β-CATENIN IS REQUIRED FOR MAINTAINING HIPPOCAMPAL MORPHOLOGY DURING THE PERINATAL PERIOD

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Abstract—In mice, the compact hippocampal primordium is formed during the prenatal stage by early-generated neurons that migrate from the lateral ventricular zone. However, despite much being understood about the formation of the hippocampus, the molecular mechanisms that maintain the morphology of the hippocampal primordium after its formation remain to be characterized. β-Catenin is a key factor of canonical Wnt signaling and also a component of adherens junctions. Previous embryonic deletion studies have demonstrated that β-catenin is required for early development and generation of granule cells. However, whether β-catenin is involved in the morphological maintenance of the hippocampus as a cell adhesion molecule is still unknown. Here, we report that perinatal deletion of β-catenin in postmitotic neurons and some radial glial cells of hippocampus using CamKII-Cre; β-cateninfloxflox conditional knockout mice, leads to disorganization of the radial glial scaffold and consequentially severe defects in hippocampal morphology. We demonstrate that β-catenin is required for maintaining radial glial scaffold possibly via its well-known role in cell adhesion during the perinatal period. These findings provide essential advances into our understanding of the maintenance of the hippocampal primordium during the perinatal period. © 2014 IBRO. Published by Elsevier Ltd. All rights reserved.

Key words: β-catenin, hippocampal primordium, radial glial scaffold, ectopic cell, perinatal stage, GFAP.

INTRODUCTION

The hippocampus, which is composed of the Cornu Ammonis (CA) fields (CA1–CA3) and the dentate gyrus (DG), plays essential roles in learning and memory, mood regulation and other functions (Li and Pleasure, 2005). Developmental defects affecting the hippocampus not only disturb the process of learning and memory, but are also associated with such neurological and psychiatric disorders as temporal lobe epilepsy, depression and others (Malykhin et al., 2010).

Considerable efforts have been made toward characterizing the molecular framework underlying hippocampal morphogenesis (Li and Pleasure, 2007). In mice, the earliest born granule neurons start their radial migration along the radial glial scaffold to form the compact primordial granular layer by E17.5 (Li and Pleasure, 2007; Tian et al., 2012). Cajal–Retzius cells in the developing brain secrete the protein reelin, which controls granule cell migration in the DG through its effect on the radial glial scaffold (Frotscher et al., 2003). The lack of reelin in the reeler mouse has been shown to produce severe morphological defects in the hippocampus: the radial glial scaffold fails to form, and granule cells disperse throughout the DG (Forster et al., 2006a). In addition, some adhesion molecules and the components of their downstream signaling pathway such as integrin, dystroglycan, integrin-associated kinase and focal adhesion kinase (fak) are involved in basement membrane assembly and radial glial end-feet anchorage (Beggs et al., 2003). Genetic deletion of these molecules in the brain results in local basement membrane disruption, disorganization of the radial glial scaffold and consequential abnormal morphology of the hippocampus (Forster et al., 2002; Moore et al., 2002; Beggs et al., 2003; Niewmierzycka et al., 2005).

β-Catenin is a central component of canonical Wnt signaling, which plays several important roles in embryonic development (MacDonald et al., 2009). It has previously been shown that inactivation of β-catenin in the mouse hippocampus at about E10.5 results in the loss of the hippocampal CA1 and CA2 fields, and in a reduction in the size of the CA3 field and the DG (Machon et al., 2003). These findings clearly illustrate that β-catenin-dependent canonical Wnt signaling is required for early hippocampal development and granule cell generation (Zhou et al., 2004; Li and Pleasure, 2005). However, whether or not β-catenin-dependent canonical Wnt signaling is involved in the perinatal development of the hippocampus has not been reported to date (Skutella and Nitsch, 2001; Forster et al., 2006b). In addition to being a key component of the Wnt pathway, β-catenin also plays a role in cell adhesion through its...
interaction with the cytoplasmic domain of cadherin and with cytoskeletal elements (Gumbiner, 1996). Thus, we aimed to discern whether β-catenin is involved in the morphological maintenance of the hippocampus as a cell adhesion molecule.

Here we generated CamKIIα-iCre; β-catenin<sup>flox/flox</sup> conditional knockout (β-catenin CKO) mice in which β-catenin expression is specifically deleted from forebrain cells during the perinatal period to examine the role of β-catenin in hippocampal development (Casanova et al., 2001). We found that the pyramidal and granular layers were disorganized and many ectopic cellular clusters were present in the hippocampus of β-catenin CKO mice. Further investigation revealed that the deletion of β-catenin expression in some radial glial cells might result in their displacement, which led to ectopic migration of hippocampal cells. These novel findings provide the first evidence that β-catenin is required for maintaining hippocampal morphology during the perinatal period.

