

miRNAs as Novel Antidepressant Targets: Converging Effects of Acute Ketamine, Electroconvulsive Shock Therapy and Chronic Fluoxetine Treatments

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1. INTRODUCTION

- The vast majority of antidepressant drugs used today rely on the same basic mechanism of increasing the levels of monoaminergic neurotransmitters available for signalling in the synaptic cleft. Despite being of benefit in a large number of patients, these antidepressants are hampered by a slow onset of action, side-effects and a significant percentage of non-responders [1].
- Acute administration of the NMDA receptor antagonist ketamine and electro-convulsive shock (ECS) therapy have been shown to induce a rapid and persistent antidepressant effect [2]. Despite their effectiveness, the molecular mechanisms underlying the mechanism of action of these treatments are not fully understood.
- microRNAs (miRNAs) are short non-coding endogenous RNA species that negatively regulate gene transcription [3] (Fig. 1). They are key regulators of gene expression, regulating an estimated ~30% of mammalian genes. They are involved in various neurobiological processes including neuronal development, behaviour, neurogenesis and synaptic plasticity. Furthermore, miRNA levels in various brain regions have already been shown to be sensitive to psychoactive drugs [4, 5]. miRNAs are increasingly seen as attractive drug targets given their ability to affect the expression of multiple genes.
- The effect that ECS and ketamine have on miRNAs in the brain remains completely unexplored. We hypothesised that certain miRNAs would be differentially and others similarly regulated by ketamine and ECS. To this end Sprague Dawley rats were administered repeated ECS, acute ketamine and for comparison chronic (21 days) treatment with the selective serotonin reuptake inhibitor fluoxetine, after which the hippocampus was dissected out, RNA was isolated and microarray based miRNA profiling was conducted

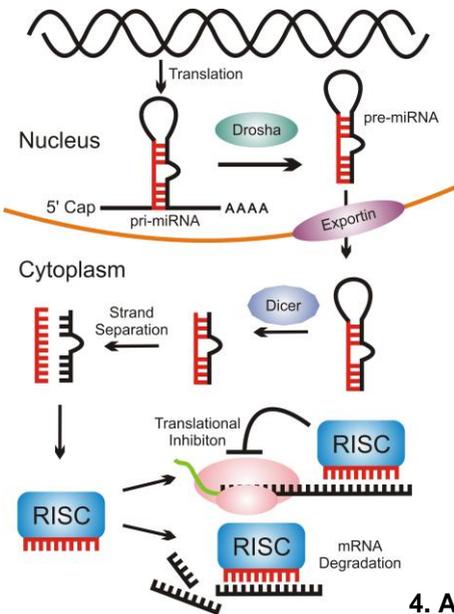


Figure 1: Biogenesis of miRNAs. miRNA genes are transcribed by RNA polymerase II producing a pri-miRNA. The pri-miRNA is then processed by Droscha to produce a hairpin structures called pre-miRNAs. Pre-miRNAs are then exported from the nucleus to the cytoplasm via the exportin5-Ran complex. In the cytoplasm they are further processed by Dicer producing the mature miRNA. The mature miRNA is then incorporated into the RNA induced silencing complex (RISC). The miRNA-RISC complex is then able to repress translation in a sequence specific manner either through degradation of the target mRNA or by blocking access of the cells translational machinery to the mRNA.

2. Methods

- Male Sprague Dawley rats received 21 days fluoxetine administration (10 mg/kg, i.p.) repeated (10 days) ECS treatment (85 mA for 0.5 ms) and a single ketamine injection (10 mg/kg, i.p.). All animals not undergoing a particular treatment regime received corresponding sham treatment to ensure all groups could be compared to each other (control animals received sham treatments for all antidepressant regimes).
- 24 hours following final treatment animals were sacrificed and the hippocampus was dissected out and placed in RNAlater @ 24 hours later RNA later was removed and tissue was stored at -80°C until further processing.
- Total RNA (including small RNAs) was isolated from the hippocampus using the mirVana™ RNA isolation kit from Applied Biosystems. miRNA profiling was conducted using agilent microRNA oligonucleotide arrays.
- Comparative analysis was conducted by calculating the difference of the miRNA hybridisation intensities between two different groups. Differentially expressed miRNAs were identified using standard criteria (Log Ratio p-value < 0.05, Absolute Fold Change > 1.3).

