Cortical neurogenesis in fragile X syndrome

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1. ABSTRACT

The absence of fragile X mental retardation 1 protein (FMRP) results in fragile X syndrome (FXS) that is a common cause of intellectual disability and a variant of autism spectrum disorder. There is evidence that FMRP is involved in neurogenesis. FMRP is widely expressed throughout the embryonic brain development and its expression levels increases during neuronal differentiation. Cortical neural progenitors propagated from human fetal FXS brain show expression changes of genes which encode components of intracellular signal transduction cascades, including receptors, second messengers, and transduction factors. The absence of functional FMRP enhances transition of radial glia to intermediate progenitor cells. Radial glial cells provide scaffolding for migrating neurons and express functional receptors for metabotropic glutamate receptors. The absence of FMRP results in alterations of neuronal differentiation and migration, which contribute to developmental changes in brain structure and function in FXS. Here, cortical neurogenesis in FXS is reviewed and the putative contribution of brain-derived neurotrophic factor to defects of FXS neurogenesis is discussed.

2. INTRODUCTION

Brain development consists of an array of dynamic and adaptive processes which are regulated by gene expression and environmental input. The process of neurogenesis includes cell divisions, fate determination, and differentiation into neuronal phenotype, and migration of neurons to their final location in the brain. During development of the central nervous system, neurogenesis defines the brain structure and function by formation of neurocircuits (1, 2). The size and folding of the mammalian cerebral cortex influence cognitive abilities and sensorimotor skills (3). Emergence and differentiation of new neural structures are highly constrained and genetically defined to support the emergence of a mature functional human brain. Interaction of many kinds of processes at different levels gives support to a series of events and defines brain development. Neurogenesis exists throughout the brain during prenatal life. Several mechanisms can underlie disrupted neurogenesis that is associated with neurodevelopmental disorders. Improved understanding of the pathways and common mechanisms involved in the regulation of neurogenesis may reveal novel molecular targets for multiple disorders.

Fragile X syndrome is an X-linked disorder that is caused by mutations in the FMR1 gene that encodes Fragile X Mental Retardation 1 protein (FMRP) (4). In most FXS individuals expansion of the trinucleotide repeats >200 in the 5´ untranslated region of the FMR1 gene leads to methylation of the promoter and the trinucleotide repeat region which results in transcriptional silencing and absence of FMRP. FMRP is a RNA-binding protein that regulates local translation of specific transcripts. FMRP contributes to the events of complex, interactive signaling cascades during brain development and there is evidence that it is an essential element in neurogenesis (5-8). The absence of FMRP changes intrinsic properties of neural stem cells in a way that the process of neurogenesis is altered both in developing and mature brain (9).

3. EARLY BRAIN DEVELOPMENT AND NEURAL PROGENITORS

The pluripotent stem cells of the blastocyst give rise to the three primary germ layers in the very early
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The neural plate forms out of the ectoderm and folds to the first human brain structure, the neural tube, during the third week of gestation (10, 11). At the fourth week of gestation, the rostral portion of the neural tube forms three vesicles that give rise to the forebrain, the midbrain, and the hindbrain (11, 12). The most rostral forebrain vesicle forms two vesicles, which will become the telencephalon (cerebral cortex) and the diencephalon (thalamus, hypothalamus, and other structures). Several morphogens, including signaling of FGF, BMP, WNT, and SHH, are involved in the dorso-ventral patterning (12).

Neuroepithelial cells lining the ventricles progressively get features associated with glial cells (13) and all neurons, astrocytes, and oligodendrocytes originate ultimately from these progenitors, radial glial (RG) cells (14, 15). Cells proliferate rapidly by gestation weeks 5-6 within the ventricular zone that lines the cerebral vesicles (16, 17). Neuron production in humans begins 6 weeks post conception (embryonic day 42) and the peak of neurogenesis is past by midgestation. Some of the multipotent stem cells generate both neurons and astrocytes, but many rodent studies have demonstrated that neuronal and astrocyte restricted progenitors coexist suggesting heterogeneity of molecular characteristics and mitotic behavior of RG (see review (13)). Multiple progenitors in human developing cerebral cortex are shown in Figure 1. RG cells divide asymmetrically, giving rise to one RG cell and one postmitotic neuron, or one RG and one intermediate progenitor (IP) (18). Two types of IPs are found; apical IPs (aIPs) reside in the ventricular zone (VZ) and basal IPs (bIPs) migrate to the subventricular zone (SVZ) where they usually divide symmetrically to generate two postmitotic neurons (19, 20). IPs are a major neurogenic cell population like RG. In addition, a new type of asymmetrically dividing RG, basal RG cells (bRGCs) have been identified in the outer SVZ in the human cortex (21, 22).

