DISC1 Regulates New Neuron Development in the Adult Brain via Modulation of AKT-mTOR Signaling through KIAA1212

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SUMMARY
Disrupted-in-schizophrenia 1 (DISC1), a susceptibility gene for major mental illnesses, regulates multiple aspects of embryonic and adult neurogenesis. Here, we show that DISC1 suppression in newborn neurons of the adult hippocampus leads to overactivated signaling of AKT, another schizophrenia susceptibility gene. Mechanistically, DISC1 directly interacts with KIAA1212, an AKT binding partner that enhances AKT signaling in the absence of DISC1, and DISC1 binding to KIAA1212 prevents AKT activation in vitro. Functionally, multiple genetic manipulations to enhance AKT signaling in adult-born neurons in vivo exhibit similar defects as DISC1 suppression in neuronal development that can be rescued by pharmacological inhibition of mammalian target of rapamycin (mTOR), an AKT downstream effector. Our study identifies the AKT-mTOR signaling pathway as a critical DISC1 target in regulating neuronal development and provides a framework for understanding how multiple susceptibility genes may functionally converge onto a common pathway in contributing to the etiology of certain psychiatric disorders.

INTRODUCTION
Neurogenesis in the dentate gyrus of the hippocampus begins during the embryonic stage and continues throughout life (Ming and Song, 2005; Zhao et al., 2008). Newborn dentate granule cells become integrated into the hippocampal neuronal circuitry through a stereotypic process, including neuronal morphogenesis, migration, axon/dendritic growth, and synapse formation (Duan et al., 2008; Ge et al., 2008). One distinct feature of adult hippocampal neurogenesis is its prolonged course of development by new neurons, which is subjected to prominent activity-dependent regulation (Duan et al., 2008). For example, neuronal activation through seizures accelerates the tempo of adult neurogenesis (Overstreet-Wadiche et al., 2006), and prolonged seizures induce inappropriate integration of new neurons into the adult hippocampal circuitry (Jessberger et al., 2007). The speed of new neuron development during adult neurogenesis also appears to be crucial for spatial memory in rodents (Farioli-Veccchioli et al., 2008). While these and other studies have implicated the importance of tempo regulation in adult neurogenesis, the underlying molecular mechanism is not well understood.

Our previous studies have revealed a key role of disrupted-in-schizophrenia 1 (DISC1) in the tempo regulation of multiple developmental steps during adult neurogenesis (Duan et al., 2007; Faulkner et al., 2008). DISC1 was originally identified at the breakpoint of a balanced (1;11) (q42;q14) chromosome translocation that cosegregates with schizophrenia, bipolar disorder, and recurrent major depression in a large Scottish family (Blackwood et al., 2001; Millar et al., 2000). Genetic lineage and association studies have further suggested DISC1 as a general risk factor for schizophrenia, schizoaffective disorder, bipolar disorder, and recurrent major depression in a large Scottish family (Blackwood et al., 2001; Millar et al., 2000). Genetic lineage and association studies have further suggested DISC1 as a general risk factor for schizophrenia, schizoaffective disorder, bipolar disorder, major depression, autism, and Asperger syndrome (Chubb et al., 2008). How DISC1 dysfunction contributes to a wide spectrum of psychiatric and mental disorders remains unknown (Hennah and Porteous, 2009). Certain schizophrenia and/or depression-related phenotypes have been observed in behavioral analysis of mice with DISC1 dysfunction, including missense mutations (Clapcote et al., 2007), overexpression of truncated forms (Hikida et al., 2007; Li et al., 2007; Pletnikov et al., 2008; Shen et al., 2008), deletion of certain isoforms (Ishizuka et al., 2007; Koike et al., 2006; Kivo et al., 2008), and lentivirus-mediated expression of short-hairpin RNA (shRNA) against Disc1 in the adult dentate gyrus (Mao et al., 2009). In vitro studies with PC12 cells and primary neurons showed that blocking DISC1 function impairs neurite outgrowth (Kamiya et al., 2006; Ozeki et al., 2003; Taya et al., 2007). In utero electroporation-mediated knockdown of DISC1, or expression of a truncated form of DISC1, in E14.5 embryos leads to retarded migration and misoriented dendrites of cortical neurons (Kamiya et al., 2005), whereas electroporation of shRNAs against Disc1 in
E13 embryos leads to premature cell cycle exit and neuronal differentiation (Mao et al., 2009). In contrast, retrovirus-mediated knockdown of DISC1 by shRNAs specifically in proliferating neural progenitors in the adult hippocampus leads to soma hypertrophy, ectopic dendrites, and increased tempo of development of newborn dentate granule cells, including overextended migration, accelerated axon and dendrite development, as well as synapse formation (Duan et al., 2007; Faulkner et al., 2008). The signaling mechanisms by which DISC1 regulates different aspects of neuronal development in vivo remain elusive.

