Analysis Of miRNome Expression Profiles In Hippocampus Of Rats Treated With Antidepressants

P.4.022

M. Pelizzari[1], D. Tardito[1], A. Mallei[1], S. Corna[1], G. Treccani[1], L. Bocchio-Chiavetto[2], G. Racagni[1], M. Popoli[1]

[1]Center of Neuropharmacology, Dept. of Pharmacological Sciences, University of Milan, Italy
[2]Neuropharmacology Unit, IRCSS San Giovanni di Dio Fatebenefratelli, Brescia, Italy

e-mail: mariagrazia.pelizzari@unimi.it, mariagrazia.pelizzari@gmail.com

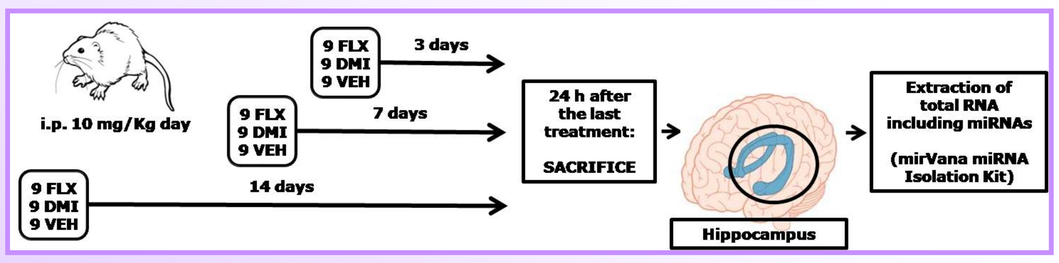
Introduction

MicroRNAs (miRNAs), small non-coding RNAs, are emerging as key regulators of complex time and spatial patterns of gene/protein expression changes and, thereby, synaptic and neural plasticity [1, 2, 3]. miRNAs, by interacting with complementary regions mainly within the 3'-UTR of target mRNAs, can inhibit mRNA translation and/or may induce mRNA degradation. A single mRNA may be regulated by multiple miRNAs and, on the other hand, a single miRNA can regulate several mRNAs, thus regulating protein expression of several genes. Converging evidence has suggested that the expression of the miRNome (the complex of all miRNAs expressed in a tissue at a given time) is highly regulated in a time- and region-specific manner both in the central nervous system and in peripheral tissues [1, 2]. It has also been shown that the expression and the function of brain miRNAs is influenced by external cues, including pharmacological agents. Pilot studies reported alterations in miRNAs regulation in psychiatric disorders, such as schizophrenia and mood disorders [4, 5, 6]. Moreover, it has been reported that some miRNAs and their effectors are modulated by the mood stabilizers lithium and valproate [7, 8]. Furthermore, it has been found that fluoxetine modulates in raphe nuclei miR-16 expression, which in turn regulates the serotonin transporter (SERT) expression, suggesting a possible role of miR-16 in the therapeutic action of selective serotonin reuptake inhibitors (SSRI) [9].

Aim of the study was to analyze the modulation of the miRNome in hippocampus (HPC) of rats after treatment with two different antidepressants (ADs): fluoxetine (FLX), a selective serotonin reuptake inhibitor (SSRI), and desipramine (DMI), a tricyclic AD with predominant action on the noradrenaline reuptake. Moreover, in order to assess the time course of the effects of AD treatments on the miRNome, rats were treated for different time lengths: 3, 7 and 14 days.