Progenitors in the cortical ventricular zone generate cortical projection neurons which are glutamatergic, excitatory neurons. The other major class of neurons in the mammalian neocortex consists of local circuit neurons which are inhibitory and GABAergic. The majority of murine cortical interneurons originate from progenitors of the ganglionic eminences in the ventral telencephalon outside the cortex (24). The origin of human inhibitory neurons appears to be controversial; a population of human GABAergic neurons origins from ventral telencephalon like in rodents but there
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is also evidence that a significant fraction of human interneurons could be derived from progenitors of the neocortical VZ and SVZ of the dorsal forebrain (25). Intrinsic programming together with external cues determines the fate specification of neurons and their laminar fate. Several transcription factors and signaling molecules are involved in specification programming. Neurogenesis precedes gliogenesis (26). The birthdate of progenitors correlates with their laminar fate and developmental potential in the six layered neocortical structure (27, 28). Early born cortical progenitors are multipotent and competent to generate both deep and upper layer neurons of the six layered mammalian cortex whereas late born neurons are more restricted and can only produce neurons of upper layers.

In adult mammalian brain, neurogenesis persists in two major brain regions; within the SVZ of the lateral ventricles and in the subgranular layer of the hippocampal dentate gyrus. Newly born neuroblasts in the hippocampus primarily replace granule cells of the dentate gyrus. The adult hippocampal neurogenesis and the integration of new neurons into the circuitry of the adult hippocampus play an important role in learning and memory (29). New cells generated in the SVZ migrate long distances to the olfactory bulb and there is evidence that olfactory bulb neurogenesis with long-term neuronal survival is very limited in human when compared to that in rodents (30).

4. THE ROLE OF FMRP DURING EARLY BRAIN DEVELOPMENT

FMRP is widely expressed throughout the embryonic brain development (31-33) and its expression levels are high in regions where neural progenitors are located (34, 35). The expression of FMRP increases during neuronal differentiation indicating the importance of FMRP for neurogenesis. Differentiating neurons in cultures of neural progenitors derived from human fetal brain express high levels of FMRP (34). FMRP is also detectable in differentiating mouse neural progenitors in agreement with the role of FMRP in the regulation of the differentiation of neuronal cell populations during development of the central nervous system (36, 37).

Unaffected organogenesis and macroscopically normal brain structures in FXS suggest that FMRP is not essential for very early stages of embryonic development. Indeed, there is evidence that in chorionic villi samples taken from FXS fetuses, FMRP is absent only at week 12 of pregnancy and, thus, many weeks after the beginning of neurogenesis (38). The phenotype of individuals with a deletion of the FMR1 has been reported to be more severe than that of individuals with the dynamic mutation but disturbed organogenesis has not been described and methylation changes of the FMR1 gene in neuronal cells during development of the central nervous system are not well understood. Since FMRP is expressed in early neural progenitors propagated from brains of human fetuses and Fmr1 knockout (KO) mice, the mouse model for FXS, compensatory mechanisms during early development could be activated and restore the initial processes of brain formation in the absence of FMRP. Two proteins that are homologous to FMRP have been identified, FXR1 and FXR2 proteins (39, 40). These homologous proteins form the small family of FXR proteins, which show similar cellular function and could partly complement one another. FMRP can form homodimers and heterodimers with other FXR proteins and the dimerization may provide a mechanism for functional autoregulation. Studies of double transgenic Fmr1/Fxr2 knockout mice have revealed that FMRP and FXR2 act in a cooperative way (41).

5. FMRP IN ADULT NEURAL PROGENITORS

The expression of FMRP is high in neurons of the adult hippocampus (42). The studies of Guo et al. demonstrated by inducible gene recombination that ablation of FMRP in adult nestin-expressing progenitor cells reduces hippocampal neurogenesis and impairs hippocampal dependent-learning in mice (8). Restoration of neurogenesis by FMRP expression rescued the learning deficits supporting a role for FMRP-dependent hippocampal neurogenesis in learning. The absence of FMRP in adult neural progenitors derived from the dentate gyrus of the hippocampus and the forebrain of Fmr1 KO mice has been shown to result in increased progenitor proliferation and fate determination toward glial lineages via the Wnt/beta-catenin signaling pathway and subsequently the downregulation of a downstream effector, neurogenin1 (7).

