Schizophrenia Research 129 (2011) 74–79

Contents lists available at ScienceDirect

Schizophrenia Research

journal homepage: www.elsevier.com/locate/schres

Genetic inactivation of *GSK3α* rescues spine deficits in *Disc1*-L100P mutant mice

Frankie H.F. Lee [a,b], Oksana Kaidanovich-Beilin [c], John C. Roder [c,d], James R. Woodgett [c,d], Albert H.C. Wong [a,b,e,f,*]

[a] Neuroscience Division, Centre for Addiction and Mental Health, 250 College St, Toronto, Ontario, Canada M5T 1R8
[b] Department of Pharmacology, University of Toronto, Toronto, Ontario, Canada M5S 1A8
[c] Samuel Lunenfeld Research Institute, Mount Sinai Hospital, Toronto, Ontario, Canada M5G 1X5
[d] Department of Molecular Genetics, University of Toronto, Toronto, Ontario, Canada M5S 1A8
[e] Department of Psychiatry, University of Toronto, Toronto, Ontario, Canada M5T 1R8
[f] Institute of Medical Sciences, University of Toronto, Toronto, Ontario, Canada M5S 1A8

ARTICLE INFO

Article history:
Received 1 February 2011
Received in revised form 26 March 2011
Accepted 29 March 2011
Available online 17 April 2011

Keywords:
Disrupted-in-schizophrenia 1 (DISC1)
Glycogen synthase kinase 3 (GSK3)
Mutant mice
Neuronal morphology
Spine density

ABSTRACT

Disrupted-in-Schizophrenia 1 (*DISC1*), a strong candidate gene for schizophrenia and other mental disorders, regulates neurodevelopmental processes including neurogenesis, neuronal migration, neurite outgrowth and spine development. Glycogen synthase kinase-3 (GSK3) directly interacts with DISC1 and also plays a role in neurodevelopment. Recently, our group showed that the *Disc1*-L100P mutant protein has reduced interaction with both GSK3α and β. Genetic and pharmacological inhibition of GSK3 activity rescued behavioral abnormalities in *Disc1*-L100P mutant mice. However, the cellular mechanisms mediating these effects of GSK3 inhibition in *Disc1* mutant mice remain unclear. We sought to investigate the effects of genetic inactivation of *GSK3α* on frontal cortical neuron morphology in *Disc1* L100P mutant mice using Golgi staining. We found a significant decrease in dendritic length and surface area in *Disc1*-L100P, *GSK3α* null and L100P/*GSK3α* double mutants. Dendritic spine density was significantly reduced only in *Disc1*-L100P and L100P/*GSK3α* +/− mice when compared to wild-type littermates. There was no difference in dendritic arborization between the various genotypes. No significant rescue in dendritic length and surface area was observed in L100P/*GSK3α* mutants versus L100P mice, but spine density in L100P/*GSK3α* mice was comparable to wild-type. Neurite outgrowth and spine development abnormalities induced by *Disc1* mutation may be partially corrected through *GSK3α* inactivation, which also normalizes behavior. However, many of the other dendritic abnormalities in the *Disc1*-L100P mutant mice were not corrected by *GSK3α* inactivation, suggesting that only some of the anatomical defects have observable behavioral effects. These findings suggest novel treatment approaches for schizophrenia, and identify a histological read-out for testing other therapeutic interventions.

1. Introduction

Schizophrenia (SZ) is a severe and chronic psychiatric disorder characterized by psychotic, negative and cognitive symptoms (Wong and Van Tol, 2003; Tamminga and Holcomb, 2005). There is substantial evidence that SZ is a neurodevelopmental disorder with many genes affecting susceptibility (Wong and Van Tol, 2003; Ross et al., 2006). Disrupted-in-Schizophrenia 1 (*DISC1*) is a prominent risk gene, first identified in a large Scottish family with a balanced chromosomal translocation (1q42.1:11q14.3) co-segregating with major mental illnesses including SZ, bipolar disorder and major depression (Millar et al., 2000; Blackwood et al., 2001). The *DISC1* locus shows genetic linkage with SZ and polymorphic variants in the *DISC1* gene are associated with SZ (Nakata et al., 2009; Rastogi et al., 2009; Schumacher et al., 2009). DISC1 acts as a scaffold protein that interacts with and regulates many other proteins that are involved in cytoskeletal structure and signaling. Thus, DISC1 is involved in brain development and functions such as neurogenesis, neuron migration, neurite outgrowth, spine development and neurotransmitter signaling (Camargo et al., 2007; Brandon et al., 2009).

