Interactions of human truncated DISC1 proteins: implications for schizophrenia

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Numerous genetic linkage and association reports have implicated the Disrupted-in-Schizophrenia (DISC1) gene in psychiatric illness. The Scottish family translocation, predicted to encode a C-terminus-truncated protein, suggests involvement of short isoforms in the pathophysiology of mental disorders. We recently reported complex alternative splicing patterns for the DISC1 gene and found that short isoforms are overexpressed in the brains of patients with schizophrenia and in carriers of risk-associated alleles. Investigation into the protein–protein interactions of alternative DISC1 isoforms may provide information about the functional consequences of overexpression of truncated forms in mental illness. Human embryonic kidney (HEK293) cells were transiently co-transfected with human epitope-tagged DISC1 variants and epitope-tagged NDEL1, FEZ1, GSK3β and PDE4B constructs. Co-immunoprecipitation assays demonstrated that all truncated DISC1 variants formed complexes with full-length DISC1. Short DISC1 splice variants L.A78, L.A3 and Esv1 showed reduced or no binding to NDEL1 and PDE4B proteins, but fully interacted with FEZ1 and GSK3β. The temporal expression pattern of GSK3β in the human postmortem tissue across the lifespan closely resembled that of the truncated DISC1 variants, suggesting the possibility of interactions between these proteins in the human brain. Our results suggest that complexes of full-length DISC1 with truncated DISC1 variants may result in cellular disturbances critical to DISC1 function.

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Introduction

The Disrupted-in-Schizophrenia 1 (DISC1) gene has been linked to schizophrenia (SZ) and psychiatric phenotypes in a multitude of genetic linkage and association studies. The original finding arose from the discovery of a balanced chromosomal translocation (1;11) in a Scottish family with a history of mental illness. This translocation is suggested to lead to truncation of the C-terminus portion of the DISC1 protein. DISC1 acts as a scaffolding protein, forming complexes with proteins involved in neurodevelopment, centrosome and microtubule orientation, signal transduction and neurite outgrowth. Thus, truncation of DISC1 may affect cellular function, alter brain development and result in behavioral changes.

The biological consequences of DISC1 truncation and their implications for susceptibility to SZ have been examined in a number of previous studies using various hypothetical constructs. In cell culture, a truncated mutant DISC1 modifies the cellular distribution from a punctate perinuclear pattern to diffuse cytoplasmic expression. Subcellular fractionation shows enrichment of short DISC1 protein in the cell nucleus of the orbitofrontal cortex of patients with SZ and bipolar disorder. Expression of the mutant DISC1 in PC-12 cells decreases extension and overall neurite number. Furthermore, animal models have simulated the Scottish family translocation. The behavioral abnormalities of transgenic mice with DISC1 mutations are reminiscent of aspects of SZ and/or other neuropsychiatric disorders.

We recently demonstrated that the human DISC1 gene encodes a number of isoforms that are translated into truncated DISC1 proteins. Importantly, the expression of these alternatively spliced transcripts is increased in the hippocampus of patients with SZ and in individuals carrying alleles associated with increased risk for mental disorders. Moreover, the truncated variants show preferential expression in the fetal cerebral cortex compared with other postnatal ages, suggesting their role in the developing brain.

This is the first study to investigate interactions of DISC1 splice variants confirmed to be enriched in patients with SZ. We examined their interactions with full-length DISC1 and with binding partners that regulate critical developmental processes, including neurite architecture, centrosome and microtubule positioning, as well as cAMP signaling. FEZ1, NDEL1, PDE4B and GSK3β were selected because of their cellular function importance and thorough binding-site characterization, as well as their involvement in SZ. We hypothesized that binding of these partners to the short DISC1 isoforms may be altered because of the absence of interaction sites or changes in three-dimensional structures of novel variants as compared with the full-length DISC1. Conversely, if short transcripts do retain binding capabilities, their overexpression in the brains of subjects who develop SZ may lead to inappropriate activation of signaling processes.
We also examined whether short variants have the ability to form complexes with the full-length DISC1, hypothesizing that if they do, they may interfere with the formation of full-length homodimers/multimers important for binding with NDE1 and other partners.14

