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Dendritic spine actin cytoskeleton in autism spectrum disorder

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ABSTRACT

Dendritic spines are small actin-rich protrusions from neuronal dendrites that form the postsynaptic part of most excitatory synapses. Changes in the shape and size of dendritic spines correlate with the functional changes in excitatory synapses and are heavily dependent on the remodeling of the underlying actin cytoskeleton. Recent evidence implicates synapses at dendritic spines as important substrates of pathogenesis in neuropsychiatric disorders, including autism spectrum disorder (ASD). Although synaptic perturbations are not the only alterations relevant for these diseases, understanding the molecular underpinnings of the spine and synapse pathology may provide insight into their etiologies and could reveal new drug targets. In this review, we will discuss recent findings of defective actin regulation in dendritic spines associated with ASD.

1. Synaptic deficiency in autism spectrum disorder

Autism spectrum disorder (ASD) comprises a range of neurological conditions that affect the ability of individuals to communicate and interact with others. ASD is characterized by impaired social interactions, communication deficits, and repetitive behaviors. ASD is typically diagnosed during the first 3 years of life, a period of extensive neurite formation, synaptogenesis and refinement (Huttenlocher & Dabholkar, 1997; Zoghbi & Bear, 2012; Stamou et al., 2013; McGee et al., 2014). Family and twin studies have revealed that ASD has a strong genetic component, with numerous genes being affected. In addition to mutations inherited from parents, many ASD-associated mutations are rare protein disrupting *de novo* mutations that have arisen in the germline. Mutations can be copy-number variants (CNVs) or single-base-pair mutations (de la Torre-Ubieta et al., 2016). By definition, CNVs are deleted or duplicated segments of DNA (> 1000 basepairs) that are thought to be involved in the pathogenesis of a wide range of human diseases, including ASD. At least six recurrent CNVs are among the most frequently identified genetic contributors to ASD (Sanders et al., 2015). Overall, there seems to be an enrichment in 'likely gene-disrupting' mutations (LGDs; nonsense, frameshift and splice site mutations that often result in the production of truncated proteins) in individuals with ASD as compared to their healthy relatives or to other unaffected individuals (de la Torre-Ubieta et al., 2016).

Multiple ASD susceptibility genes converge on cellular pathways that intersect at the postsynaptic site of glutamatergic synapses (Bourgeron, 2015; Peca & Feng, 2012), the development and maturation of synaptic contacts (Gilman et al., 2011) or synaptic transmission

Abbreviations: Abi-1, Abelson interacting protein 1; Abp1, actin binding protein 1; ACTN4, α-actinin-4; ADF, actin-depolymerizing factor; ADHD, attention deficit/hyperactivity disorder; ADNP, activity-dependent neuroprotective protein; ANK, ankyrin; ARHGEF9/PEM2, RhoGTPase exchange factor 9/posterior end mark 2; ASD, autism spectrum disorder; Atf3, stress-induced transcription factor-3; BAR, Bin1/amphiphysin/Rvs167; CaMKII, calcium-calmodulin kinase II; CH1/2, calponin homology; CNS, central nervous system; CNV, copy-number variants; CSWSS, continuous spike wave in slow-wave sleep; CTTNBP2, cortactin-binding protein 2; CYFIP1, Cytoplasmic FMRP-interacting protein 1; Dbl, diffuse B-cell lymphoma; DH, Dbl homology; DIAPH3, diaphanous homolog 3; DISC1, disrupted in schizophrenia; DLGAP2, Disk large-associated protein 2; DMD, dystrophin; Dp427, full-length dystrophin containing actin binding domain; E, embryonic day; FDR, false discovery rate; FMRP, fragile X mental retardation protein; FRAP, fluorescence recovery after photobleaching; FXS, fragile X syndrome; GAP, GTPase activating protein; GEF, guanine-exchange factor; GRAF, GTPase regulator associated with focal adhesion kinase; GSN, gelsolin; IRSp53/BAIAP2, insulin receptor substrate p53/Brain-specific angiogenesis inhibitor 1-associated protein; Kal7, kalirin-7; LGD, likely gene-disrupting; LKS, Landau-Kleffner syndrome; LTP, long-term potentiation; MAPK, Mitogen-activated protein kinases; mDia2, mammalian Diaphanous-related formin 2; mEPSC, miniature excitatory postsynaptic currents; mGluR, metabotropic glutamate receptors; mTOR, mammalian target of rapamycin; mTORC1, mTOR complex 1; MYH4/MyHC-IIb, myosin heavy chain IIb; MYO16, Myosin XVI; MYO9B, myosin IXb; NL, Neuroligin; NMD, Nonsense-mediated mRNA decay; NMDA(R), *N*-methyl-D-aspartate (receptor); NMJ, neuromuscular junction; OPHN1/ARHGAP41, Oligophrenin1/RhoGTPase activating protein 41; PAK, p21-activated kinase; PH, pleckstrin homology; PI3K, phosphatidylinositol 3-kinase; PSD, postsynaptic density; Rac1, Ras-related C3 botulinum toxin substrate 1; ROCK, Rho-associated coiled-coil containing protein kinase; SAM, sterile alpha motif; SAPAP, PSD-95-associated protein; SH3, Src homology 3; SHANK3, SH3 and multiple ankyrin repeat domains; sLTP, structural LTP; SR1-4, spectrin repeats; SRA1, specific Rac1-activated; STRN, striatin; STXBP5, syntaxin binding protein 5; SynGAP1, synaptic GTPase activating protein 1; SV, Synaptic vesicle; TADA, transmission and de novo association; TCGAP, TC10β/Cdc42-GAP; TSC, tuberous sclerosis complex; WRC, WAVE regulatory complex; Zn, zinc; α-Pix/Cool2, α-Pak interactive exchange factor/Cloned out of library 2

