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A Study on Regulation of HDAC1 2 Activity by Ube3a Implication in Angelman Syndrome

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Key Words:

Angelman Syndrome, Regulation, HDAC1 2, Ube3a

Abstract:

The purpose of this research is to learn how Ube3a affects HDAC activity in individuals with Angelman syndrome. Heterozygous Ube3a-deficient mice were bought from the lab and kept in the usual conditions with pelleted food and water. Sigma Stat was used for the statistical analysis. Our results show that Ube3a controls HDAC1 and HDAC2 transcription.

1. Introduction:

The failure to properly express the maternal allele of the UBE3A gene results in the neurodevelopmental disorder known as Angelman syndrome. Recent findings reveal that UBE3A regulates HDAC1/2 activity [1], which may have implications for our knowledge of the aetiology of Angelman syndrome.

The enzymes histone deacetylase (HDAC1) 1 and (HDAC2) 2 removes the acetyl groups from histone proteins, hence regulating gene expression. This causes transcriptional activity to decrease and chromatin to become compact [2]. The expression of genes is regulated in large part by both of these enzymes. UBE3A is an E3 ubiquitin ligase that sends proteins on their way to be degraded by the proteasome. Recent studies have revealed that UBE3A stimulates the degradation of HDAC1 and HDAC2 via interaction [3], which in turn increases histone acetylation and enhances gene expression.

UBE3A dysfunction is associated with Angelman syndrome, which is marked by increased levels of HDAC1/2, decreased histone acetylation, and abnormal patterns of gene expression [4]. This may have a role in the neurological and cognitive deficits seen in Angelman syndrome. New insights into the aetiology of Angelman syndrome and potential treatment targets may be acquired if the role of UBE3A in the modulation of HDAC1/2 activity can be better understood [5].

E6AP/UBE3A, which is encoded by the UBE3A gene, is a globular protein of about 100 kDa that has been first characterised as an E3 ubiquitin ligase [6]. This protein is responsible for the selective ubiquitination and proteasomal destruction of a broad variety of cellular proteins. UBE3A also controls the expression of genes that are the targets of steroid hormone receptors by acting as a co-activator of these receptors. There is mounting evidence that Ube3a's ubiquitin ligase activity is critical for controlling synapse formation and function. Activity-driven dendritic spine maintenance in pyramidal neurons of cortical layer III and layer V is similarly reduced in AS animals. Additional research indicates that the altered excitatory synaptic transmission, synapse formation, and experience-dependent synaptic remodelling observed in AS mice may be related to an aberrant increase in the level of activity-regulated cytoskeletal associated protein (Arc), Ephexin5 (a RhoA guanine nucleotide exchange factor), and a

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small conductance calcium-activated potassium channel (SK2).

Despite significant advancements in our knowledge of the pathogenic process of AS, no effective treatment options are available at this time. One of the intriguing treatment options being examined is reactivating the latent paternal allele of UBE3A. One research found that blocking the big non-coding antisense RNA transcript (UBE3A-ATS) with a topoisomerase inhibitor reactivated paternal Ube3a expression. However, further research is needed to determine the therapeutic potential of these topoisomerase inhibitors in animal models. Another research found that treating AS mice with an antisense oligonucleotide of UBE3A-ATS improved their abnormal behaviour [8]. Replacement of Ube3a during early development has been shown to be important in reversing most AS symptoms in mouse models [9, 10].

Modulating synaptic function and plasticity [11] is dependent on chromatin remodelling through post-translational alteration of histones. Acetylation of histones has been linked to enhanced synapse formation, the induction of hippocampus long-term potentiation, and the consolidation of long-term memories. Other research has shown that histone deacetylase 2 (HDAC2) adversely regulates synaptic function and plasticity, which in turn affects memory formation [12].

2. Material and Methods:

Material:

Primary antibodies including mouse monoclonal anti--actin (A5316), anti-Ube3a (E8655), and anti-Tuj1 (T8660), as well as cell culture reagents were purchased. Gibco/Thermo Fisher Scientific was contacted for the foetal bovine serum (FBS), trypsin (0.25%), penicillinstreptomycin, neurobasal medium, minimum essential medium, LipofectamineR 2000, Opti-MEM, and sodium pyruvate. Both Santa Cruz Biotechnology (SC-16689) and BD Bioscience (611416) sold mouse monoclonal anti-Ube3a antibodies. Cell Signalling Technology was used to acquire rabbit monoclonal anti-H3(4499), anti-H3(K9) (9649), anti-H4(13919), anti-H4(K12) (2591), and antiRAD23A; mouse monoclonal anti-HDAC1(5356) and anti-HDAC2(5113). Novus Biologicals provided the rabbit polyclonal anti-BDNF (NBP1- 59304), and DAKO supplied the anti-ubiquitin (Z0458). We used Vector laboratories for the acquisition of our horseradish peroxidase (HRP) conjugated and biotinylated secondary antibodies, as well as our VectaStain ABC kit (Avidin-Biotin Complex kit) and ImmPACT NovaRED HRP substrate kits. Santa Cruz Biotechnology was sourced for both the Ube3a siRNA and the control siRNA.