Glycogen synthase kinase 3β (GSK3β) has been shown to regulate neuronal proliferation (Kim et al., 2009), and it also interacts with DISC1 (Mao et al., 2009; Lipina et al., 2010). GSK3 is a highly-conserved serine/threonine kinase expressed in two paralogous proteins (GSK3α and β) that share similar DNA sequence (Woodgett, 1990). GSK3 activity is inhibited by several mechanisms, such as phosphorylation, complex formation and cellular translocation (Frame and Cohen, 2001; Jope and Roh, 2006; Kockeritz et al.,

* Corresponding author at: Neuroscience Division, Centre for Addiction and Mental Health, 250 College Street, Room 711, Toronto, ON, M5T 1R8. Tel.: +1 416 535 8501x4010 (office).
E-mail address: albert.wong@utoronto.ca (A.H.C. Wong).

0920-9964/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.schres.2011.03.032

2006). GSK3 is widely expressed in the brain (Perez-Costas et al., 2010) and as part of a large molecular network has key roles in many neurodevelopmental processes including neurogenesis, neuron growth and synaptic plasticity (Zhou et al., 2004; Kim and Kimmel, 2006; Peineau et al., 2008; Kim et al., 2009; Mao et al., 2009; Hur and Zhou, 2010; Lange et al., 2011).

Accumulating evidence implicates disruption of GSK3 signaling in psychiatric disorders. Genetic studies have demonstrated that *GSK3* polymorphisms are associated with SZ (Souza et al., 2008; Benedetti et al., 2010). Multiple susceptibility genes for SZ including *DISC1*, neuregulin-1 (*NRG1*) and *ERBb4* have been shown to affect GSK3 regulators such as Akt, or pathways downstream from GSK3 such as β-catenin and the canonical Wnt signaling pathway (Koros and Dorner-Ciossek, 2007; Lovestone et al., 2007; Freyberg et al., 2010). Post-mortem brain studies of patients with SZ show reduced GSK3β phosphorylation, decreased Akt protein, and β- and γ-catenin expression (Cotter et al., 1998; Emamian et al., 2004). The GSK3 pathway is also targeted by a number of important drugs used in psychiatry, such as lithium, valproic acid and antipsychotics such as haloperidol and clozapine, which provides further evidence that GSK3 is important for understanding mental illnesses (Kang et al., 2004; Bibb, 2005; Li et al., 2007; Rosenberg, 2007; Beaulieu et al., 2008; Li and Jope, 2010).

Since the N-terminus of DISC1 can directly interact with and suppress GSK3β activity and also reduce signaling downstream through the Wnt/β-catenin pathway, a change in DISC1 expression may affect neurodevelopment through GSK3. Indeed, recent studies have reported a direct interaction between DISC1, GSK3β (Mao et al., 2009) and the Akt-binding partner Girdin (Enomoto et al., 2009) affecting neurogenesis. Furthermore, administration of a GSK3 inhibitor, SB216763 rescued the behavioral deficits in mice with reduced DISC1 expression in the dentate gyrus of the hippocampus of adult animals (Mao et al., 2009). These data support a role for DISC1 in modulating the multiple cellular functions of the GSK3 signaling pathway.

Our group previously described a mouse carrying a single point mutation in *Disc1* (*Disc1*-L100P) with abnormalities related to SZ, including pronounced deficits in prepulse inhibition (PPI), latent inhibition (LI) and working memory. Their behavioral phenotype was reversed by using antipsychotic drugs (Clapcote et al., 2007), as well as by administration of TDZD-8 (a GSK3 inhibitor) (Lipina et al., 2010). The morphology of frontal cortical pyramidal neurons from these mice is also abnormal, with reduced dendritic length, dendrite surface area and spine density (Lee et al., 2011). Moreover, genetic deletion of one allele of *GSK3α* was equally effective in rescuing abnormal *Disc1*-L100P behaviors (Lipina et al., 2010). Therefore, we sought to investigate whether the beneficial effect of *GSK3α* gene deletion on *Disc1*-L100P behaviors is associated with changes in neuronal morphology. Our findings confirmed the morphological deficits previously reported in *Disc1*-L100P mutants and we also found similar abnormalities in *GSK3α* null mice. Genetic inactivation of *GSK3α* in *Disc1*-L100P mice significantly rescued spine density, but had no effect on dendritic length, surface area and arborization. These results show that GSK3α is involved in neurite outgrowth and spine development, since inactivation of *GSK3α* can partially restore some deleterious effects of the *Disc1* mutation. However, the precise relationship between these two molecules in regulating dendritic development and morphology remains unclear. Further studies are required to understand how specific components of the complex GSK3 signaling cascade may interact with other important disease genes to mediate neural development.