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(Li et al., 2014). The majority of excitatory glutamatergic synapses are located in small dendritic protrusions known as spines. The formation, maturation and elimination of dendritic spines lie at the core of synaptic transmission and memory formation (Roberts et al., 2010; Yang et al., 2009). Many of the ASD risk genes encode synaptic scaffolding proteins, receptors, cell adhesion molecules or proteins that control actin cytoskeleton dynamics, all of which directly affect synaptic strength and number and, ultimately, neuronal connectivity in the brain. In addition, ASD risk genes encode proteins that are involved in chromatin remodeling, transcription, and protein synthesis or degradation, all of which can act as upstream controllers of the expression levels of synaptic proteins. Changes in any of these proteins can affect dendritic spine density in the brain. When deleterious mutations occur, an individual's risk for ASD is further affected by how well the defects of a single mutation can be compensated in the brain (Bourgeron, 2015).

Increased spine density has been observed in the frontal, temporal, and parietal lobes of human ASD brains (Hutsler & Zhang, 2010) and recent studies indicate a defect in dendritic spine pruning from 13–18 years of age (Tang et al., 2014). Tang et al. proposed that this pruning deficit may contribute to abnormalities in the cognitive functions that humans acquire in their late childhood, teenage, or early adult years, including the acquisition of executive skills such as reasoning, motivation, judgment, language, and abstract thinking (Goda & Davis, 2003). Many children diagnosed with ASD reach adolescence and adulthood with functional disability in these skills, in addition to social and communication deficits (Seltzer et al., 2004). Although human ASD studies have reported increased dendritic spine density (Hutsler & Zhang, 2010; Tang et al., 2014), only a few genetic ASD animal models display this effect, making it unclear whether the increased spine density causes ASD-like behavior or whether any change in the number or function of synapse leads to atypical brain connectivity and symptoms of ASD (Bourgeron, 2015).

One of the mouse models that does mimic the spine-pruning defect seen in humans has a constitutively overactive mammalian target of rapamycin (mTOR) (Tang et al., 2014). In this model, the mechanism behind the defective spine pruning is an autophagy deficiency in synapses. Autophagy is an evolutionarily conserved cellular process that provides nutrients during starvation and eliminates defective proteins and organelles via lysosomal degradation. Hyperactive mTOR inhibits autophagy at an early step in autophagosome formation (Kim et al., 2011). Inhibition of neuronal autophagy produces ASD-like inhibition of normal developmental spine depletion, without affecting the rate of spine formation, leading to increased spine density and ASD-like behaviors (Tang et al., 2014). This model offers one mechanism underlying increased spine density and ASD-type behavior, but considering the heterogeneity of ASD genetics, it is likely that other mechanisms affecting spine pruning are defective in different ASD patients. Interestingly, it has been reported that mutations of five autism-risk genes with diversified molecular functions all lead to a similar ASD phenotype of behavioral inflexibility, indicated by impaired reversal-learning in *Drosophila* (Dong et al., 2016). These reversal-learning defects result from an inability to activate Ras-related C3 botulinum toxin substrate 1 (Rac1)-dependent forgetting (Dong et al., 2016). In mammalian neurons, Rac1 affects spine structure, regulates synaptic plasticity in the hippocampus and is required for proper hippocampus-dependent spatial learning (Haditsch et al., 2009; Bongmba et al., 2011). Active Rac1, when targeted to potentiated spines, is able to deplete targeted synapses (Hayashi-Takagi et al., 2015). Rac1 is one of the main regulators of the actin cytoskeleton and, therefore, one of the possible mechanisms underlying the defective spine pruning is deficient regulation of the synaptic actin cytoskeleton. It is also possible that spine pruning is normal, but spine formation is overactive, leading to increased spine density.

