Dendritic Spine Structural Anomalies in Fragile-X Mental Retardation Syndrome

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Fragile-X syndrome is the most common single-gene inherited form of mental retardation. Morphological studies suggest a possible failure of the synapse maturation process. Cerebral cortical spine morphology in fragile-X syndrome and in a knockout mouse model of it appears immature, with long, thin spines much more common than the stubby and mushroom-shaped spines more characteristic of normal development. In human fragile-X syndrome there is also a higher density of spines along dendrites, suggesting a possible failure of synapse elimination. While variously misspoken spines are characteristic of a number of mental retardation syndromes, the overabundance of spines seen in fragile-X syndrome is unusual. Taken with evidence of neurotransmitter activation of the synthesis of the fragile-X protein (FMRP) at synapses in vitro and evidence for behaviorally induced FMRP expression in vivo, and with evidence compatible with a role for FMRP in regulating the synthesis of other proteins, it is possible that FMRP serves as an ‘immediate early protein’ at the synapse that orchestrates aspects of synaptic development and plasticity.

Introduction

In investigating molecular mechanisms of synaptic plasticity, it was discovered that the fragile-X mental retardation protein (FMRP), the protein absent in those afflicted with fragile-X syndrome, is synthesized in synaptoneurosome preparations in response to neurotransmitter stimulation (Weiler et al., 1999). This was a revolutionary discovery which suggested that this protein is made in vitro at synapses in response to afferent activation, possibly for involvement in synaptic plasticity processes (Weiler and Greenough, 1999). This chapter briefly describes fragile-X syndrome, summarizes developmental neuronal plasticity processes, and details dendritic structure anomalies found associated with fragile-X syndrome. It then compares these structural deficits with those of other mental retardation syndromes, suggests a possible therapeutic approach, and reviews possible roles of FMRP in the nervous system and other tissues.

Fragile-X syndrome is a common form of mental retardation affecting nearly one in 2000 males and roughly half as many females (Brown, 1996). It is caused by the insertion of extra DNA into the X chromosome that silences the gene encoding FMRP. Phenotypic traits include facial abnormalities, macro-orchidism, developmental delay, mental retardation and autistic-like behaviors (Hagerman and Cronister, 1996).

FMRP: Role in Synapse Maturation and Pruning?

During normal brain development, forebrain sensory systems generate more neuronal processes and connections than will ultimately survive. This is followed by an activity-mediated competition process in which some synapses are eliminated while others are stabilized and strengthened (LeVay et al., 1980), so that patterned neural circuitry arises from experience-associated neural activity (Goodman and Shatz, 1993; Hata and Stryker, 1994). The strengthening of synapses appears to involve a transition from small immature synapses associated with long, thin, dendritic spines early in development to larger synapses associated with more ‘mushroom-shaped’ dendritic spines (Greenough and Chang, 1985, 1988a; Hwang and Greenough, 1986; Schultz, 1986; Galafre et al., 1987; Horner, 1993; Greenough et al., 1994). Synaptic overproduction, pruning and strengthening may also underlie postdevelopmental experience/learning associated synapse addition.

The synaptic selection/maturation process may be grossly impaired in cases of clinical fragile-X syndrome. A qualitative study of rapid-Golgi-stained human autopsy material from a single fragile-X patient described long, thin, tortuous, dendritic spines with prominent heads and irregular dilations on apical dendrites of pyramidal cells in layers III and V of the parieto-occipital neocortex (Rudelli et al., 1985; Wisniewski et al., 1991). Reduced mean synaptic contact area was also reported using electron microscopy; however, no other major neuropathologies were noted. Two additional fragile-X patients were added to this study by Hinton et al. (Hinton et al., 1991). Similar dendritic spine characteristics were noted, and no differences in neuronal density between fragile-X patients and controls were found, possibly suggesting normal neurogenesis and cell migration in fragile-X patients; however, a stereological method that would have overcounted larger cells was used.