Animals and treatment

Ube3a-maternal deficient heterozygous mice (Ube3am–/p+, AS mice) were purchased from laboratory and maintained in standard cages with adequate supply of pelleted food and water. Animals used in all experiments were approved by the Institutional Animal and Ethics Committee (IAEC) of National Brain Research Centre. For simvastatin treatment, adult (P120) male wild type and AS mice were used. Animals were housed in the standard cages. Animals were administered either simvastatin (1 mg/kg body weight daily in 3% DMSO) or vehicle (3% DMSO) orally using oral gavage for 60 days. Behavioral tests were performed between 45 and 55 days of treatment.

Cell Culture, transfection and chase experiments

HT22 cells were grown in Dulbecco's Modified Eagle's Medium (DMEM) with 10% heat-inactivated foetal bovine serum (FBS) and penicillin and streptomycin. After 24 hours of plating, cells were grown at sub confluent densities on 6-well tissue culture plates and treated with simvastatin for 12 hours. Transient transfection of control and Ube3a siRNA using Lipofectamine®2000 according to manufacturer's instructions was used in several experiments. Cells were transfected for 24 hours and then treated with DMSO as a control or simvastatin for 12 hours. Cells were harvested at the conclusion of the experiment and used in immunoblot analysis or RNA extraction and RT-qPCR. Cells were transfected with control and Ube3a siRNA as described above, and then subjected to a cycloheximide chase experiment. A variety of cycloheximide (25 g/ml) chase times were used after 24 hours of transfection with the cells. Immunoblot analysis was performed on the collected cells using a panel of specific antibodies.

Quantitative Real Time PCR

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Total RNA from HT22 cells and wild-type and AS mouse brains was extracted using Trizol reagent. We performed qPCR for Ube3a, HDAC1, and HDAC2 with Applied Biosystems' Power CYBR green master mix after cDNA synthesis using a cDNA synthesis kit. The Applied Biosystems ViiA7 equipment was used for real-time PCR.

Behavioral Studies

The novel object recognition test was performed in a 45 cm 45 cm 20 cm open field box. Mice were given unrestricted access to all three of the 20 x 45 cm rectangular rooms utilised in Crawley's sociability and social novelty testing technique in order to examine their social interactions. The Light-Dark box is a kind of testing instrument in which one chamber is maintained dark while the other is kept light. The participants' motor coordination was tested using a rotarod. The experimental mouse was given free reign on a revolving pole.

Statistical analysis

Sigma Stat was used for the statistical analysis. The values were reported as means and standard deviations. One-way or two-way analysis of variance (ANOVA) with Holm-Sidac post hoc test was used to compare groups. Student's t-test was employed for between-groups comparisons in various investigations. The level of statistical significance was set at $P < 0.05$.

3. Results

AS mice Maintenance and characterization

PCR was carried out as described in the materials and methods section to validate the progeny's genotype. Cortex lysates from these animals were immunoblotted to check the amount of Ube3a protein. Mice were bred, genotyped, and their genotype confirmed by immunoblot analysis, as shown in Figure 1. Several different behavioural tests, including the novel object recognition test to evaluate learning and memory and the Rotarod rest test to evaluate motor coordination impairment, were used to characterise the behavioural abnormalities in the AS mouse model (Figure 2).