Adult neurogenesis occurs in a special microenvironment, named “niche” (Alvarez-Buylla and Lim, 2004; Lledo et al., 2006; Ming and Song, 2005; Ninkovic and Gotz, 2007; Zhao et al., 2008). Within the niche, a plethora of extracellular factors regulate adult neural progenitors and their development through activation of diverse intracellular signaling cascades (Schmidt and Duman, 2007; Zhao et al., 2008). How DISC1 participates in these signaling pathways in regulating different steps of adult neurogenesis is largely unknown. One clue came from studies of PTEN (phosphatase and tensin homolog deleted on chromosome ten), a lipid phosphatase that counteracts the kinase function of phosphatidylinositol-3-kinase (PI3K) and suppresses AKT activation (Maehama and Dixon, 1998). AKT is a major mediator of signaling pathways in response to a large spectrum of extracellular stimuli. Upon its activation in neurons, AKT phosphorylates different substrates, which in turn regulate diverse processes of neuronal development, including morphogenesis, dendritic development, synapse formation, and synaptic plasticity (Atwal et al., 2000; Jaworski et al., 2005; Jiang et al., 2005; Kuruvilla et al., 2000; Markus et al., 2002; Sanna et al., 2002; Yoshimura et al., 2006). Interestingly, conditional deletion of PTEN in differentiated dentate granule cells leads to soma hypertrophy, ectopic dendrites, and much elongated dendritic process in the adult mice (Kwon et al., 2006). The phenotypic similarities between suppression of PTEN and DISC1 in the adult brain suggest a potential connection between signaling regulated by these two molecules. Among a large number of proteins identified as potential DISC1-interacting partners in a recent yeast two-hybrid screen (Camargo et al., 2007), KIAA1212 directly binds AKT and enhances its kinase activity in vitro (Anai et al., 2005). Also known as AKT-phosphorylation enhancer (APE) (Anai et al., 2005), Gz-interacting vesicle-associated protein (GIV) (Le-Niculescu et al., 2005), girders of actin filaments (Girdin) (Enomoto et al., 2005), and Hook-related protein1 (HkRP1) (Simpson et al., 2005), KIAA1212 regulates cytoskeletal dynamics in response to extracellular cues in nonneuronal cells (Enomoto et al., 2006). KIAA1212 is expressed in the developing nervous system, and its expression is retained in dentate granule cells of the adult hippocampus (Thompson et al., 2008). The physiological interaction between KIAA1212 and DISC1 has not been characterized and the function of KIAA1212 in neuronal development is unknown.

To investigate the molecular mechanism underlying DISC1 functions in adult neurogenesis, we examined AKT signaling and observed marked increase in phosphorylation levels of both AKT and its downstream effector S6 ribosomal protein (S6) in newborn dentate granule cells with DISC1 knockdown during their maturation process. Further biochemical analysis suggests a model that DISC1 directly interacts with KIAA1212 and prevents the activation of AKT signaling. The physiological significance of this model in neuronal development is supported by in vivo findings that multiple genetic means of increasing AKT signaling in newborn neurons all leads to similar developmental defects as DISC1 suppression and these defects are rescued by pharmacological inhibition of AKT downstream effector mTOR.

**RESULTS**

**Increased AKT Signaling in Newborn Neurons with DISC1 Knockdown in the Adult Brain**

Given the phenotypic similarities between suppression of DISC1 and PTEN in dentate granule cells of the adult brain (Duan et al., 2007; Kwon et al., 2006), we examined AKT signaling in newborn neurons with antibodies raised specifically against phosphorylated AKT (pAKT at Ser473), a marker of AKT activation (Manning and Cantley, 2007). We have previously developed a specific shRNA against mouse Disc1 (DISC1-shRNA) and confirmed its efficacy and specificity in knocking down endogenous DISC1 in hippocampal neurons in vitro (see Figure S1 in the Supplemental Data) (Faulkner et al., 2008) and in dentate granule cells in vivo (Duan et al., 2007). We stereotaxically injected engineered retroviruses coexpressing GFP and DISC1-shRNA, or a control-shRNA, into the dentate gyrus of the adult mouse hippocampus (see Experimental Procedures). This “single-cell genetic” approach allows birth-dating, lineage tracing, and genetic manipulation of proliferating neural progenitors in a cell-autonomous fashion in the adult brain (Ge et al., 2006). Immunostaining of pAKT and DCX, an immature neuronal marker, showed a significant increase of pAKT in GFP+DCX+ new neurons expressing DISC1-shRNA, but not control-shRNA, at 14 days post-viral injection (dpi) in comparison to unlabelled GFP+DCX+ new neurons in the same preparation (Figures 1A and 1B). Time course analysis further showed that the increase of pAKT level was initiated after 7 dpi (Figure 1B), a time when almost all GFP+ cells became postmitotic (Ge et al., 2006). S6 is a well-characterized downstream target of AKT-mTOR pathway, and phosphorylation of S6 (pS6 at Ser235/236) has been commonly used as an indicator for the activation of AKT signaling (Manning and Cantley, 2007). Similar time course of pS6 elevation was found in GFP+DCX+ neurons expressing DISC1-shRNA in comparison to GFP+DCX+ new neurons expressing control-shRNA or uninfected GFP+DCX+ neurons in the same section (Figures 1C and 1D). Taken together, these results suggest that AKT signaling is tightly controlled and that DISC1 may be essential in maintaining AKT signaling at low levels in newborn neurons in the adult brain.

