1009 Modeling Rett syndrome using immortalized human neural cells expressing different MeCP2 mutants

Susan A. Finniss[1], Amotz Ziv Av[2], Cunli Xiang[1], Hae Kyung Lee[1], Bruria Ben Zeev[3] and Chaya Brodie[1,2]
[1]Department of Neurosurgery, Henry Ford Hospital, MI, [2]Faculty of Life Sciences, Bar-Ilan University, Israel and [3]Child Neurology Unit Edmond and Lilly Safra Pediatric Hospital Israel

Rett syndrome (RTT) is an X- linked dominant neurodevelopmental disorder causing severe intellectual disability accompanied by autistic regression. The primary cause of RTT is de novo germline mutations in the methyl CpG-binding protein 2 (MECP2) gene. Different mutations influence phenotype severity and may distinguish between classical and atypical milder phenotypes. However, the role of specific MeCP2 mutations and the molecular mechanisms involved in their effects are not yet understood. Recent data also suggest a role of glial cells in the pathogenesis of RTT in a non-cell autonomous manner. Here, we describe the generation of in vitro RTT models using immortalized human primary neural cells silenced for MeCP2 or expressing specific MeCP2 mutants. Silencing of MeCP2 in human neural stem cells inhibited β3 tubulin and increased GFAP expression. Human astrocytes silenced for MeCP2 exhibited lower levels of GFAP, the glutamate transporter EAAT2 and IGF-1 secretion. Silencing of MeCP2 increased the secretion of glutamate, decreased BDNF secretion and induced the M1 phenotype in microglia cells. Expression of the MeCP2 R168X, T158M, R306C and R133C mutants induced a differential impact on the function of the NSCs and the glial cells. Analyzing miRNA expression in the MeCP2 silenced astrocytes and microglia identified novel altered miRNAs and related pathways. We conclude that immortalized human neural cells expressing different MeCP2 mutants can serve as a novel model system for studying molecular mechanisms involved in the pathogenesis of RTT in both neurons and glial cells and for analyzing the impact of specific MeCP2 mutations. In addition, the in vitro models can be also employed for the high throughput screening of novel and repurposed drugs for the treatment of this disease.

1010 Securinine, the GABA receptor antagonist, enhances SMN2 exon 7 inclusion and SMN protein expression in spinal muscular atrophy

Jan-Gowth Chang[1], Yu-Chia Chen[2], Yuh-Jyh Jong[2], Wei-Lin Cheng[2], Chung-Yee Yuo[3]
1. Epigenome Research Center, China Medical University Hospital, Taichung, Taiwan, 2. Graduate Institute of Medicine, Kaohsiung Medical University, Kaohsiung, Taiwan, 3. Department of Biomedical Science and Environmental Biology, Kaohsiung Medical University, Kaohsiung, Taiwan

Spinal muscular atrophy (SMA) is an autosomal recessive disease characterized by the degeneration of motor neurons in the spinal cord, leading to muscular atrophy. SMA is caused by deletions/mutations in the telomeric copy of the *survival motor neuron* gene (*SMN1*) on chromosome 5q13. A second centromeric copy of the *SMN* gene (*SMN2*) also exists on chromosome 5. However, due to the alternative splicing of the exon 7, the majority of SMN protein produced by *SMN2* is truncated and unable to compensate for the loss of *SMN1*. Increasing full-length SMN protein production by promoting the exon 7 inclusion in *SMN2* mRNA or increasing *SMN2* gene transcription could be a therapeutic approach for SMA. We found that securinine can increase *SMN2* exon 7 inclusion by using *SMN2* minigene-luciferase reporter system. We also found that securinine increased full-length *SMN2* mRNA expression and SMN protein expression in primary fibroblast cells from SMA patients. To investigate the mechanism of securinine on changing *SMN2* splicing, we compared the protein levels of relevant splicing factors. We found that securinine treatment in SMA primary fibroblast cells downregulated hnRNP A1 and upregulated Tra2-β1 protein expression levels. However, securinine had no effect on enhancing *Tra2-β1* transcription, indicating a post-transcriptional mechanism of Tra2-β1 d upregulation. In addition, we treated SMA-like mice with securinin and found that securinine treatment can slightly increase SMN2 exon 7 inclusion in the brain and spinal cord. According to our result, securinine might have the potential to become a therapeutic drug for SMA disease.