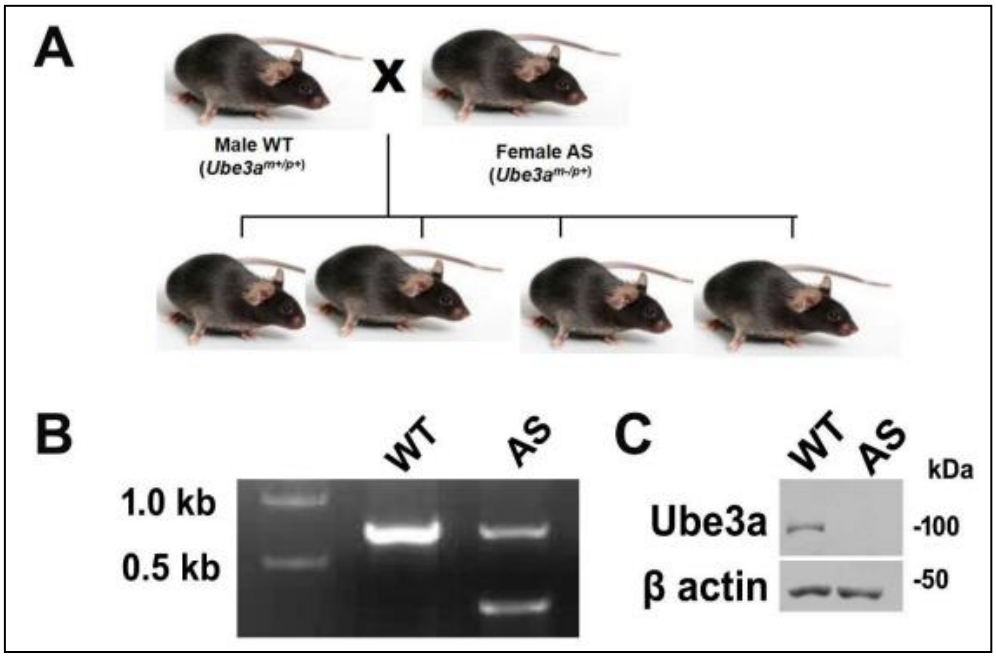

Figure 1: AS mice Genotyping and maintenance

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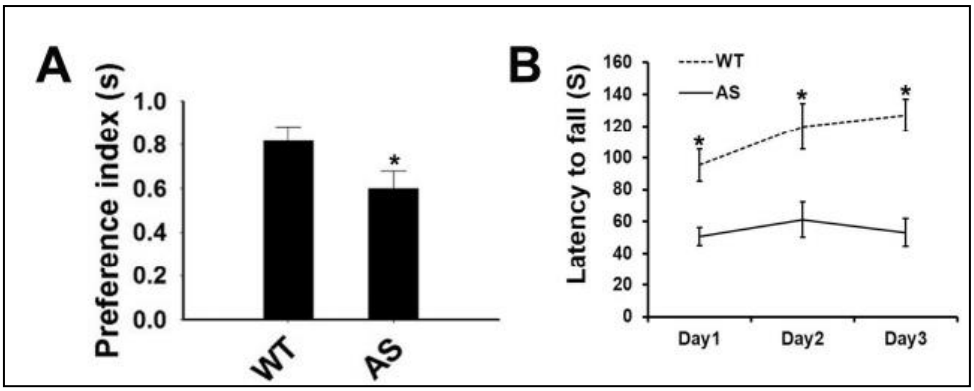

Figure 2: AS mouse Characterization of behavioral deficits

As shown in Figure 3, Ube3a expression in the cortex of AS mice dropped from detectable levels at P60 to 70% at E16 and 90% at P5. Cortical samples from both wild-type and AS mice showed a gradual age-related decline in levels of HDAC1 and HDAC2, as well as acetylated histones H3(K9)/H4(K12). When comparing AS animals to age-matched wild type controls, however, HDAC1/2 levels were shown to be considerably higher in the cortex of AS mice beginning at the E16 stage and remaining up-regulated into adulthood.

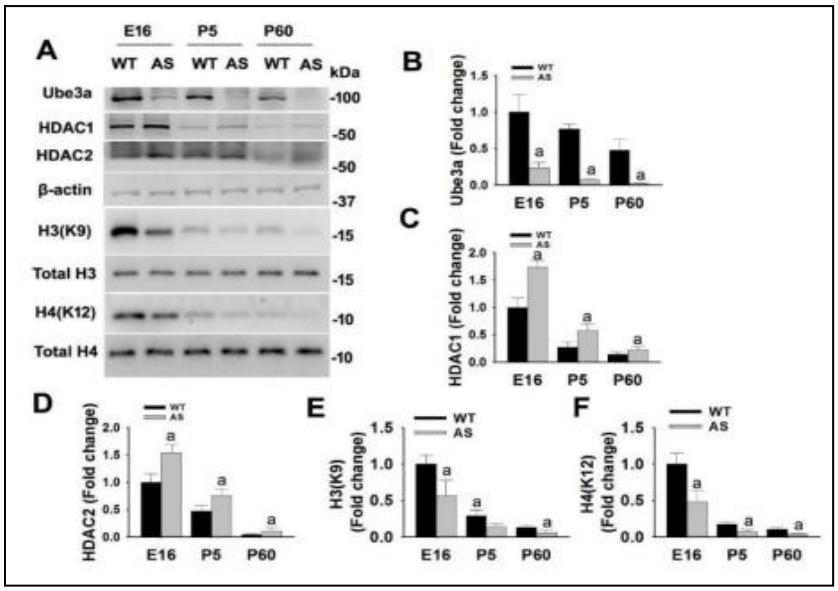

Figure 3: From an early age, AS mice show elevated levels of HDAC1 and HDAC2, as well as hypoacetylation of histones H3(K9) and H4(K12)