**Direct Association between DISC1 and KIAA1212**

KIAA1212, a potential binding partner of DISC1 identified from a previous yeast two-hybrid screen (Camargo et al., 2007), has been shown to directly interact with AKT and strengthen AKT activation in cell lines (Anai et al., 2005). We first characterized the expression of KIAA1212 in proliferating adult neural progenitors and their progeny in culture (Figure S2). Interestingly,
very little KIAA1212 was detected in proliferating adult neural progenitors at both mRNA and protein levels. Instead, there was a gradual increase of KIAA1212 expression during neural differentiation of adult neural progenitors. In situ hybridization confirmed high level of KIAA1212 expression in the dentate granule cell layer of the adult hippocampus (Figure S2). Thus, KIAA1212 appears to function during differentiation and maturation of adult neural progenitors, but not during the proliferation stage.

We next examined whether physiological interaction between DISC1 and KIAA1212 occurs in hippocampal neurons. Endogenous DISC1 and KIAA1212 were communoprecipitated (co-IPed) from lysates of cultured hippocampal neurons using antibodies against either DISC1 or KIAA1212 (Figures 2A, S3A, and S3B). Similarly, endogenous DISC1 and KIAA1212 were co-IPed from lysates of acutely dissected adult hippocampal tissues (Figures 2B, S3C, and S3D). Thus, DISC1 and KIAA1212 form a complex in hippocampal neurons in vivo.

KIAA1212 binds to several proteins through its C terminus (CT; Figure 2C), including AKT (Anai et al., 2005; Enomoto et al., 2005), Gαi/s (Le-Niculescu et al., 2005), and actin (Enomoto et al., 2005). To map the region(s) of KIAA1212 involved in association with DISC1, we generated a series of Flag-tagged N-terminal deletion mutants of KIAA1212 and coexpressed them with DISC1 in HEK293 cells (Figure S4A). Co-IP results suggested that the CT2 domain of KIAA1212 is sufficient to associate with DISC1 in the same protein complex (Figure S4B). To determine whether KIAA1212 and DISC1 directly bind to

Figure 1. Overactivation of AKT Signaling in Newborn Neurons with DISC1 Knockdown in the Adult Brain

(A) Sample confocal images of immunostaining for GFP, phosphorylated AKT (pAKT), an immature neuronal marker DCX, and DAPI. Retroviruses coexpressing GFP and DISC1-shRNA, or a control-shRNA, were used to infect proliferating neural progenitors in the adult brain. GFP+ neurons were examined at 14 days post-viral injection. Scale bar, 25 μm.

(B) A summary of quantifications of pAKT levels in the cytosol of GFP+DCX+ immature neurons at different stages of development in the adult brain. The pAKT fluorescence intensity of individual GFP+DCX+ new neuron was first normalized to the GFP+DCX+ immature new neurons in the same image. Values represent mean ± SEM (n = 10–33 cells from at least four animals; *p < 0.01, ANOVA).

(C and D) Same as in (A) and (B), except that phosphorylated S6 (pS6) was examined.
we generated recombinant GST-tagged KIAA1212 fragments (Figure 2C) and performed an in vitro pull-down assay using His-tagged DISC1. Consistent with the co-IP result, the CT2 domain of KIAA1212 exhibited direct interaction with DISC1 (Figure 2D). In addition, the M1 and M2 domains of KIAA1212 also directly bind to DISC1 (Figure 2D). We further examined the affinity between the CT2 domain of KIAA1212 and DISC1 in vitro (Figure 2E). Dose-response analysis estimated a dissociation constant (Kd) at 1.0 μM, which is very similar to the reported Kd between KIAA1212 and actin (Enomoto et al., 2005). It is possible that the affinity between the full-length KIAA1212 protein and DISC1 is much higher, given that multiple domains of KIAA1212 showed direct association with DISC1.

We also mapped the DISC1 domain(s) involved in KIAA1212-DISC1 association. We generated a series of HA-tagged deletion mutants of DISC1 (Figure S4C) and coexpressed them with KIAA1212 in HEK293 cells. Co-IP results suggested that both CT and MD domains of DISC1 are involved in association with KIAA1212 in the same protein complex (Figure S4D). Taken together, these results indicate a direct and complex interaction between DISC1 and KIAA1212 through multiple domains.

