Gene 480 (2011) 34–41

Contents lists available at ScienceDirect

Gene

journal homepage: www.elsevier.com/locate/gene

MicroRNA-146a is linked to pain-related pathophysiology of osteoarthritis

Xin Li [a], Gary Gibson [b], Jae-Sung Kim [a], Jeffrey Kroin [c], Shunbin Xu [d], Andre J. van Wijnen [e], Hee-Jeong Im [a,f,g,h,*]

[a] Department of Biochemistry, Rush University Medical Center, Chicago, IL, USA
[b] Henry Ford Hospital, Detroit, MI, USA
[c] Department of Anesthesiology, Rush University Medical Center, Chicago, IL, USA
[d] Department of Neurological Sciences, Rush University Medical Center, Chicago, IL, USA
[e] Department of Cell Biology, University of Massachusetts, Amherst, MA, USA
[f] Department of Internal Medicine, Section of Rheumatology, Rush University Medical Center, Chicago, IL, USA
[g] Department of Orthopedic Surgery, Rush University Medical Center, Chicago, IL, USA
[h] Department of Bioengineering, University of Illinois at Chicago, IL 60612, USA

ARTICLE INFO

Article history:
Accepted 3 March 2011
Available online 10 March 2011

Received by A.J. van Wijnen

Keywords:
miR-146a
Osteoarthritis
Pain
Chondrocyte
Synovial cells
Glial cells

ABSTRACT

Because miR-146a is linked to osteoarthritis (OA) and cartilage degeneration is associated with pain, we have characterized the functional role of miR-146a in the regulation of human articular cartilage homeostasis and pain-related factors. Expression of miRNA 146a was analyzed in human articular cartilage and synovium, as well as in dorsal root ganglia (DRG) and spinal cord from a rat model for OA-related pain assessment. The functional effects of miR-146a on human chondrocytic, synovialm and microglia cells were studied in cells transfected with miR-146a. Using real-time PCR, we assessed the expression of chondrocyte metabolism-related genes in chondrocytes, genes for inflammatory factors in synovial cells, as well as pain-related proteins and ion channels in microglial cells. Previous studies showed that miR-146a is significantly upregulated in human peripheral knee OA joint tissues. Transfection of synthetic miR-146a significantly suppresses extracellular matrix-associated proteins (e.g., Aggrecan, MMP-13, ADAMTS-5, collagen II) in human knee joint chondrocytes and regulates inflammatory cytokines in synovial cells from human knee joints. In contrast, miR-146a is expressed at reduced levels in DRGs and dorsal horn of the spinal cords isolated from rats experiencing OA-induced pain. Exogenous supplementation of synthetic miR-146a significantly modulates inflammatory cytokines and pain-related molecules (e.g., TNFα, COX-2, iNOS, IL-6, IL8, RANTS and ion channel, TRPV1) in human glial cells. Our findings suggest that miR-146a controls knee joint homeostasis and OA-associated algesia by balancing inflammatory responses in cartilage and synovium with pain-related factors in glial cells. Hence, miR-146a may be useful for the treatment of both cartilage regeneration and pain symptoms caused by OA.

1. Introduction

Joint degeneration associated with osteoarthritis (OA) affects a large number of individuals (>100 million) throughout the world and leads to chronic and severe pain in knee joints. Development of OA and one of its key debilitating symptoms, pain, is associated with global changes in gene expression in damaged peripheral tissues and neurons. A mechanistic understanding of OA-related pain requires elucidation of how peripheral tissue injury alters gene expression in sensory neurons and how these changes contribute to the development and maintenance of pain in OA patients.

