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Review

Cell type-specific roles of RAS-MAPK signaling in learning and memory: Implications in neurodevelopmental disorders

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ABSTRACT

The RAS-mitogen-activated protein kinase (MAPK) signaling pathway plays critical roles in brain function, including learning and memory. Mutations of molecules in the RAS-MAPK pathway are associated with a group of disorders called RASopathies, which include Noonan syndrome, neurofibromatosis type 1, Costello syndrome, Noonan syndrome with multiple lentigines, Legius syndrome, and cardio-facio-cutaneous syndrome. RASopathies share certain clinical symptoms, including craniofacial abnormalities, heart defects, delayed growth, and cognitive deficits such as learning disabilities, while each individual syndrome also displays unique phenotypes. Recent studies using mouse models of RASopathies showed that each disorder may have a distinct molecular and cellular etiology depending on the cellular specificity of the mutated molecules. Here, we review the cell-type specific roles of the regulators of the RAS-MAPK pathway in cognitive function (learning and memory) and their contribution to the development of RASopathies. We also discussed recent technical advances in analyzing cell type-specific transcriptomes and proteomes in the nervous system. Understanding specific mechanisms for these similar but distinct disorders would facilitate the development of mechanism-based individualized treatment for RASopathies.

1. Introduction

The RAS-mitogen-activated protein kinase (MAPK) pathway transduces signals from membrane receptors to the cytoplasm and nucleus and plays important roles in multiple biological processes such as cell proliferation and differentiation (Boguski & McCormick, 1993). The small GTPase protein RAS activates downstream effectors such as the Ser/Thr kinase RAF, which in turn activate MEK, which subsequently activates MAPK proteins (Boguski & McCormick, 1993; Weiss, Bollag, & Shannon, 1999). This biochemical cascade is tightly regulated by multiple regulatory proteins such as GTPase activating proteins (GAPs) which suppress the activation of RAS, and guanine nucleotide exchange factors (GEFs), which activate the pathway (Bernards & Settleman, 2004; Ye & Carew, 2010).

The RAS-MAPK pathway is critically involved in regulating cognitive functions, including learning and memory (Lee & Silva, 2009; Sweatt, 2001; Thomas & Huganir, 2004; Ye & Carew, 2010). In addition, the requirement for RAS-MAPK signaling in synaptic plasticity and learning is evolutionarily conserved (Ye & Carew, 2010). MAPK is activated after learning or the induction of long-term potentiation (LTP) in the involved brain areas, including the hippocampus and amygdala in rodents (Blum, Moore, Adams, & Dash, 1999; English & Sweatt, 1996; Schafe et al., 2000). Importantly, pharmacological inhibition of MAPK activation impairs LTP and learning (Blum et al., 1999; English & Sweatt, 1997; Schafe et al., 2000; Selcher, Atkins, Trzaskos, Paylor, & Sweatt, 1999). Pharmacological inhibition of MAPK activation impairs long-term memory, but leaves short-term memory intact (Blum et al., 1999; Schafe et al., 2000). Studies using genetically modified mutant mice also support key roles for the RAS-MAPK pathway in synaptic plasticity and cognitive function. For example, a mutant mouse in which ERK2 expression is partially reduced showed deficits in long-

Abbreviations: CFC, cardio-facio-cutaneous; CS, Costello syndrome; FACS, fluorescence-activated cell sorting; GAPs, GTPase activating proteins; GEFs, guanine nucleotide exchange factors; GFP, green fluorescent protein; HCN, hyperpolarization-activated cyclic nucleotide-gated channel; iPSCs, induced pluripotent stem cells; LS, Legius syndrome; LTP, long-term potentiation; MAPK, mitogen-activated protein kinase; NF, neurofibromin; NMDA, *N*-methyl-D-aspartate; NS, Noonan syndrome; NSML, Noonan syndrome with multiple lentigines; PSD, postsynaptic density; PV, parvalbumin; SPRED, Sprouty-related proteins with an EVH-1 domain; ID, intellectual disability; NF1, neurofibromatosis type 1.

