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Rescue of altered HDAC activity recovers behavioural abnormalities in a mouse model of Angelman syndrome

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ABSTRACT

Angelman syndrome (AS) is a neurodevelopmental disorder characterized by severe intellectual and developmental disabilities. The disease is caused by the loss of function of maternally inherited *UBE3A*, a gene that exhibits paternal-specific imprinting in neuronal tissues. *Ube3a*-maternal deficient mice (AS mice) display many classical features of AS, although, the underlying mechanism of these behavioural deficits is poorly understood. Here we report that the absence of Ube3a in AS mice brain caused aberrant increase in HDAC1/2 along with decreased acetylation of histone H3/H4. Partial knockdown of Ube3a in cultured neuronal cells also lead to significant up-regulation of HDAC1/2 and consequent down-regulation of histones H3/H4 acetylation. Treatment of HDAC inhibitor, sodium valproate, to AS mice showed significant improvement in social, cognitive and motor impairment along with restoration of various proteins linked with synaptic function and plasticity. Interestingly, HDAC inhibitor also significantly increased the expression of Ube3a in cultured neuronal cells and in the brain of wild type mice but not in AS mice. These results indicate that anomalous HDAC1/2 activity might be linked with synaptic dysfunction and behavioural deficits in AS mice and suggests that HDAC inhibitors could be potential therapeutic molecule for the treatment of the disease.

1. Introduction

Angelman syndrome (AS) is a neurodevelopmental disorder characterized by severe developmental delay, cognitive and motor deficits, lack of speech and epileptic seizures along with multiple other associated features particularly excessive laughter and sleep disturbances (Williams et al., 2006; Williams et al., 2010). Genetic studies have revealed that the AS is caused by the loss of function of the maternally inherited *UBE3A* allele (Fang et al., 1999; Kishino et al., 1997; Matsuura et al., 1997). Because the paternally inherited *UBE3A* is epigenetically silenced in the neuronal tissue through cell type specific imprinting, the defect in maternally inherited *UBE3A* results its loss of function in the brain (Albrecht et al., 1997; Mabb et al., 2011; Yamasaki et al., 2003). The *UBE3A* gene encodes a 100 kDa protein that has been characterized as an E3 ubiquitin ligase (involved in targeting proteins for ubiquitination) and transcriptional co-activator for steroid hormone receptors (Huibregtse et al., 1995; Ramamoorthy and Nawaz, 2008). Therefore, it is hypothesized that loss of ubiquitin ligase activity or co-activator function of Ube3a might be linked with the AS phenotypes.

To gain deeper insight into the AS patho-mechanism, several mouse models has been generated by disrupting the maternally inherited Ube3a (Jana, 2012). Mouse model generated by Jiang et al. reproduced many characteristic features of AS and is widely used to understand the disease pathogenesis (Jiang et al., 1998). These mice not only exhibit classical cognitive and motor deficits, but also display audiogenic seizure, anxiety-like behaviour, disturbance in circadian clock and sleep homeostasis (Godavarthi et al., 2012; Heck et al., 2008; Jiang et al., 1998; Mulherkar and Jana, 2010; Shi et al., 2015). Moreover these AS mice also become obese (Meng et al., 2015; Shi et al., 2015). In depth study in this mouse model further demonstrates defect in hippocampal calcium/calmodulin dependent protein kinase-II and long-term potentiation, experience-dependent synaptic plasticity and imbalance of excitatory/inhibitory circuitry (Jiang et al., 1998; Sato and Stryker, 2010; Wallace et al., 2012; Weeber et al., 2003; Yashiro et al., 2009). These results strongly indicate that Ube3a plays a crucial role in regulating synaptic function.

Although there have been considerable progress in understanding AS pathogenesis, currently there is no effective therapy. It has long been thought that activation of silenced paternal allele of *UBE3A* could be an attractive therapeutic strategy. Because the silencing is mediated by the expression of large noncoding antisense RNA transcript (UBE3A-ATS), several attempts have been made to suppress the expression of UBE3A-ATS. Recently, antisense oligonucleotide of UBE3A-ATS

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has been shown to activate the paternal Ube3a and consequently improve the behavioural abnormalities in AS mice (Meng et al., 2015). Furthermore, topoisomerase inhibitors also have been reported to unsilence the paternal Ube3a expression by inhibiting the UBE3A-ATS (Huang et al., 2012). However, the therapeutic benefit of these inhibitors in animal models have not been established so far.

Histone acetylation and deacetylation regulated by histone acetyl transferase (HAT) and histone deacetylase (HDAC) plays a significant role in regulating the gene transcription (Delcuve et al., 2012; Haberland et al., 2009). Differential histone acetylation has been reported in the key imprinted gene locus in Prader-Willi Syndrome (PWS), but not in the AS imprinting centre or promoter region of *UBE3A* (Saitoh and Wada, 2000). Therefore, we aimed to investigate the role of HDAC inhibitor in the regulation of Ube3a expression using AS mouse model. We found that HDAC inhibitors significantly increased the expression of Ube3a in the brain of wild type mice, but had no effect on the expression of Ube3a in AS mice brain. During our study, we surprisingly noticed that the acetylation of histones H3(K9) and H4(K12) was significantly lower along with increased levels of HDAC1/2 in AS mice brain compared to the wild type counterpart. Finally, we demonstrate significant improvement of various behavioural abnormalities upon treatment with the HDAC inhibitor, sodium valproate to AS mice.