**Modulation of AKT Signaling by DISC1 through Its Interaction with KIAA1212**

We next examined the potential role of DISC1 and KIAA1212 association in regulating AKT signaling. Treatment of HEK293 cells with insulin led to increased activation of AKT signaling as shown by significant elevation of pAKT and pS6 levels (Figure 3A). Insulin treatment also led to increased phosphorylation of KIAA1212 (pKIAA; Figure S5). Consistent with a role of KIAA1212 in potentiating AKT activation, overexpression of KIAA1212 led to an elevation of pAKT and pS6 levels in the absence of insulin treatment (Figure 3A). Interestingly, coexpression of DISC1 and KIAA1212 significantly suppressed pAKT and pS6 levels in response to either insulin treatment or after KIAA1212 expression (Figure 3A). Furthermore, phosphorylation of KIAA1212 was also diminished under these conditions (Figure S5). In contrast, expression of DISC1 alone without KIAA1212 overexpression did not appear to change the levels of pAKT and pS6 with or without insulin treatment (Figure 3A).
suggesting a requirement of KIAA1212 for DISC1-dependent modulation of AKT signaling.

How does DISC1 binding to KIAA1212 suppress the activation of AKT signaling? We examined interaction among KIAA1212, AKT and DISC1. Consistent with previous reports (Anai et al., 2005; Enomoto et al., 2005), co-IP analysis suggested that HA-KIAA1212 expressed in HEK293 cells was associated with endogenous AKT (Figure 3B). HA-KIAA1212 and DISC1 were also associated with each other with or without insulin treatment (Figure 3C). Interestingly, coexpression of DISC1 and KIAA1212 inhibited the association between KIAA1212 and endogenous AKT in HEK293 cells (Figure 3B). In addition, no strong association between DISC1 and endogenous AKT was detected in the presence or absence of either insulin treatment or KIAA1212 overexpression in HEK293 cells (Figure 3D), suggesting a lack of high-affinity direct interaction between DISC1 and AKT.

Collectively, the biochemical results suggest that DISC1 directly interacts with KIAA1212 and prevents its activation of AKT signaling. This model is consistent with the immunohistochemical evidence of increased pAKT and pS6 levels in newborn neurons with DISC1 knockdown in the adult brain (Figure 1). The model further predicts that an increase of AKT signaling would mimic DISC1 knockdown in affecting development of new neurons in the adult brain, whereas inhibition of AKT downstream signaling may rescue neuronal developmental defects from DISC1 knockdown.

**Role of KIAA1212 and AKT Signaling in Regulating Morphogenesis of Adult-Born Neurons**

To directly test our model, we carried out a series of in vivo “single-cell genetic” analysis to compare phenotypes between DISC1 knockdown and multiple means of activating AKT signaling in newborn neurons, including overexpression of KIAA1212, overexpression of a constitutively active form of AKT1 (CA-AKT) (Kohn et al., 1996), and knockdown of PTEN. We used a previously characterized shRNA against Pten (PTEN-shRNA) (Jaworski et al., 2005) and confirmed its efficacy and specificity in knocking down endogenous PTEN in primary hippocampal neurons (Figure S1).

We first examined morphogenesis of newborn neurons in the adult brain at 14 dpi under different manipulations. Similar to DISC1 knockdown, GFP+ neurons overexpressing KIAA1212 exhibited a significant increase in both soma size and number of primary dendrites, in comparison to those expressing GFP alone (pCUXIE overexpression control; Figures 4A–4C). In addition, GFP+ neurons expressing PTEN-shRNA or CA-AKT also exhibited soma hypertrophy and ectopic primary dendrites.
Collectively, these experiments showed that three different means of genetic manipulations to activate AKT signaling in adult-born new neurons all resulted in similar defects as DISC1 knockdown in their morphological development.

The retrovirus-based “single-cell genetic” approach is not best suited to investigate the proliferation phase of adult neurogenesis in vivo because of the delay in transgene/shRNA expression after stable viral integration into the host genome and rapid cell cycle exit of infected transient amplifying cells (Ge et al., 2006). However, such an approach has been shown to be effective in affecting the fate choice of adult neural progenitors in vivo (Jessberger et al., 2008). To ensure that our genetic manipulations of the AKT signaling pathway do not perturb neuronal subtype differentiation of adult neural progenitors, we examined the expression of Prox-1, a dentate granule cell marker, and parvalbumin, an interneuron marker (Duan et al., 2007). Immunostaining showed that at 14 dpi, all GFP+ neurons expressing KIAA1212 or CA-AKT also expressed Prox-1, but not parvalbumin (Figure S6). Thus, the observed defects on soma hypertrophy and ectopic dendrites under these conditions were not due to changes in neuronal subtype differentiation of adult neural progenitors in the adult brain.

