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Review

Fragile X Syndrome: From molecular pathology to therapy

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ABSTRACT

Fragile X Syndrome (FXS) is the most common form of inherited intellectual disability due to the silencing of the *FMR1* gene encoding FMRP (Fragile X Mental Retardation Protein), an RNA-binding protein involved in different steps of RNA metabolism. Of particular interest is the key role of FMRP in translational regulation. Since the first functional characterizations of FMRP, its role has been underlined by its association with actively translating polyribosomes. Furthermore, a plethora of mRNA targets of FMRP have been identified. In the absence of FMRP the deregulation of translation/transport/stability of these mRNAs has a cascade effect on many pathways, resulting into the final phenotype. We review here a set of targets of FMRP (mRNAs and proteins) that may have an impact on the FXS phenotype by deregulating some key cellular processes, such as translation, cytoskeleton remodeling and oxidative stress. The manipulation of these abnormal pathways by specific drugs may represent new therapeutic opportunities for FXS patients.

Contents

1. Introduction

The causes of intellectual disability (ID) are extremely heterogeneous, ranging from environmental to genetic and even to combinations of the two. In our laboratory we focus our studies on the molecular and cellular basis of Fragile X Syndrome (FXS), a form

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of ID due to the silencing of the expression of Fragile X Mental Retardation gene 1 (*FMR1*). This gene encodes a set of proteins involved in the regulation of RNA metabolism at the post-transcriptional level playing important roles in synaptic plasticity, in the development of dendrites and axons and in underlying learning and memory. FXS is the most common cause of inherited ID, affecting 1/4000 males and 1/7000 females, and is characterized by moderate to severe ID in affected males, whereas 60% of carrier females present with mild to moderate ID. Affected boys often manifest hyperactive and/or autistic behavior symptoms. They can be affected by epileptic seizures, attention deficit and sleep disorders. FXS males are also characterized by facial dysmorphism, post-pubertal macroorchidism and mild connective tissue dysplasia (Bardoni et al., 2006; Bardoni et al., 2000). Pathological examination of brains from FXS patients has shown an increased density of long and tortuous dendritic spines, suggesting a delay in spine maturation. This alteration is considered the cellular abnormality underpinning ID in FXS patients and in FXS animal models, and in mice, it is also associated with some altered forms of synaptic plasticity (Bassell and Warren, 2008) (Liu-Yesucevitz et al., 2011). The lack of FMRP has also been shown to interfere with mechanisms underlying mGluR-dependent Long Term Depression (LTD) and epileptogenesis. Indeed, mGluR-dependent LTD in the hippocampus is amplified in the absence of FMRP, whereas NMDA receptor-dependent LTD is not. mGlu5R-dependent Long Term Potentiation (LTP) is instead reduced in the cerebral cortex of *Fmr1* null mice. Hippocampal epileptogenesis is another form of synaptic plasticity that depends on group I mGlu receptor activation and protein synthesis and is altered in *Fmr1* null mice. In wild-type animals, long-term modification of excitability occurs only in response to activation of group I mGluRs and subsequent protein synthesis (Bardoni et al., 2006; Bassell and Warren, 2008; D'Antoni et al., in press; Liu-Yesucevitz et al., 2011).

