Time-dependent effects of antidepressant treatments on miRNome expression profile in hippocampus of rats

M. Seguini[1], D.Tardito[1], A. Mallei[1], I. Merelli[2], D. Corrada[3], L. Milanesi[2], G. Racagni[1], M. Popoli[1]

1 Laboratory of Neuropsychopharmacology and functional Neurogenomics, Dept. of Pharmacological and Biomolecular Sciences, University of Milano, Milano, Italy;
2 Institute for Biomedical Technologies, National Research Council, Segrate, Italy
3 Institute of Chemistry of Molecular Recognition, National Research Council, Segrate, Italy

In the last few years it has become clear that in addition to traditional regulatory mechanisms, several novel gene-regulatory systems can produce short-term changes in gene expression and may be potentially involved in the pathophysiology of mood disorders. MicroRNAs (miRNAs), involved in a great number of cellular processes, have emerged as major players in the posttranscriptional regulation of gene expression. Recently, it has been hypothesized that miRNAs could have a role in brain disorders, and emerging evidence suggest that they regulate neuropathology associated processes, such as brain development, dendritic spine morphology and neurite outgrowth. Furthermore, miRNAs appear to be therapeutically relevant effectors of currently used pharmacotherapies including the mood stabilizers lithium and sodium valproate and the SSRI antidepressant (AD) fluoxetine (FLX) (1, 2). Moreover, a recent clinical study reported changes in blood expression of 30 miRNAs after 12 weeks of treatment with the SSRI escitalopram (3). Aim of our study was to analyze the effects of two different ADs, FLX and desipramine (DMI, a tricyclic AD with predominant action on noradrenaline reuptake), on rat hippocampal miRNome expression profile. Moreover, in order to assess the time course of AD effects, treatments were performed for different time lengths: 3, 7 and 14 days. To this aim, total RNA including miRNAs was isolated from each hemi-hippocampus and reverse transcribed. Quantitative Real Time PCR (qRT-PCR) amplification was carried out using TaqMan Array Rodent MicroRNA A+B Card set v3.0 using the ddCt method. A total of about 450 miRNAs were detected in all samples. The analysis of miRNome expression revealed a significant effect of AD treatments at all time points. After 3 days of treatment, FLX down-regulated the expression of 8 miRNAs while DMI up-regulated the expression of 9 miRNAs. A more pronounced effect was found after 7 days of treatment; indeed, at this time point FLX modulated the expression of 35 miRNAs (28 up-regulated and 7 down-regulated) and DMI down-regulated the expression of 13 miRNAs. Interestingly, 8 of them were similarly up-regulated by both drugs, suggesting common targets for FLX and DMI. Finally, after 14 days of treatment FLX modulated the expression of 4 miRNAs (1 up-regulated and 3 down-regulated) and DMI down-regulated the expression of 18 miRNAs. Bioinformatic analysis was performed in order to identify putative target genes of miRNAs significantly modulated by ADs and enriched signalling pathways. The predicted target genes of miRNAs are mainly involved in pathways related to neuronal brain function; moreover, many of them have been previously associated to both depression pathophysiology and to AD mechanism of action. A number of putative miRNA target genes have been selected for validation studies by means of mRNA/protein expression studies. Moreover, in order to better clarify the mechanisms through which ADs can modulate miRNome expression, we have measured the expression of some of the most important components of microRNAs biogenesis pathway, by means of qRT-PCR. The results of this work, showing that AD treatments induce early and time-dependent modifications in hippocampal miRNome expression, could be of help to better understand AD mechanism of action and could represent the starting point for the identification of novel targets for development of new drugs for the treatment of mood disorders.

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