Role of KIAA1212 and AKT Signaling in Regulating Positioning of Adult-Born Neurons
Previous studies have established that newborn neurons generated during adult hippocampal neurogenesis contribute mostly to the inner granule cell layer and to a less extent to the middle granule cell layer (Figure 4D) (Duan et al., 2007). At 14 dpi, GFP+ neurons expressing KIAA1212 exhibited moderate overextended migration phenotype with some cells into the outer granule cell layer and the molecular layer (Figure 4E). GFP+ neurons expressing CA-AKT exhibited similar positioning defects to those expressing KIAA1212 (Figure 4E). On the other hand, GFP+ new neurons expressing DISC1-shRNA exhibited stronger migration phenotype with a significant percentage of cells into the outer granule cell layer and molecular layer (Figure 4E). These results suggest that the AKT pathway may be only part of the signaling pathway controlling the positioning of new neurons in the adult brain.

Role of KIAA1212 and AKT Signaling in Regulating Dendritic Development of Adult-Born Neurons
We next examined dendritic development of new neurons by reconstructing the dendritic arborization of individual GFP+
neurons using confocal microscopy. Similar to neurons expressing DISC1-shRNA, GFP+ neurons overexpressing KIAA1212 exhibited accelerated dendritic growth at 14 dpi (Figure 5A), as shown by a significant increase in the total dendritic length (Figure 5B). GFP+ neurons expressing CA-AKT or PTEN-shRNA also exhibited significantly accelerated dendritic growth (Figures 5A and 5B). Sholl analysis further showed much increased dendritic complexity of these newborn neurons at 14 dpi (Figure 5C). Among these manipulations, CA-AKT overexpression led to the largest increase of dendritic growth. Such a stronger effect on the maturation of newborn neurons, including both dendritic development (Figure 5) and soma size (Figure 4B), is consistent with the role of AKT as a downstream effector in the DISC1 signaling cascade. Taken together, these results further support our model that DISC1 regulates AKT signaling through KIAA1212 during development of new neurons in the adult brain.

Figure 5. Role of KIAA1212 and AKT Signaling in Regulating Dendritic Development of Newborn Neurons in the Adult Brain

(A) Sample projected confocal images of GFP+ new neurons in the adult brain. Same as in Figure 4, GFP+ neurons with retrovirus-mediated genetic manipulation were examined at 14 dpi. Scale bar, 50 μm.

(B) A summary of total dendritic length of GFP+ neurons at 14 dpi under different conditions. Shown is the cumulative plot with each symbol representing data from an individual GFP+ neuron from at least four animals for each condition. (p < 0.01, Kolmogorov-Smirnov test).

(C) Analysis of dendritic complexity of GFP+ neurons using Sholl analysis. The same sets of GFP+ neurons as in (B) were used. Values represent mean ± SEM (p < 0.01, student t test).

Rescue of Developmental Defects of New Neurons from DISC1 Knockdown and KIAA1212 Overexpression by Pharmacological Inhibition of mTOR, but Not by Inhibition of GSK3β

AKT signaling regulates cellular responses in neurons through a number of downstream effectors, including activation of mTOR pathway and inhibition of GSK3β (Manning and Cantley, 2007). Given the increased mTOR signaling as shown by the elevated pS6 levels in newborn neurons expressing DISC1-shRNA (Figure 1D) or KIAA1212 (Figure 6B), we first examined the effect of pharmacological inhibition of mTOR with rapamycin (Figure 6A). The effectiveness of in vivo application of rapamycin was confirmed by western blot analysis of pS6 levels in various brain tissues from injected animals (Figure S7) and by immunostaining of pS6 in GFP+ newborn neurons expressing DISC1-shRNA or KIAA1212 in the adult brain (Figure 6B). Interestingly, application of rapamycin completely rescued defects of new neurons with DISC1 knockdown or KIAA1212 overexpression, including soma size, number of primary dendrites, total dendritic length and complexity (Figures 6C–6G). On the other hand, rapamycin has no significant effects on both pS6 levels and morphogenesis of control GFP+ neurons expressing DISC1-shRNA or KIAA1212 in the adult brain (Figure 6B). These results indicate that mTOR is a major downstream mediator of DISC1-dependent regulation of AKT signaling in development of newborn neurons in the adult brain.

We also examined the potential role of GSK3β in DISC1-dependent regulation of new neuron development. A recent
study showed that pharmacological inhibition of GSK3β by SB-216763 normalized the decreased proliferation of adult neural progenitors from lentivirus-mediated expression of DISC1-shRNA in the adult dentate gyrus (Mao et al., 2009). We focused our study on the neuronal developmental stage of newborn dentate granule cells beyond the proliferation stage. After daily application of SB-216763 for 14 days, GFP+ neurons expressing DISC1-shRNA or KIAA1212 still exhibited significant developmental defects, including soma hypertrophy, ectopic dendrites and increased dendritic length and complexity (Figure S8). There was a moderate increase in the number of BrdU labeled cells examined at 2 hr after BrdU injection on 14 dpi (data not shown), suggesting an increased proliferation of adult neural progenitors in the dentate gyrus.

