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miR-9/MCPIP1 axis mediated regulation of IL-6 expression in osteoarthritis chondrocytes

Tariq M Haqqi, Mohammad S Makki and **Abdul Haseeb**
Northeast Ohio Medical University, USA

Background/Purpose: Post-transcriptional regulation of cytokine expression is important for maintaining tissue integrity. MCPIP1 was identified as a novel protein, which destabilizes inflammatory cytokines mRNAs via their 3' UTR. IL-6 has recently gained attention because of its high levels in synovial fluid in Osteoarthritis (OA) and ability to induce high levels of MMP-13 in OA. In the present study we determined whether MCPIP1 regulates IL-6 expression and evaluated the role of miR-9/MCPIP1 axis in the regulation of IL-6 in human OA chondrocytes.

Methods: Human chondrocytes were prepared from OA cartilage by the enzymatic digestion. TaqMan assays were used for gene expression analysisusing RNA isolated from cultured primary chondrocytes or from damaged or smooth regions of OA cartilage or RNA immunoprecipitation (RIP). RNA fluorescent in-situ hybridization (ISH) for IL-6 and MCPIP1 expression was performed using RNAScope. Transfection was done using Amaxa kit. Knockdown experiments were performed using Trisilencer-27 human siRNA. For RIP, lysates from IL-1β-stimulated chondrocytes were incubated overnight with anti-MCPIP1 antibody or with isotype control IgG followed by RNA purification.

Results: MCPIP1 expression was low in damaged cartilage compared to smooth cartilage while the expression of IL-6 was high in damaged cartilage and low in smooth cartilage, suggesting that lower expression of MCPIP1 may be contributing to the excessive expression of IL-6 in OA. Expression of miR-9 predicted by Target scan to bind the seed sequence in MCPIP1 mRNA was high in damaged cartilage compared to smooth cartilage and was also upregulated by IL-1β in OA chondrocytes. Over expression of miR-9 mimic or inhibitor in OA chondrocytes altered the expression of MCPIP1 and IL-6. IL-1β-mediated induction of IL-6 was initially low in OA chondrocytes but was significantly accelerated 8 h post-stimulation. On the other hand, expression of MCPIP1 was high initially in IL-1β-stimulated OA chondrocytes but started to decline 8 h post-stimulation. Over expression of wild type MCPIP1, but not of mutant MCPIP1, in OA chondrocytes reduced the expression of IL-6 mRNA and protein significantly ($p<0.05$). Importantly siRNA-mediated knockdown of MCPIP1 elevated the IL-6 mRNA expression in OA chondrocytes. TaqMan analysis of the immunoprecipitated mRNAs showed that anti-MCPIP1 antibody pulled down larger amount of IL-6 mRNA than control IgG antibody did thus demonstrating the binding of MCPIP1 with IL-6 mRNA in OA chondrocytes.

Conclusions: In this study for the first time expression of MCPIP1 and miR-9 in human OA cartilage and chondrocytes is shown. The data also demonstrate miR-9/MCPIP1/IL-6 interactions and provide evidence of miR-9/MCPIP1 axis as an important regulator of IL-6 expression in OA.

Biography

Tariq M Haqqi completed his PhD from Aligarh Muslim University, India and postdoctoral studies from Marseille-Luminy, France and Mayo Clinic, Rochester, MN. He is Professor of Anatomy and Neurobiology at the Northeast Ohio Medical University, Rootstown, OH. He has published over 80 papers in highly reputed journals and has served as Editor-in-Chief and Editorial Board Member of reputed journals.

thaqqi@neomed.edu