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term memory while the short-term memory was intact (Satoh et al., 2007). Furthermore, a transgenic mouse line expressing dominant-negative MEK1, the upstream activator of MAPK ERK1/2, exhibits impaired spatial learning and fear conditioning (Kelleher, Govindarajan, Jung, Kang, & Tonegawa, 2004). Consistent with the results from pharmacological manipulations, short-term memory was intact in this transgenic mouse, suggesting that MAPK pathway is critically involved in memory consolidation (Kelleher et al., 2004). This transgenic mouse also displays a deficit in the protein synthesis-dependent form of long lasting LTP in the hippocampus (Kelleher et al., 2004). Mice lacking neuron-specific GEF also show deficits in long-term fear memory and amygdalar LTP (Brambilla et al., 1997), demonstrating that the regulation of RAS-MAPK pathway is critical for synaptic plasticity and learning.

Mutations in the RAS-MAPK pathway are associated with a group of developmental disorders termed RASopathies (Aoki, Niihori, Inoue, & Matsubara, 2016; Bentires-Alj, Kontaridis, & Neel, 2006; Rauen, 2013; Zenker, 2011). RASopathies include Noonan syndrome (NS), neurofibromatosis type 1 (NF1), Costello syndrome (CS), cardio-facio-cutaneous (CFC) syndrome, LEOPARD syndrome (also called Noonan syndrome with multiple lentigines, NSML), and Legius syndrome (LS) (Rauen, 2013). In most cases, the activation of the RAS-MAPK pathway is enhanced in RASopathies (Bentires-Alj et al., 2006; Rauen, 2013). As a group, RASopathies affect approximately 1 out of 1000 live births worldwide (Rauen, 2013). RASopathies share a molecular etiology (i.e., mutations in the RAS pathway) and common clinical features, including heart defects, delayed growth, craniofacial abnormalities, predisposition to developing cancers, and neurocognitive impairments (Bentires-Alj et al., 2006; Rauen, 2013; Zenker, 2011). However, each disease also displays distinct symptoms depending on the specific mutated genes (Jindal, Goyal, Burdine, Rauen, & Shvartsman, 2015). The most intuitive strategy for treating RASopathy would be to target the RAS-MAPK pathway directly. However, considering the ubiquitous roles of RAS signaling in the human body, it is of great interest to develop individualized treatments that are specific for each disease by understanding the disease-specific mechanism.

Recent studies suggest that RAS-MAPK signaling plays distinct roles in different cell types in mammalian brain depending on its specific interactions with other regulatory proteins such as different GAPs and GEFs (Cui et al., 2008; Jindal et al., 2015; Lee et al., 2014). Also in *Caenorhabditis elegans*, different RasGAP proteins are differentially involved in different types of associative memory (Gyurko, Csermely, Soti, & Stetak, 2015). This review focuses on the cell type-specific roles of the molecules in RAS-MAPK signaling, which are associated with RASopathy and related neurodevelopmental disorders.

2. Postsynaptic neuron – excitatory synapse

At the glutamatergic excitatory synapse, neurotransmitter receptors and signaling proteins are tightly organized on the postsynaptic neurons. This postsynaptic density (PSD) functions not only as a structural scaffold but also specifically couples the activation of neurotransmitter receptors to downstream signaling pathways at the site of the excitatory synapse on postsynaptic neurons (Husi, Ward, Choudhary, Blackstock, & Grant, 2000; Scannevin & Huganir, 2000; Sheng & Kim, 2011). These complexes may direct the activation of distinct biochemical signaling cascades in response to different upstream stimuli such as different neurotransmitters or different patterns of neuronal activity (Husi et al., 2000; Komiyama et al., 2002). Accordingly, activation of the RAS-MAPK pathway is also tightly controlled near PSD (Hardingham, Arnold, & Bading, 2001; Husi et al., 2000; Thomas & Huganir, 2004). Classically, the neuronal RAS-MAPK pathway is thought to be activated mainly through receptor tyrosine kinases in response to neurotrophins such as brain derived neurotrophic factor (Margolis & Skolnik, 1994; Park & Poo, 2013). In addition, calcium influx through *N*-methyl-D-aspartate (NMDA) receptors or voltage-gated calcium channels in response to glutamate or membrane depolarization also activates the RAS-MAPK pathway in postsynaptic neurons (Hardingham et al., 2001; Thomas & Huganir, 2004). Neurotrophin- and glutamate-mediated RAS activation are controlled by different regulators, such as RasGEFs and RasGAPs.