Fig. 1. Expression analysis of β-catenin from P0 to P15 in control and β-catenin CKO mice. (A–F), In situ hybridization revealed that, as age increases, so does the extent to which β-catenin mRNA expression is inactivated in the hippocampus of CKO mice. (A, B), In P0 CKO mice compared to controls, β-catenin is absent from a cluster of cells in the DG and from a stripe of cells in the CA1, as indicated by arrowheads in B. (C, D), At P7, the absence of β-catenin expression has expanded to CA2 and CA3 (double arrowheads in D). In addition, the absence of β-catenin mRNA expression is also expanded to a small area within the CA1 field from a stripe of cells at P0 (arrowhead in D). (E, F), By P15, β-catenin expression has been conditionally inactivated from almost all hippocampal cells, except for a few cells in the DG (arrowhead in F). (G), Real-time polymerase chain reaction (PCR) analysis showing β-catenin mRNA levels in the cortex and hippocampus of control and CKO P15 mice. Data represent mean ± SEM, n = 3. Differences between groups were determined by unpaired t-test. **, P ≤ 0.01 compared with control mice. Scale bars = 100 μm in B (for A–B), 200 μm in F (for C–F).
EXPERIMENTAL PROCEDURES

Generation, genotyping and maintenance of animals

CaMKIIα-iCre transgenic mice were a kind gift from Dr. Schutz’s lab in the Deutsches Krebsforschungszentrum (DKFZ) and the β-catenin floxed mice were obtained from Jackson Laboratory (strain name: B6.129-Ctnnb1tm2Kem/KnwJ; stock number: 004152). These two lines were first crossed to obtain CaMKIIα-iCre; β-catenin^flox/+ progeny, which were then crossed with each other to obtain CaMKIIα-iCre; β-catenin^flox/flox mice. The CaMKIIα-iCre; β-catenin^flox/+ mice appeared normal both behaviorally and physiologically, and were thus labeled as controls, along with wild-type mice. CaMKIIα-iCre; β-catenin^flox/flox mice were labeled as β-catenin CKO mice. Animal care and experimental protocols were approved by the East China University of Technology Animal Care and Use Committee.

Primers for the β-catenin gene were: 5'-AAGGTAGGTGGATGAGGTAGTTGGT-3' and 5'-CACCAGTGCCTCTCGTCTATT-3'. Primers for the iCre gene were: 5'-TGGCCAAGAAGAGGAA-3' and 5'-TTGCGAGTAGCATGAGGATGTC-3'. Polymerase chain reaction (PCR) amplifications for β-catenin and iCre were performed separately. The PCR temperature cycle conditions for β-catenin and iCre were 94°C for 30 s, 55°C for 30 s and 72°C for 30 s, for 35 cycles. PCR products were resolved in 1.0% agarose gel.

Nissl staining and immunofluorescence

Forebrain anatomy was analyzed using Nissl (cresyl violet) staining. Coronal brain sections of 30-μm thickness were placed in xylene for 20 min to remove lipids from the tissue, then rehydrated in 100%, 95%, 75% and 50% ethanol and dH2O, for 3 min per wash, and then stained in 0.5% cresyl violet staining solution for approximately 5 min. Cresyl violet staining solution was made freshly by dissolving 2.5 g of cresyl violet (Sigma) in 250 ml of distilled water and adding 1.5 ml of glacial acetic acid, stirring for 20 min and filtering before use. After staining, the slices were rinsed quickly in dH2O, differentiated in 75% ethyl alcohol for 2–30 min and checked microscopically for optimal results. Finally, sections were dehydrated in 95% and 100% ethanol (20 s each), immersed in xylene twice for 10 min, mounted with DPX and dried at room temperature overnight.

Immunofluorescence staining was carried out as described previously (Dai et al., 2008). Briefly, brain sections were incubated with the following primary antibodies: rabbit anti-Cre (1:200; Covance, Princeton, NJ, USA), rabbit anti-calbindin (1:200; Sigma, St. Louis, MO, USA), rabbit anti-glial fibrillary acidic protein (GFAP; 1:1000; Chemicon, Temecula, CA, USA). Single or double immunofluorescence labeling was detected with corresponding secondary antibodies conjugated to Alexa fluorochromes (Molecular Probes, Eugene, OR, USA). Vectastain ABC Kits (Vector Labs Inc, Burlingame, CA, USA) were used for co-immunolabeling. Cell nuclei were stained with Hoechst 33258 (Sigma, St. Louis, MO, USA).

