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Research report

Interaction between DISC1 and CHL1 in regulation of neurite outgrowth

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ABSTRACT

Disrupted-in-schizophrenia 1 (*DISC1*), a gene susceptible for major mental illnesses, including schizophrenia, plays multiple roles in neural development, including neuronal proliferation, maturation, migration and neurite outgrowth. DISC1 regulates neurite length *via* interaction with several intracellular proteins, such as NDEL1, FEZ1 and dysbindin. However, the signal transduction mechanism upstream of DISC1 in regulating neurite outgrowth remains to be elucidated. Here we show that DISC1 interacts with the intracellular domain of close homolog of L1 (CHL1), a member of the L1 family of neural cell adhesion molecules. DISC1 and CHL1 proteins co-localize in growth cones of cortical neurons. Moreover, in neurite outgrowth assay, CHL1 rescues the inhibitory effect of DISC1 on the initial phase of neurite outgrowth. Considering the fact that CHL1 also plays crucial roles in neural development, and its coding gene is associated with schizophrenia, our findings indicate that DISC1 and CHL1 may engage in physical and functional interaction in neural development, supporting the notion that DISC1 regulates neurite outgrowth with a receptor belonging to the neural cell adhesion molecules, and disruption of such interaction may contribute to increased risk for schizophrenia.

1. Introduction

Schizophrenia is one of the most devastating mental disorders marked by hallucinations, delusions, impaired cognition and poor emotional responses. It affects around 1% of the world's population (Cardno and Gottesman, 2000). Given the fact that the heritability of this multi-factorial disorder is up to 80%, researchers have been focused on identifying genetic variations associated with schizophrenia (Sullivan et al., 2003). It has been shown that many genes associated with schizophrenia participate in neural development such as neuronal proliferation, migration, neurite outgrowth, synaptic formation and myelination (Lewis and Levitt, 2002; Stefansson et al., 2008; Walsh et al., 2008). However, the pathogenesis of schizophrenia at the molecular level still needs further investigations.

The discovery of schizophrenia-associated gene *DISC1* (*disrupted-in-schizophrenia 1*) has provided us an opportunity to investigate the link of neural development to this disease (St Clair et al., 1990). The *DISC1* gene was originally identified in a Scottish pedigree with multiple mental disorders including schizophrenia (Hashimoto et al., 2006; Millar et al., 2000; Sachs et al., 2005). As a scaffold protein, DISC1 plays crucial roles in multiple processes during neural development (Duan et al., 2007; Ishizuka et al., 2011; Kamiya et al., 2006; Kang et al., 2011; Kim et al., 2009; Millar et al., 2005; Schurov et al., 2004). Dysfunction or mutations of *DISC1* in several mouse models lead to abnormalities in neuronal morphology and neurite length (Kamiya et al., 2006; Kim et al., 2012; Lee et al., 2015; Niwa et al., 2010; Shinoda et al., 2007). Consistent with these findings, DISC1 regulates neurite length *via* interaction with several intracellular proteins, such as NDEL1, FEZ1 and dysbindin (Kamiya et al., 2006; Kang et al., 2011; Lee et al., 2015). However, cell surface proteins that interact with DISC1 in regulating neural development remains to be elucidated.

CHL1 is a transmembrane protein belonging to the L1 family of cell adhesion molecules (CAMs) (Chen et al., 1999). Its coding gene in *Homo sapiens*, *CALL*, has been associated with 3p-syndrome and schizophrenia (Chen et al., 2005; Frints et al., 2003; Montag-Sallaz et al., 2002; Sakurai et al., 2002; Wei et al., 1998). As an adhesion molecule, CHL1 regulates axon guidance, neurite outgrowth and dendrite orientation in the developing brain (Demyanenko et al., 2004; Hitt et al., 2012; Katic et al., 2014). Several studies concerning its function in neurite outgrowth indicate that CHL1 can promote neurite outgrowth as a

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substrate, inhibit neurite elongation *via* homophilic binding, or exert diverse effects *via* heterophilic interactions with other extracellular matrix (ECM) proteins (Buhusi et al., 2003; Demyanenko et al., 2004; Hillenbrand et al., 1999; Jakovcevski et al., 2007; Jakovcevski et al., 2009; Katic et al., 2014). CHL1 also serves as a receptor for integrins and growth factors to mediate morphogenetic events during neural development (Buhusi et al., 2003; Wright et al., 2007).

A few studies have revealed the similar spatiotemporal expression and function of DISC1 and CHL1 in neural development. DISC1 is expressed at a high level in the developing brain from embryonic day 10 (E10) until 6 months of age, preferentially in hippocampus, cerebral cortex and olfactory bulbs and peaks between E13.5 and postnatal day 35 (P35) (Schurov et al., 2004). In lysates of the mouse forebrain, CHL1 becomes weakly detectable from E13, and peaks from E18 to P7 (Hillenbrand et al., 1999). DISC1 shows multiple intracellular distributions in the cytoplasm, including the centrosome and microtubule fractions, the dendrites, the actin cytoskeletal fractions, and the neurite growth cone (Hayashi-Takagi et al., 2010; Miyoshi et al., 2003; Morris et al., 2003). CHL1 also has multiple localizations, such as cell body, dendrite, axon and growth cone (Hillenbrand et al., 1999; Leshchyns'ka et al., 2006; Schlatter et al., 2008). In this study, we provide evidence supporting the existence of physical interaction between DISC1 and CHL1 and their functional collaboration in neurite outgrowth.

