Cell PRESS
Neuron
Previews

Fragile X Syndrome Therapeutics: Translation, Meet Translational Medicine

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http://dx.doi.org/10.1016/j.neuron.2013.01.009

Fragile X syndrome, a common cause of intellectual disability and autism, is thought to occur due to abnormal regulation of neuronal protein synthesis. A study by Osterweil et al. (2013), in this issue, demonstrates that the HMG-CoA reductase inhibitor lovastatin can normalize protein synthesis and also reduce audiogenic seizures in Fmr1 knockout mice.

The autism spectrum disorders (ASDs) are characterized by a suite of neuropsychiatric phenotypes that include abnormal social behavior, restricted behavioral repertoires, and dysfunctions of language. Although they are not considered a core component of ASDs, approximately 30% of ASD patients have a seizure disorder, a marked increase over the incidence of epilepsy in the general population. Diagnosis of ASDs has been on an alarming rise, with recent epidemiological estimates of up to 1/54 males being affected (Baio, 2012). Because most ASDs have not been pathophysiologically attributable to single biological lesions, the therapy for ASD patients is primarily supportive, and the development of mechanism-based therapeutics has remained a major challenge. One approach toward getting a biological foothold into complex neurological conditions such as ASDs has been to study single gene disorders, of which ASD phenotypes are a common component. Approximately 5%–7% of ASDs occur as part of so-called "syndromic autism" and include fragile X syndrome (FXS), tuberous sclerosis complex (TSC), and PTEN-associated macrocephaly/autism, among others. Remarkably, many of these disorders are caused by mutations in pathways that regulate mRNA translation, strongly suggesting that the regulation of protein synthesis is implicated in ASD pathophysiology. In this issue of *Neuron,* Osterweil et al. (2013) have exploited this approach, building on years of mechanistic investigations, to test a rationally conceived, though unexpected, therapeutic for neurophysiological derangements in a mouse model of FXS: the HMG-CoA reductase inhibitor, lovastatin.

FXS is estimated to affect 1/4000 males, representing the most common inherited form of intellectual disability worldwide (Santoro et al., 2012). FXS results from expansions of a CGG trinucleotide repeat in the 5′ untranslated region of the fragile X mental retardation gene 1 (*FMR1*) resulting in the silencing and loss of expression of the FMRP protein. FMRP is an RNA binding protein that is highly expressed in neurons and is estimated to bind ~4% of all brain RNAs (Ashley et al., 1993; Darnell et al., 2011). Animal models for FMRP have been made in mice, flies, and zebrafish with convergent streams of data pointing to a role for FMRP as a translational repressor. Indeed, FMRP knockout mice ($Fmr1^{-/y}$) demonstrate a significant increase in global protein synthesis in the brain, also reflected by changes in protein synthesis at the synapse ("local translation") (Bassell and Warren, 2008). These findings are supported by the demonstration of impaired synaptic plasticity, alterations in dendritic morphology, and ultimately, neurocognitive deficits in FMRP mutants. Overall, these data have established a major role for FMRP in the regulation and maintenance of synaptic function. Long-term depression (LTD) is a form of hippocampal synaptic plasticity that requires the function of a family of metabotropic glutamate receptors (mGLuRs), most conspicuously, mGluR5. This has previously led the authors, and others, to directly test the role of mGluRs in mediating FMRP-related synaptic phenotypes (Bear et al., 2004). Indeed, genetic reduction of mGluR5 normalized the increase in protein synthesis in $Fmr1^{-/y}$ mice, suggesting that FMRP mediates its effects through mGluR signaling (Dölen et al., 2007). The precise mechanisms of FMRP-mediated translational control (through mGluR or otherwise) remain unclear; however, experiments from several labs have suggested that both the mTOR and MAP kinase signaling cascades may be involved (Gross et al., 2012).

The phosphorylation of the Ras-dependent MAP kinases ERK1/2 can be suppressed pharmacologically with the HMG-CoA reductase inhibitor lovastatin. Statins block the mevalonate pathway thus inhibiting cholesterol biosynthesis and the farnesylation of Ras (and other proteins). Farnesylation is important for Ras' association with the membrane and, subsequently, its full activation. Suppression of ERK1/2 can normalize increases in protein synthesis in $Fmr1^{-/y}$ mice, supporting a role for ERK activity in mediating the suppressive effects of FMRP on translation. Despite the normalization of FMRP-related phenotypes by suppression of activated ERK1/2, the kinases do not appear to be overactivated in $Fmr1^{-/y}$ mice. Because of the effectiveness of ERK1/2 suppression on protein synthesis in $Fmr1^{-/y}$ mice, Osterweil et al. (2013) hypothesized that suppression of farnesylation might effectively block the neurological phenotypes of $Fmr1^{-/y}$ mice, presumably through Ras-related changes in ERK1/2 activation. The authors show that in hippocampal slices, either farnesyl thiosalicylic acid