In situ hybridization (ISH)

ISH on cryosections was carried out as described elsewhere (Thissen and Thissen, 2008). In vitro transcribed, digoxigenin-labeled antisense RNA probes were produced for the following genes: CTNNB1 (encoding β-catenin; NM_007614), NRP2 (encoding neuropilin 2; NM_010939), PROX1 (encoding prox1; NM_008937), RELN (encoding Reelin; NM_005045). The sequences for these probes were obtained from the Allen Brain Atlas ISH database and prepared following standard protocols (Wilkinson, 1998). Thirty-micrometer coronal brain sections were mounted on microscope slides (Fisher Scientific, Waltham, MA, USA), fixed for 20 min with 4%
**RESULTS**

As traditional knockout of the β-catenin gene in embryonic stem cells leads to embryonic lethality (Haegel et al., 1995), we instead used CamKII\_x:iCre transgenic mice, which express iCre recombinase under the control of the CamKII\_x promoter, to specifically delete β-catenin in forebrain cells during the perinatal stage (Casanova et al., 2001). To verify the effective deletion of β-catenin from the forebrain of β-catenin CKO mice, we conducted ISH analyses on coronal brain sections at different developmental stages, from P0 to P15 (Fig. 1). We found that, starting at P0, β-catenin mRNA expression is absent from a stripe of cells in the hippocampal CA1 field, and from a cluster of cells at the border between CA3 and the DG in β-catenin CKO mice (arrowheads in Fig. 1B). By P7, the absence of β-catenin mRNA expression has expanded into CA2 and CA3 (double arrowheads in Fig. 1D), as well as parts of the DG, cortex and thalamus. The absence of β-catenin mRNA expression is also expanded to a small area within the CA1 field from a stripe of cells at P0 (arrowhead in Fig. 1D). As expected, ISH analysis at P15 revealed that β-catenin mRNA expression had been successfully deleted in almost all hippocampal cells of β-catenin CKO mice; however, there remained some detectable expression within the DG (arrowhead in Fig. 1F), which may be due to the lack of CamKII\_x promoter-driven iCre recombinase expression in the

![Fig. 3. NRP2 and Prox1 mRNA expression in control and β-catenin CKO mice at P7. (A-F) In situ hybridization experiments revealed that ectopic cells in both the CA1 (arrowheads in D and F) and DG (arrows in D and F) are all negative for NRP2 (C, D) and Prox1 (E, F). In addition, β-catenin expression is missing from the CA2 and CA3 fields (double arrowheads in B), but NRP2 expression is not (D). Scale bar = 200 μm.](image)

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**Statistical analysis**

Statistical analysis was carried out using SPSS 17.0. Data in the figures and text were expressed as mean ± standard error of the mean and statistically analyzed using unpaired two-tailed Student's t test. P < 0.05 was regarded as being statistically significant.
progenitor cells. P15 brain tissue samples obtained from \(\beta\)-catenin CKO mice and their littermate controls were also subjected to RT-PCR analysis, revealing that \(\beta\)-catenin mRNA levels are markedly reduced in the cortex and hippocampus of \(\beta\)-catenin CKO mice compared with controls (Fig. 1G).

Hippocampal malformations in \(\beta\)-catenin CKO mice
To characterize the role of \(\beta\)-catenin in postnatal hippocampal development, we examined Nissl-stained coronal sections from \(\beta\)-catenin CKO and control mice (Fig. 2). At P0, no major differences in hippocampal morphology were observed between \(\beta\)-catenin CKO and control mice (Fig. 2A, B). The only visible abnormalities are an ectopic cluster of cells present above the DG (arrow in Fig. 2B'), which create a cellular cleft at the border between CA3 and the DG (arrowhead in Fig. 2B'). By P7, more severe hippocampal malformations become apparent in \(\beta\)-catenin CKO mice (Fig. 2C, D). We observed that the pyramidal neuron layer of the CA1 field is disrupted and irregularly bent, exhibiting a ribbon-like appearance (Fig. 2D). Ectopic cell clusters (arrowheads in Fig. 2D), seemingly derived from the pyramidal neuron layer, are visible immediately adjacent to the disrupted CA1 sites. The DG is also disorganized in the \(\beta\)-catenin CKO mice, with some additional ectopic cell clusters seen adjacent to it (double arrowhead in Fig. 2D'). Hippocampal morphology phenotypes at P15 are similar to those observed at P7 (Fig. 2E, F). We therefore concluded that perinatal inactivation of \(\beta\)-catenin causes ectopic migration of hippocampal cells and the disruption of overall hippocampal morphology. It is important to note, however, that no differences were observed in the six-layer structure or neuronal density of the cerebral cortex between \(\beta\)-catenin CKO and control mice, as revealed by Nissl staining (data not shown).