2. Results

2.1. DISC1 interacts with CHL1

The highly overlapped expression pattern, their effects on neural development and their association with schizophrenia raise the probability of a functional linkage between DISC1 and CHL1. To investigate the possible interaction between these two proteins, we generated two recombinant constructs encoding a Myc-tagged human DISC1 (DISC1-Myc) and an HA-tagged mouse CHL1 (CHL1-HA), respectively. Co-immunoprecipitation assay was performed using transfected HEK293T cells. Cells overexpressing either DISC1-Myc, CHL1-HA, or both proteins were lysed and immunoprecipitated with an anti-HA antibody. The precipitated samples were detected using immunoblotting. DISC1-Myc was detected in the precipitates from DISC1-Myc/CHL1-HA co-expressing cells, but not from mock- or single-transfected cells (Fig. 1A). Reciprocal co-immunoprecipitations were carried out, in which CHL1-HA was detected in immunoprecipitates using *anti*-Myc to pull down DISC1-Myc (Fig. 1A). To determine the interacting domain of CHL1 and DISC1, we made a construct of mutant CHL1 with the transmembrane domain intact and the intracellular domain deleted (CHL1^{Mut}) (Fig. 1B, D). When HEK293T cells were co-transfected with DISC1 and CHL1^{Mut}, neither DISC1 nor CHL1^{Mut} could be detected in the co-immunoprecipitation experiments (Fig. 1C), indicating that DISC1 and CHL1 proteins engage in physical interaction *via* the intracellular domain of CHL1.

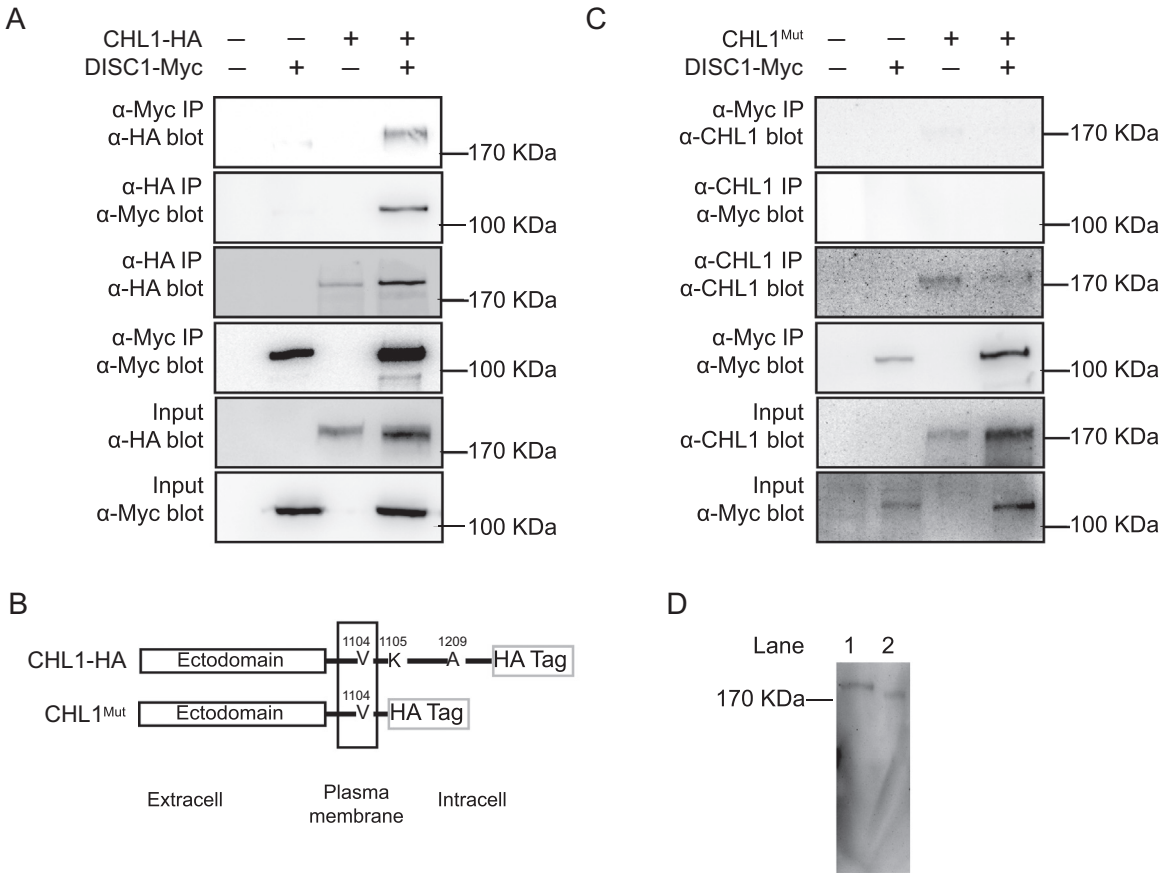

Fig. 1. Interaction of DISC1-Myc and CHL1-HA in HEK293T cells. A. HEK293T cells were transfected with either DISC1-Myc, CHL1-HA, or both, and immunoprecipitated with *anti*-Myc or anti-HA antibodies. Immunoblotting detected DISC1-Myc and CHL1-HA in the precipitates from co-expressing cells. IP, immunoprecipitation. B. Schematic representation of CHL1-HA and CHL1^{Mut} constructs. CHL1^{Mut}, mouse CHL1 without the intracellular domain. C. Neither DISC1-Myc nor CHL1^{Mut} was detected in the immunoprecipitates from co-transfected HEK293T cell lysates. D. Immunoblotting showed different molecule weights of CHL1-HA (lane 1) and CHL1^{Mut} (lane 2) using anti-CHL1 antibody.