Despite a lack of evidence for changes in neuronal number or other gross structural deficits, fragile-X patients have been shown to display subtle abnormalities in gross brain anatomy. Two groups (Reiss et al., 1991; Mostofsky et al., 1998), using magnetic resonance imaging (MRI), showed that fragile-X males exhibited a reduction in the size of the posterior cerebellar vermis and an enlargement of the fourth ventricle. An increased volume in fragile-X patients as compared with controls has also been reported in the hippocampus (Reiss et al., 1994; Katz et al., 1997), caudate nucleus (Reiss et al., 1995), and lateral ventricles (Reiss et al., 1995). Furthermore, an age-related increase in the volume of the hippocampus and an age-related decrease in the volume of the superior temporal gyrus were found in fragile-X patients (Reiss et al., 1994). It should be noted that the changes in fragile-X patients found using MRI were subtle and not replicated by physical measurement of autopsy material, but this data was derived from only two different fragile-X patients (Reyniers et al., 1999).

Neuronal Morphology in the Fragile-X Brain

In order to examine the effects of a deficiency of FMRP on dendritic structure, a series of quantitative anatomical studies was undertaken by the Dutch–Belgian Fragile-X Consortium of both human autopsy material from fragile-X patients and of transgenic mice in which the FMR-1 gene was knocked out.
It should be noted that this knockout model is less susceptible to a common criticism of knockout models, as both fragile-X patients and these transgenic mice undergo development without a functional FMR-1 gene.

A quantitative neuroanatomical study of Golgi–Kopsch-stained human autopsy material from three male adult fragile-X patients and three male age-matched controls was carried out in order to quantify and further describe dendritic spine characteristics in fragile-X patients by comparing dendritic spine length, morphology and density along apical shaft, apical oblique and basilar dendrites on layer V pyramidal cells in the temporal cortex of these subjects (Irwin et al., 1999) (S.A. Irwin et al., submitted for publication). The results showed that fragile-X patients had significantly more long spines and significantly fewer short spines than controls on apical shaft, apical oblique and basilar dendrites (Figures 1 and 2). These two findings suggest an impairment of normal spine maturation in fragile-X syndrome that is persistent throughout the entire life span of these patients. Fragile-X patients also exhibited a significantly greater spine density on distal segments of apical dendritic shafts and on apical oblique and basilar dendrites (Figure 1), suggesting a persistent failure of normal synapse pruning processes as well. No differences in dendritic diameter were found for any dendritic branch type.

Increased spine density and increased numbers of long and immature spines are the most salient neuropathologies described in association with fragile-X syndrome thus far and are likely to play a role in the cognitive deficits associated with it. This is the first finding of increased dendritic spine density in fragile-X patients. Furthermore, these findings of higher numbers of longer and more immature type spines in fragile-X patients agree with the qualitative neuronal morphological findings of other groups (Rudelli et al., 1985; Wisniewski et al., 1991; Hinton et al., 1991), and extend them to the entire dendritic tree of cortical layer V pyramidal neurons.

These findings suggest that synaptic pruning and maturation may be grossly impaired in cases of fragile-X syndrome, and further imply a role in these processes for FMRP. Further support for a role of FMRP in synaptic plasticity comes from in vitro studies suggesting that this protein is made at synapses in response to synaptic activation (Weiler et al., 1997) (Figure 3) and in vivo studies demonstrating increased FMRP immunoreactivity in brain regions known to be undergoing: (i) active synaptogenesis in response to motor-skill learning or complex environment rearing or (ii) increased activation in response to repetitive motor activity (Irwin et al., 1998, 2000) (S.A. Irwin et al., in preparation) (Figure 4).

A mouse model of fragile-X syndrome (FraX mice) was developed by the Dutch–Belgian Fragile-X Consortium (Consortium, 1994). These mice initially exhibited macro-orchidism, mild learning deficits on a reversal condition in the Morris water-maze task and hyperactivity. More extensive studies of the FraX mice were carried out by Kooy et al. (Kooy et al., 1996), which again demonstrated macro-orchidism [caused by an increase in the rate of Sertoli cell proliferation from postnatal day 12 to 15 (Slegtenhorst-Eegdeman et al., 1998)] and subtle learning deficits in the Morris water-maze; however, there was no indication of hyperactivity upon retesting. No impairment of short- or long-term potentiation was found in hippocampal slices from these mice (Godfraind et al., 1996). Furthermore, despite a possible role for FMRP in the transport of mRNA to synapses (see below), no disruptions in the dendritic localization of the mRNAs for MAP2, CaMKII or dendrin, or in the rapid dendritic transport of the mRNA for activity-regulated cytoskeletal protein (ARC) were found in FraX mice (Steward et al., 1998).