Consistently with the fact that the main morphological alteration in FXS brain is the presence of abnormal (immature) dendritic spines, to date most of the studies on FXS have been focused on the role of FMRP in mature neurons (Bardoni et al., 2006). However, other morphological abnormalities in the brain of FXS patients and of *Fmr1* knockout mice are emerging. Indeed an increased volume of the grey matter in the caudate and fusiform gyrus in adult as well young (1–3 years old boys) FXS patients has been reported (Gothelf et al., 2008; Hoeft et al., 2010). Furthermore, a reduction of cell body density in the septal dentate gyrus of adult *Fmr1* null mice when compared with wild type animals was also observed (Nahirney et al., 2011). These abnormalities are likely to be generated early during development. They could be associated to defects in proliferation and/or differentiation of neural progenitors, pointing out the role of FMRP not only in neuronal maturation but also in neurogenesis. Jin and co-workers showed an increased level of proliferation of neural progenitors around 9–10 days after birth and later apoptosis (Luo et al., 2010). However, this defect is not present three weeks later, suggesting that a complex regulation is set up. They also showed an implication of FMRP in neuron and glia differentiation from adult neural progenitors by modulating the expression of neurogenin (Luo et al., 2010). In the developing brain of *Fmr1*-null mice, a significant accumulation of progenitor cells in the subventricular zone was observed, suggesting that this abnormal cell population contributed to changes in glutamatergic neurogenesis (Castren, 2012). In addition it was reported that the cortical neural progenitors derived from the embryonic brain of *Fmr1* null mice generated an increased number of cells responsive to the activation of mGluRs signal. These progenitors generated a reduced number of astroglial cells (Castren, 2012). This result is particularly important for the pathophysiology of this syndrome; indeed FXS astrocytes induce a delay in dendritic patterning and in development of excitatory synapses in hippocampal neurons (Pacey and Doerig, 2007; Jacobs and Doerig, 2010). More recently, the function of FMRP was investigated in adult neurogenesis by silencing *Fmr1* expression in neural stem and progenitor cells (aNSCs). In these conditions a reduction of neurogenesis *in vivo* was observed as well as an impaired hippocampus-dependent learning in mice. These abnormalities were rescued by restoring a normal expression level of FMRP in aNSCs (Guo et al., 2011).

The functional characterization of FMRP during the last years has revealed the specific dynamics of this protein: FMRP enters the nucleus and interacts with pre-messenger ribonucleoprotein (pre-mRNP) complexes to escort them to the cytoplasm. FMRP-containing mRNPs are massively associated to polyribosomes and involved in translational control both in soma and in dendritic spines (Bardoni et al., 2006). FMRP has also been found to be a component of P-bodies and stress granules, suggesting a role for this protein in transporting RNAs in different cellular structures involved in post-transcriptional regulation (Didiot et al., 2009; Wang et al., 2008). In addition, in neurons, some of the FMRP-mRNP complexes are selectively translocated to distant locations as component of RNA-granules, where they mediate the binding between mRNAs and molecular motors, such as kinesins, promoting transport upon specific stimuli (Davidovic et al., 2007). This mechanism probably also influences the synaptic abundance of a subset of mRNAs (Dictenberg et al., 2008; Miyashiro et al., 2003).

Decreased capacity to transport mRNA and control local translation into distal processes may result in an abnormal level of their protein products with consequences on the structure and synaptic plasticity, as observed in FXS patients and animal models. Furthermore this abnormality could alter the ability to respond to injury or local stimuli within axons and dendrites.

Starting from these milestones, we will describe here the main function of FMRP, its ability to bind a subset of mRNAs modulating their expression and the consequences of its absence on different pathways having pathophysiological importance in FXS.

2. Fragile X Mental Retardation Protein is an RNA-binding protein

Consistent with its ability to bind RNA, FMRP contains three different motifs known to mediate protein/RNA interaction: two hnRNP-K-homology (KH) domains and an arginine–glycine–glycine (RGG) box (Bardoni et al., 2006; Bassell and Warren, 2008). The search for the RNA targets of FMRP has been very active. To this purpose, several approaches have been used during the last twenty years, according to the evolution of techniques employed to study protein/RNA interaction.

2.1. mRNA targets and protein partners of FMRP

The first example is represented by the study of Ashley et al. (1993) that, by measuring the amount of radioactively-labeled-FMRP able to bind biotinylated RNAs, was able to show that FMRP interacts with approximately 4% of human fetal brain messages and binds *in vitro* to its own mRNA. In the same laboratory, brain ribonucleoprotein complexes were coimmunoprecipitated with FMRP, and mRNA obtained from these FMRP-containing complexes was used to interrogate microarrays. A total of 432 FMRP-associated mRNAs from mouse brains were identified by this study, suggesting that deregulation of a subset of mRNAs underpins the FXS phenotype. Among candidate genes relevant to the ID/autistic phenotype, the role of *MAP1B* was highlighted (see also Section 3.2.1) (Brown et al., 2001).

Other studies used SELEX (Darnell et al., 2005; Darnell et al., 2001; Sung et al., 2000), the yeast three-hybrid system (Dolzhanskaya et al., 2003) and, more recently, CLIP (cross-link and

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