2. Materials and methods

2.1. Mice

ENU-mutagenized *Disc1*-L100P homozygous (−/−), GSK3α null (−/−) mutant mice and wild-type (WT) littermates on a C57BL/6 background were generated as previously described (Clapcote et al., 2007; Kaidanovich-Beilin et al., 2009). Similarly, *GSK3α* heterozygous (+/−) mice were backcrossed to C57BL/6 mice and bred with *Disc1*-L100P mutants. The resultant offspring were then intercrossed to obtain WT and *Disc1*-L100P −/− with either none or one copy of the *GSK3α* gene (Lipina et al., 2010). Mice of both sexes for each group were used for all analyses. All animal protocols were approved by the TCP Animal Care Committee.

2.2. Golgi-Cox staining

Golgi-Cox staining was performed as previously described (Gibb and Kolb, 1998). In summary, adult mice (age 6–8 weeks) were anesthetized with xylazene/ketamine (10ml/kg) and intracardially perfused with 0.9% saline. Brains were removed and immersed in Golgi-Cox solution in the dark for 14 days before transferring to 30% sucrose solution for 5 days. Sections of 200 μm were sliced using a microtome (Leica VT1000S, Germany) and were placed on 2% gelatinized microscope slides. The slides were stored in a humidified chamber for 3 days prior to further staining and fixation.

2.3. Neuron morphology and dendritic spines

For morphometric analyses of individual neurons, Golgi images at 40× magnification were captured under bright-field illumination with a Nikon Eclipse E600 microscope. Neurons were chosen based on the following criteria: (i) fully visible and showing clear, distinct morphology, (ii) all dendrites visible within the 40× magnification field, and (iii) pyramidal neurons in layers III and V of the frontal cortex, as demarcated in the Golgi Atlas Of The Postnatal Mouse Brain (Valverde, 1998). 15 neurons from 4 to 6 mice per group were randomly selected for analysis of dendritic length, surface area and arborization. A z-stack of different focal lengths was generated for each neuron to capture the three-dimensional dendritic branching tree in different planes. Acquisition parameters were kept the same for all images. The neurites of each neuron were traced, and the length and surface area were estimated using Neuromantic software (http://www.rdg.ac.uk/neuromantic). All parameters were then normalized to soma surface area for comparison.

Sholl analysis provides a quantitative measure of the radial distribution of neuronal dendritic arborization (Sholl, 1953). Using ImageJ, we created 15 concentric circles (each with 8 μm larger radius than the previous circle) centered at the perikaryon and then counted the number of dendritic intersections with each. The log of the number of intersections per circle area versus the circle radius was plotted (the semi-log Sholl method). The slope of the regression line (κ = Sholl regression coefficient) is a measure of the decay rate of the number of branches with increasing distance from the soma (Sholl, 1953). The Schoenen ramification index, R_I (maximum number of intersections/number of primary dendrites), a measure of the ramification richness for each neuron (Schoenen, 1982) also provides an indicator of the degree of dendritic branching complexity.

Dendritic spine density was measured with Golgi-stained images captured at 100× magnification (Nikon Eclipse E600). 45 neurons from 4 to 6 mice per group were selected for spine analysis. Spines were counted only on the apical dendrites of pyramidal neurons in layers III and V of frontal cortex. Spine density was expressed as the number of spines per dendritic length (μm). All images for quantification were blinded prior to analysis.

2.4. Statistical analysis

All parameters were analyzed by comparing the average values for each group of mice sharing the same genotype. Statistical differences among WT, *Disc1*-L100P, L100P/*GSK3α* +/− and L100P/*GSK3α* −/− were assessed using one-way ANOVA (SPSS 13.0), followed by the