DEFINING THE ROLE AND MECHANISMS OF MICRORNAS IN OSTEOARTHRITIS

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Introduction

Knee osteoarthritis (OA) is one of the most common age-related joint diseases in humans, characterized by articular cartilage degeneration and joint inflammation. Treatment is only symptomatic and most patients with late-stage OA undergo knee replacement surgery. MicroRNAs (miRNAs) are a class of small non-coding RNAs which function at the post-transcriptional level as important regulators of gene expression. There is increasing evidence of a role for miRNAs in cartilage ageing and OA. In this study we undertook a microarray analysis approach to determine alterations in the miRNA expression profile between young and old OA human knee cartilage and investigate how these changes can contribute to the pathogenesis of OA. This study could uncover novel molecular players and lead to potential therapeutic targets in OA.

Materials and Methods

Femoral articular cartilage was collected from the knee of young patients (n=9, 24±3.8 years) undergoing anterior cruciate ligament repair surgery and old OA patients (n=10, 63±7.3 years) undergoing total knee replacement, at Maastricht University Medical Centre under appropriate ethical approval. For the old OA group two samples were collected per patient; one cartilage sample from an area that was less affected, representing early-stage OA and one sample from an area that was heavily affected, representing late-stage OA. OA severity was confirmed histologically by two independent scorers using a modified Mankin’s scoring system. For microarray analysis, total RNA was extracted and hybridized to Affymetrix GeneChip 125 miRNA 4.0 arrays. MiRNA log fold change (logFC) values were adjusted for multiple testing and significantly differentially expressed (DE) miRNAs were identified. Selected DE miRNAs were validated through quantitative Reverse Transcription Polymerase Chain Reaction (qRT-PCR) analysis in the same samples (dependent cohort) as well as in an independent cohort of cartilage samples (n=8-10 per group). Treatment of human articular chondrocytes with interleukin 1 beta (IL-1b) confirmed differential expression of selected miRNAs in an OA-like in-vitro model.

Results

Mankin’s histological score showed a significant difference between early and late-stage OA samples (mean±SD; Early-OA: 2.6±1.3; Late-OA: 4.8±2.6). Microarray analysis revealed 484 DE miRNAs in young vs early-stage OA and 318 DE miRNAs in young vs late-stage OA. In contrast to histological findings, early and late-state OA groups showed a similar set of DE miRNAs when compared to young group, suggesting there is a similar miRNA expression profile in different stages of OA progression. MiRNAs with logFC > 4 and predicted significance in OA were selected for further validation. Among these, miRNA-361-5p, -379-5p, -107 and -143-3p were significantly decreased in OA cartilage compared to young cartilage. QRT-PCR analysis confirmed reduced expression of these miRNAs, both in dependent and independent cohorts of cartilage samples, validating microarray results. Finally, treatment of human articular chondrocytes with IL-1b decreased the expression of the selected miRNAs in the treated group compared to control, suggesting a potentially important role of these miRNAs in OA.

Discussion

Using microarray technology we identified several DE miRNAs, between young and old OA human cartilage of different disease stages. Early and late-stage OA showed a similar pattern of DE miRNAs indicating that dysregulation of important miRNAs in cartilage seems to be an early event during OA pathogenesis that precedes major histological changes. Differential expression of selected miRNAs was validated in an independent cohort of cartilage tissue samples as well as in an OA-like in-vitro model suggesting a potentially important role for these miRNAs in knee OA.