6. DEFECTS OF CORTICAL NEUROGENESIS IN THE ABSENCE OF FMRP

Developmental abnormalities of specific regions in human FXS brain suggest regional and temporal alterations of neurogenesis in FXS brain. The FXS full mutation associates with abnormal profile of cortical lobe volumes, increased size of the caudate nucleus, and decreased size of the posterior vermis, amygdala, and superior temporal gyrus in human brain (43, 44). Involvement of several gene products with unique temporal and spatial expression patterns as well as many potential environmental factors increase the complexity of the signaling cascades during brain development and effects of the absence of FMRP may be specific for distinct progenitor populations (Figure 1). Cortical neural progenitors derived from a fragile X full-mutation male fetus at 14 week of gestation display expression changes of genes which encode components of intracellular signal transduction cascades, including receptors, second messengers, and transduction factors (45). The neuronal differentiation of progenitors at that developmental stage
has been shown to be normal while progenitors derived from an 18-week-old fragile X fetus were found to give rise to more neurons with short neurite phenotype than the age-matched control NPCs (34) suggesting alterations of neurogenesis in specific neuronal populations and/or during distinct phases of development in FXS.

The expression levels of FMRP are relatively high in RG of embryonic mouse neocortex and FMRP has been shown to be important for maintaining the pool of RG during neocortical development (35). The absence of functional FMRP causes depletion of RG by enhanced transition of RG to IP cells by an actin-dependent mechanism in the brain of Fmr1 KO mice. RG cells participate in neurogenesis and provide scaffolding for migrating neurons. RG expresses functional receptors for metabotropic glutamate receptors which are known to be dysregulated in FXS. RG lacking FMRP show alterations of the intracellular calcium responses (34) and since migration of IP cells is a Ca-dependent process, changes in the intracellular calcium dynamics likely contribute to the aberrant differentiation of IP cells. Enhanced production of IP cells combined with impaired migration and differentiation of new born neurons lacking functional FMRP is seen as an accumulation of new born neurons expressing a mutated FMRP with gain of function properties in the SVZ of developing brain of Fmr1 KO mouse after in utero electroporation of a vector expressing the mutant protein (5). The IP cells express T-box transcription factor, Tbr2 that has an essential role in specifying and expanding cortical IPs (19, 46). The number of Tbr2-immunopositive cells is increased in the SVZ of embryonic brain of Fmr1 KO mice (5). The expression of Tbr2 is temporally followed by the expression of Tbr1 (47, 48). The number of Tbr1-positive cells is increased in supragranular layers of embryonic brain of Fmr1 KO mice. Tbr1 is expressed in almost all postmitotic glutamatergic neurons in the embryonic neocortex and changes of Tbr2 and Tbr1 expression indicate disturbed glutamatergic neurogenesis in FXS. Although the number of Tbr1 cells does not differ in the somatosensory cortex of wild type and Fmr1 KO mice at the time of the birth (P0) (personal observation, not published data), changes of glutamatergic neurogenesis likely reflect maturation defects of glutamate signaling observed at early postnatal days during critical period (49).

There is evidence that BDNF/TrkB signaling contributes to the perturbations of FMRP-deficient progenitor differentiation and brain development in FXS (67). TrkB receptor expression is increased in undifferentiated cortical progenitors derived from Fmr1 KO mouse and intracellular calcium responses to BDNF are enhanced in differentiated FMRP-deficient progenitors (67). In addition, progenitors lacking FMRP generate more cells with catalytic activity (52) and migration (INM) (52) that is thought to be involved in cell fate determination of neural progenitors. Nuclear migration is linked to cell cycle progression and nuclei in RG undergoing S phase form a layer at the apical side of the VZ, while nuclei in the M phase stay along the surface of the ventricle. Perturbation of INM by down-regulation of centrosome- and microtubule-associated proteins disrupts maintenance of the neural progenitor pool and enhances neurogenesis at the expense of neural progenitor cells (53, 54). INM is also affected in FXS and the velocity of the nuclear movement in RG of differentiating neurospheres derived from Fmr1 KO mice is enhanced when compared with that of wild type controls (55) in agreement with intrinsic changes in FXS RG.

