Osteoarthritis (OA) is a disease that progresses from degeneration of the articular cartilage to remodeling of the underlying subchondral bone over many years. While miRNAs have been identified with the inflammatory pathogenesis of rheumatoid arthritis (RA), only a few studies have been performed on OA tissue (1,2). Here we performed a systematic analysis of the articular cartilage from varus OA knee replacements, comparing multiple tissue samples from the lateral (spared) and medial (diseased) compartments. Before proceeding to a miRNA profiling, each sample was analyzed for expression of a small set of miRNAs that have been reported in association with RA, OA and cartilage formation. These preliminary findings have identified a spectrum of changes in surface cartilage between control and diseased tissue.

## Methods

**Human tissues:** 6 individual articular cartilage samples were harvested from a total of 5 osteoarthritic varus human knees. Cartilage samples were exempt from IRB review as they are discarded materials. Samples were removed with a biopsy punch and were approximately 6 x 2mm (diameter x thickness). Cartilage specimens were harvested from the more normal-appearing lateral ('spared') compartments and from the more OA-affected, medial compartments of the knees. This sampling technique allows direct comparison of more significantly OA-affected cartilage samples with those of lower OA grade from the same set of individuals. Knee ages ranged from 53-74 years old and averaged 65 years old.

**RNA and miRNA Isolation:** Each osteochondral specimen was placed in RNA Later (Sigma) immediately following surgical removal, in order to preserve the integrity of the total RNA. Specimens were transported to the lab where individual samples were removed carefully with RNase-treated tools and were transferred into fresh RNA Later solution and incubated overnight at 4C to allow penetration and maximal inhibition of RNase activity. Samples were then removed from RNA Later, blotted briefly and frozen in liquid N<sub>2</sub>, and then pulverized using a Bessman tissue pulverizer (Fisher). The pulverized samples were immediately placed into Trizol (Invitrogen) and homogenized using a polytron device. Total RNA was isolated to include small RNAs of >17 nucleotides, according to the manufacturer's protocol (Invitrogen). Purified RNA was obtained using precipitated total RNAs filtered through glass columns according to the manufacturer's protocol (Zymo Research). RNAs were reverse-transcribed into DNA using 900ng of each purified RNA sample using the TaqMan microRNA Reverse Transcription Kit (Applied Biosystems). TaqMan qPCR analysis for small RNAs was performed using the following human primer-probe sets from Applied Biosystems: hsa-miRs-9, 22, 27a, 29a and 34a. Human U6 was used to normalize all qPCR data and data was plotted as normalized relative values. Normalized relative values were averaged for each of the complement of medial vs. lateral samples.

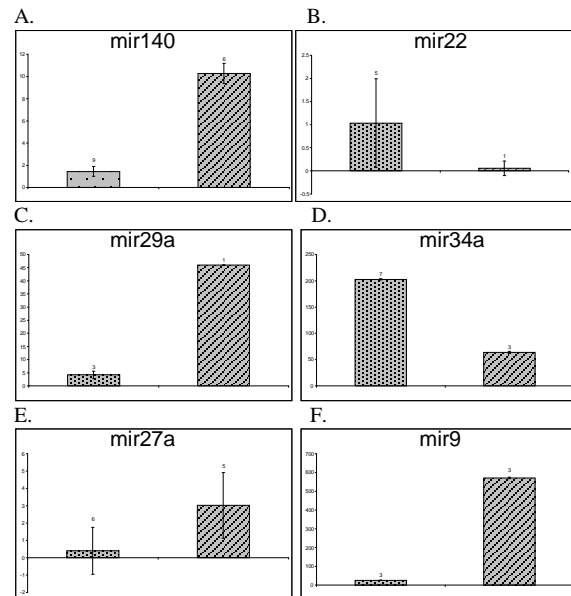
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## Significance

The aims of this project are to provide an internally-controlled platform of study for the miRNAs of OA using the natural disease differences inherent in spared vs. non-spared cartilage compartments from a varus

OA knee. Such efforts may provide an alternative methodology when compared to the significant barrier of obtaining age-matched, non-OA control knee cartilage.



**Figure 1.** qRT PCR comparison of miRNAs in medial (dotted) vs. lateral (cross-hatched) articular cartilage samples from OA varus knees.

## Results

MiRs were found to be either up- or down-regulated in a manner that suggests a mechanism of de-repression of pro-inflammatory cytokine signaling and repression of pro-inflammatory events in medial vs. lateral varus knee OA cartilage samples, respectively. MiRs 9, 27a, and 29a were found to up-regulated in lateral varus knee cartilage samples vs. medial varus knee cartilage samples (Fig.1, A,C,E,F). Conversely, miRs 22 and 34a were found to upregulated in medial vs. lateral cartilage samples (Fig.1, B, D).

## Discussion

The functional characterization of global gene expression patterns through miRNAs in OA is lacking. Particularly, the roles of miRs in OA disease development, as biomarkers, and in disease outcomes are at question. A few large-scale microarray approaches have previously identified expression signatures of potential OA-involved miRNAs (2). By comparing cartilage samples that derive from more advanced (medial) vs. less advanced (lateral) OA stages in varus human knees, we seek to combine miRNA expression analysis with clinicopathologic features. MiRs -9, -22 and -34a are known to be involved in regulating pro-inflammatory events in OA. Higher levels of miRs, -9, -27a & -140 in less-affected lateral compartment cartilage are consistent with previous reports of reduced TNF $\alpha$ , MMP-13 & ADAMTS-5 expression events, respectively (Fig.1, F, E & A) (3,4,5). MiRs -22 and -34a have been shown to be associated with promoting tissue catabolism by their presence and are here shown to be increased in more affected medial compartment cartilage (Fig.1, B, D) (4). In addition, miR-34a deficiency has been previously shown to inhibit chondrocyte apoptosis, consistent with the lower expression level found in lateral cartilage (Fig.1, D) (6). MiR-29a was found in a previous microarray analysis to be the highest-fold down-regulated miRNA in OA vs. normal cartilage, consistent with our finding of under-expression in medial cartilage samples (Fig.1, C) (1). The goal of these studies is to begin to understand how miRNAs can both contribute to and protect against OA. Here we show that the comparison of cartilage-derived miRNAs in medial and lateral compartment pairs from the same knee may facilitate validation of candidate OA miRNAs.