Chronic pain in OA is caused by inflammatory responses that modulate gene expression in a manner dependent of Toll-like receptor (TLR) and nuclear factor kappa B (Liu-Bryan and Terkeltaub, 2010). These inflammatory responses are modulated by specific microRNAs, which are small RNAs that bind to 3'-UTR (untranslated region) of target mRNAs to inhibit protein translation and reduce mRNA stability (Taganov et al., 2006). For example, miR-146 has been shown to regulate TLR pathways and NFκB-dependent targets, while expression of miR-146a is transcriptionally induced by inflammatory

Abbreviations: OA, osteoarthritis; RA, rheumatoid arthritis; DRG, dorsal root ganglia; MMP, matrix metalloproteinase; ADAMTS, a disintegrin and metalloproteinase with thrombospondin motifs; UTRs, untranslated regions; TLR, Toll-like receptor; NFkB, nuclear factor-kappa B; LPS, lipopolysaccharide; IL-1, interleukin-1; MIA, monosodium iodoacetate; RT, reverse transcription; PCR, polymerase chain reaction; BCA, bicinchoninic acid; TNF, tumor necrosis factor; TRAF 6, TNF receptor-associated factor 6; IRAK1, IL-1 receptor-associated kinase 1; COX-2, cyclooxygenase-2; iNOS, inductible nitric oxide synthase; TRPV1, transient receptor potential cation channel, subfamily V, member 1.

* Corresponding author at: Cohn Research BD 516, 1735 W. Harrison, Rush University Medical Center, Chicago, IL 60612, USA. Tel.: +1 312 942 3091; fax: +1 312 942 3053.
E-mail address: Hee-Jeong_Sampen@rush.edu (H.-J. Im).

0378-1119/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.gene.2011.03.003

cytokines (Taganov et al., 2006; Bhaumik et al., 2008). Together, these two processes constitute a negative feedback loop that controls immune activation and may modulate OA-related inflammation.

Recent evidence suggests that miR-146a is expressed in OA cartilage at significantly higher levels than in normal cartilage (Yamasaki et al., 2009). Expression of miR-146a/b is induced in response to lipopolysaccharide (LPS) and proinflammatory mediators in THP-1 cells and this induction is regulated by NFκB (Taganov et al., 2006) Furthermore, miR-146a is more strongly expressed in synovial tissues of patients with rheumatoid arthritis (RA) compared to normal individuals, and its expression in synovial fibroblasts from RA patients is stimulated by inflammatory cytokines such as tumor necrosis factor α (TNFα) and Interleukin-1 β (IL-1β) (Nakasa et al., 2008). Expression of miRNAs in neural cells is altered after spinal cord injury (Liu et al., 2009). Bioinformatics analysis of potential targets for these miRNAs reveals that genes involved in inflammation, oxidation, and apoptosis may contribute to the pathogenesis of spinal cord injury. In a mammalian spinal nerve ligation model for chronic neuropathic pain, changes in miRNA expression contribute to pain sensation through translational regulation of genes relevant to pain-related pathways (Aldrich et al., 2009). In this study, we examined pathological links between miR146a, knee OA and neural pathways associated with OA-induced pain. While altered expression of miR146a in degenerating cartilage is associated with OA and controls inflammatory responses, we find that miR146a is also expressed in neural cells and perhaps may modulate genes involved in OA-related pain.

2. Materials and methods

2.1. Generation of a rat model for knee joint OA

We generated an animal model for OA-induced pain by mono-sodium iodoacetate (MIA) injection as described previously (Im et al., 2010). This MIA-induced OA pain model demonstrates pathological features similar to those observed in human knee joint OA, including symptomatic chronic pain, cellular alterations in synovial tissues and disorganization of chondrocytes. Briefly, Sprague–Dawley rats were anesthetized with isoflurane (Abbott Laboratories, North Chicago, IL, USA) in oxygen and given a percutaneous single intra-articular injection of 0.5 mg of MIA (Sigma, St. Louis, MO, USA; cat #I2512) or saline vehicle through the infra-patellar ligament of the left knee ($N=8$ for each group). MIA was dissolved in physiologic saline and administered in a volume of 25 μl using a 26-gauge, 0.5-in. needle. The right contra-lateral knee was used as a behavioral and histological control. Animal behavioral tests (knee pressure hyperalgesia, knee extension hyperalgesia, mechanical allodynia (von Frey) and knee edema) were performed to confirm that the MIA injection increased knee joint discomfort in rats as described previously (Im et al., 2010).