7. THE ROLE OF BDNF IN THE IMPAIRED CORTICAL NEUROGENESIS IN FXS

Brain-derived neurotrophic factor (BDNF) is implicated in control of neurogenesis during brain development and in adult brain (56-63). The ability of exogenous BDNF to increase the number of new born neurons has been shown in several studies using intraventricular infusion or viral overexpression of BDNF (57, 64, 65), but the effect of BDNF on neurogenesis has not been consistent in all studies (66) and the mechanisms of BDNF action on neurogenesis are not fully understood. During cortical development neurotrophin-mediated signalling via tropomyosin-related kinase (Trk) receptors plays a cell-autonomous role that is essential for control of the proliferation and differentiation of neural precursors (57, 59). Decreased TrkB signaling reduces embryonic precursor proliferation and inhibition of TrkB/C delays production of neurons. BDNF and TrkB/C signaling also contributes to the regulation of the laminar fate of cortical neurons.

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Cells isolated from embryonic cortex and grown in culture are multipotent and sequentially generate neuronal and glial progenitors according to the in vivo developmental sequence (50). Neural progenitors propagated from brains of FXS fetuses and the mouse model for FXS show unique properties when compared with the wild-type progenitors (7, 34, 45). The differentiation of neural progenitors has been shown to be affected by the absence of FMRP in human, mouse, and fly (34, 45, 51). RG cells display cell cycle-dependent nuclear movement referred to as interkinetic nuclear
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BDNF in neural progenitors generated from double transgenic mice deficient of both FMRP and BDNF prevents the short neurite phenotype characteristic of new born FXS neuroblasts (68). Reduction of BDNF expression has also beneficial effects on motor activity and motor skill learning suggesting that some behavioural abnormalities in Fmr1 KO mice may be induced by increased BDNF signaling and could be counteracted by reducing extracellular BDNF levels. However, impaired fear learning and spatial learning defects in the adult mice deficient in both BDNF and FMRP are more pronounced than those with a deletion of only one of the genes encoding these proteins. Disturbed hippocampal neurogenesis underlies learning deficits in adult Fmr1 KO mice (8) and mechanisms linked to the effects of BDNF on the survival of new born cells in the hippocampus could explain the negative effects of reduced BDNF on the cognition of Fmr1 KO mice.

8. SUMMARY AND PERSPECTIVE

FXS is caused by the absence of functional FMRP, a RNA protein that regulates translation and is required for normal cortical neurogenesis that underlies normal synapse formation and maturation, and neuronal network development and function. FMRP plays a role in the specification and differentiation of cortical neurons. Alterations of BDNF signaling are involved in defects of neurogenesis in the absence of FMRP. Enhanced TrkB signaling could explain at least some of the alterations of the processes during development of brain lacking FMRP. However, spatial and temporal changes of neurotrophin receptors, including receptor splice variants and the low-affinity receptors, and their ligands during cortical neurogenesis in FXS are not well understood. BDNF is a critical molecule in the regulation of plasticity during embryonic and perinatal period, and it also appears to promote plasticity in adult brain (69). Some existing pharmacological interventions such as selective serotonin reuptake inhibitors (SSRIs) enhance plasticity through BDNF/TrkB signaling. The effects of these drugs may depend on the developmental stage and could be modulated by intrinsic factors. Indeed, alterations of BDNF/TrkB signaling caused by the absence of FMRP result in distinctive cellular and behavioral responses to fluoxetine in adult FXS mice (70). The impact of BDNF on developmental deficits in FXS remains to be further studied in detail for identification of new treatment strategies.

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Abbreviations: aNPCs, adult neural progenitor cells; BDNF, Brain-derived neurotrophic factor; FMR1 gene, fragile X mental retardation 1 gene; Fmr1 KO, fragile X mental retardation 1 knockout; FMRP, FMR1 protein; FXS, Fragile X syndrome; GABA, gamma-aminobutyric acid; mGluR, metabotropic glutamate receptor; IP, intermediate progenitor; RG, radial glia; SVZ, subventricular zone; SSRI, selective serotonin reuptake inhibitor; TrkB receptor, tropomyosin-related kinase B receptor; VZ, ventricular zone

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