1011 Complex neurological phenotype in mutant mice lacking *Tsc2* in excitatory neurons of the developing forebrain

Beth Crowell[a], Gum Hwa Lee[a], Valentina Dal Pozzo[a,b], Ina Nikolaeva[a,c] and Gabriella D'Arcangelo[a,b,c]
[a]Department of Cell Biology and Neuroscience, Graduate Programs in [b]Neuroscience and in [c]Molecular Biosciences, Rutgers, the State University of New Jersey, Piscataway, NJ, USA

Tuberous Sclerosis Complex (TSC) is a genetic disease resulting from mutations in either the *TSC1* or the *TSC2* gene. The disease is characterized by a high frequency of epilepsy, intellectual disability and autism, and by the frequent presence of brain malformations (tubers), tumors and lesions in other organs. Cognitive dysfunction and tuber formation may result from abnormalities in different cell types. However, it is not presently clear how TSC mutations affect neuronal and non-neuronal cell populations during brain development. To better understand the role of TSC2 in excitatory neurons during forebrain development, we generated a conditional mutant mouse line (NEX-*Tsc2*), which lacks *Tsc2* expression specifically in these cells. Homozygous mutant mice exhibited neuroanatomical abnormalities in the cerebral cortex and hippocampus, became runt and mostly die during the second-third postnatal week. These mutants also displayed expected abnormalities in Akt and mTOR signaling, whereas NEX-*Tsc2* heterozygous mice appeared normal. Homozygous mutants exhibited not only neuronal abnormalities, but also striking abnormalities in non-neuronal cell populations of the forebrain, which were not directly affected by our gene knock out strategy. These studies may provide insights into the role of Tsc2 in the development of forebrain structures and molecular mechanisms affecting neuron-glia communication during brain development.

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1012 GABAergic/ glutamtergic imbalance relative to excessive neuroinflammation in autism spectrum disorders

Prof. Afaf El-Ansary
Biochemistry Department, Science College, King Saud University, Riyadh, Saudi Arabia, Autism Research and Treatment Center, Riyadh, Saudi Arabia

Background: Autism spectrum disorder (ASD) is characterized by three core behavioral domains: social deficits, impaired communication, and repetitive behaviors. In pathological conditions, microglia release large amounts of cytokines as an important component of the so-called neuroinflammatory response that is associated with several neurological disorders. These cytokines can potentiate glutamate-mediated cytotoxicity either by inhibiting glutamate transport on astrocytes, or by rapidly triggering the surface expression of Ca^{+2} permeable-AMPA receptors and NMDA receptors, while decreasing inhibitory $GABA_A$ receptors on neurons. Thus, resulting in a higher synaptic excitatory/inhibitory ratio. This study aimed to elucidate the relationship between Glutamatergic/GABAergic imbalance and neuroinflammation as two recently discovered autism-related etiological mechanisms.

Methods: Twenty autistic patients aged 3-15 years and 19 age- and gender-matched healthy controls were included in this study. The plasma levels of glutamate, GABA and glutamate/ GABA ratio as markers of excitotoxicity together with TNF-α, IL-6, IFN-γ and IFI16 as markers of neuroinflammation were determined in both groups using ELIZA diagnostic kits.

Results: Autistic patients exhibited glutamate excitotoxicity represented as much higher plasma glutamate compared to control subjects. Unexpected higher GABA and lower glutamate/GABA level were recorded in autistic compared to control. TNF-α and IL-6 were significantly lower, whereas IFN-γ and IFI16 were remarkably higher in the autistic patients than in the control subjects.

Conclusion: Multiple regression analysis revealed associations between high plasma GABA level, neuroinflammation and glutamate excitotoxicity which suggest that autism could be a disorder of synaptic modulation or maintenance showing imbalance in GABAergic and glutamatergic synapses as a consequence of neuroinflammation that might lead to less number or dysfunctional neuronal GABA receptors. The role of excitotoxicity and the mechanism behind its action in autistic subjects could be used as target to treat this disorder.