(FTS)—a direct farnesylation inhibitor—or lovastatin (an upstream metabolite inhibitor) reduces ERK1/2 phosphorylation and normalizes both global protein synthesis and the defective hippocampal LTD in *Fmr1*$^{-/y}$ mice. Consistent with previous experiments, the authors found no increase in basal activation of ERK1/2 levels in the *Fmr1*$^{-/y}$ background. Thus, the authors attribute lovastatin's effectiveness to a hypersensitivity of *Fmr1*$^{-/y}$ mice to ERK1/2 activity.

As alluded to above, epilepsy is comorbid in about 30% of patients with ASDs but only in about 14% of FXS patients—still a substantial increase over the general population. In FXS patients, seizures are typically of the complex partial type, start between 4 and 10 years of age, and resolve during childhood. Importantly, the presence of seizures appears to correlate with increased risk of ASD (Berry-Kravis et al., 2010). The pathophysiological relationships between ASD and epileptiform activity remain largely unclear. Are epilepsy and neuropsychiatric features of ASD independently adverse outcomes of a concomitant neuropathological mechanism or, alternatively, do the two phenotypes synergize to exacerbate overall neurological outcomes? Increased ERK1/2 activity has been previously shown to increase spontaneous discharges in the CA3 region of the hippocampus (Zhao et al., 2004). In a set of complementary experiments, Osterweil et al. (2013) show that lovastatin could block most of the epileptiform bursting in the CA3 region of the hippocampus and reduce spontaneous action potentials in the visual cortex of *Fmr1*$^{-/y}$ mice.

Finally, the authors show that lovastatin administered either orally or by injection was able to inhibit the expression of audiogenic seizures (AGS) in *Fmr1*$^{-/y}$ mice. An AGS is an evoked brainstem reflex consisting of a running fit driven by activation of brainstem nuclei with no concordant EEG hyperexcitability at the cortical level. Thus, AGS may be a reflection of hyperexcitability, but it is of unclear relation to most forms of human epilepsy. Nevertheless, AGS represents a persuasive phenotypic manifestation of the overall hyperexcitability in *Fmr1*$^{-/y}$ mice, and the response to lovastatin in *Fmr1*$^{-/y}$ mice lends further credence to the potential effectiveness of the drug in this context.

This study opens up many interesting areas of further inquiry; for example, the detailed mechanisms by which lovastatin works to reduce hyperexcitability are not yet clear. The authors hypothesize that the suppression of ERK1/2 is at least partially responsible. Given that ERK1/2 levels are not elevated in *Fmr1*$^{-/y}$ mice and that FTS or lovastatin affects multiple targets, this suggests that the observed suppression of ERK1/2 may not fully explain the efficacy of either compound. The greatest morbidity associated with FXS is intellectual disability and behavioral problems. Future studies will be required to address the responsiveness of the neurocognitive phenotypes in *Fmr1*$^{-/y}$ mice to lovastatin treatment. It will be very interesting to see whether lovastatin can correct exaggerated mGluR-LTD, increased dendritic spine density, and impaired learning in the *Fmr1*$^{-/y}$ mouse model. Ultimately, the findings reported in Osterweil et al. (2013) will be best judged by the results of a clinical trial in children with FXS. Indeed, lovastatin has already been used in clinical trials for neurofibromatosis I (NF1), a condition in which Ras is dysregulated (Li et al., 2005; Acosta et al., 2011). Lovastatin is particularly exciting as a potential therapy in FXS because the drug has already been in wide use for years and is approved for use in children with hypercholesterolemia, thus expediting the tests of its effectiveness in FXS patients.

Based on other clinical trials in neurodevelopmental disorders, it is likely that subgroups of patients will respond better to different targeted agents, even in a homogeneous disorder such as FXS. One would predict, then, that ERK hyperactivity could potentially be a biomarker for subsets of FXS patients that could benefit from lovastatin treatment (Weng et al., 2008). These results suggest that ERK activity could potentially act as a surrogate marker of drug responsiveness during treatment with lovastatin and related drugs.

In summary, rational therapies for ASDs are greatly needed. Several clinical trials are ongoing in syndromic causes of ASD including FXS, TSC, and Rett syndrome. By linking FMRP-mediated increase in protein synthesis to the ERK1/2 pathway, Osterweil et al. (2013) have harnessed rational hypotheses based on animal models toward testing a relatively seasoned and safe therapeutic for a novel and previously unconsidered translational application. This potential treatment may hold great promise for patients with FXS and perhaps those with other forms of hyperexcitability and ASD as well.

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