Methods

Animals



Q-RT-PCR

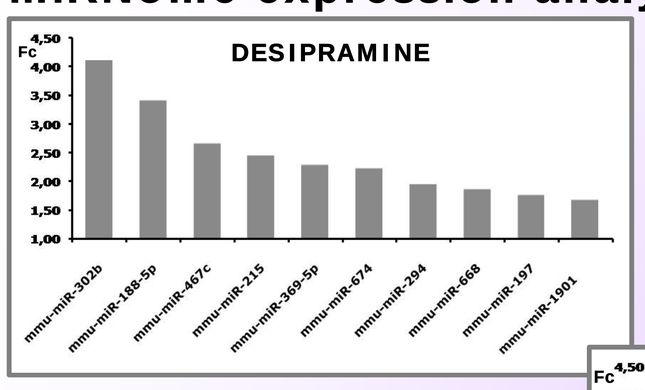
Total RNA from each sample was reverse transcribed using MegaplexTM RT Primers and TaqMan MicroRNA Reverse Transcription Kit. The cDNA were then preamplified by using Megaplex PreAmp Primers (Applied Biosystem). Quantitative Real Time PCR (qRT-PCR) was carried out using TaqMan Array rodent MicroRNA A+B Cards Set v3.0 using the comparative CT (ΔΔCT) method. Raw Ct values were extracted from filtered SDS files using the Applied Biosystems SDS 2.3 software, with a threshold value of 0.1 and automatic baseline. Ct values were normalized by the ΔCt method on endogenous controls U6B, U87, Y1 and snoRNA135.

Bioinformatic analysis

Bioinformatic analysis were performed in order to identify miRNA target genes and molecular pathways potentially altered by the expression of single or multiple miRNAs by means of the most known prediction tools for miRNA targets recognition: miRanda, TargetScan, DIANA-microT. The DIANA mir-Path software, a web-based computational tool, was used to identify molecular pathways potentially altered by the expression of single or multiple microRNAs comparing each set of microRNA targets to all known KEGG pathways.

RESULTS

miRNome expression analysis after 3 days of ADs

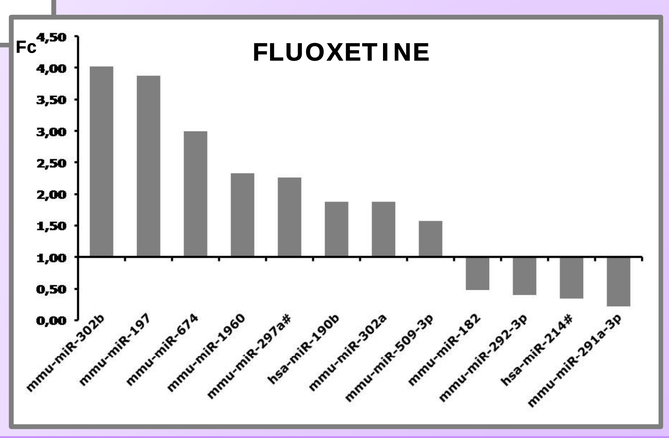


DESIPRAMINE led to an increased expression of 10 miRNAs (FC≥1.5).

FLUOXETINE increased the expression of 8 miRNAs and decreased other 4 miRNAs (FC≤0.5).

Interestingly, 3 miRNAs were similarly modulated by both drugs:

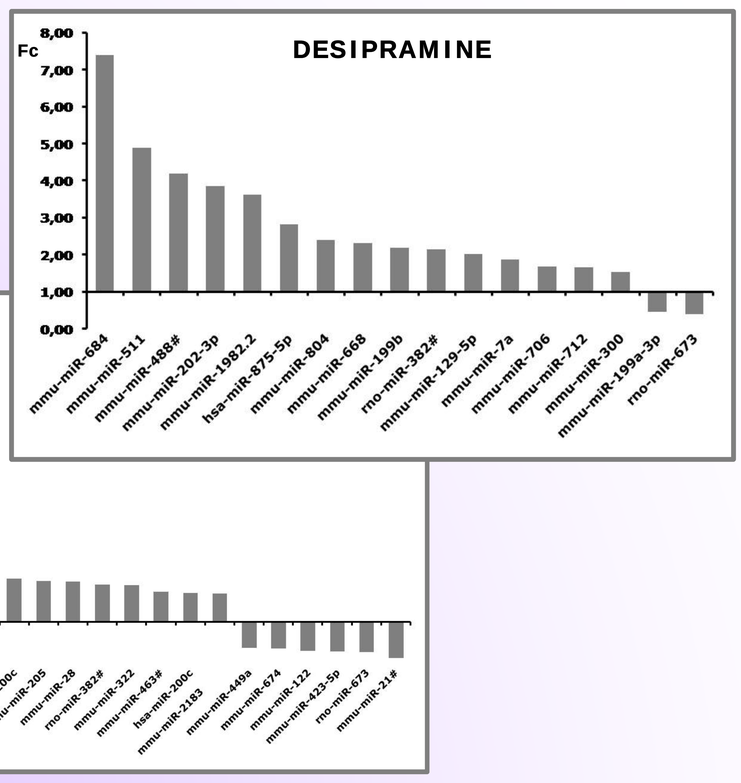
miR-302b
miR-197
miR-674



miRNome expression analysis after 7 days of ADs

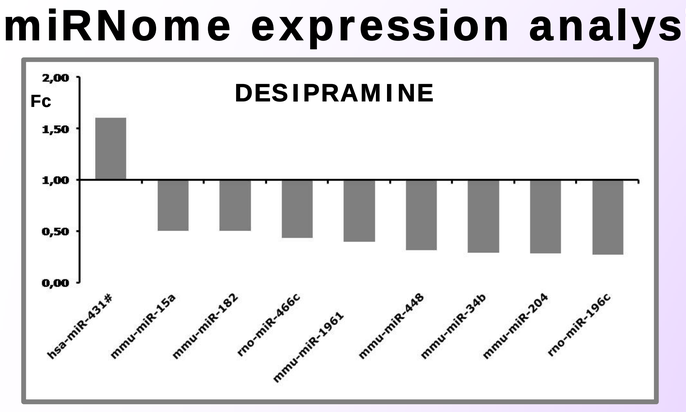
DESIPRAMINE led to an increased expression of 15 miRNAs and down-regulated 2 miRNAs.

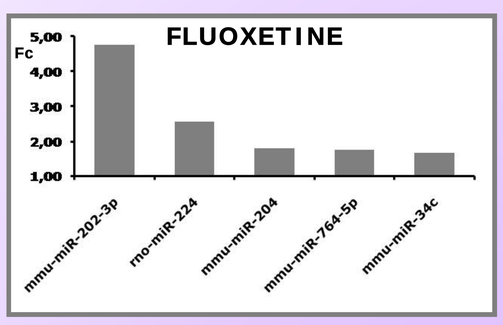
FLUOXETINE increased the expression of 23 miRNAs and decreased the levels of 6 miRNAs.



Interestingly 6 miRNAs were similarly modulated by both drugs:
5 were up-regulated (**miR-511, miR-202-3p, miR-1982.2, miR-382# and miR-129-5p**) and 1 was down-regulated (**miR-673**).

miRNome expression analysis after 14 days of ADs





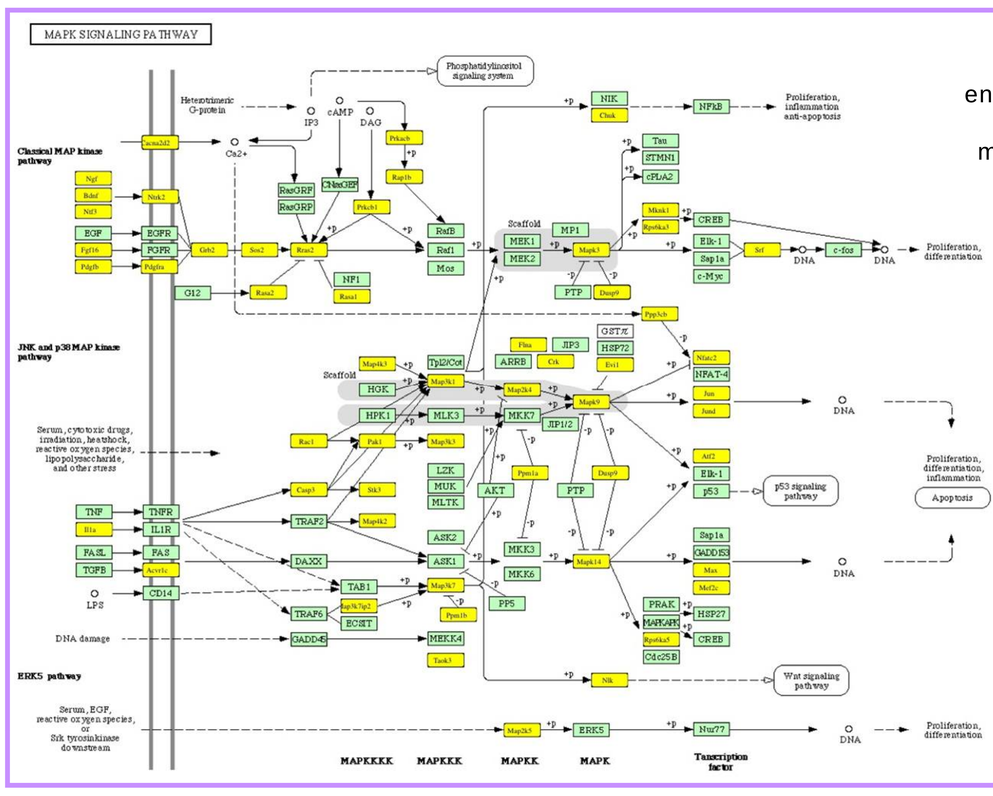
DESIPRAMINE up-regulated 1 miRNA and down-regulated 8 miRNAs. **FLUOXETINE** up-regulated 5 miRNAs. Interestingly, miR-204 was modulated in an opposite way by the two drugs.