**DISCUSSION**

Emerging evidence support the notion that DISC1 functions as a key regulator for a number of developmental processes in the nervous system (Ishizuka et al., 2006; Mackie et al., 2007). During adult neurogenesis, DISC1 controls the tempo of cell cycle exit of neural progenitors and multiple processes of neuronal development of newborn neurons (Ming and Song, 2009). In this study, we identified AKT-mTOR signaling as a previously unexpected target of DISC1 in regulation of morphogenesis and dendritic development of new neurons in the adult brain (Figure 7). We further pinpointed KIAA1212, a protein binding to DISC1 and AKT, as the critical mediator in linking these two molecules into the same signaling pathway. Our results suggest that DISC1 modulates AKT signaling in newborn neurons.
levels of pAKT and pS6 are very low in both DCX+ immature neurons and DCX+ mature neurons (Figure 1), indicating the existence of an active mechanism to keep AKT signaling in check. Our in vitro biochemical study and in vivo “single-cell genetic” analysis suggest that DISC1 serves as a key intrinsic modulator of AKT signaling in regulating the morphogenesis and tempo of dendritic development of new neurons in the adult brain.

Neurogenesis occurs in a specialized local “niche” that not only houses neural stem cells but also regulates their development through extrinsic factors (Ma et al., 2005; Ming and Song, 2005). A number of growth factors have been implicated in regulating different steps of the adult neurogenesis process, especially in response to activation of the existing neuronal circuitry, including brain-derived neurotrophic factor (BDNF), fibroblast growth factors (FGFs), vascular endothelial growth factor (VEGF), and neuregulins (NRGs) (Ghashghaei et al., 2006; Jin et al., 2002; Li et al., 2008; Ma et al., 2009; Schmidt and Duman, 2007). AKT signaling is a major pathway in response to these growth factors, and recent studies have implicated its role in morphogenesis and dendritic development of postmitotic dentate granule cells in the adult hippocampus (Kwon et al., 2006; Zhou et al., 2009). In the adult dentate gyrus, the basal levels of pAKT and pS6 are very low in both DCX+ immature neurons and DCX+ mature neurons (Figure 1), indicating the existence of an active mechanism to keep AKT signaling in check. Our in vitro biochemical study and in vivo “single-cell genetic” analysis suggest that DISC1 serves as a key intrinsic modulator of AKT signaling in regulating the morphogenesis and tempo of dendritic development of new neurons in the adult brain.

Previous biochemical analysis and yeast two-hybrid screen have identified a large number of DISC1-interacting proteins, named “DISC1 interactome” (Camargo et al., 2007). The functional significance of these potential interactions is largely unexplored. Our current study provided novel insight into the function of KIAA1212 as a critical mediator, through which DISC1 modulates AKT signaling in neuronal development. The time course of KIAA1212 expression during differentiation of adult neural progenitor (Figure S2) correlates with the gradually elevated AKT signaling in newborn neurons with DISC1 knockdown in vivo (Figure 1). The lack of KIAA1212 expression in adult neural progenitors raises a possibility of developmental stage-dependent regulation by DISC1 during adult neurogenesis. In support of this notion, DISC1 was shown to regulate proliferation of adult neural progenitors through inhibition of GSK3β, a downstream target of AKT (Mao et al., 2009), whereas our genetic and pharmacological studies demonstrated a role of DISC1 in regulating development of postmitotic newborn neurons through mTOR (Figure 7). Whether GSK3β plays a cell-autonomous role in regulating development of newborn neurons in the adult brain remains to be fully characterized. The distinct functions and signaling mechanisms of DISC1 at different cellular stages further substantiate the notion that DISC1 regulates multiple aspects of neural development in a temporal and cell-type-specific manner.

Figure 7. A Model of DISC1 Signaling Pathways in Regulating Different Aspects of Adult Hippocampal Neurogenesis

RTKs, receptor tyrosine kinases; GPCRs, G protein-coupled receptors; D2 receptor, dopamine type 2 (D2) receptor.
inbred substrains, may have certain DISC1 isoforms deleted (Clapcote and Roder, 2006; Ishizuka et al., 2007; Koike et al., 2006). Interestingly, the phenotypes of DISC1 suppression, KIAA1212 overexpression or knockdown in newborn neurons in the adult brain were similar between 129S6 and C57BL/6 mice (Figure S10), further supporting our conclusions.

