# The role of miR199a-3p and NEDD4 on the development of methamphetamine addiction

S.-J. JIAN, J.C. Chen, C.C. Chun, Z.H. WANG

Chang Gung University, Taiwan

**Background**

Drug addiction, a chronic disorder with frequent relapse characteristics, could readily trigger physical and psychological dependence in drug addicts. Upon drug withdrawal, patients usually experience compulsive drug-seeking and severe withdrawal syndromes that engage further drug taking. Of abused psychostimulants, methamphetamine (METH) blocks the dopamine transporter that leads to an enhanced synaptic dopamine transmission, in particular in the nerve terminals of mesolimbic and mesocortical dopamine systems, two of essential neural regions involved in brain reward system that functions intimately with drug addiction.

**Aims & Objectives**

Recently, several reports indicated the significance of miRNA on development of neuroplasticity as well as impact on behavioral manifestation, including the behavioral sensitization and conditioned place preference (CPP), a characteristic of behavioral up-regulation or conditioning upon repetitive METH administration or pairing in rodents. Therefore, the aim of this study is to explore the altered miRNA species in METH sensitization and CPP paradigms.

**Methods**

METH sensitization mice were received systemic daily METH (2 mg/kg, i.p.) treatment for 8 consecutive days, and then conducted behavioral sensitization or CPP assessment. The behavioral positive mice were selected and compared the amount difference of designated miRNAs in various brain regions with their pre-test vs. post-treated sampling. The brain tissues of ventral tegmental area, ventral striatum (nucleus accumbens; NAc), infralimbic cortex (IL), and prelimbic cortex were dissected and stored in freezer at -80°C. Trizol solution was used to extract the total RNA (1 mg/ml) from tissue, following the manufacturer’s protocol. Afterwards, miRNAs and mRNA were reversely transcribed followed by PCR reaction to generate cDNA products and quantified by quantitative PCR.

**Results**

We established behavioral METH sensitization model in male B6 mice and found several miRNA species in the NAc were altered. Of those miRNAs, we consistently found the miR-199a-3p expression increased and miR-181a-5p reduce significantly in NAc of METH sensitized mice. In addition, we selected 11 mRNAs that could be targeted by miR-199a-3p and measured their amount of expression in the NAc of behaviorally sensitized mice. Of which, we found NEDD4 mRNA and protein was significantly reduced in the nucleus accumbensof behaviorally sensitized mice, consistent with the change of mir-199a-3p. We chose miR-199a-3p and miR181a-5p as potential targets for further analyses. In addition, miR-199a-3p and miR-181a-5p was decreased at acquisition phase but increased at extinction phase in the NAc of METH-CPP mice. miR-199a-3p and miR-181a-5p were decreased at acquisition phase, whiles miR-199a-5p, miR-199a-3p and miR-181a-5p were significantly decreased at extinction phase in the IL METH-CPP mice.

**Discussion & Conclusion**

We discovered that mir-199a-3p expression was significantly changed in behaviorally sensitized and METH-CPP mice. Whether miR-199a-3p also plays a role in METH-CPP extinction memory is currently not clear. More important, we identified a reciprocal change in amount of NEDD4 mRNA in the striatum of METH-sensitized mice that implicate a miR-199a-3p/NEDD4-dependent protein ubiquitination would be involved in the development of METH addiction. The results suggest miR-199a-3p would be a novel therapeutic target in the treatment of METH addiction.