miRNAs target prediction and pathways analysis

✓ A preliminary analysis of target prediction for the miRNAs modulated by antidepressant treatments produced lists of several potentially regulated genes known to be involved in both the pathophysiology of mood disorders and in the action of antidepressants: BDNF, MAPK1, GSK3Beta, AMPA receptor subunit 2, CaM kinase II and IV, Calmodulin 1, TrkB, Rap1, Atf2, Protein Phosphatase 3, among the others.

✓ Interestingly, some proteins involved in miRNA biogenesis are putative target genes of miRNAs affected by treatments with fluoxetine or desipramine, thus suggesting a modulation of miRNA processing by antidepressants.

✓ The analysis for functional annotation based on KEGG terms (DIANA mir-Path) showed a significant enrichment in many pathways linked to neuronal functions, in particular to neuronal plasticity: axon guidance, regulation of actin cytoskeleton, Wnt-signaling, MAPK signaling, LTP and LTD, among others.



An example of **pathway analysis** enriched in target genes of the 29 miRNAs modulated by FLX after 7 days of treatment

MAPK signaling pathway: 81 target genes p value: –log(29,88)

CONCLUSIONS

This is the first study of miRNome expression profiles in hippocampus of rats following treatment for different times with two ADs endowed with different mechanisms of action.

✓ Both fluoxetine and desipramine modulated the hippocampal miRNome expression at all time points of treatment: the effects are early (3 days) and more marked after 7 days.

✓ Interestingly, a number of miRNAs is modulated in the same way by FLX and DMI, after 3 or 7 days of treatment, suggesting common targets in the mechanism of action of the two antidepressants.

✓ Predicted gene targets of the identified miRNAs (and associated pathway) are known to play diverse and relevant roles in brain functions, mainly in neuronal plasticity (i.e. axon guidance, regulation of actin cytoskeleton, LTP/LTD, etc).

✓ Some of the target genes belong to the biogenetic and functional pathways related to miRNA generation, maturation, and action, suggesting an intriguing effect of antidepressants on miRNA regulation.

Overall, these findings suggest that some miRNAs and their gene targets may contribute to the molecular, cellular, and behavioral actions of ADs. Further investigation might lead to new insights into the pathophysiology and novel targeted therapies for mood disorders.

References
1) Fiore et al. (2008) Biochim Biophys Acta 1779: 471-478; 2) Gao (2010) Neural Dev 5:25 1-9; 3) Millan (2011) Curr Opin Pharmacology 11: 11-22; 4) Beveridge et al. (2008) Human Molecular Genetics 17(8): 1156-1168; 5) Mellios et al. (2008) Hum Mol Genet 17(9): 3030-3042; 6) Dwivedi (2011) J Chem Neuroanatomy 42:142-56; 7) Zhou et al. (2009) Neuropsychopharmacol 34: 1395-1405; 8) Chen et al. (2009) Int J Neuropsychopharmacol 12: 975-981; 9) Baudry et al. (2010) Science 329: 1537-1541

This study was supported by a grant from Fondazione Cariplo to D.T. e L. BC. - No potential conflicts of interest