Substantial evidence has implicated the involvement of AKT signaling in major mental and mood disorders (Beaulieu et al., 2008). Both AKT1 and its upstream activator NRG1 have been identified as susceptibility genes for schizophrenia (Emamian et al., 2004; Mei and Xiong, 2008; Stefansson et al., 2002; Tosato et al., 2005). Interestingly, a number of studies have suggested hyperactivation of NRG signaling in schizophrenia patients, including increased expression of type IV NRG1 in the hippocampus resulted from a mutation in the promoter region (Law et al., 2006) and upregulation of specific ErbB4 splice isoforms that activate PI3K (Law et al., 2007). At the functional level, NRG1 signaling through ErbB4 receptors promotes neuronal maturation and dendritic growth (Mei and Xiong, 2008). The similarities in neuronal phenotypes among genetic manipulations of multiple AKT signaling components and DISC1 further support the notion that they work in the same pathway in regulating neuronal development. While it remains an intriguing hypothesis that defects in adult hippocampal neurogenesis may contribute to psychiatric disorders (Ming and Song, 2009), the signaling mechanism identified here provides an important framework to understand how multiple susceptibility genes functionally converge onto a common pathway that may underlie the etiology of psychiatric disorders. Future characterization of a core molecular pathway with a focus on DISC1 and other schizophrenia susceptibility genes may lead to a profound advance in understanding schizophrenia and the development of therapeutic interventions.

**EXPERIMENTAL PROCEDURES**

**Constructs, Cell Culture, and Biochemical Analysis**

Full-length KIAA1212 was cloned from a mouse cDNA library and inserted into pCRII zero (Invitrogen) or pRK5 vector (BD PharMingen) for the HA tag. Fragments of KIAA1212-NT (amino acids 1–240), M1 (amino acids 241–1000), M2 (amino acids 1001–1383), C1 (amino acids 1384–1600), C2 (amino acids 1601–1845) were cloned into pCRII zero (Invitrogen) or pRK5 vector for the HA tag. For GST pull-down experiments, pGST-KIAA1212 and pET28-DISC1 were introduced into E. coli (BL21) and the fusion proteins were induced with 1 mM IPTG for 4–5 hr. For binding assay, ~5 µg of GST-KIAA1212 was incubated with about the same amount of His-tagged DISC1 and protein complex was purified using glutathione-Sepharose beads. For KD calculation, a fixed amount of His-tagged DISC1 was mixed with various amounts of GST-CT2 (0–10 µM) and GST pull-down was performed. Amounts of the free and bound DISC1 were quantified by western blot analysis. The percentage of bound DISC1 was plotted against the concentration of GST-CT, and the Kd value was calculated by the Scatchard analysis.

**Adult neural progenitors were isolated from adult mice hippocampi (C57BL/6)** and cultured as monolayer on plastic dishes in DMEM/F12 medium supplemented with N2, FGF-2 (20 ng/ml), heparin (5 µg/ml) and EGF (20 ng/ml) as previously described (Ma et al., 2008). Neuronal differentiation of these adult neural progenitors was induced by withdrawal of growth factors and treatment of retinoic acid (0.5 µM), forskolin (1 µM), and fetal bovine serum (0.5%) and cultures were continued for 2–12 days.

**In Vivo Birth-Dating, Genetic Manipulation of Neural Progenitors with Engineered Retroviruses, and Pharmacological Treatments**

Engineered self-inactivating murine oncoretroviruses were used to coexpress shRNA under the U6 promoter and GFP under the EF1α promoter (pUEG vector), or to coexpress a transgene under the U6Ubiquitin promoter and GFP under IRES sequence (pCUXIE vector) specifically in proliferating cells and their progeny. Specific shRNAs against mouse disc1 (DISC1-shRNA) (Duan et al., 2007) and mouse pten (PTEN-shRNA) (Jaworski et al., 2005) have been characterized previously, and we further confirmed their specificity and efficacy in knocking down endogenous proteins in hippocampal neurons using lentiviruses. Three different shRNAs against mouse KIAA1212 were cloned into the pUEG vector with the following sequences: shRNA-K1: 5’-GGTACCAAT CAGTTATTA-3’; shRNA-K2: 5’-CCTAGCAA TAGAATAT-3’; shRNA-K3: 5’-GAAAGGAGA GCAACTGAT-3’. The efficacy of knocking down endogenous KIAA1212 was confirmed in hippocampal neurons using lentiviruses. The shRNA-K3 sequence is identical to the one previously characterized (Kitamura et al., 2008). High titers of engineered retroviruses were produced by cotransfection of retroviral vectors and vesicular stomatitis virus envelope into 293 gp cells followed by ultracentrifugation of viral supernatant as previously described (Duan et al., 2007).

