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

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INTRODUCTION

MicroRNAs (miRNAs) play a key role in post-transcriptional regulation of gene expression in almost every biological process. By interacting with complementary regions mainly within the 3'-UTR of target mRNAs, miRNAs interfere with translation and/or stability of the mRNA, thus leading to inhibition of protein synthesis. A single mRNA may be regulated by multiple miRNAs and, on the other hand, a single miRNA can regulate several mRNAs, thus modulating protein expression of several different genes simultaneously (1).

MiRNAs have a fundamental role in nervous system development and function, with major involvement in neurogenesis, neuronal differentiation and survival, as well as in neuroplasticity (2). Recent studies suggest a possible contribution of miRNAs in the pathophysiology of neuropsychiatric disorders, including major depression (3, 4). Studies also suggested a possible involvement of miRNAs into the action of psychotropic drugs, such as the mood stabilizers lithium and valproate and antidepressants (ADs) (5-9).

Aim of our study was to analyze whether treatment with two different ADs, fluoxetine (FLX), a selective serotonin reuptake inhibitor (SSRI), and desipramine (DMI), a tricyclic AD with predominant action on the noradrenaline reuptake, modulate rat hippocampal miRNome expression. Moreover, in order to assess the time course of AD treatments on the miRNome expression profile, treatments were performed for different time lengths: 3, 7 and 14 days.

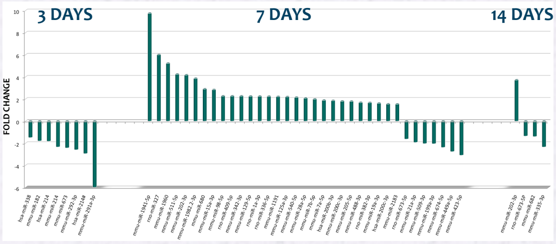
METHODS

9 rats for each experimental group were treated by i.p. injections with 10 mg/kg of drugs or vehicle for 3, 7 or 14 days. MiRNA expression analysis was carried out by Quantitative Real Time PCR (qRT-PCR) reactions by using TaqMan Array rodent MicroRNA A+B Cards Set v3.0, according to the manufacturer's protocol (Life Technologies). Briefly, total RNA including miRNAs was isolated from each hemi-hippocampus (randomly right or left) using mirVana miRNA Isolation Kit (Life Technologies) and then 500 ng of total RNA was retrotranscribed by means of MegaplexTM RT Primers and TaqMan MicroRNA Reverse Transcription Kit. The cDNA was then preamplified by using Megaplex PreAmp Primers (Life Technologies). qRT-PCR was carried out by 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. Statistical analysis was carried out with SAM (Significance Analysis of Microarrays software, version 4.0, Stanford University, http://www-stat.stanford.edu/≈tibs/SAM/, False Discovery Rate <5%). Bioinformatic analyses were performed in order to identify miRNA putative target genes and molecular pathways potentially involved by means of MyMir (10). For each miRNA the 100 most significant targets were selected and included in the annotation analysis performed with Gene Ontology subcategories (Biological Processes, Molecular Function and Cellular Component) and KEGG pathways (exact Fisher test, p<0,05).

RESULTS

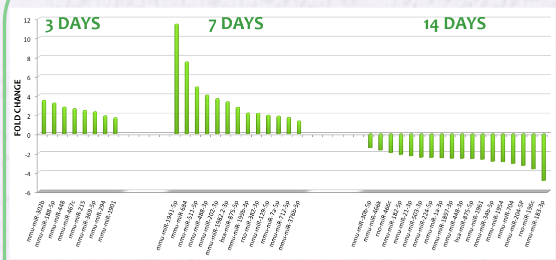
➢ MiRNome EXPRESSION ANALYSIS

FLUOXETINE



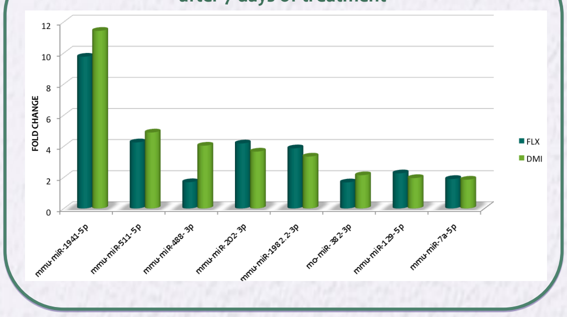
FLX treatment induced significant and time-dependent modifications on miRNome expression. Minor effects were found after 3 days (8 miRNAs) and 14 days (4 miRNAs), whereas 7 days of treatment modulated 35 miRNAs.

