CONVERGENT SEQUENCING AND MICROARRAY DATA ANALYSES SUGGEST THE INVOLVEMENT OF MIRNAS AND TARGET MRNAS IN LITHIUM RESPONSE IN BIPOLAR DISORDER

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Introduction: After more than 60 years of use, lithium (Li) is still a first line treatment for bipolar disorder (BD). Li is effective in at least 60% of patients but its use is characterized by high interindividual variability and important side effects. These features have stimulated intensive research to identify molecular predictors of response and disentangle its complex biological mechanisms. Genetic studies have explained only a small proportion of the observed variability, suggesting that factors other than DNA variants could be implicated. Recent findings showed that Li interferes with the expression of microRNAs (miRNA) and their targeted genes, suggesting that non-coding RNAs could play a role in modulating its clinical efficacy.

Material and methods: We used next generation sequencing (NGS) to investigate genome wide miRNAs expression in lymphoblastoid cell lines (LCLs) derived from BD patients excellent responders (ER, n=12) and non-responders (NR, n=12) to Li with the "Retrospective Criteria of Long-Term Treatment Response in Research Subjects with Bipolar Disorder" (Alda scale). These data were matched with genome wide expression data obtained from the same LCLs. The lists of miRNAs and mRNA differentially expressed between ER and NR were analyzed with MiRComb to identify couples of miRNA/mRNA inversely and significantly correlated. This analysis was integrated with results from miRNA target prediction using 7different databases.

Results: Twenty-four miRNAs were significantly differentially expressed in ER vs NR and inversely correlated with genes showing significant differential expression between the two groups (FDR $q < 0.05$). The 24miRNAs were involved in 146negative correlations which included 125unique genes. Two miRNAs (miR-320a and miR-155-3p) were selected for qRT-PCR validation based on the strength and p-values of the correlations with mRNAs. Both were successfully validated. miR-320a and miR-155-30 were inversely correlated with 24and 19genes predicted by at least 1database, respectively. Four genes for miR-320a and three genes for miR-155-3p were selected for validation. CAPNS1(Calpain Small Subunit 1) and RGS16(Regulator of G Protein Signaling 16) for miR-320a and SP4(Sp4Transcription Factor) for miR-155-3p were successfully validated.

Discussion and conclusions: miR-320a was suggested to be involved in major depression, Alzheimer disease and dementia. Its target gene CAPN1is involved in dendritic branching, spine density, and hippocampal long-term potentiation, and has been reported to be hypermethylated in the prefrontal cortex of schizophrenia patients. RGS16encodes for an indispensable protein for the circadian regulation of cAMP in the superchiasmatic nucleus. Circadian genes have been strongly suggested to be involved in BD and in Li response. miR-155-3p is involved in processes related to the immune system. Its target gene SP4encodes a transcription factor involved in the development of the hippocampus. It has been associated with schizophrenia, major depression, and BD in several studies, and its encoded protein was shown to be stabilized by Li in rat cerebellar granule neurons. Our data suggest that miRNAs and their targeted genes involved in key processes of neuronal functioning and circadian rhythm might be involved in Li response in BD.