

Involvement of Calcium, Ras, MAPK, PI3K-Akt and mTOR Signaling Pathways in Autism Spectrum Disorders

Wen Y^{1, 2, 3*}

¹Department of Neurology, Massachusetts General Hospital, USA

²Harvard Medical School, Harvard University, USA

³Higher Synthesis Foundation, Cambridge, USA

***Corresponding author:** Ya Wen, Department of Neurology, Harvard University, Boston, Massachusetts, USA, E-mail: ywen3@mgh.harvard.edu

Mini Review

Volume 2 Issue 1

Received Date: February 22, 2017

Published Date: April 04, 2017

Abstract

Autism Spectrum Disorders (ASDs) are a highly variable spectrum of behaviorally defined neuro-developmental conditions. The involvement of cell signaling pathways such as MAPK (mitogen-activated protein kinases, also known as ERK, extracellular signal-regulated kinases) signaling, PI3K (phosphatidylinositol 3-kinase) -Akt signaling, mTOR (mammalian target of rapamycin) signaling, Ras signaling and calcium signaling pathways have been observed in ASDs using methodologies from several disciplines, including genetics, bioinformatics and biological studies. These pathways interact with each other and profoundly influence a number of cellular activities including cell differentiation, proliferation, metabolism, and response to environmental signals. To understand how these signaling pathways may contribute to the mechanisms that may underlie ASDs, it is important to look at their interactions and function networks. This article discussed the involvement of these signaling pathways in ASDs and proposed a pathway network analysis for further studies.

Keywords: Autism spectrum disorders; Metabolism; Signal transduction; Cell signaling; Pathway network

Introduction

ASDs are characterized by social impairments, communication difficulties, and restricted, repetitive, and stereotyped patterns of behavior. While people with the diagnosis meet the same defining behavioral criteria, the spectrum involves great heterogeneity between individuals and across multiple levels - genes, brain characteristics, and comorbidities. A growing number of published studies have identified many conditions that comorbid to ASDs such as anxiety, sleep disorders,

gastrointestinal symptoms, epilepsy, diabetes, etc. [1-3]. Although the discovery of these comorbid multisystem conditions adds further complexity to ASDs, it greatly extends the Pathophysiology of ASDs and begin to render it a systemic rather than strictly neurological phenotype. The mechanisms underlie the spectrum conditions possibly include a functional cellular network which would trigger multisystem dysfunction when disturbed. From reviewing existing findings in published literatures, it is worth noting that cell signaling pathways such as MAPK, PI3K-Akt, mTOR, Ras and calcium signaling

pathways are all reported to be linked to ASDs and many other conditions that comorbid to ASDs. These pathways response to extracellular and intracellular cues and regulate a variety of cellular activities. To understand how they function in concert with regard to ASDs may shed lights to understand the pathophysiology.

Involvement of Cell Signaling Pathways to ASDs

Studies have uncovered many potential ASD candidate genes. As of December 2016, the SFARI (Simons Foundation Autism Research Initiative) Gene-Human

Gene Module recorded 859 human genes implicated as relevant to ASDs. The signaling transduction pathways, Calcium, Ras, MAPK, PI3K-Akt, and mTor signaling pathways, have some of these ASD associated genes as components (Table 1). This suggests that these pathways may be influence by the genetic defects that associated with ASDs and their function may be disturbed in ASDs. Published studies from various disciplines and perspectives have identified links between these pathways and ASDs.

