



Free as a BRD4: Bromodomain Inhibition Ameliorates Disease Phenotypes in a Model of MECP2 Deficiency and Is a Potential Therapy for Rett Syndrome

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Dysregulation of BRD4 Function Underlies the Functional Abnormalities of MeCP2 Mutant Neurons

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Rett syndrome (RTT), mainly caused by mutations in methyl-CpG binding protein 2 (MeCP2), is one of the most prevalent intellectual disorders without effective therapies. Here, we used 2D and 3D human brain cultures to investigate MeCP2 function. We found that MeCP2 mutations cause severe abnormalities in human interneurons (INs). Surprisingly, treatment with a BET inhibitor, JQ1, rescued the molecular and functional phenotypes of MeCP2 mutant INs. We uncovered that abnormal increases in chromatin binding of BRD4 and enhancer-promoter interactions underlie the abnormal transcription in MeCP2 mutant INs, which were recovered to normal levels by JQ1. We revealed cell-type-specific transcriptome impairment in MeCP2 mutant region-specific human brain organoids that were rescued by JQ1. Finally, JQ1 ameliorated RTT-like phenotypes in mice. These data demonstrate that BRD4 dysregulation is a critical driver for RTT etiology and suggest that targeting BRD4 could be a potential therapeutic opportunity for RTT.

Commentary

Rett syndrome (RTT) is an X-linked neurodevelopmental disorder primarily affecting girls, caused by *de novo* pathogenic *MECP2* variants. Individuals with RTT develop impairments in ambulation, speech, and respiratory function, and the majority have epilepsy.¹ As RTT is an X-linked disorder, random X-inactivation leads to mosaicism in RTT patients, which contributes to a wide range of disease severity. There are currently no effective pharmacological interventions available for individuals with RTT. One of the main barriers to therapy development has been the inaccessibility of viable neurons from patients with RTT for study. In recent years, human embryonic stem cells (hESCs) and induced pluripotent stem cells (iPSCs) have been used to model RTT in human neurons; however, most studies focus on glutamatergic cortical neurons.^{2,3} Female hESC lines demonstrate variable states of X inactivation, which can depend on culture conditions, and show random X inactivation upon differentiation.⁴ Induced pluripotent stem cells, however, retain an inactive X chromosome in a nonrandom pattern, making them a valuable resource in which to study X-linked genetic disorders.⁵ In the past several years, hESC/iPSC-derived cerebral organoid protocols have been developed to generate 3D “mini-brains,” which recapitulate features of human brain development, such as cortical layering, neuronal migration, and electrical activity. Organoids are

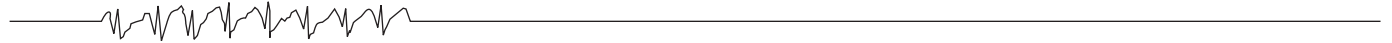
composed of diverse cell types, including neurons and glia, and can be differentiated to represent different brain regions, such as forebrain, midbrain, or hypothalamus.⁶

MeCP2 (methyl-CpG binding protein 2) DNA binding is associated with both transcriptional activation and repression. MeCP2 is expressed in many cell types, including glutamatergic cortical neurons, GABAergic interneurons, astrocytes, and oligodendrocytes.⁷ Cell-type-specific deletions of *MECP2* in animal models show distinct phenotypes and different alterations to gene expression, indicating that *MECP2* functions differently depending on cell type.⁸ Xiang et al hypothesized that disruption of *MECP2* in distinct neuronal subtypes in humans will have cell-type-specific effects.⁷ To test this hypothesis, the authors utilize hESCs to generate GABAergic interneurons, the major inhibitory neurons of the central nervous system, and medial ganglionic eminence organoids (MGEs), which represent the region of the brain responsible for the genesis of interneurons.⁷ To avoid the unreliable X-inactivation effects of hESCs, the authors chose the commonly used male hESC line, H1, and used genome editing to generate patient-specific pathogenic *MECP2* variants.

Interneurons expressing pathogenic *MECP2* variants underwent an exhaustive downstream analysis including calcium imaging, electrophysiology, bulk and single-cell RNA-sequencing, genome-wide DNA methylation profiling,



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quantitative proteomics, chromatin accessibility assays, and chromatin interaction assays. Most assays focus on p.R133C, a pathogenic missense variant in the methyl-CpG-binding domain. Interneurons expressing this variant show defects in electrophysiology, abnormal calcium signaling, and misregulation of gene expression. Strikingly, these defects can be almost completely rescued with JQ1, a small molecule that inhibits bromodomain proteins such as BRD4. JQ1 interacts with acetylated histones and has broad effects on gene expression, which was the rather vague justification for the use of this compound. The authors go on to show that BRD4 binding is increased genome-wide in p.R133C interneurons, but this aberrant binding is corrected with JQ1 treatment.