In order to determine the cell type of ectopically migrating hippocampal cells in \(\beta\)-catenin CKO mice, we examined the expression of neuropilin-2 (NRP2), a receptor for semaphorin 3C and 3F thought to be expressed throughout the entire hippocampus (Chen et al., 1997), and the homeobox gene Prox1, which is selectively expressed in the granule cells of the DG (Lavado et al., 2010) at P15. Interestingly, we found that ectopic cells from either the CA3–DG border (arrows in Fig. 3D, F) or from the CA1 field (arrowheads in Fig. 3D, F) lack NRP2 and Prox1 expression in \(\beta\)-catenin CKO mice. Co-immunostaining of NeuN (a pan-neuronal

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Fig. 4. Co-expression of NeuN and Calbindin in some ectopic cells. (A–G) Co-immunostaining experiments revealed that all neurons in the CA fields and the DG were labeled with NeuN, whereas all granule cells of the DG and most CA pyramidal neurons were labeled with calbindin. In \(\beta\)-catenin CKO mice, the stereotypical horseshoe shape of the DG was interrupted by ectopic cell clusters, forming instead a closed-loop structure (B, D, F, H). Normal DG lamination, however, was maintained in the CKO mice (B’, D’, F’, H’). Most ectopic cells were labeled with NeuN (B, B’) and only several ectopic cells were labeled with both NeuN and calbindin (arrowheads in B’, D’ F’). Scale bars = 200 \(\mu\)m in H (for A–H), 200 \(\mu\)m in H’ (for A–H').
Radial glial cells, especially at P7 (Fig. 6I). These results suggest that the deletion of β-catenin, calbindin, GFAP or reelin between control and β-catenin CKO mice (data not shown). These findings demonstrate that primary radial glial scaffold and compact hippocampal primordium are normally formed in E17.5 β-catenin CKO mice.

**β-Catenin deletion leads to ectopic projection and migration of radial glial cells**

We found that at E18 in the wild-type hippocampus, GFAP+ radial glial cells form a very clear horseshoe-shaped scaffold around the DG (Fig. 5C, E), and GFAP+ fibers have begun to extend from the lower to the upper blade at the blade tip. In the β-catenin CKO mice, a strand of GFAP+ fibers extends ectopically to the upper blade of DG in the middle of the lower blade (arrowheads in Fig. 5D, F). Calbindin and Hoechst staining revealed that the cells surrounding the ectopic GFAP+ fibers have begun to disperse, leaving behind an empty space (arrowhead in Fig. 5B, H), however there are still no differences in the distribution of Reelin+ Cajal–Retzius cells at E18 between control and β-catenin CKO mice (Fig. 5I, J). These findings indicate that the ectopic projection and migration of radial glial cells observed in β-catenin CKO mice are not mediated through reelin-dependent pathways.

To determine the mechanism underlying the ectopic projection and migration of radial glial cells in β-catenin CKO mice, we first conducted double immunostaining of β-catenin and GFAP to detect whether β-catenin is expressed by these radial glial cells. We found that some GFAP+ cells coexpress β-catenin at P0 (arrows in Fig. 6C). We then performed double immunostaining of Cre and GFAP at E18 and P7 to examine whether Cre recombinase is expressed in radial glial cells in the CaMKIIz-iCre transgenic mice we used. Expression of Cre recombinase in CamKIIz-iCre transgenic mice was mainly observed in granule cells (arrowhead in Fig. 6E, H) and pyramidal cells (arrow in Fig. 6E, H), which is consistent with previous reports (Casanova et al., 2001). Interestingly some of GFAP+ radial glial cells, especially at the DG, also coexpress Cre recombinase at E18 (arrowheads in Fig. 6F), while there is no colocalization between Cre+ cells and GFAP+ cells at P7 (Fig. 6I). These results suggest that the deletion of β-catenin expression in some radial glial cells might lead to disorganization of radial glial scaffold possibly via the well-known role of β-catenin in cell adhesion in the developing brain.

