Functional alteration in gating behavior of IP3R channel mediating Calcium Signaling as common biomarker in Autism Spectrum Disorder

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Abstract

Background: Current clinical measures used in diagnosing Autism Spectrum Disorder (ASD) have been adapted to screen core characteristics that range widely in severity. However, even at its most refined, ASD remains a group of developmental disorders that is defined by behavior quality, not by its pathogenesis. Studies on the genetic architecture of ASD and related neurological disorders have brought light to the central role of calcium channelopathies and its effects on calcium signaling pathways rationality implicated in diverse aspects of ASD pathogenesis.

Objective: We propose that depressed function in IP3R-mediated calcium release channels in the ER may be a reliable diagnostic biological marker for monogenic and ‘sporadic’ forms of ASD.

Methods: Functional components of calcium signaling were dissected in ASD and a super-resolution STORM system was used to obtain molecular-resolution optical patch clamp analysis on monogenic ASD models. Fibroblasts derived from skin biopsies of healthy, unaffected individuals, and patients with rare monogenic forms or ‘sporadic’ autism were cultured and monosorted for agonist-evoked calcium signals using a high-throughput FLIPR assay. Human induced pluripotent stem cells (hiPSCs) were generated from primary fibroblasts using the Thomson-Fisher Sendai virus protocol. hiPSCs were differentiated into neuronal progenitors, and measured with UV-activated caged IP3.

Results: Local IP3-mediated calcium signaling was decreased in fibroblasts derived from patients with monogenic forms of ASD when compared to those derived from healthy, control patients. Likewise, IP3-mediated calcium signaling was repeatedly decreased in fibroblasts derived from patients with monogenic or ‘sporadic’ forms of ASD when compared to healthy, control patients. hiPSC-derived neuronal precursors from patient fibroblasts also share this signaling defect.

Conclusion: Our results strongly implicate deregulated calcium signaling in the pathogenesis of ASD and supports IP3-mediated calcium signaling as a diagnostic biological marker for ASD. Furthermore, a high-throughput FLIPR assay may be used as a highly reproducible diagnostic that is able to capture differences in IP3-mediated calcium signaling.

Introduction

Autism Spectrum Disorders (ASD) is a group of neurodevelopmental disorders characterized by impaired social communication and interaction, and repetitive and stereotyped behaviors. Genomic sequencing technology has improved our understanding of ASD, and have pointed towards calcium ion channel variants as a possible mechanism of disease. Calcium signaling is involved in many cellular functions that span multiple physiologic systems. In most cells, intracellular calcium is released by inositol trisphosphate (IP3) receptor calcium ion channels on the endoplasmic reticulum, a major calcium store. This release is spatially and temporally coordinated to elicit specific functions including neuronal excitability, neurotransmitter release, cell secretion, gene expression, and apoptosis.

Figure 1. Single channel gating is altered in ASD. Ca2+ ion signaling is initiated by IP3 from G protein coupled membrane receptor, first at a single channel. It is propagated by Ca2+ within a cluster, then spreads in waves throughout a cell by a positive feedback mechanism that involves diffusion of neighboring Ca2+.

Methods and Materials

FLIPR Ca2+ imaging

Skin fibroblasts were seeded in 96-well plates and loaded with 2 μM of Fluo-8 AM. Cultures were incubated with 100 μM ATP or 1 μM ionomycin in Ca2+-free HBSS in triplicates.

Single-cell Ca2+ imaging

Cells seeded in glass-bottomed dishes were loaded with 4 μM Fluo-8 AM and 1 μM caged IP3 (c-IP3) for 45 min. [Ca2+]i changes were imaged with a 40x oil objective at 30 frames sec−1. A single flash of UV light was used to uncage c-IP3. For local Ca2+ signals, cells were loaded with Ca2+ indicator Cal520, c-IP3, and 10 μM EGTA-AM for an hour. [Ca2+]i changes were imaged using an Apo ThF-100x (NA=1.49) objective at 129 frames sec−1.

Human Induced Pluripotent Stem Cells

Human induced pluripotent stem cells (hiPSCs) were generated from the fibroblasts using the Thomson-Fisher Sendai virus protocol. For the differentiation, hiPSCs form EBs in suspension culture for the first 7 days and then are plated and develop into colonies containing rosette, neuroepithelial cells. At day 16, neural progenitors can be observed in the edge and the rosette-containing colonies are detached and grown in suspension to form neural epithelial sphere.

Discussion

Currently, ASD is diagnosed using subjective, clinical, behavioral assessments that delay diagnosis until at least 2 years. Our project suggests that intracellular calcium signaling is a likely ASD biomarker that can be detected using in vitro high throughput assay techniques. An ROC curve evaluates parameters to separate affected from unaffected individuals for diagnostic purposes. The area under the curve (AUC) suggests that our assay is quite robust in discriminating between syndromic and sporadic ASD samples and controls.

Figure 2. Local IP3-mediated Ca2+ signaling is decreased in FXS and TS

Figure 3. High-throughput FLIPR screen to monitor IP3-mediated Ca2+ signaling changes in response to purinergic activation

Figure 4. IP3-mediated Ca2+ response is significantly depressed across monogenic and sporadic forms of ASD

Figure 5. Step-by-step illustration of iPSC differentiation.

Figure 6. IP3-mediated Ca2+ signaling is decreased in neuronal progenitors from a FXS patient, similar to fibroblasts.

Conclusions

• ASD has no defined biomarkers for diagnostics or novel drug discovery
• In rare forms of monogenic ASD syndromes, a molecular defect in IP3 channel gating is resolved showing all forms have a short flicker open time.
• A high-throughput screen was developed to capture this defect in the monogenic ASD and typical, sporadic ASD samples.
• iPSC-derived neuronal precursors from patient fibroblasts share this signaling defect.
• Therefore, IP3R signaling appears to be at a node in a signaling pathway at which many forms of ASD are unified into a shared defective output.
• ROC curves can distinguish, with high sensitivity and specificity, between syndromic and sporadic ASD samples, which signal similarly in this assay, and neurotypical controls.
• This biomarker may come to be useful as an adjunct diagnostic and potentially in a screen for novel therapeutics for ASD.