Pathway	ASD Associated Genes in the Pathway
Calcium Signaling Pathway	ATP2B2, CHRM3, ADORA2A, ADRB2, DRD1, HTR7, GNAS, PLN, CACNA1C, CACNA1D, CACNA1F, CACNA1A, CACNA1B, CACNA1E, CACNA1G, CACNA1H, CACNA1I, CHRNA7, P2RX4, P2RX5, GRIN1, GRIN2A, GRM1, GRM5, GRPR, HTR2A, OXTR, AVPR1A, PTGER3, ERBB4, GNA14, PLCD1, PLCB1, CD38, CAMK4, NOS1, PDE1C, PRKCB
Ras Signaling Pathway	KIT, FLT1, MET, PTPN11, HTR7, GRIN1, GRIN2A, GRIN2B, HRAS, NF1, SYNGAP1, RASSF5, PIK3R2, MAPK1, MAPK3, PRKCB
MAPK Signaling Pathway	CACNA1A, CACNA1B, CACNA1C, CACNA1D, CACNA1E, CACNA1F, CACNA1G, CACNA1H, CACNA1I, CACNA2D3, CACNB2, PRKCB, NF1, BDNF, NTRK1, HRAS, BRAF, MAPK1, MAPK3, RPS6KA3, RPS6KA2, IL1R2, GADD45B, MAPK8IP2, MAPK12, MEF2C
PI3K-Akt Signaling Pathway	KIT, FLT1, MET, HRAS, MAPK1, MAPK3, IL6, LAMA1, LAMB1, LAMC3, RELN, THBS1, TNN, ITGA4, ITGB3, ITGB7, PIK3R2, PIK3CG, TSC1, TSC2, MTOR, EIF4E, PTEN, PPP2R1B, PPP2R5D, GSK3B, CDKN1B, YWHAE, BCL2
mTOR Signaling Pathway	MTOR, EIF4E, DEPDC5, TSC1, TSC2, TBC1D7, WNT1, WNT2, DVL3, DVL1, GSK3B, HRAS, BRAF, MAPK1, MAPK3, RPS6KA3, RPS6KA2, PIK3R2, PTEN, PRKCB

Table 1: ASD associated genes participate in the cell signaling pathways.

Calcium Signaling Pathway

Calcium ions are essential for signal transduction, and impact all kinds of cellular activities. Calcium signaling dysregulation has been studied in ASDs for many years. Mutation of the calcium channel gene *CACNA1C* was found to be associated with a syndromic autism, Timothy syndrome [4]. Later, more calcium channel genes were reported to be associated with ASDs by studying genome-wide association study (GWAS) data [5]. It has been suggested that disturbances in calcium signaling pathway

may contribute greatly to the underlying molecular mechanism of autism [6,7].

MAPK Signaling Pathway

MAPK signaling pathway is a chain of proteins that response to extracellular signals and transduce the signals to cell nucleus. It is involved in variety of fundamental cellular processes such as differentiation, proliferation, gene expression, mitosis, cell survival and apoptosis [8]. Recently several studies have suggested or

supported a link between MAPK signaling and ASDs [7-9,11].

PI3K-Akt Signaling Pathway

PI3K-Akt signaling pathway regulates cellular functions such as transcription, translation, proliferation, growth, and survival. PI3K catalyzes the production of phosphatidylinositol-3,4,5-triphosphate (PIP3) which in turn serves as a second messenger that helps to activate Akt. Once active, Akt controls key cellular processes by phosphorylating substrates involved in apoptosis, protein synthesis, metabolism, and cell cycle. Dysregulated PI3K-Akt signaling has been shown to be associated with ASDs [12]. Mutations in genes *TSC1*, *TSC2*, *NF1* and *PTEN* that participate in the PI3K-Akt signaling pathway have also been found to be associated to ASDs [13-15].

mTOR Signaling Pathway

mTOR is a serine/threonine protein kinase, which exists in two complexes termed mTOR complex 1 (mTORC1) and 2 (mTORC2). mTOR signaling response to both intracellular and extracellular signals and regulate several biological processes, including protein synthesis, cell metabolism, proliferation and survival [16]. Over activated mTOR signaling was found to be linked to ASDs [17], and mTOR was proposed to be a potential target for treating ASDs [18].

Ras Signaling Pathway

The Ras signaling pathway also regulates cell proliferation, survival, growth, migration, and differentiation. Ras proteins transduce signals from extracellular growth factors. It regulates multiple cellular functions through effectors including Raf, MAPK and PI3K. *HRAS* gene that encodes small GTPase H-Ras in the Ras family, is associated with ASDs [19,20]. Higher prevalence and severity of autism traits were found in RASopathies (a group of genetic conditions caused by mutations in genes of the Ras-MAPK pathway) compared to unaffected siblings [21]. This suggests a link between dysregulation of Ras/MAPK signaling and ASDs.