**Normal primary radial glial scaffold and hippocampal primordium development in E17.5 β-catenin CKO mice**

The secreted protein reelin, which is produced and released by Cajal–Retzius cells, is known to act upon the radial glial scaffold to control the migration of dentate granule cells (Forster et al., 2006b). In order to determine what causes hippocampal malformations in β-catenin CKO mice, we first examined the distribution of reelin+ Cajal–Retzius cells and GFAP+ radial glial cells at late embryonic stages. At E17.5, when the primary radial glial scaffold is apparent and calbindin+ cells have densely aggregated to form the hippocampal primordium, we found no differences in the expression levels of β-catenin, calbindin, GFAP or reelin between control and β-catenin CKO mice (data not shown). These findings demonstrate that primary radial glial scaffold and compact hippocampal primordium are normally formed in E17.5 β-catenin CKO mice.

**β-Catenin domain is occupied by GFAP+ radial glial cells at P0 in CKO mice**

By P0, the ectopic projection and migration of radial glial cells in β-catenin CKO mice had become more apparent.
(Fig. 7). We found a dense column of GFAP\(^{+}\) fibers migrating through the calbindin\(^{-}\) domain to the upper blade, forming an expression pattern complementary to that of calbindin\(^{+}\) cells in the DG (arrowheads in Fig. 7E–H, E’–H’). Since the calbindin\(^{-}\) domain is equivalent to the \(\beta\)-catenin\(^{-}\) domain at the border between CA3 and the DG at P0 (Figs. 1B, 7E), we speculated that the cells in the \(\beta\)-catenin\(^{-}\) domain were not neurons but radial glial cells. We further examined the expression of various hippocampal markers at P0, starting with neurogenin 2 (Ngn2), a bHLH transcription factor expressed in immature hippocampal precursors that is essential for neuronal specification (Pleasure et al., 2000; Parras et al., 2002). In our CKO mice, we found that Ngn2 mRNA expression is absent in the same cluster of hippocampal cells from which \(\beta\)-catenin expression was missing (Fig. 8B, D). We also looked at two other markers: NRP2 and Prox1, whose expression is also missing from the same \(\beta\)-catenin\(^{-}\) domain, as was the case for Ngn2 (arrows in Fig. 8B, D, F, H). These findings demonstrate that \(\beta\)-catenin\(^{-}\) domain is probably occupied by GFAP\(^{+}\) radial glial cells at P0 in \(\beta\)-catenin CKO mice.

**DISCUSSION**

Hippocampal development is divided into prenatal and postnatal stages. During the prenatal stage, early-generated pyramidal and granule cells migrate all the way from the lateral ventricular zone to form the hippocampal primordium. Later on, during the early postnatal stage, new granule cells are primarily generated in the primordial granular layer, and they combine with the migrated cells to form the highly radially organized DG (Li and Pleasure, 2005; Tian et al., 2012). Maintaining the hippocampal primordium is thus an essential aspect of normal postnatal hippocampal

![Fig. 6. Double immunostaining of GFAP and \(\beta\)-catenin or Cre recombinase in CaMKII\(^{\alpha}\)-iCre transgenic mice. Co-immunostainings of GFAP and \(\beta\)-catenin at P0 (A–C) or GFAP and Cre recombinase at E18 (D–F, D’–F’) and P7 (G–I) were conducted respectively. It was noted that \(\beta\)-catenin is expressed by some GFAP\(^{+}\) cells (arrows in C) and Cre is mainly expressed in granule cells (arrowheads in E, H) and pyramidal cells (arrow in E, H). However, at E18, Cre can be detected in some GFAP\(^{+}\) radial glial cells in the DG of CaMKII\(^{\alpha}\)-iCre transgenic mice (arrow heads in F’). D’–F’ show magnified views of D–F respectively. Scale bars: 100 \(\mu\)m in C (for A–C), 100 \(\mu\)m in F (for D–F), 50 \(\mu\)m in F’ (for D’–F’), 100 \(\mu\)m in I (for G–I).]
development. However, the mechanisms that maintain the morphology of the primordium remain to be characterized. In the present study, we found that β-catenin is required for the maintenance of hippocampal primordium morphology during the perinatal period. Deleting β-catenin from some radial glial cells at approximately E18 might result in the displacement of radial glial cells and consequential ectopic migration of hippocampal cells, particularly in the DG.

