INTRODUCTION: The discovery of miRNAs is a remarkable breakthrough in the field of molecular genetic fields, as miRNAs are key actors that regulate gene expression in diverse cellular process from unicellular yeast to human. Typically, they bind to the 3'-untranslated region of their target mRNAs and repress protein expression by mRNA destabilization. By doing so, miRNAs are emerging as key regulators of innate and adaptive immune responses, inflammation and chronic pain. The objective of this study is to characterize the functional role of miR-146a in human articular cartilage homeostasis and OA pain by regulating pain-related molecules.

METHODS: Generation of knee joint OA animal model with behavioral hyperalgesia assessments (In et al., A&R, 2010, in press). Cell culture and Transfection: Human articular cartilage and synovium were obtained from the Gif of Hope Organ and Tissue Donor Network. The chondrocytes and synovium were released by enzymatic digestion and plated onto 12-well plates at 5x10^5 cells/cm². After 4 wks, the cells were split into 12 well plates at 2X10^5 cells/cm². Human Astrocyte cell line were plated at 5X10^5 cells/cm² .Cells were transfected with 10-20 pmol of miR-146a (Dharmacon) using lipofectamine (LF) plus (Invitrogen). Total RNA isolation, Reverse transcription and real-time PCR: Total RNA was isolated using the Trizol reagent (Invitrogen) following the instructions provided by the manufacturer. Reverse transcription (RT) was carried out with 1ug total RNA using ThermoScript TM RT-PCR system (Invitrogen) for first strand cDNA synthesis. For real-time PCR, cDNA was amplified using MyQ Real-Time PCR Detection System (Bio-Rad Hercules, CA). β-actin was used as internal control. Western blotting analyses were performed for examining protein levels as previously described.

RESULTS:

Spinal dorsal horn was harvested from intra-articular MIA-induced knee OA animal models. Comparative analysis by miRCURYT™ LNA Array miR profiles demonstrate significant alterations of a group of miRs that regulate theNFκB-dependent pathway and inflammatory cytokine genes in the spinal dorsal horn.

Overexpression of synthetic miR-146 significantly regulates inflammatory cytokines, MMP-13, ADAMTS-5, matrix associated molecules and pain-related molecules in human articular chondrocytes, knee joint synovium and glial cells.

CONCLUSION & DISCUSSION:

miR-146a plays a role in knee joint homeostasis and OA symptom, pain, by balancing the inflammatory response and pain-associated molecules in cartilage, synovium, glial activity. miR-146a may represent effective therapeutic agent for the treatment of both cartilage regeneration and treatment of its symptom, pain.

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