Multisystem Involvement of Cell Signaling Pathways

All these signaling pathways are involved in multiple systems and impact many aspects of cellular life. For example, calcium ion (Ca^{2+}) is likely the most common intracellular messenger in neurons, and calcium signaling is critical for neuronal development [22]. Calcium signaling also plays an important role in immune cells [23], regulates metabolism [24], and is involved in the digestive system (gastrointestinal tract, pancreas, and liver) [25]. Ras, MAPK, PI3K-Akt and mTOR signaling

pathways are best known for their central roles in cell differentiation, proliferation, metabolism, mitosis, survival and apoptosis, etc. However, they also play roles in neural systems such as regulating dendritic morphogenesis [26]. MAPK and Ras pathways respond to neurotransmitters which are the chemicals that allow the transmission of signals from one neuron to the next across synapses [27]. MAPK signaling regulates synaptic plasticity [28], PI3K/Akt/mTOR signaling pathways are involved in mental illnesses such as depression in regard to their functions in neuronal cells [29].

Overall, Calcium/Ras/MAPK/PI3K/Akt/mTOR signaling pathways are involved in multiple systems and influence a wide range of cellular activities. Together they form a complicated function network which would lead to a variety of consequences when disturbed. The complexity of the signaling pathway network could fit into the great heterogeneity and diverse comorbidities of ASDs. However, how may these pathways specifically contribute to the underlying mechanism of ASDs is unclear. Further studies that emphasize on exploring the links between these signaling pathways and the common features of the spectrum conditions (e.g. social impairments and stereotyped patterns of behavior) may help develop biomarkers and treatment strategies for ASDs.

Signaling Pathway Network

The signaling pathways regulate each other and co-regulate downstream functions. To gain better understanding of how this pathway works together, it is important to put them together and look at them in a framework. (Figure 1) illustrates these pathways in a cellular model. On the cell surface, the calcium channel mediates the influx of calcium ions (Ca^{2+}). Intracellular changes in Ca^{2+} concentration modulate protein kinase C (PKC) and Ras, which activate proteins including MAPK, PI3K, Akt and Mtor [30]. The receptor tyrosine kinases (RTKs) on the cell membrane activate Ras/MAPK/PI3K/Akt/mTOR signaling too, by binding to extracellular growth factors, and intracellular signal molecules Grb2 (growth factor receptor-bound protein 2), Sos (son of sevenless) and IRS-1 (insulin receptor substrate 1). Activated Ras triggers a phosphorylation cascade Raf-MEK-MAPK. PI3K activation can be accomplished by three different ways: by binding to the phosphorylated intracellular domain of RTK, or by binding to IRS-1 which binds to RTK, or by binding to activated Ras. PI3K activation triggers activation of Akt by phosphorylating PIP3. Akt activates TOR, and eventually leads to series reactions such as protein synthesis [27].

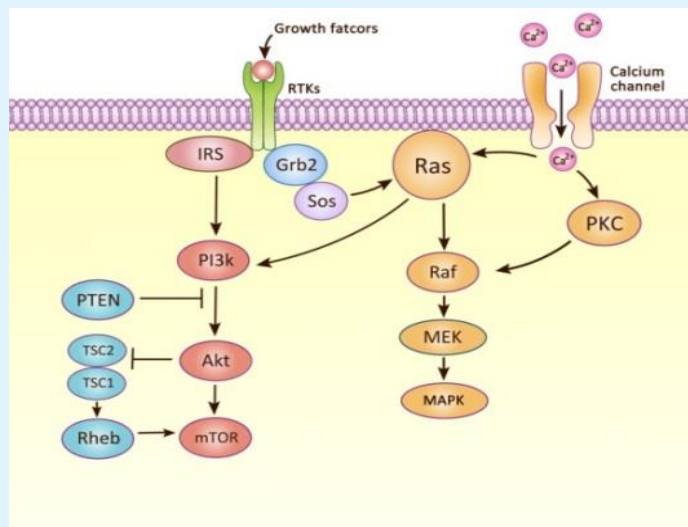


Figure 1: Calcium/Ras/MAPK/PI3K/Akt/mTOR signaling pathways in a cellular model.