The results of Comery et al. (Comery et al., 1997) in these mice support the involvement of FMRP in normal spine

Figure 1. Examples of typical spine morphologies on Golgi-impregnated dendrites from (A) a human afflicted by fragile-X syndrome and (B) an unaffected control. Dendrites are at extremes of density differences and not intended to depict the norm.
development, as they demonstrated an increase in spine density and spine length on layer V pyramidal cells in the visual cortex of FraX mice. This has been the only brain abnormality so far reported in these mice (Consortium, 1994; Godfraind et al., 1996; Kooy et al., 1996); however, it was later determined that an unknown number of the mice used in that study may have been homozygous for a recessive retinal degeneration gene that would have been likely to affect cell morphology in the visual cortex of these animals. A quantitative neuroanatomical study of Golgi–Cox–stained material from ten young-adult male Frax mice and ten young-adult wild-type control mice was carried out in order to characterize the structure of dendrites and dendritic spines of layer V pyramidal cells in the visual cortex of non-blind FraX mice (Irwin et al., 1999) (S.A. Irwin et al., in preparation). The purpose of these investigations was: (i) to confirm the results of Comery et al. (Comery et al., 1997); (ii) to determine if abnormalities similar to those of fragile-X patients were present; (iii) to examine dendritic-tree characteristics [as dendrites of some cell populations also undergo an overproduction and pruning developmental process (Falls and Gobel, 1979; Brunjes et al., 1982; Murphy and Magness, 1984; Greenough and Chang, 1988b)]; and (iv) to further evaluate these mice as a model of the human condition.

FraX mice were found to have longer, more immature dendritic spines than wild-type control mice. These mice exhibited more longer and fewer shorter spines than controls on segments of apical dendritic shafts, apical oblique dendrites and basilar dendrites. Similarly, FraX mice exhibited more immature spine shapes and fewer mature shapes on segments of apical dendritic shafts and apical oblique dendrites. No differences in the frequencies of spine shapes along basilar dendrites were found. FraX and wild-type mice did not exhibit statistically significant spine density or dendritic diameter differences along any of the branch types; however, a trend toward a higher spine density in FraX mice along both apical shaft and apical oblique dendrites was evident. For characteristics of apical and basilar dendritic trees, no significant differences in the number of branches at each order, the chance of bifurcation at each order or the amount of dendritic material at constant intervals away from the soma were found between FraX and wild-type mice for layer V pyramidal visual cortical cells.

These findings indicate that FraX mice exhibited similar dendritic spine abnormalities to those of humans afflicted with fragile-X syndrome; however, the differences may be more subtle. These findings also partially replicate those of Comery et al. (Comery et al., 1997), but the absence of a significant spine density difference suggests that the mice in that study may have been affected by the retinal degeneration mutation. The fact that dendritic spine abnormalities are found in FraX mice suggests that they are a viable model for the study of the human condition; however, these mice, unlike the fragile-X patients and the Comery et al. mice, did not exhibit statistically significant abnormalities in spine density. It should be noted that both apical shafts and apical oblique dendrites of these FraX mice exhibited a tendency toward increased spine density. The abnormal spines found in these mice might be associated with their behavioral deficits, and the fact that the spine abnormalities were more subtle in mice than in humans might explain why this subtlety appears to be so, thus far, for the behavioral deficits found in these mice as well. The fact that FraX mice do not show any dendritic arborization abnormalities suggests that the role of FMRP in neurons is limited to the spine/synaptic structure and does not play a crucial regulatory role affecting overall neuronal morphology of pyramidal neurons in the cerebral cortex.

A lack of FMRP leads to abnormal dendritic spine structure and number on layer V pyramidal cells in the temporal cortex of humans and abnormal dendritic spine structure on layer V pyramidal cells in the visual cortex of FraX mice, and the neuronal structural abnormalities in fragile-X appear to be limited to dendritic spines. This presents strong evidence for the involvement of FMRP in synaptic structural transformation processes across brain regions and points to a cause of the behavioral
deficits observed in both humans and mice lacking FMRP. What role FMRP plays in this process remains unknown. A lack of a 'housekeeping' protein that would lead to altered metabolism could certainly affect neuronal morphology, as could the lack of a cytoskeletal protein involved in synaptic shape. Where FMRP fits into this spectrum of proteins affecting neuronal morphology also remains a mystery. Comparisons with other forms of mental retardation demonstrate that FMRP is not alone in having morphological effects, as several syndromes have been reported to be associated with neuronal structural abnormalities. However, the deficits associated with these syndromes have not been limited to dendritic spines, nor have any of these syndromes been found to exhibit an increase in dendritic spine number.