**Adult female C57BL/6 mice (7–8 weeks old) housed under standard conditions were anesthetized, and concentrated retroviruses were stereotaxically injected into the dentate gyrus at four sites (0.5 µl per site at 0.25 µl/min) with the following coordinates (posterior = 2 mm from Bregma, lateral = ±1.6 mm, ventral = 2.5 mm; posterior = 3 mm from Bregma, lateral = ±2.6 mm, ventral = 3.2 mm) as previously described (Duan et al., 2007). In one set of experiments (Figure S10), adult female 129S6 mice were used. All animal procedures used in this study were performed in accordance with the protocol approved by the Institutional Animal Care and Use Committee at Johns Hopkins University School of Medicine.

For pharmacological treatments, rapamycin (LC Laboratories) was first dissolved in ethanol at a stock concentration of 25 mg/ml and was further
diluted with a final concentration of 1 mg/ml in 4% ethanol, 5% Tween 80, and 5% PEG 400 as previously described (Zhou et al., 2009). Rapamycin was intra-peritoneally delivered to mice (20 mg/kg body weight), SB-217673 (Tocris bioscience) was dissolved in DMSO at a stock concentration of 75 mM and intraperitoneally delivered to mice (2 mg/kg body weight) daily during the course of experiments (Mao et al., 2009).

**Immunohistochemistry, In Situ Hybridization, Confocal Imaging, and Analysis**

Coronal brain sections (40 μm thick) were prepared from injected mice and processed for immunostaining as described (Ge et al., 2006). The following primary antibodies were used: DCX (goat, 1:250, Santa Cruz), GFP (rabbit, 1:1000, Abcam), Prox-1 (rabbit, 1:1000, Abcam), parvalbumin (mouse, 1:2000, Sigma), pAKT (rabbit, 1:150, Cell Signaling), pS6 (rabbit, 1:1000, Cell Signaling). Effective immunostaining of pAKT and pS6 required an antigen retrieval protocol. The sections were incubated for 30 min in 4',6-diamidino-2-phenylindole (DAPI, 1:5000) before washing and mounting. Images were acquired on a META multiphoton confocal system (Zeiss LSM 510) using a multitrack configuration. For comparison of pAKT and pS6 levels, sections were processed in a series of concentric circles at 10 m intervals from the cell soma as previously described (Duan et al., 2007). All quantifications were carried out in a blind fashion to experimental conditions. Statistic significance was determined by ANOVA.

For analysis of cell morphology, Z series stacks of confocal images were taken and a single confocal image slice with the largest soma area for individual GFP+ neurons was used for quantification using NIH ImageJ program. For analysis of neuronal positioning, single-section confocal images of GFP+ neurons with DAPI staining were used to determine the cell localization within four areas defined in Figure 4D. All quantifications were carried out in a blind fashion to experimental conditions. Statistic significance was determined by ANOVA.

For analysis of dendritic development, 3D reconstruction of entire dendritic processes of each GFP+ neuron was made from Z series stacks of confocal images. The 2D projection images were traced with NIH ImageJ. All GFP+ dentate granule cells with largely intact dendritic trees were analyzed for total dendritic length as described (Duan et al., 2007; Ge et al., 2006). The measurements did not include corrections for inclinations of dendritic process and therefore represented projected lengths. Sholl analysis for dendritic complexity was carried out by counting the number of dendrites that crossed a series of concentric circles at 10 μm intervals from the cell soma as previously described (Duan et al., 2007). All quantifications were carried out in a blind fashion to experimental conditions. Statistic significance was determined with the Kolmogorov-Smirnov test and Student’s t test.

For analysis of cell proliferation in the dentate gyrus, adult mice at the end of 14 day treatment of SB-217673 or saline were injected with BrdU (200 mg/kg body weight, i.p.) and sacrificed 2 hr later. Coronal brain sections (40 μm thick) were prepared and processed for immunostaining using anti-BrdU antibodies (rat; 1:400; Accurate). Images were acquired on a Zeiss LSM 510 Live confocal system (Carl Zeiss). Stereological quantification of BrdU+ cells within the subgranular zone and granule cell layer were carried out as previously described (Ma et al., 2009).

For in situ hybridization analysis, brains were fixed with 4% paraformaldehyde and 20 μm thick brain sections were prepared using cryo-stat. Antisense riboprobe for full-length KIAA1212 was generated by in vitro transcription and labeled by digoxigenin. Hybridization of the riboprobes on sections was performed at 65°C overnight and followed by washing once with 5x SSC and 1% SDS, then twice with 2x SSC for 30 min each at 65°C. The incubation of alkaline phosphatase-conjugated anti-digoxigenin antibody was performed at 4°C for overnight, and sections were visualized using nitroblue tetrazolium (NBT, 35 μg/ml) and 5-bromo-4-chloro-3-indoly phosphate 2 (BCIP, 18 μg/ml) color reaction at room temperature.

**SUPPLEMENTAL DATA**

Supplemental Data include ten figures and can be found with this article online at http://www.cell.com/neuron/supplemental/S0896-6273(09)00617-5.

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**REFERENCES**