There are ASD associated genes involved in the processes (Table 1). For example, *HRAS* encodes protein H-Ras which belongs to Rasfamily; *PRKCB* encodes PKC; *BRAF* encodes protein B-Raf which is activated by Ras and which then activate MAPK; *MAPK1/3* encode MAP kinases (also known as ERKs) which belong to MAP Kinase family; *PTEN* encodes phosphatase and tensin homolog (PTEN) which inhibits Akt activity by dephosphorylating PIP3; *MTOR* encodes mechanistic target of rapamycin (mTOR) which belongs to a family of phosphatidylinositol kinase-related kinases regulates phosphorylation. *TSC1/2* encodes the tuberous sclerosis hetero dimer TSC1/2, which negatively regulates mTORC1 through Rheb (Ras homologue enriched in brain), and which are inhibited by Akt inhibits [27].

Based upon the discussion above, it is clear that these signaling pathways are linked to ASDs. But the mechanism regarding how they may be involved is far from clear. A systematic biological analysis that includes the signaling pathways and ASD associated genes should help push the research further.

Conclusion

In summary, the signaling transduction pathways: Calcium, Ras, MAPK, PI3K-Akt, and mTOR signaling pathways, are all involved in a wide range of biological activities, and are all linked to ASDs. These pathways share a common feature which is response to environmental signals, and process the information. They interact with each other and impact all kinds of cellular activities. Together they form a complex network. There

are a number of ASD associated genes involved in the signal transduction pathway network. Alterations in their function or imbalance in the pathway network may lead to a variety of consequences in multiple systems. Further pathway network studies that put these pathways and ASD associated genes into one picture and focusing on their function in neurodevelopment should help illuminate their contribution to ASD pathophysiology, and even help develop more targeted treatment plans.

References

1. Doshi Velez F, Ge Y, Kohane I (2014) Comorbidity clusters in autism spectrum disorders: an electronic health record time-series analysis. *Pediatrics* Jan 133(1): 54-63.
2. Kohane IS, McMurphy A, Weber G, Mac Fadden D, Rappaport L, et al. (2012) The co-morbidity burden of children and young adults with autism spectrum disorders. *PLoS One* 7(4): 33224.
3. Mannion A, Leader G (2013) Comorbidity in autism spectrum disorder: A literature review. *Res Autism Spectr Disord* 7(12): 1595-1616.
4. Splawski I, Timothy KW, Sharpe LM, Decher N, Kumar P, et al. (2004) Ca V1.2 Calcium Channel Dysfunction Causes a Multisystem Disorder Including Arrhythmia and Autism. *Cell* 119(1): 19-31.
5. Lu AT H, Dai X, Martinez Agosto JA, Cantor RM (2012) Support for calcium channel gene defects in autism spectrum disorders. *Mol Autism* 3(1): 18.

