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Research Report

Expression of fragile X mental retardation protein in neurons and glia of the developing and adult mouse brain

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ABSTRACT

Fragile X syndrome is the most common inherited form of mental retardation and autism. It is caused by a reduction or elimination of the expression of fragile X mental retardation protein (FMRP). Because fragile X syndrome is a neurodevelopmental disorder, it is important to fully document the cell type expression in the developing CNS to provide a better understanding of the molecular function of FMRP, and the pathogenesis of the syndrome. We investigated FMRP expression in the brain using double-labeling immunocytochemistry and cell type markers for neurons (NeuN), astrocytes (S100β), microglia (Iba-1), and oligodendrocyte precursor cells (NG2). The hippocampus, striatum, cingulate cortex, retrosplenial cortex, corpus callosum and cerebellum were assessed in wild-type C57/BL6 mice at postnatal days 0, 10, 20, and adult. Our results demonstrate that FMRP is ubiquitously expressed in neurons at all times and brain regions studied, except for corpus callosum where FMRP was predominantly present in astrocytes at all ages. FMRP expression in Iba-1 and NG2-positive cells was detected at postnatal day 0 and 10 and gradually decreased to very low or undetectable levels in postnatal day 20 and adult mice. Our results reveal that in addition to continuous and extensive expression in neurons in the immature and mature brain, FMRP is also present in astrocytes, oligodendrocyte precursor cells, and microglia during the early and mid-postnatal developmental stages of brain maturation. Prominent expression of FMRP in glia during these crucial stages of brain development suggests an important contribution to normal brain function, and in its absence, to the fragile X phenotype.

1. Introduction

Fragile X syndrome (FXS) is a genetic disorder and a leading cause of cognitive impairment and autism. The disorder is caused by triplet repeat expansion in the 5' untranslated region of the FMR1 gene which induces a dramatic reduction or elimination of the expression of the encoded protein, fragile X mental retardation protein (FMRP). FMRP is an mRNA binding protein that controls the expression of hundreds of genes in the CNS through multiple mechanisms including modulating

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ribosome stalling (Darnell et al., 2011). However, FMRP has been shown to also act as a positive modular of protein translation (Bechara et al., 2009), likely by enhancing mRNA stability (Zalfa et al., 2007). The FMR1 gene undergoes alternative splicing and possesses alternative transcription start sites such that at least 12 isoforms are generated (Brackett et al., 2013; Tassone et al., 2011). Isoform 1 is the longest form and codes for a protein of 632 amino acids and a molecular weight of 71kilodaltons.

In the adult brain FMRP is abundantly expressed in most neurons throughout the CNS. In neurons, the protein is primarily located in the cytosol and in synaptic spines where it plays a role in spine maturation (Cruz-Martín et al., 2010). A portion of total cellular FMRP is also present in the nucleus where its role is less well characterized, and nuclear expression is isoform dependent (Dury et al., 2013). FMRP is highly abundant in "fragile X granules" in neuronal axons and presynaptic terminals where it apparently regulates recurrent neuronal activity (Akins et al., 2012). FMRP is also present in neural stem cells (Luo et al., 2010) where it has been shown to control hippocampal-dependent learning in the mature brain (Guo et al., 2011).

In contrast to the expression of FMRP in neurons of the adult brain, relatively little is known about the types of glial cells that express FMRP during CNS development. FMRP was reported in primary cultures of rodent astrocytes (Yuskaitis et al., 2010), and in the mouse hippocampus, FMRP is expressed in astrocytes within the first week of birth and then declines to low or undetectable levels (Pacey and Doering, 2007). It is also present in oligodendroglia (Wang et al., 2004) and oligodendrocyte precursor cells (OPCs) in the immature cerebellum where it appears to be a factor in the proper progression of myelination (Pacey et al., 2013).

The purpose of the present study was to more fully document the cell type expression of FMRP in neurons and glia in selected brain regions of the developing and mature mouse CNS. Our analysis encompassed the cerebral cortex, striatum, hippocampus, cerebellum, and the corpus callosum, and extended from postnatal days (PNDs) 0, to PNDs 10, 20, and adult. Our findings reveal that FMRP is extensively expressed in glia in the developing postnatal brain and that with the exception of the corpus callosum, glial expression declines at different rates in different brain regions to low levels in the adult brain.

2. Results

Immunocytochemical analysis was used to map the expression of FMRP in selected brain regions at PND 0 (day of birth), 10, 20, and young adult (8–12 weeks old) wild-type C57/BL6 mice. Sections from cingulate cortex, hippocampus, striatum, corpus callosum, and cerebellum were immunolabeled for FMRP as well as a cell-type specific marker for neurons, astrocytes, oligodendrocyte precursor cells, and microglia. The 5C2 monoclonal antibody used here has previously been shown to be specific for FMRP (LaFauci et al., 2013). Brain sections treated with secondary antibody only showed no fluorescence. We quantified the total number of cells expressing FMRP per visual field, as well as cells co-expressing FMRP with one of the four cell-specific markers, and calculated the percent of total FMRP-positive cells that co-expressed each of the four cell markers in the cingulate cortex and corpus callosum (Table 1).

2.1. *Gradual reduction in the number of FMRP-positive cells from the early postnatal period to adulthood*

FMRP+ cell counts in the cingulate cortex and corpus callosum revealed that the number of FMRP+ cells in both brain regions was highest at PND 0 (see "Total FMRP+" column in Table 1). In the cingulate cortex there was a gradual decline over time such that the adult level was approximately 57% of that at birth (P0). In the corpus callosum there was a modest decline whereby adult mice showed about 85% of the level at P0. Overall, these observations are consistent with previous reports suggestion developmentally declining levels of FMRP protein, peaking at first postnatal week and declining thereafter (Lu et al., 2004; Pacey et al., 2013).

2.2. *Predominant neuronal expression of FMRP throughout development*

At all postnatal times examined, FMRP expression was predominantly neuronal in all of the brain regions analyzed, except for corpus callosum (Fig. 1, Tables 1 and 2) where only a few NeuN+ cells were present and most FMRP expression was observed in S100β+ cells (Fig. 2, Table 2). Quantitative analysis of cell type-specific expression of FMRP revealed

Table 1 – Quantitative analysis of cell type-specific expression of FMRP in the cingulate cortex and corpus callosum. The analysis was performed by counting the FMRP-positive neurons (NeuN), astrocytes (S100β), microglia (Iba-1) and oligodendrocyte precursor cells (NG2) in the cingulate cortex and corpus callosum. The results are reported as the average number of total FMRP-positive cells per visual field, and the percentage of FMRP-positive cells that co-localized with NeuN, S100β, Iba-1, and NG2. Data are presented as mean±S.E.M. Standard error values smaller than 1.0 are not listed.

Brain region	Age	Total FMRP+	% NeuN+	% S100β	% Iba-1	% NG2
Cingulate cortex	**PND 0**	154±5	85	5	4	6
	PND 10	123±5	89±1	2	5	3
	PND 20	86±5	94±1	2	2	4
	Adult	88±7	94±1	3	2	5±1
Corpus callosum	**PND 0**	82±3	2	27	20	18
	PND 10	75±3	2	33±3	19±3	14
	PND 20	76±3	2	33±2	10±1	11
	Adult	70±3	2	22±3	3	7