Neuronal Morphological Abnormalities in Other Forms of Mental Retardation

Several other disorders are known to exhibit aberrations in both dendritic arbor and dendritic spine structure. In the most common form of genetically related mental retardation, Down syndrome, increases in dendritic arbor and dendritic length on visual cortical pyramidal cells were found in infants (Becker et al., 1986; Becker, 1991), but decreases in both of these parameters have been found in juveniles and up through adulthood (Becker et al., 1986; Becker, 1991; Takashima et al., 1994). In addition, neurons of Down syndrome patients were found to exhibit decreased spine density in a number of brain regions (Marin-Padilla et al., 1972; Marin-Padilla, 1976; Suetsugu and Mehrain, 1980; Takashima et al., 1981; Ferrer and Gullotta, 1990). Decreases in spine number and/or dendritic length have similarly been found in cases of general mental retardation (Huttenlocher et al., 1974; Purpura, 1974). Rett syndrome (Armstrong, 1992; Belichenko et al., 1994; Armstrong et al., 1995) and autism (Williams et al., 1980). Various forms of metabolic storage diseases due to inborn (genetic) errors of metabolism have also been found to exhibit decreases in dendritic material and/or decreases in spine density; syndromes with such findings include Type C Niemann-Pick disease (Braak et al., 1983), neuronal ceroid lipofuscinosis (Purpura and Suzuki, 1976), infantile Tay-Sachs B variant (Purpura and Suzuki, 1976; Takashima et al., 1985), Hurler's syndrome (Takashima et al., 1985), infantile type 2 sialidosis (Takashima et al., 1985) and Sanfilippo type A syndrome (Takashima et al., 1985). Thus, while dendritic spine numbers were found to be lower and/or dendritic tree characteristics were found to be affected in many mental retardation disorders, fragile-X syndrome appears to be the only form of mental retardation that exhibited dendritic spine abnormalities and increased numbers of dendritic spines; unlike many other syndromes, there are no alterations in the dendritic arbor. This contrast with other syndromes further supports the hypothesis that FMRP is somehow necessary for developmental maturation and pruning of dendritic spines.

Other Evidence Regarding FMRP Function

While it may be that the dendritic spine abnormalities seen in fragile-X syndrome are due directly to the absence of FMRP, particularly at synapses, it must be kept in mind that altered afferent input can also lead to abnormal neuronal structure. For example, it is possible that the lack of this protein leads to abnormal afferent input by way of corrupting a neurotransmitter system, which in turn would lead to these structural abnormalities. It may prove very difficult to disentangle the effects of this (or any) genetic defect from those of altered afferent input on the nervous system, and both may be involved in a vicious spiral leading to aberrant neuronal structure.

It is perhaps a little curious that FraX mice show more subtle and slightly different abnormalities relative to the fragile-X patients. It must be kept in mind that mice can never be a perfect human model, and that the mice used in this study were young adults as opposed to the middle- to old-aged subjects used in the human study. However, a closer examination of both the human and mouse data side by side might suggest another explanation for these phenomena. The data from the control mice appear more similar than those from the knockout mice to fragile-X syndrome humans. Perhaps housing mice in standard laboratory cages makes them more like a 'mental retardation' model, whereas animals raised in toy and object filled complex environments, which exhibit increased brain connectivity (Turner and Greenough, 1985) and learning capacity (Hebb, 1947; Greenough et al., 1970; Black et al., 1998) when compared with animals raised in standard laboratory cages, are more like 'normal' mice. It may be the case that complex environment rearing may produce a more profound difference between control and FraX mice. Evidence for this comes from longitudinal studies that described declines in IQ and in adaptive behavior of fragile-X patients. These scores were reported not to reflect a decline in intellectual or social skills, but rather a widening gap with age between these fragile-X and normally developing individuals (Fisch et al., 1996). Although experience may widen the gap between normally developing and fragile-X affected humans or animals, it may also serve to ameliorate the deficits. Success along these lines has been found in a rat model of fetal alcohol syndrome (Klintsova et al., 1997, 1998). Similarities, others have reported amelioration of genetically related behavioral deficiencies by exposing mentally retarded children to an 'enriched' toy and object filled environment (Horner, 1980) or rearing animals in a more 'normal' environment (Caston et al., 1999; Rampon et al., 2000). Studies are underway in our laboratory to investigate how experience affects neuronal structure and learning performance in FraX mice, and these studies may give us a better model and understanding of the human condition and how experience may affect it.