DESIPRAMINE



Modifications in miRNome expression were found also after DMI treatments but with a different time-dependent profile: minor effects after 3 and 7 days, with 8 and 13 miRNAs modulated respectively, and a greater effect after 14 days, with 18 miRNAs down-regulated.

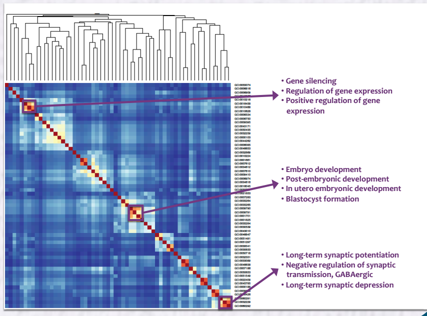
8 miRNAs were similarly modulated by both FLX and DMI after 7 days of treatment



➢ MiRNA PATHWAY ANALYSIS

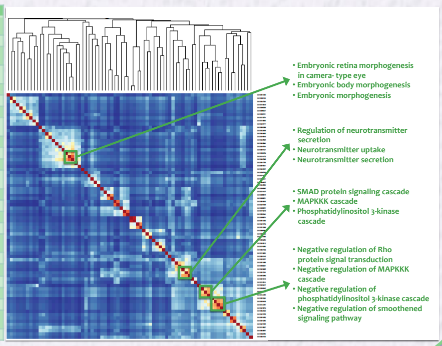
3 DAYS OF TREATMENT with FLX – GO: BIOLOGICAL PROCESSES

PATHWAY DESCRIPTION	ENRICHED TARGET GENES	P-VALUE
Calcium ion homeostasis	Cav, Ccdc47, Grin1	0.0015
Cellular response to insulin stimulus	Ahsg, cdk2, D1a, Insig1, Ppat, Pkcl, Tc10, Ube2b, Vldlr	0.0020
Response to DNA damage stimulus	Atm, Bcl6, Ercc6, Nfatc2, Obfc2b, Parg, Ube2b, vav3, Xpc	0.0033
Long term synaptic depression	D1a, Slc24a2, Stxbp1	0.0035
Positive regulation of gene expression	Alox122, Cited2, Ccr5, Dnmt1, Gja1, Nfatc2, Prdm1, Qki, Rarg	0.0082
G-protein coupled receptor protein signaling pathway	Adra2b, Adrb2, Xcr1, Ccr5, F2r, D1a, Ednra2, Gpr146, Gpr37l1, Gpr61, Galr1, Gng11, Hcrtr2, Lphn2, Lgr4, Edg8	0.0098
Calcium ion transport	Atp2a3, Cacnb3, Cav1, NMDAR1, Itpr1, Itpr2, Pplca, Pkcb, Slc24a2	0.0106
Behavior	D1a, Glut, Plcl1, Usp46	0.0119
Learning	D1a, NMDAR1, Prkar2b, Slc24a2, Slc6a1	0.0126
Maintenance of DNA methylation	Dnmt1, Hells	0.0152
Negative regulation of JAK-STAT cascade	Cav1, Neurod1	0.0152
Negative regulation of signal transduction	Adh7, Cav1, Ihh, Camki2, Rgs8	0.0156
Positive regulation of cell adhesion	Alox122, Fgf1, Pld2, Vav3	0.0157
Hippo signaling cascade	Lats2, Mobkl1a, Sav1	0.0165
Positive regulation of cell-cell adhesion	Cited2, Ccr5, Kif26b	0.0234
Gene silencing	Chmp1a, Dnmt1	0.0290
Actin filament polymerization	Arpc4, mtss1	0.0290
Cerebral cortex GABAergic interneuron migration	D1a, Lhx6	0.0290
Adrenergic receptor signaling pathway	Adra2b, Adrb2	0.0290
Long-term synaptic potentiation	D1a, Slc24a2	0.0290
Response to cholesterol	Smad2, Tgfbr1	0.0290
Regulation of gene expression	Cyp1a2, Dnmt1, Irf1, Mitf, Rarg, Tgfbr1	0.0392
Regulation of inflammatory response	Pp63, Bcl6, Zyx	0.0412
Methylation	Mtr, Suv39h2	0.0460
Negative regulation of synaptic transmission, GABAergic	Slc6a1, Stxbp1	0.0460


