Differentially expressed miRNAs in Multiple Sclerosis patients stratified for NEDA status

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BACKGROUND

Multiomics is gaining increasing attention as a promising approach for studying complex diseases like Multiple Sclerosis (MS). In this context, microRNAs (miRNAs), small non-coding RNA molecules involved in post-transcriptional gene regulation, represent an attractive field of investigation due to their potential as diagnostic biomarkers, therapeutic targets and tools for elucidating disease-related biological pathways.

AIM

To identify miRNAs relevant for MS disease activity in a population of patients with RRMS

METHODS

Patients

Cohort: 69 treatment-naïve patients with relapsing-remitting MS. At 2-year follow-up, patients were classified as NEDA or EDA. PBMCs were collected and total RNA was extracted.

miRNA data

miRNA sequencing profiles: SMARTer smRNA kit (Takara). Tools used: Cutadapt and miRDeep2 miRNA-seq for transforming data into counts per transcript and DESEq2 for comparison between EDA and NEDA patients. Predicted mRNA target of relevant miRNAs were selected according to miRTarBase (release 2025).

mRNA data

mRNA sequencing profiles: Truseq stranded mRNA (Illumina). Tools used: featureCounts on GenCode v19 for transforming data into counts; DESEq2 for comparison between EDA and NEDA patients. Correlation analysis between miRNAs and target genes expression profiles was conducted and only negative correlations were retained ($p < 0.05$ and correlation coefficient $\rho < -0.3$).

Network and functional enrichment analysis

Network analysis and functional enrichment analysis were performed on validated target genes using miRNet 2.0 and KEGG as reference pathway database.

RESULTS

Differentially expressed miRNAs

15 miRNAs were found to be differentially expressed between EDA and NEDA patients (adjusted P < 0.05)

miRNA	log2FC	P adj
hsa-miR-7-1-3p	0.68	4.78E-03
hsa-miR-146b-5p	0.56	4.78E-02
hsa-miR-590-3p	1.18	4.78E-02
hsa-miR-142-5p	0.58	4.78E-02
hsa-miR-4286	-0.54	4.78E-02
hsa-let-7g-5p	0.4	2.42E-02
hsa-miR-744-5p	-0.51	3.20E-02
hsa-miR-424-5p	0.8	3.20E-02
hsa-miR-30e-3p	0.5	3.20E-02
hsa-miR-335-5p	0.63	3.87E-02
hsa-miR-19a-3p	0.74	4.57E-02
hsa-miR-3665	-0.87	4.97E-02
hsa-miR-15b-3p	0.64	4.97E-02
hsa-miR-4516	-0.58	4.97E-02
hsa-miR-25-3p	0.32	4.97E-02

Table 1. miRNAs associated with NEDA status. Log2FC: fold comparing the EDA vs the NEDA patients, P adj: adjusted P value

Correlation analysis

Predicted target mRNAs according to miRTarBase

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Confirmation of miRNA/mRNA negative correlation (P<0.05 and ρ <-0.3)

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114 significant negative correlations

Figure 1. Predicted targets of the 15 differentially expressed miRNAs were selected according to miRTarBase and were validated in the same samples. We identified 114 significant negative correlation (P<0.05 and ρ <-0.3) between miRNAs and their target genes.
Three genes, ARPC5L, PELP1, and ZNF598, were targeted by more than one miRNA, specifically hsa-miR-25-3p and hsa-miR-335-5p. Among the miRNAs, hsa-miR-335-5p exhibited the highest number of negatively correlated targets (n = 49).

Network and functional enrichment analysis

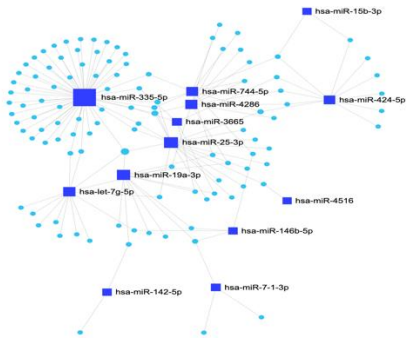

Figure 2. Network analysis performed on NEDA status-associated miRNAs and their negatively correlated target genes. The dimension of the node reflects the centrality of the corresponding miRNA or gene in the network.

Network analysis

Network analysis performed on NEDA status-associated miRNAs and their negatively correlated target genes identified a network of 122 nodes (13 miRNAs and 109 genes) connected by 147 edges. One miRNA and two genes formed a separate component due to the absence of links with the main network. In this network, hsa-miR-335-5p emerged as the main hub.

Functional enrichment analysis

The resulting network was then integrated with the corresponding protein-protein interaction (PPI) network, to gain further insights into the underlying regulatory relationships.

Functional enrichment analysis identified 49 KEGG pathways (FDR < 0.05)

Pathway	FDR
Neurotrophin signaling pathway	4.77E-06
NOD-like receptor signaling pathway	2.99E-04
Epstein-Barr virus infection	4.68E-04
RIG-I-like receptor pathway	2.60E-03
MAPK signaling pathway	2.60E-02
ErbB signaling pathway	1.05E-02
Toll-like receptor	1.48E-02
B cell receptor	3.74E-02

Table 2. KEGG pathways relevant for MS resulting from functional enrichment analysis. FDR: false discovery rate

CONCLUSIONS

We identified 15 miRNAs differentially expressed between active and non active MS patients, 13 of which have been previously associated with MS or with immunological pathways in the literature, with hsa-miR-335-5p emerging as the main hub. Functional enrichment analysis performed on validated gene targets suggests their involvement in immunological function, Epstein-Barr infection and neuronal development, survival and function.