It should be mentioned that FMRP is expressed in a number of tissues, especially early in development, and may play a similar role in structural transformations occurring in tissues other than the brain. Evidence for this comes from studies demonstrating high levels of FMRP expression in a number of tissues during embryonic development (Abitbol et al., 1993; Hinds et al., 1993), a period of massive cellular proliferation. A role for FMRP in cellular remodeling is further supported by its increased levels after quiescent mouse kidney cells were stimulated to resume proliferation (Khandjian et al., 1995). In addition, Devys et al. (Devys et al., 1993) found that dividing layers of epithelial tissues as well as cells during wound healing and pathogenesis that involved active cellular remodeling, such as in heart myocytes of patients with ischemic cardiopathy, exhibited strong FMRP expression. In non-neuronal tissue FMRP may be involved in cellular structural transformations accompanying cellular proliferation, which may have mechanisms in common with neuronal structural transformations, such as synaptogenesis and/or spine maturation and pruning.

Molecular Roles of FMRP

Clearly FMRP plays a role in the central nervous system and is likely to play an important one that affects synaptic plasticity. Since FMRP was found to bind mRNA and associate with ribo-
somes in an RNA dependent manner (Tamanini et al., 1996; Willemse et al., 1996; Corbin et al., 1997; Feng et al., 1997a), it may play a role in many aspects of mRNA processing, such as alternative splicing, nucleocytoplasmic shuttling (Feng et al., 1997b; Ceman et al., 1999), ribosomal complex targeting and/or docking (Dreyfuss et al., 1988; Corbin et al., 1997), translation initiation or regulation, or dendritic localization. Which RNAs FMRP may be regulating is still unknown; however, recent evidence suggests that it may be a large set of RNAs and not just a select few. 1]. Weiler et al. (submitted for publication) have demonstrated that synaptoneurosomes taken from FraX mice, although exhibiting normal basal protein translation, did not exhibit stimulation-induced protein synthesis or normal levels of postsynaptic polyribosomal aggregates in vitro. It is possible that FMRP acts as an ‘immediate early protein’, and much like immediate early genes that are transcribed rapidly in response to neuronal activity, FMRP may be rapidly translated in response to neuronal activity in order to regulate the translation of other activity-dependent proteins [immediate early genes are reviewed by Sheng and Greenberg and by Clayton (Sheng and Greenberg, 1990; Clayton, 2000)]. Since activity drives nervous system organization, it makes sense that these proteins might be involved in synaptic plasticity processes, and the fact that a lack of FMRP leads to selective structural deficits in dendritic spines is compatible with this view. Possibly FMRP is the ‘synaptic tag’ proposed by Frey and Morris (Frey and Morris, 1997), that it sets in motion a process at the synapse which synthesizes new proteins for interaction with those being shipped from the soma in support of synaptic plasticity processes. This takes into account that synaptic plasticity has a longer time course than would be expected if it were dependent on local processes alone, and it would provide synapse specificity and perhaps require transcription, which have been shown to be important characteristics for some forms of synaptic plasticity (Huang and Kandel, 1994; Kurotani et al., 1996).

Conclusions

No matter what specific function FMRP turns out to have in the nervous system, the protein appears to be essential to processes necessary for the maturation and pruning of dendritic spines, either during development or throughout the lifetime of an organism. While immature appearing dendritic spines are relatively common among mental retardation syndromes, increased spine density in adulthood appears to be unique to fragile-X syndrome. This, along with in vitro evidence that FMRP is synthesized at synapses in response to synaptic activity and in vivo evidence that FMRP is synthesized during times of active synapse addition, suggests that the function of this protein may be intimately tied to these processes. Future work needs to focus on the synaptic and other functional roles of FMRP in the nervous system as well as in other organ systems.

Notes

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