• Gene silencing
• Regulation of gene expression
• Positive regulation of gene expression
• Embryo development
• Post-embryonic development
• In utero embryonic development
• Blastocyst formation
• Long-term synaptic potentiation
• Negative regulation of synaptic transmission, GABAergic
• Long-term synaptic depression

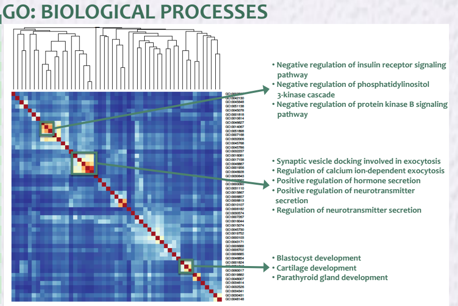
7 DAYS OF TREATMENT with DMI – GO: BIOLOGICAL PROCESSES

PATHWAY DESCRIPTION	ENRICHED TARGET GENES	P-VALUE
Regulation of synaptic transmission, glutamatergic	Cnr11, Grm1, Grm2, Serpine2	0,0002
Cytoplasmic mRNA processing body assembly	Dnch1, Limd1, Lsm14a	0,0019
Cellular calcium ion homeostasis	Atp2a2, Atp2c1, cacnb4, Cav1, Pgm11, Prkcb1, Slc24a2, Slc37a4, Stim2, Sypl2, Trpv4	0,0031
MAPKKK cascade	Adra2b, Cav1, Fgfr3, Itpkb, Map3k7, Nf1, Pbp, Smad1, Raf1	0,0039
Regulation of cell-matrix adhesion	Myf5, Nf1, Hnf6	0,0069
Phosphatidylinositol 3-kinase cascade	Htr2b, Edn1, Nf1	0,0069
Long-term synaptic potentiation	Serpine2, Slc24a2, Snca	0,0069
Positive regulation of dendritic cell antigen processing and presentation	Nod1, Bcg	0,0153
DNA integration	Baf, Ppfibp2	0,0153
Fatty acid elongation, saturated fatty acid	Elovl3, Elovl4	0,0153
Response to corticosterone stimulus	Ccnd1, c-Fos, Gpx3, Hsd3b11, Pbp, Star	0,0232
Cell-cell signaling	Bhlha15, Edn1, Fgfr3, Fzd1, Cx40, Gja5, Gjb2, Panx2, Sdc4	0,0259
Negative regulation of ERK1 and ERK2 cascade	Cnksr3, Kif4, Ndrg2, Slc9a3r1	0,0266
Cytosolic calcium ion homeostasis	Cav1, Ptgdr1, Tgfa	0,0284
Neurotransmitter secretion	Nrxn1, Nrxn2, Rab15, Rab3c, Snap25, Syt1	0,0298
Negative regulation of MAP kinase activity	Bmp7, Cav1, Inppl1, Nf1, Spyr4	0,0299
Negative regulation of inflammatory response	Nt5e, Adora2a, CB2, Cd276, Il2ra	0,0299
Regulation of neurotransmitter secretion	Cplx3, Grm2, TRKB1, Snca	0,0377
Glucose metabolic process	Crem, H6pd, Ship2, Hnf6, Pgm11, Kcnj11, Serp1	0,0418
DNA synthesis involved in DNA repair	Pole, Polh	0,0422
Neurotransmitter uptake	Atp1a, Crebbp	0,0422
Regulation of glutamate secretion	Grm1, Snca	0,0422
Calcium ion transport	Accn2, Atp2a2, Atp2c1, Bspry, Cacnb2, Cacnb4, Cav1, Pkcb, P2xrx4, Slc24a2, Vroac	0,446
SMAD protein signal transduction	Bmp7, c-Fos, T	0,0452