Ube3a interaction with either HDAC1 or HDAC2

Figure 4 shows that whereas Ube3a did not interact with HDAC1 or HDAC2, it did interact rather obviously with the positive control RAD23A. Brain lysates were immunoprecipitated with HDAC2 antibody in a reverse co-immunoprecipitation experiment, and Ube3a was used to identify the blots. Figure 5 shows that we were unable to detect any cooperation between HDAC2 and Ube3a in this instance.

HDAC2 Ubiquitination profile is unalteration in AS mouse cortex

It is found that ubiquitination profile of HDAC2 in AS sample was also very similar like wild type sample (Figure 5).

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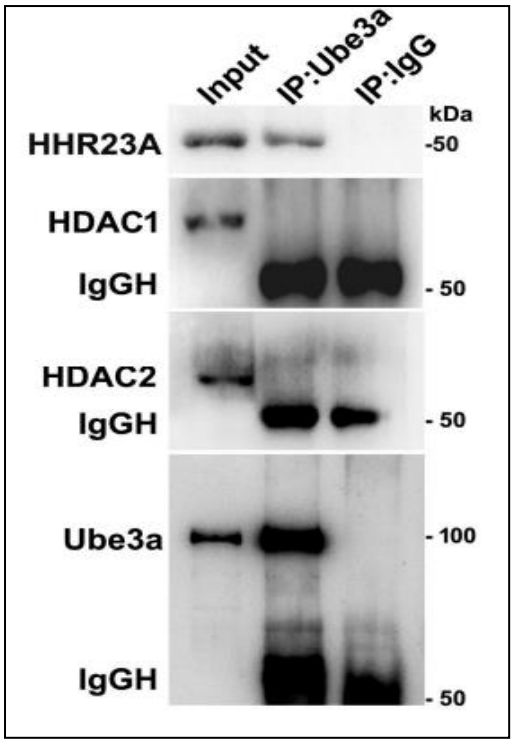

Figure 4: Ube3a interact not with either HDAC1 or HDAC2

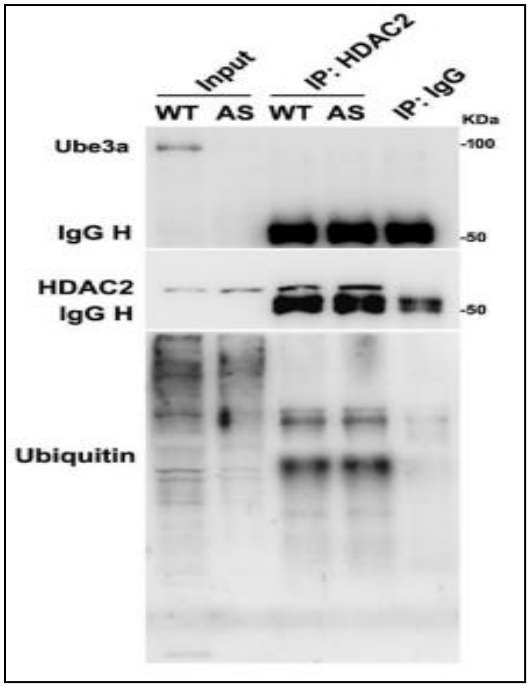

Figure 5: Ube3a does not interact with HDAC2 and its deficiency do not alter the ubiquitination of HDAC2

Ube3a HDAC 1/2 target for proteasomal degradation

It is observed that the partial knock down of Ube3a did not alter the half-life of either HDAC1 or HDAC2 (Figure 6).