2. Materials and methods

2.1. Materials

Sodium valproate, sodium butyrate, trizol reagent and mouse monoclonal antibody against β-actin and synaptophysin were purchased from Sigma. Rabbit polyclonal anti-Ube3a, and anti-BDNF, anti-phosphoThr286 CaMKIIα and anti-CaMKIIα antibodies were purchased from Santa Cruz Biotechnology. Mouse specific Ube3a siRNA oligoneucleotides (a pool of 3 target specific 20–30 nucleotide siRNA) and control siRNA (scrambled sequences) were also purchased from Santa Cruz Biotechnology. Rabbit polyclonal anti-HDAC1 and HDAC2, PSD95, anti-H2A, H2B, H3, H4 and their acetylated antibodies were procured from Cell Signalling Technology. Rabbit polyclonal GluR1 and GluR2 antibodies were obtained from Millipore and mouse monoclonal anti-Ube3a was from BD Biosciences. Biotinylated anti-rabbit IgG, HRP-conjugated anti-rabbit and anti-mouse IgG, VECTASTAIN-Elite ABC reagent and ImmPact Novared staining kit were purchased from Vector Laboratories.

2.2. Ethic statement

All animal experiments were approved by the Institutional Animal and Ethics Committee (IAEC) of the National Brain Research Centre. Mice were handled strictly according to guidelines defined by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Environment and Forestry, Government of India.

2.3. Animal treatment

Heterozygous mice for *Ube3a* gene were obtained from the Jackson Laboratory (Jackson code: 129-*Ube3a*tm1Alb/J) and maintained in animal house facility of the Institute. The test for genotyping was performed from the isolated genomic DNA of mice tails using PCR as described previously (Jiang et al., 1998). *Ube3a* heterozygous female (Ube3am−/p+) mice were crossed with wild type male (Ube3am+/p+) to obtain the wild type as well as Ube3a-maternal deficient or AS mice (Ube3am−/p+). Just after weaning (at 21 days), male wild type and AS mice were taken to experimental room, divided into different groups and acclimatized for about one week. Animals were then intraperitoneally injected sodium valproate (300 mg/kg body weight daily) for 60 days. Control group received same volume of saline. Each group was having 10–12 animals. Behavioural studies were conducted between 50 and 60 days of drug treatment.

2.4. Animal behavioural study

2.4.1. Test for social interaction

Crawley's sociability and preference for social novelty test protocol was used to study the social interaction (Kaidanovich-Beilin et al., 2011). The test used a 3-chamber rectangular box (20 × 45 cm) with removable dividing walls (made in plexiglass) and two identical cup-like container made up of metal wires to hold a single mouse that can freely breathe and move around. These cups were placed inside the left or right side chamber of the rectangular box to keep the naive mice. Initially, the test mouse was placed at the center of the middle chamber with closed left and right chamber and allowed to familiarize for 5 min. Subsequently, one of the wild type mice (stranger 1) was kept inside the cup of left side chamber (keeping empty cup in the right side chamber) and plexiglass walls between the compartments were removed. Interaction time of test mice with the stranger 1 as well as with empty cup was monitored for 10 min using a digital camera mounted above the apparatus. This experiment was conducted to evaluate the sociability of the test mice which is directly proportional with its interaction time on stranger. In the second session, another wild type mouse (stranger 2) was placed in the right side chamber that was empty during session 1 and the interaction time of the test mice with both strangers was monitored for 10 min duration. The tendency of the test mice to spend time with stranger 2 (previously un-encountered mouse) with regard to stranger 1 (familiar mouse) was used to assess social novelty. The experiment for session 1 and session 2 for each test mouse (from saline and valproate treated wild type, AS mice) were conducted in similar manner, interaction time for stranger 1 and stranger 2 were analyzed and plotted. After each trial all chambers were cleaned with 70% ethanol to prevent olfactory cues bias.

2.4.2. Test for motor function

Balance beam test was used to assess the fine motor skill of AS mice (Mulherkar and Jana, 2010). Mice were allowed to walk 80 cm long beam (either square or round with 8, 12 and 20 mm diameter) that was kept 50 cm above base and at the end of the beam, a dark wooden box was kept to rest the mice. Mice were initially trained on 20 mm diameter round or square beam for two days and on the following day, they were tested for round or square beam walk on 8 and 12 mm diameter beam. Each mouse was used for 3 trials. Round bean and square beam experiments were conducted on different days. All experiments were recorded using a digital camera. Amounts of time to cross the beam for each mice was calculated and average values were used for statistical analysis.

2.4.3. Test for cognitive function and anxiety

Novel object recognition test was used to assess the cognitive function in wild type and AS mice. The test for anxiety was conducted using light/dark box test**.** Detailed procedures for these tests have been described in our earlier paper (Godavarthi et al., 2012).

2.5. Neuronal cell culture and transfection

Mouse neuro 2a cells were cultured in Dulbecco's modified Eagle's medium supplemented with 10% heat-inactivated fetal bovine serum and antibiotics penicillin/streptomycin. For experimental purpose, cells were plated into 6-well tissue culture plate at sub-confluent density. After 24 h of plating, cells were transfected with Ube3a and control siRNA oligonucleotides using Lipofactamine® 2000 (Invitrogen) according to the instruction of the manufacturer. Cells were harvested 48 h of post transfection, lysates were made and then processed for immunoblot analysis using various antibodies. In another experiment, cells were treated with different doses of either sodium valproate or