Although ASD is considered one of the most heritable neurodevelopmental disorders (El-Fishawy & State, 2010; Geschwind, 2011) and majority of the research on ASD has focused on the genetics of the disorders (Autism Genome Project, C, 2007; Buxbaum & Hof, 2011), single causative gene anomalies account for only a small proportion of ASD cases (Herbert, 2010; Landrigan et al., 2012). To date, inheritance of multiple gene variants, rare *de novo* single gene mutations and copy number variants have been proposed to be liable for ASD. In addition, a multitude of environmental risk factors have been proposed to contribute to the development of ASD (Herbert, 2010). Prenatal environmental conditions, such as maternal infections during pregnancy, have been linked to social communication difficulties in children with ASD-associated CNVs (Vijayakumar & Judy, 2016). Other environmental factors that have been proposed to interact with risk genes include preterm birth, folate deficiency, and exposure to certain toxins (Vijayakumar & Judy, 2016). One of these risk factors is lead exposure, which can cause dose-dependent changes in DNA methylation (Dosunmu et al., 2012), and among the hypomethylated genes caused by lead exposure, some affect actin cytoskeleton (Senut et al., 2014).

Genes associated with autism are listed at publicly accessible websites SFARI Gene (https://gene.sfari.org/database/human-gene/) and AutismKB (http://autismkb.cbi.pku.edu.cn/index.php). In this review, we evaluate the current literature from SFARI listed genes, which encode proteins that regulate actin dynamics and have been linked with autism (Tables 1 and 2). The review aims to discuss the current literature to unravel whether the actin regulators encoded by ASD-associated genes regulate synaptic structure and function, and if yes, how? Additionally, the review aims to identify any common pathways on synaptic or actin regulation among these ASD-associated actin regulators.

2. The role of actin cytoskeleton and actin binding proteins on the imbalanced spine formation and plasticity in ASD

Although a wide range of autism-associated genes has been identified, it is becoming increasingly evident that many of these genes converge into common cellular pathways associated with neurite outgrowth, synaptogenesis, synaptic plasticity and spine stability, which together regulate the structural stability of neurons. Actin is the building block of cells and regulates the shape and density of dendritic spines (Hotulainen & Hoogenraad, 2010). Perturbing actin cytoskeleton and its regulators can lead to an imbalance in cytoskeleton dynamics, which in turn, can cause alterations in dendritic spine size, shape and number, as well as neural arborization. The correct morphology of synapses is key to the formation of functional neuronal circuitry and deficits in the structural stability of dendritic spines have been reported in several neurological disorders, including fragile X syndrome (FXS) (Scotto-Lomassese et al., 2011), schizophrenia (Cahill et al., 2012; Kalus et al., 2000) and autism (Raymond et al., 1996).

2.1. Regulation of F-actin polymerization and depolymerization

The dynamic remodeling of the actin cytoskeleton is a critical part of most cellular activities, and the transition between two forms of actin, monomeric globular (G)-actin and filamentous (F)-actin, is tightly regulated in time and space by a large number of signaling, scaffolding and actin-binding proteins. Most of these proteins integrate actin binding, protein-protein interaction, membrane binding, and signaling domains. In response to extracellular signals, often mediated by Rho family GTPases, actin-binding proteins control the cellular actin cytoskeleton by regulating actin filament nucleation, elongation (= polymerization) and treadmilling, maintenance, severing, capping, and depolymerization.

Cortactin-binding protein 2 (*CTTNBP2*) is highly expressed in dendritic spines where it locally interacts with proteins to control the formation and maintenance of the spines. The dendritic spine head typically contains a dense network of short, cross-linked, branched F-actin (Hotulainen & Hoogenraad, 2010), and CTTNBP2 has been shown to regulate the mobility and distribution of cortactin which, in turn,