

Abstract

Mutations in the X-linked gene, methyl-CpG binding protein 2 (*MECP2*), underlie a wide range of neuropsychiatric disorders, most commonly Rett syndrome (RTT), a severe neurodevelopmental disorder. Despite numerous studies, the question of why the loss of MeCP2 results in RTT remains largely unanswered, and it represents a major challenge from both basic biological and therapeutic standpoints. Our previous studies, based on mouse models, advanced the knowledge of the disease and of the specific cell types involved in the RTT neuropathology. Initially, RTT was attributed solely to neuronal dysfunction, but our recent studies have challenged this view and suggested that astrocytes are an integral part of RTT neuropathology. However, mouse models do not represent properly human RTT, which is known to be more severe than in mouse models. Importantly, human astrocytes are significantly different from mouse astrocytes, both in their structure, which is much more complex, and in the gene expression landscape, making it highly important to use human-based models to investigate the cellular and molecular mechanisms underlying RTT.

In the past few years, my lab has transitioned from mouse models to human-based models to study how RTT-causing *MECP2* mutations impact the developmental maturation and function of human astrocytes and thereby their key role in providing structural and metabolic support to the neurons (currently funded by NIH R21 grant). For this purpose, we used male human embryonic stem cells (hESCs) in which *MECP2* mutations were inserted via CRISPR/Cas9 technology and developed several platforms for human astrocyte differentiation and their neuron-dependent and independent maturation. We showed for the first time that *MECP2* mutations in human astrocytes greatly impact their transcriptional landscape, affecting pathways pertaining to structure and energy metabolism. Indeed, our data showed that the neuron-induced mature stellate morphology of human astrocytes, normally occurring during postnatal brain development, as well as their energy metabolism and neurotransmitter homeostasis, which the neurons rely on, were severely compromised. Importantly, we showed that the mitochondrial morphology and respiration were severely impaired in mutant human astrocytes, suggesting that mitochondrial dysfunction mediates their impaired energy metabolism and likely their inability to properly support the neurons. A manuscript describing these studies is under revision.

Our future goal is to continue to investigate how *MECP2* mutations in human astrocytes impact their central role, which is to support the neurons in the brain, structurally and metabolically, and whether mitochondrial dysfunction lies at the heart of this disorder. To achieve this goal, it is necessary to generate compelling data that will help develop the current R21 grant to an R01 grant. While we have generated ample significant data using male human models for RTT brain development, it is highly important to generate and investigate also female human models for RTT as the majority of RTT syndrome patients are females. Female models will allow us not only to verify our studies which were based on male models, but also to model the mosaicism for *MECP2* mutations occurring in the female RTT brain, due to random X-chromosome inactivation. We will use female RTT patient-derived induced pluripotent stem cells (iPSCs) with different *MECP2* mutations to model astrocyte development and maturation and investigate whether normal astrocytes are also affected by the mutations in a non-cell autonomous manner as suggested by our morphological analysis of astrocytes in postmortem brains of female RTT patients.

Modeling the development and maturation of human RTT astrocytes and analyzing their cellular and molecular properties, especially at a stage that parallels the onset of RTT, will provide important insights into the mechanism of human RTT and serve as a platform for developing therapeutic strategies to reverse the impaired human RTT neuronal networks.