In 3D models, human MGEs (hMGEs) expressing p.R133C also exhibit transcriptional dysregulation and abnormal network activity that is rescued with JQ1 treatment. This abnormal network activity consisted of a lack of synchronization of calcium surges as compared to wild-type, suggesting a developmental defect. What exactly these variant-specific electrophysiological alterations mean in the context of seizure-like activity is not clear. For instance, modeling of another genetic epilepsy, *UBE3A*, in knockout cortical organoids showed synchronization of neural networks was rare in control organoids, and synchronization was only observed in *UBE3A*-deficient organoids.⁹ While varied methodologies may account for the differences in the results of these 2 studies, standardization of techniques and future studies in multiple models of genetic epilepsies are required to understand how developmental defects and hypersynchronous activity relate to seizure-like phenotypes in a dish.

At the molecular level, comparison of hMGEs with human cortical organoids (hCOs) showed distinct sets of genes, associated with different functions, are misregulated in lines expressing p.R133C. The transcriptional dysregulation of p.R133C hCOs is also rescued with JQ1 treatment but to a lesser extent than in hMGEs. Importantly, p.R133C hCOs do not exhibit abnormal network activity, as the p.R133C hMGEs do, indicating that some RTT phenotypes are cell-type-specific. Finally, the authors test JQ1 treatment in a mouse model of RTT and find that JQ1 is able to increase survival and ameliorate RTT severity. Overall, these results point to bromodomain inhibition as a promising new avenue for RTT therapeutics.

While these results are indeed encouraging, one of the primary limitations of this study was the use of male hESC lines. Males with the same pathogenic variants as females with RTT are rare, with only a handful of male mosaic individuals with RTT reported.¹⁰ In general males with RTT-causing pathogenic variants in females, do not have RTT, but rather a more severe and progressive encephalopathy.¹⁰ Thus, the pathogenic mechanisms uncovered in the study by Xiang et al. represent the most severe consequence of *MECP2* loss and how well these mechanisms mimic RTT remain to be determined. There are also known differences between gene expression and genetic mechanisms in the sexes as well as in the reprogramming of somatic cells to hESCs/iPSCs, including lower levels


of DNA methylation in female iPSC lines.¹¹ This male-based approach is not restricted to human iPSC models, indeed male mouse models have also been used extensively in *Mecp2* research. These mice recapitulate many of the clinical features of RTT, whereas female mice have a subtler phenotype. In the future, because female human iPSCs retain an inactive X chromosome, it is conceivable that “mixing” experiments could be performed, where the active X chromosome expressing the variant and invariant *MECP2* alleles are mixed at 50:50 ratios to recapitulate what is more commonly occurring in the brains of individuals with RTT. *MECP2* is a good model for such experiments given the breadth of knowledge of protein function and dysfunction. Such research would be beneficial for the epilepsy field more broadly as many of the more commonly implicated genes in the pediatric epilepsies are on the X-chromosome, including *PCDH19*, *ARX*, *NEXMIF*, and *SMC1A*.¹²

The rationale for selecting JQ1 as a candidate therapeutic agent was not apparent in the present study. JQ1 is a potent inhibitor of all members of the BET family of bromodomain families and has been used in preclinical studies on cancer. However, other than it being a broad regulator of gene expression, and multiple genes being dysregulated in *MECP2* models, the choice of this compound as a therapeutic agent is not clear. The omission of the rationale limits the more broad-scale applicability of this approach to other neurodevelopmental disorders. The breadth of experiments performed by Xiang et al is impressive; however, most experiments were performed with the p.R133C variant, and not all phenotypes were confirmed for the other 2 variants studied (p.R270X and p.R306C). Moreover, aberrant gene expression and electrophysiological properties were rescued with JQ1 to a greater extent in RTT model hMGEs than hCOs, and hCOs do not exhibit abnormal network activity. These findings are similar to conditional *Mecp2* mutations in mice, where different *Mecp2* variants have varied impacts on gene expression in different cell types.⁸ Collectively, this would suggest that different variants may require different therapeutic options, including those individuals who may benefit from BRD4 inhibition in the future. Finally, as noted by the authors, the short half-life of JQ1 precludes its use as an effective treatment in individuals with *MECP2*-associated neurodevelopmental disorders and additional research is required to detect an effective analog.


Overall, this extensive study utilized an impressive array of model systems and molecular and physiological assays to demonstrate BRD4 inhibition as a possible therapeutic strategy for individuals with RTT. The models and approach serve as a template for other epilepsy-associated genes that control gene expression. These include another methyl-DNA binding protein, *MBD5*, the transcription factors *ARX*, *FOXP1*, *CUX2*, the chromatin remodelers *CHD2* and *ACTL6B*, as well as histone modifying enzymes *KDM5C*, *KMT2E*, and *SETD1B*.¹³ Indeed, though speculative, it is possible that these gene transcription regulators may have overlapping pathophysiology and thus therapeutic targets. Finally, given that many of the dysregulated genes in the RTT model neurons are ion channels, which account for a high proportion of pediatric genetic epilepsy, it is



also an attractive hypothesis that epigenetic-based therapies may be effective in epilepsy more broadly.

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