3. EXPERIMENTAL DESIGN

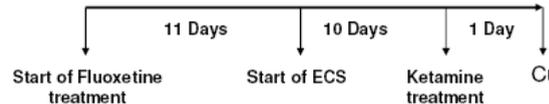


Figure 2. Schematic representing when the animals underwent the different antidepressant regimes.

4. Antidepressant induced changes to Hippocampal miRNA expression

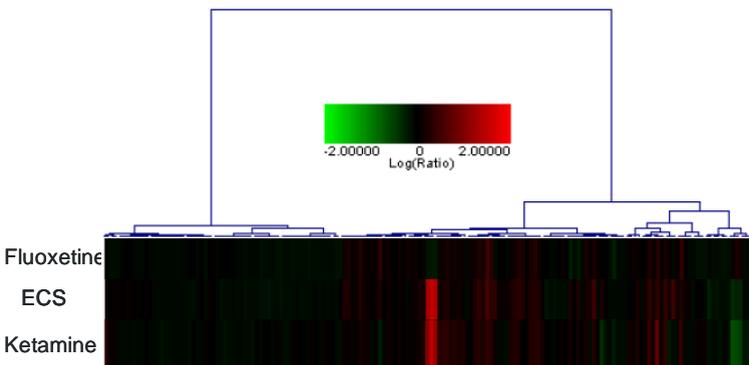


Figure 3: Heat map representing changes to hippocampal miRNA expression. Treatment groups are represented on the x-axis while miRNAs are divided along the y-axis with closely related miRNAs being closer to each other.

CONCLUSIONS

- The antidepressant treatments chronic fluoxetine, repeated ECS and acute ketamine have the capacity to alter hippocampal miRNA levels. These changes may represent previously unknown therapeutic mechanisms of these antidepressant treatments.
- Interestingly, all three antidepressant treatments share one common miRNA target suggesting changes to levels of this miRNA may be an important molecular change underlying the therapeutic benefit of these antidepressants
- Furthermore, ECS and ketamine share four common miRNA targets. These antidepressant treatments are more rapidly acting than traditional antidepressants and, moreover are effective in cases of treatment-resistant depression. This data suggests these two antidepressants strategies may share overlapping molecular mechanisms of action.
- Targeting miRNAs may represent a novel strategy for the treatment of depression which may induce a more rapid antidepressant effect and also may be effective in a larger number of cases than traditional monoaminergic based therapies. Further work will need to be conducted in which the levels of these miRNAs are modulated in pre-clinical models of depression to further assess their antidepressant potential

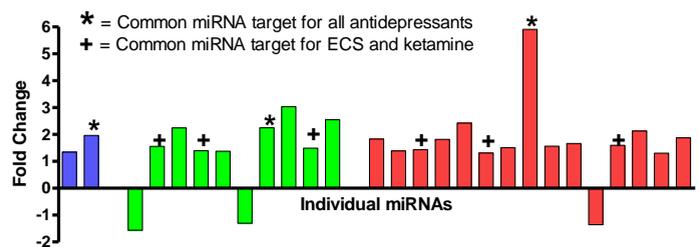
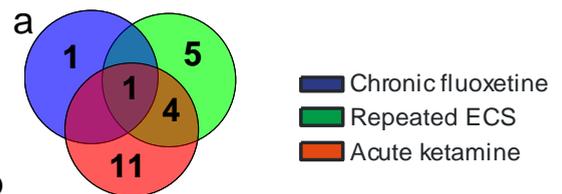


Figure 4. (a) Venn Diagram displaying the number of miRNAs altered by each antidepressant strategy. (b) Bar chart displaying the fold change induced by the individual antidepressant strategies on hippocampal miRNA levels

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