A complex DISC1 interaction network has been proposed from yeast two-hybrid studies,2,15 and cell lines have been manipulated with truncated versions of mutant DISC1 to delineate protein interactors. We found that all truncated DISC1 isoforms, Esv1, LA3, LA78T9 and LA78T10, bind to full-length DISC1 as well as to FEZ1 and GSK3β, whereas interactions with other partners are weakened. Coexpression analysis suggests that GSK3β may be a binding target for the truncated DISC1 transcripts overexpressed in the brains of patients with SZ. These data highlight the role of DISC1 splice variants in forming unique protein complexes and suggest the mechanisms by which they may confer risk for mental illness.

Materials and methods

Transfection of cultured HEK293 cells with plasmid DISC1 cDNA variants and NDE1, PDE4B, GSK3β and FEZ1. The human embryonic kidney (HEK293) cell line was cultured at 6 × 10^5 cells per well 24 h before transfection. DISC1 cDNA variants were subcloned into the mammalian expression vector pCMV-SC-NM containing an N-terminal Myc or FLAG tag for NDE1 (transcript variant 1; NM_001025579.1), PDE4B1 (isoform 1; NM_002660.3), GSK3β isoform1; NM_002093.3), FEZ1 (transcript variant 1; NM_005103.4) and DISC1–DISC1 association (Stratagene, La Jolla, CA, USA). Previously characterized splice variants DISC1L (exons 1–13), Lv (exons 1–13), S and novel variants Esv1 (insertion at exon 3; accession ID FJ804213), LA78T9 (deletion of exons 7 and 8, termination at exon 9; accession ID FJ804202), LA78T10 (termination at exon 10; accession ID FJ804188) and LA3 (deletion of exon 3; accession ID: FJ804212) were transfected (Figure 1a). Cells were sonicated and incubated on ice for 15 min. Protein concentration was assayed by the Bradford method.

Immunoprecipitation. Lysates (100 μg protein) were incubated with anti-myc antibodies (2 μg per sample; Millipore, Billerica, MA, USA) overnight at 4 °C. Immunocomplexes were recovered by incubating with Protein G-Plus agarose beads (25 μl per sample; Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA) for 2 h at 4 °C. Immunoprecipitates were isolated through centrifugation (14,000 × g for 2 min at 4 °C) and aspiration of the supernatant from the pellet. Bead pellets were washed three to four times with the lysis buffer (T-PER, Thermo Fisher Scientific, Rockford, IL, USA, containing 25 mM bicine, 150 mM sodium chloride; pH 7.6), centrifuged and resuspended in sample buffer.

Western blot analysis. Immunoblotting was performed using 20 μg of protein per sample or 10 μl immunoprecipitant in 4 × lithium dodecyl sulfate (LDS) sample buffer containing β-mercaptoethanol. Samples were resolved on NuPage 4–12% Bis-Tris gels (Invitrogen, Carlsbad, CA, USA), transferred to nitrocellulose membranes and blocked in Tris-buffered saline containing 0.05% Tween 20 and 5% nonfat dry milk for 1 h. For detection of c-Myc and FLAG fusion proteins, an anti-Myc or FLAG antibody conjugated to horseradish peroxidase was diluted in TBSTM (Tris-buffered saline (TBS) containing 0.05% Tween-20 and 5% nonfat dry milk) at a 1:5000 concentration and incubated overnight at 4 °C. Blots were washed and developed using chemiluminescence. Densitometry of bands was quantitated using ImageJ (NIH, Bethesda, MD, USA), an open-source image program (available at http://imagej.nih.gov/ij/download.html).