6. Krey JF, Dolmetsch RE (2007) Molecular mechanisms of autism: a possible role for Ca^{2+} signaling. *Curr Opin Neurobiol* 17(1): 112-119.
7. Wen Y, Alshikho MJ, Herbert MR (2016) Pathway Network Analyses for Autism Reveal Multisystem Involvement, Major Overlaps with Other Diseases and Convergence upon MAPK and Calcium Signaling. *PLOS ONE* 11(4): 0153329.
8. Pearson G, Robinson F, Beers Gibson T, Xu B, Karandikar M, et al. (2001) Mitogen-Activated Protein (MAP) Kinase Pathways: Regulation and Physiological Functions. *Endocr Rev* 22(2): 153-183.
9. Ebert DH, Greenberg ME (2013) Activity-dependent neuronal signalling and autism spectrum disorder. *Nature* 493 (7432): 327-337.
10. Subramanian M, Timmerman CK, Schwartz JL, Pham DL, Meffert MK (2015) Characterizing autism spectrum disorders by key biochemical pathways. *Front Neurosci* 9: 313.
11. Garg S, Brooks A, Burns A, BurkittWright E, Kerr B, et al. (2017) Autism spectrum disorder and other neurobehavioural comorbidities in rare disorders of the Ras/MAPK pathway. *Dev Med Child Neurol*.
12. Chen J, Alberts I, Li X (2014) Dysregulation of the IGF-I/PI3K/AKT/mTOR signaling pathway in autism spectrum disorders. *Int J Dev Neurosci Off J* 35: 35-41.
13. Smalley SL (1998) Autism and tuberous sclerosis. *J Autism Dev Disord* 28(5): 407-414.
14. Marui T, Hashimoto O, Nanba E, Kato C, Tochigi M, et al. (2004) Association between the neurofibromatosis-1 (NF1) locus and autism in the Japanese population. *Am J Med Genet Part B Neuro psychiatr Genet* 131B (1): 43-47.
15. Goffin A, Hoefsloot LH, Bosgoed E, Swillen A, Fryns JP (2001) PTEN mutation in a family with Cowden syndrome and autism. *Am J Med Genet* 105(6): 521-524.
16. Laplante M, Sabatini DM (2009) mTOR signaling at a glance. *J Cell Sci* 122(20): 3589-3594.
17. Sawicka K, Zukin RS (2012) Dysregulation of mTOR signaling in neuropsychiatric disorders: therapeutic implications. *Neuropsychopharmacol* 37(1): 305-306.
18. Sato A (2016) mTOR, a Potential Target to Treat Autism Spectrum Disorder. *CNS Neurol Disord Drug Targets* 15(5): 533-543.
19. Hérault J, Perrot A, Barthélémy C, Büchler M, Cherpi C, et al. (1993) Possible association of c-Harvey-Ras-1 (HRAS-1) marker with autism. *Psychiatry Res* 46(3): 261-267.
20. Hérault J, Petit E, Martineau J, Perrot A, Lenoir P, et al. (1995) Autism and genetics: clinical approach and association study with two markers of HRAS gene. *Am J Med Genet* 60(4): 276-281.
21. Adviento B, Corbin IL, Widjaja F, Desachy G, Enrique N, et al. (2014) Autism traits in the RASopathies. *J Med Genet* 51(1): 10-20.
22. Rosenberg SS, Spitzer NC (2011) Calcium Signaling in Neuronal Development. *Cold Spring Harb Perspect Biol* -3(10): a004259.
23. Vig M, Kinet J-P (2009) Calcium signaling in immune cells. *Nat Immunol* 10(1): 21-27.
24. Nguyen MHT, Jafri MS (2005) Mitochondrial calcium signaling and energy metabolism. *Ann N Y Acad Sci* 1047: 127-137.
25. Nathanson MH (1994) May Cellular and subcellular calcium signaling in gastrointestinal epithelium. *Gastroenterology* 106(5): 1349-1364.
26. Kumar V, Zhang M-X, Swank MW, Kunz J, Wu G-Y (2005) Regulation of Dendritic Morphogenesis by Ras-PI3K-Akt-mTOR and Ras-MAPK Signaling Pathways. *J Neurosci* 25(49): 11288-11299.
27. Mendoza MC, Er EE, Blenis J (2011) The Ras-ERK and PI3K-mTOR Pathways: Cross-talk and Compensation. *Trends Biochem Sci* 36(6): 320-328.
28. Thomas GM, Huganir RL (2004) MAPK cascade signalling and synaptic plasticity. *Nat Rev Neurosci* 5(3): 173-183.
29. Kitagishi Y, Kobayashi M, Kikuta K, Matsuda S (2012) Roles of PI3K/AKT/GSK3/mTOR Pathway in Cell Signaling of Mental Illnesses. *Depress Res Treat* 752563.
30. Clapham DE (2007) Calcium Signaling. *Cell* 131(6): 1047-1058.