2.1. SYNGAP1 and intellectual disability

SYNGAP1 is a neuron-specific RasGAP that is largely localized to excitatory synapses via interaction with the PSD complex (Chen, Rojas-Soto, Oguni, & Kennedy, 1998). SYNGAP1 suppresses RAS signaling activation through glutamate receptor activation (Kim, Lee, Takamiya, & Huganir, 2003; Kim, Liao, Lau, & Huganir, 1998; Rumbaugh, Adams, Kim, & Huganir, 2006). Accordingly, a recent study showed that SYNGAP1 is rapidly dispersed from synaptic spines in response to LTP induction, which allows the synaptic accumulation of AMPA receptors via the activation of RAS-ERK signaling (Araki, Zeng, Zhang, & Huganir, 2015). Due to its specific expression pattern, SYNGAP1 provides a unique opportunity to study the role of RAS-MAPK signaling in excitatory postsynaptic neurons. Importantly, loss of function mutations in *SYNGAP1* have been reported to be associated with intellectual disability (ID) (Berryer et al., 2013; Hamdan, Daoud, et al., 2011; Hamdan, Gauthier, et al., 2011). SYNGAP1 contains several domains, including a PH and C2 domain, a PDZ ligand domain, and a GAP domain (Pena et al., 2008). Two lines of *Syngap1* knockout mice were generated by deleting exons encoding either C2 and GAP domains or PH and C2 domains (Kim et al., 2003; Komiyama et al., 2002). In both lines, homozygous gene knockouts were lethal, and subsequently electrophysiological and behavioral analyses have been done largely in the heterozygous knockout mice ($Syngap1^{+/-}$) (Kim et al., 2003; Komiyama et al., 2002). Both lines of mutants showed significant deficits in adult hippocampal LTP even though basal synaptic transmission was normal (Kim et al., 2003; Komiyama et al., 2002). Interestingly, Kim and colleagues showed that the number of AMPA receptor clusters was significantly increased in neuronal cultures from the knockout mice, suggesting that SYNGAP1 is involved in synaptic trafficking of AMPA receptors (Kim et al., 2003). In addition, Rumbaugh and colleagues showed that MAPK activation is enhanced in knockout mice lacking the PH and C2 domain of SYNGAP1, indicating that SYNGAP1 negatively regulates MAPK activation and that increases in MAPK activation cause increases in AMPA receptor trafficking to the synapse in the *Syngap1* knockout mice (Rumbaugh et al., 2006) (Fig. 1A). Consistently, increased MAPK activation was also found in the hippocampus of other mutant mouse lines lacking the C2 and GAP domain (Komiyama et al., 2002).

Syngap1 knockout mice showed several behavioral deficits, including spatial memory deficits in a C2 and GAP domain deletion mutant (Komiyama et al., 2002). Other behavioral conditions were also associated with *Syngap1* deletion, including schizophrenia and ID-like symptoms, such as deficits in working memory and auditory fear conditioning, sensory-motor gating, social interaction, and contextual discrimination (Clement et al., 2012; Guo et al., 2009). Spatial memory deficits in the Morris water maze were not reversed by crossing $Syngap1^{+/-}$ mice with $Hras^{-/-}$ mice, suggesting that SYNGAP1 regulates RAS signaling through another form of RAS protein other than HRAS (Komiyama et al., 2002). Interestingly, *Syngap1* haploinsufficiency accelerates the maturation of glutamatergic synapses in the early postnatal period (Clement, Ozkan, Aceti, Miller, & Rumbaugh, 2013; Clement et al., 2012). Moreover, glutamatergic synaptic transmission and the