Tissue samples. Postmortem human brains obtained from non-psychiatric controls were collected at the Clinical Brain Disorders Branch (NIMH), and at the Brain and Tissue Bank for Developmental Disorders of the National Institute of Child Health and Human Development under protocols 90-M-0142, NO1-HD-4-3368 and NO1-HD-4-3383. Toxicological analysis was performed on every case. Subjects with evidence of macroscopic or microscopic neuropathology, drug use, alcohol abuse or psychiatric illness were excluded, as described previously.16 Postmortem tissue homogenates of the prefrontal cortex (dorsolateral prefrontal cortex, BA46/9) were obtained from 272 normal controls (149 African American, 113 Caucasian, 6 Hispanic and 4 Asian) spanning human aging from gestational age 14–20 weeks (n = 38) and from day 1 after birth to 78 years (n = 234). Total RNA was extracted, reverse transcribed with oligo dT, T7 amplified and labeled with the Cy3 fluorescent dye. Reference RNA was...
pooled from all samples treated identically to sample RNAs, and was labeled with the Cy5 fluorescent dye.

**Illumina microarrays.** Two-color custom-spotted arrays from the NHGRI microarray core facility with the Illumina Oligoset (Illumina, San Diego, CA, USA; HEEBO7) were used. After scanning using an Agilent scanner (Agilent, Santa Clara, CA, USA), DeArray software (Fairfax, VA, USA; http://www.scanalytics.com) was used to export intensity data. After background correction on the linear scale, log2 ratios (sample/reference) were normalized across mean log2 fluorescent intensities using loess correction. After normalization, log2 ratios were further adjusted to reduce the impact of known and unknown sources of systematic noise on gene expression measures using surrogate variable analysis. The positions of probes used in analysis were as indicated: FEZ1 (ID 34517) chr11: 124821043–1248211123, GSK3β (ID 24272) chr3:121065012–121065081, NDEL1 (ID 5747) chr17: 8279903–8312206 and PDE4B (ID 5298) chr1: 66030780–66612850.
Results

Self-binding of DISC1 variants. To investigate DISC1 self-association propensity of short variants with full-length DISC1, myc-tagged full-length DISC1 (L) was cotransfected with FLAG-tagged short variants. All short isoforms bind with full-length DISC1, indicated by the bands present at expected sizes after immunoprecipitation (Figure 1b). Inputs of cell lysates were used as positive controls confirming appropriate molecular weight of the bands and demonstrating comparable protein levels within the experiment (Figure 1c). Quantification of the immunoprecipitated bands compared with input revealed no statistical differences in isoform-binding abilities to full-length DISC1L (Figure 1f). This suggests that the protein self-association domain is in the region not altered during splicing or that novel self-association sites are present in these short isoforms.

Binding of DISC1 variants to developmentally important proteins. To determine whether products of DISC1 alternative splicing interact with the DISC1-binding partners, FEZ1, GSK3β, NDEL1 and PDE4B, containing an N-terminus FLAG epitope tag, were coexpressed with known (L, Lv, S) and novel DISC1 splice variants (Esv1, Δ78T9, Δ78T10 and LA3) containing a myc-tag at the N-terminus. Only L and Lv isoforms bound NDEL1 (first and second lanes, third immunoblot, Figure 1d), suggesting that this binding requires regions at the carboxyl terminus. DISC1 Esv1 and DISC1 Δ78T9 weakly bound PDE4B, as indicated by a substantial reduction in band intensity (third and fifth lanes, bottom, Figure 1d; quantification in 1g). A conformational change or a lack of a critical protein fragment may contribute to the decreased PDE4B/DISC1 interaction. In contrast, all DISC1 protein isoforms retained the ability to co-immunoprecipitate with FEZ1 and GSK3β (top and second immunoblots, Figure 1d), as indicated by strong bands at 67 and 47kDa respectively.

Developmental expression patterns in the human brain. To examine whether truncated DISC1 variants are temporally coexpressed with FEZ1, NDEL1, PDE4B and GSK3β, expression levels were assessed across the lifespan in the prefrontal cortex of non-psychiatric individuals. FEZ1, NDEL1 and PDE4B were expressed at low levels during the fetal period, followed by a gradual increase in expression and a peak at young adulthood (Figures 2a, c and d). Their expression was negatively correlated with expression of the short DISC1 transcripts \( r \) ranging from \(-0.18\) to \(-0.51, P < 0.001\). In contrast, GSK3β, which bound all short DISC1 isoforms in the HEK293 cell system, was highly expressed during fetal age and declined slowly after birth (Figure 2b). The expression of GSK3β across the lifespan was significantly and positively correlated with all short DISC1 isoforms: DISC1 Δ78 (\( r = 0.37, \ P = 9 \times 10^{-5} \)), LA3 (\( r = 0.28, \ P = 2 \times 10^{-5} \)) and Esv1 (\( r = 0.2, \ P = 0.001 \)). These results suggest that GSK3β has the potential to interact with the truncated variants of DISC1, in particular during early development when they are both expressed at relatively high levels.

