

Next Generation Sequencing Analysis of Circulating MiRNAs in Rheumatoid Arthritis and Osteoarthritis

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Abstract : Introduction: Osteoarthritis is the most common form of arthritis that involves the wearing away of the cartilage that caps the bones in the joints. While rheumatoid arthritis is an autoimmune disease in which the immune system attacks the joints, beginning with the lining of joints. In this study, we aimed to study the top deregulated miRNAs that might be the cause of pathogenesis in both diseases. Methods: Eight cases were recruited in this study: 4 rheumatoid arthritis (RA), 2 osteoarthritis (OA) patients, as well as 2 healthy controls. Total RNA was isolated from plasma to be subjected to miRNA profiling by NGS. Sequencing libraries were constructed and generated using the NEBNextR Ultra™ small RNA Sample Prep Kit for Illumina R (NEB, USA), according to the manufacturer's instructions. The quality of samples were checked using fastqc and multiQC. Results were compared RA vs Controls and OA vs. Controls. Target gene prediction and functional annotation of the deregulated miRNAs were done using Mienturnet. The top deregulated miRNAs in each disease were selected for further validation using qRT-PCR. Results: The average number of sequencing reads per sample exceeded 2.2 million, of which approximately 57% were mapped to the human reference genome. The top DEMs in RA vs controls were miR-6724-5p, miR-1469, miR-194-3p (up), miR-1468-5p, miR-486-3p (down). In comparison, the top DEMs in OA vs controls were miR-1908-3p, miR-122b-3p, miR-3960 (up), miR-1468-5p, miR-15b-3p (down). The functional enrichment of the selected top deregulated miRNAs revealed the highly enriched KEGG pathways and GO terms. Six of the deregulated miRNAs (miR-15b, -128, -194, -328, -542 and -3180) had multiple target genes in the RA pathway, so they are more likely to affect the RA pathogenesis. Conclusion: Six of our studied deregulated miRNAs (miR-15b, -128, -194, -328, -542 and -3180) might be highly involved in the disease pathogenesis. Further functional studies are crucial to assess their functions and actual target genes.

Keywords : next generation sequencing, mirnas, rheumatoid arthritis, osteoarthritis

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