• Embryonic retina morphogenesis in camera-type eye
• Embryonic body morphogenesis
• Embryonic morphogenesis
• Regulation of neurotransmitter secretion
• Neurotransmitter uptake
• Neurotransmitter secretion
• SMAD protein signaling cascade
• MAPKKK cascade
• Phosphatidylinositol 3-kinase cascade
• Negative regulation of Rho protein signal transduction
• Negative regulation of MAPKKK cascade
• Negative regulation of phosphatidylinositol 3-kinase cascade
• Negative regulation of smoothened signaling pathway

7 DAYS OF TREATMENT: miRNAs modulated by both FLX and DMI GO: BIOLOGICAL PROCESSES

PATHWAY DESCRIPTION	ENRICHED TARGETS	P-VALUES
Negative regulation of platlet-derived growth factor receptor signaling pathway	Ship2, Slc9a3r1, Snca	0,0004
Negative regulation of ERK1 and ERK2 cascade	Prp4, Klf4, Ndrg2, Slc9a3r1	0,0044
Regulation of mTOR signaling cascade	Agpat9, Slc7a1	0,0055
DNA integration	Baf1, Ppfibp2	0,0055
Regulation of neurotransmitter secretion	Cplx3, Grm2, Snca, Syph1	0,0066
Negative regulation of insulin receptor signaling pathway	Grb10, Grb14, Ship2, Byb1	0,0093
Negative regulation of cytokine production	Ndrg2, Slc11a1	0,0158
Regulation of microtubule polymerization or depolymerization	Clasp2, Ska1	0,0158
Cytoplasmic mRNA processing body assembly	Limd1, Lsm14a	0,0158
Synaptic vescicle docking involved in exocytosis	Spnb3, Stx1a	0,0158
Glycosphingolipid biosynthetic process	Ugt8, B4galt6	0,0158
Phoshatidylinositol phosphorilation	Pik3ca, Pik3cd, Pik3c2g	0,0173
Potassium ion transport	Sur2, Atp1a1, Kcnk3, Kcnv2, Kcnj12, Kcns1, Kcna, Kcnd2	0,0192
Cell-cell signaling	Edn1, Gja1, Gja5, Gjb2, Panx2, Sdc4	0,0212
Response to interferon– gamma	Kynu, Slc11a1, Snca	0,0246
Antigen processing and presentation	Ap3b1, Ap3d, Erap1, Mr1	0,0269
Sphingomyelin catabolic process	Smpd1, Smpdl3a	0,0300
Regulation of calcium ion-dependent exocytosis	Sptbn2, Syt1	0,0300
Positive regulation of interferon-gamma biosynthetic process	Cebpg, Cd276	0,0300
Adrenergic receptor signaling pathway	Adra1b, Adra2b	0,0300
Positive regulation of neurotransmitter secretion	Stx1a, Snca	0,0300
Negative regulation of phosphatidylinositol 3-kinase cascade	Klf4, Slc9a3r1	0,0300
Reduction of cytosolic calcium ion concentration	Atp1a2, Kcnk3	0,0476


• Negative regulation of insulin receptor signaling pathway
• Negative regulation of phosphatidylinositol 3-kinase cascade
• Negative regulation of protein kinase B signaling pathway
• Synaptic vesicle docking involved in exocytosis
• Regulation of calcium ion-dependent exocytosis
• Positive regulation of hormone secretion
• Positive regulation of neurotransmitter secretion
• Regulation of neurotransmitter secretion
• Blastocyst development
• Cartilage development
• Parathyroid gland development

CONCLUSIONS

1. FLX and DMI significantly modulated the hippocampal miRNome expression at all time of treatment and the effects of both ADs were early (3 days);
2. FLX and DMI showed a different profile of miRNome modulation: FLX induced more marked effects after 7 days of treatment, while DMI after 14 days of treatment;
3. Interestingly, 8 miRNAs were similarly regulated by both FLX and DMI after 7 days of treatment, thus suggesting the presence of common targets;
4. Bioinformatic analysis has shown a significant enrichment of target genes in different pathways linked to neuronal functions as well as other biological processes related in particular to DNA/RNA regulation and inflammation; some of the putative target genes have been previously shown to be involved in the action of ADs.

Overall, our data show that different ADs induced time-dependent modifications in rat hippocampal miRNome. Although further work is needed, these results suggest that miRNAs can contribute to the mechanism of action of ADs 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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