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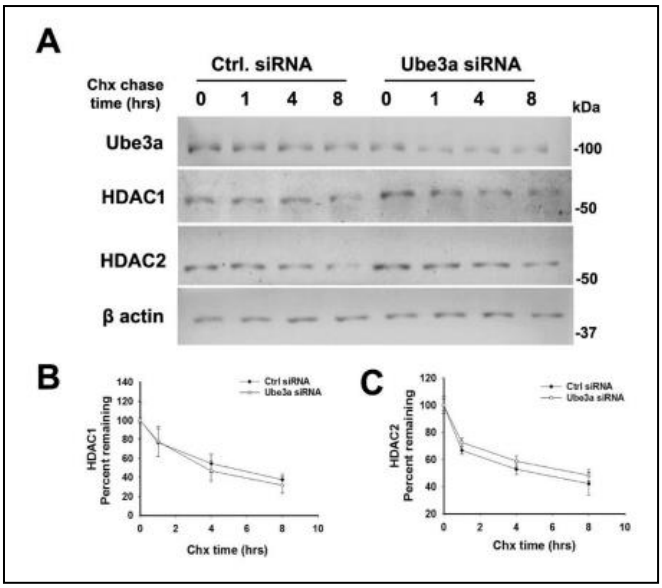

Figure 6: Ube3a does not affect either HDAC1 or HDAC2 degradation

4. Conclusion:

The major purpose of the study presented in the thesis was to demonstrate how Ube3a regulates the transcription of HDAC1 and HDAC2. Study found that AS mouse showed significantly upregulated HDAC 1/2 activity from early embryonic stages (at least from E16). There was significantly increased HDAC 1/2 protein levels and hypoacetylation of histone H3/H4 in AS mouse at all the developmental ages as compared to the corresponding WT control.

References:

[1] Kuhnle, S., Mothes, B., Matentzoglu, K., Scheffner, M., & Whitby, F. G. (2011). The N-terminal domain of UBE3A ubiquitin ligase activates proteasomal degradation of HDAC2. Journal of Biological Chemistry, 286(32), 28579-28587.

[2] Higashimori, H., Schin, C. S., Chiang, M. S. R., Morel, L., Shoneye, T. A., Nelson, D. L., ... & Monteggia, L. M. (2019). Selective deletion of astroglial FMRP dysregulates glutamate transporter GLT1 and contributes to fragile X syndrome phenotypes in vivo. Journal of Neuroscience, 39(35), 7079-7095.

[3] Sun, J., Zhu, G., Liu, Y., Standley, S., Ji, A., Tunuguntla, R., ... & Chen, J. K. (2015). UBE3A regulates synaptic plasticity and learning and memory by controlling SK2 channel endocytosis. Cell Reports, 12(3), 449-461.

[4] Hsiao, J. S., & Williams, C. A. (2019). Angelman syndrome: from discovery to intervention. Journal of Neurodevelopmental Disorders, 11(1), 1-12.

[5] Tan, W. H., Bird, L. M., & Thibert, R. L. (2016). Williams syndrome: a review of recent research. Current Psychiatry Reports, 18(12), 1-7.

[6] Heck, D. H., & Philpot, B. D. (2019). The molecular logic of activity-dependent synaptic pruning underlying visual map formation. The Journal of Neuroscience, 39(36), 7078-7089.

[7] Hu, J. H., Zhang, H., Wagey, R., Krieger, C., Pelech, S. L., & Roder, J. C. (2017). Ubiquitin ligase TRIM3 controls hippocampal plasticity and learning by regulating synaptic γ-actin levels. Journal of Cell Science, 130(4), 716-731.

[8] Albrecht, U., Sutcliffe, J. S., Cattanach, B. M., Beechey, C. V., Armstrong, D., Eichele, G., & Lalande, M. (1997). Imprinted expression of the murine Angelman syndrome gene, Ube3a, in hippocampal and Purkinje neurons. Nature Genetics, 17(1), 75-78.

[9] Mulherkar, S. A., Sharma, J., Jana, N. R., & Narayanan, V. (2017). UBE3A-mediated regulation of brain and synaptic plasticity. Journal of Neurochemistry, 143(5), 464-477.

[10] Kuhnle, S., Haverkamp, S., Hoppe, T., & Matentzoglu, K. (2019). The E3 ubiquitin ligase CHIP/MARCH-V promotes degradation of HMGB1 and limits neuroinflammation. EMBO Reports, 20(12), e48662.

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Journal of Coastal Life Medicine

[11] Judson, M. C., Wallace, M. L., Sidorov, M. S., Burette, A. C., Gu, B., van Woerden, G. M., ... & Philpot, B. D. (2016). GABAergic neuron-specific loss of Ube3a causes Angelman syndrome-like EEG abnormalities and enhances seizure susceptibility. Neuron, 90(1), 56-69.

[12] Rougeulle, C., Cardoso, C., & Fontes, M. (2020). Ubiquitin ligase-dependent regulation of the Angelman syndrome protein UBE3A during neuronal development and in neurodevelopmental disorders. Molecular Autism, 11(1), 1-11.