2.2. General tissue preparation

Human articular synovial tissues from knees were obtained from donors through the Gift of Hope Organ and Tissue Donor Network (normal tissue specimens) and Rush Orthopedic Depository Studies (surgically removed OA tissues). For normal tissues, each donor specimen was graded for gross degenerative changes based on a modified version of the 5-point scale of Collins (Muehleman et al., 1997). At 2 weeks and 4 weeks post-MIA or saline injection, the animals were euthanized with halothane anesthesia. Bilateral lumbar DRGs and dorsal horn of the spinal cords were harvested under the light microscope for further analyses (e.g., RT-PCR).

2.3. Cell culture and transfection

Human articular chondrocytes and synoviocytes were released by enzymatic digestion as previously described (Im et al., 2003). For monolayer cultures, isolated chondrocytes were counted and plated onto 12-well plates at 8×10^5 cells/cm^2 as previously described (Im et

Table 1
Primer sequences for real-time PCR.

Primer	Sequences	Tm	NCBI reference no.
TNF-α	Forward : 5'-ACCAGCTAAGAGGGAGAGAAGCAA-3' Reverse : 5'-TCAGTGCTCATGGTGTCCTTTCCA-3'	60 °C	NM_000594
Aggrecan	Forward : 5' TCTTGGAGAAGGGAGTCCAACTCT–3' Reverse : 5'-ACAGCTGCAGTGATGACCCTCAGA-3'	60 °C	NM_013227
MMP13	Forward : 5'-ACCCTGGAGCACTCATGTTTCCTA-3' Reverse : 5'-TGGCATCAAGGGATAAGGAAGGGT-3'	55 °C	NM_002427
Col2A1	Forward : 5'- GGGCCTCAAGGATTTCAAGGCAAT-3' Reverse : 5'-TCACCATCATCACCAGGCTTTCCA -3'	58 °C	NM_001844
ADAMTS5	Forward : 5'-CTGTGACGGCATCATTGGCTCAAA -3' Reverse : 5'- TTCAGGAATCCTCACCACGTCAGT-3'	60 °C	NM_007038
COX2	Forward : 5'-TTCCATTGACCAGAGCAGGCAGAT-3' Reverse : 5'-GCATCGATGTCACCATAGAGTGCT-3'	55 °C	NM_000963
iNOS	Forward :ATCACACGCCCACAGAGATCCA Reverse :GCTTCAGGCTGTTGAGCCATGT	55 °C	NM_000625
IL-6	Forward :AAGCCAGAGCTGTGCAGATGAGTA Reverse :TTCGTCAGCAGGCTGGCATTTGT	60 °C	NM_000600
IL-8	Forward :TCTTGGCAGCCTTCCTGATTTCTG Reverse :GGGTGGAAAGGTTTGGAGTATGTC	55 °C	NM_000584
IRAK1	Forward :TACCTGCCCGAGGAGTACATCAA Reverse :TCCTCTTCCACCAGGTCTTTCAGA	55 °C	NM_001569
TRAF6	Forward : 5'-AATGTTGGCCCAGGCTGTTCATAG-3' Reverse : 5'-TAAGGCGACCCTCTAACTGGTGAAT-3'	55 °C	NM_145803
GAPDH	Forward : 5'-TCGACAGTCAGCCGCATCTTCTTT -3' Reverse : 5'-GCCCAATACGACCAAATCCGTTGA -3'	55 °C	NM_031144
RANTS	Forward : 5'-ACCAGCCTGGCCAACATGATGAAA-3' Reverse : 5'-TTCACGCCATTCTCCTGCCTCA -3'	60 °C	NM_002985
TRPV1	Forward : 5''-CCGACAACACGAAGTTTGTGACGA-3' Reverse : 5'-TTCCCTTCTTGTTGGTGAGCTCCT -3'	60 °C	NM_080704