Figure 2 mRNA expression of DISC1-binding partners across the lifespan. Expression of FEZ1 (a), GSK3β (b), NDEL1 (c) and PDE4B (d) mRNA was measured in the human dorsolateral prefrontal cortex across the lifespan using custom-spotted Illumina microarrays. Each dot indicates the expression level of an individual subject reported as log2 (sample/reference). The x axis is labeled in fetal weeks, (14–20) followed by postnatal life in years (0–80). The expression trajectory (blue line) represents loess fitting of the expression data.
Discussion

We examined whether short transcript variants of DISC1 bound full-length DISC1 and several of the proteins that are integral to DISC1 function and neurodevelopment. Surprisingly, we found that all short variants bind to full-length DISC1, although self-association motifs are believed to be located around amino acids 403–504, which are absent in L\(^2\)3 and Esv1 variants (Figure 3). As previous in vitro studies suggest that coexpression of truncated and full-length DISC1 results in aberrant function, including dysfunctional subcellular localization and centrosomal orientation,\(^{10}\) it is possible that sequestering of full-length DISC1 by short variants overexpressed in patients might be a mechanism by which these proteins exert their pathological role in psychiatric disorders.

Interactions of the truncated DISC1 variants with other proteins appear to be complex and sometimes inconsistent with our predictions. For instance, our data suggest that DISC1 splice variants bind FEZ1, although a known FEZ1-binding domain (amino acids 446–633) is missing in all truncated DISC1 variants (Figure 3).\(^{9}\) On the other hand, interactions with PDE4B are altered although the N-terminus domain where PDE4B binds to DISC1 should be preserved in truncated DISC1 variants.\(^{22}\) Perhaps other binding sites are sufficient in the case of FEZ1 or conformational changes in the truncated proteins as compared with full-length DISC1 after binding to PDE4B. Although all short DISC1 variants fully bind GSK3β, as predicted from the binding domain in the intact DISC1 protein, the functionality of these interactions is unknown. It is possible that GSK3β binding with the short DISC1 isoforms may lead to changes in the activation of the GSK3β/catenin signaling pathway.\(^{23}\) As expected from previous reports, the DISC1–NUDEL1 interaction occurs only with the L and L\(^2\)v variants.\(^{4}\)

Existing evidence of altered protein and/or transcript levels of NDE1, GSK3β, FEZ1 and PDE4B in patients with SZ strongly suggests involvement of these proteins/pathways in the pathophysiology of SZ.\(^{11–13}\) Previously, we demonstrated that the expression of DISC1 variants is higher in fetal life compared with the postnatal period,\(^{6}\) emphasizing their neurodevelopmental importance. After determining that short DISC1 isoforms are capable to form protein complexes, we speculated that they may interact with critical proteins during human brain development. Examination of GSK3β expression pattern in the postmortem human brain shows that it closely resembles that of truncated DISC1 variants, and thus raises the possibility that GSK3β is capable of forming a partnership with DISC1 to affect cellular signaling during development. Co-immunoprecipitation experiments in mouse embryos for GSK3β–DISC1 have already proposed that such interactions are temporally dependent.\(^{19}\) Perhaps enrichment of short isoforms in SZ, expressed in parallel with GSK3β, may interfere with the GSK3β pathway in development.

In summary, our results raise the possibility that truncated DISC1 proteins, enriched in patients with SZ and carriers of DISC1 risk alleles, might exert their pathological effects through their binding to full-length DISC1 and other neurodevelopmentally important molecules.

Conflict of interest

The authors declare no conflict of interest.

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