It was noted that the Reichardt laboratory had also investigated CamKIIα-Cre-mediated β-catenin CKO mice and observed no obvious phenotypes in the hippocampus (Bamji et al., 2003). These contrasting results may be explained by the large difference between

![Fig. 7. Ectopic projection and migration of the radial glial cells in P0 β-catenin CKO mice. (A–H) Calbindin and GFAP protein expression was examined by co-immunostaining analysis in P0 control and β-catenin CKO mice. Calbindin immunoreactivity was found to be generally strong in postmitotic cells of the hippocampus; the one exception being a cluster of calbindin+ cells at the border between CA3 and the DG in β-catenin CKO mice (E and arrowhead in E). A dense column of GFAP+ fibers was observed migrating through the calbindin+ domain to the upper blade, thus forming an expression pattern complementary to that of calbindin+ cells in the DG (F and arrowhead in F). In addition, GFAP+ cells in the upper blade of the radial glial scaffold are much more dispersed in β-catenin CKO mice than in controls (arrows in B and F). Hoechst staining revealed that, in the CKO mice, some cells had left the upper blade of the hippocampus (arrow in H), and an empty, cell-less space was observed in the lower blade (dotted lines in H). A–H show magnified views of A–H, respectively. Scale bars = 200 μm in H (for A–H), 100 μm in H’ (for A’–H’).](image)

![Fig. 8. Absence of various hippocampal markers in the β-catenin domain. In situ hybridization analysis showed that, in β-catenin CKO mice, the expression of Ngn2 (C–D), NRP2 (E–F) and Prox1 (G–H) mRNA is lost in the same cluster of hippocampal cells (arrows in D, F, H) where β-catenin expression is also missing (arrow in B). Scale bars = 100 μm in H (for A–H).](image)
the two Cre lines. The expression of Cre recombinase in the CaMKIIα-Cre mice used by the Reichardt laboratory is under the control of an 11-kb fragment containing the promoter for CaMKIIα (Xu et al., 2000). Almost complete Cre-mediated recombination was initiated as early as E17 and observed in the hippocampus, including CA1, CA2, and CA3 pyramidal neurons as well as dentate granule neurons (Bamji et al., 2003). However, the expression of iCre recombinase in the CaMKIIα-iCre mice that we used was driven by CaMKIIα BAC transgene (Casanova et al., 2001). In addition, iCre is Codon-improved Cre recombinase that can improve Cre expression in mammalian cells (Shimshek et al., 2002). Consequently the Cre recombinase expression in the CaMKIIα-iCre mice was initiated as early as E18 and was observed in the whole hippocampus, and even in some radial glial cells, constituting a significant difference to those used by the Reichardt’s laboratory. It is therefore reasonable that the phenotypes in the hippocampus differ in the two different β-catenin CKO mice.

**Function of β-catenin in the maintenance of hippocampal radial glial scaffold**

A number of previous studies have shown that radial glial scaffold formation is essential for the proper development of the mature hippocampus (Forster et al., 2006a). In the present study, double immunostaining of β-catenin/GFAP or Cre/GFAP revealed that β-catenin could be conditionally knocked out in some GFAP+ radial glial cells (Fig. 6). It was therefore hypothesized that the disruption of β-catenin–cadherin-mediated cell adhesion would result in ectopic projection and migration of radial glial cells in β-catenin CKO mice. Then the disorganization of the radial glial scaffold ultimately resulted in the disruption of hippocampal morphology. This hypothesis is in agreement with and supported by a previous study showing that in mice where N-cadherin has been conditionally knocked out, the organization of the pyramidal neuron layer is significantly disrupted, in a fashion similar to that seen in the hippocampus of β-catenin CKO mice (Kadowaki et al., 2007). In addition, Bamji’s paper revealed that the deletion of β-catenin in neurons did not result in the malformation of hippocampus (Bamji et al., 2003), which also supports our hypothesis.

Despite our aforementioned hypothesis that radial glial scaffold dysfunction underlies hippocampal defects in β-catenin CKO mice, it is difficult to definitively ascertain cell autonomous versus non-cell autonomous mechanisms from the data. Many questions such as when the radial glial cells begin to express β-catenin and whether it is possible that a neuronal loss of adhesion leads to a non-cell autonomous glial ectopia remain to be answered. More Cre-specific expression mice are required to investigate these possibilities and to elucidate the full mechanism underlying our observations.

**Function of β-catenin-dependent Wnt signaling in hippocampal cell differentiation**

β-Catenin is a major structural component of adherens junctions, but also plays a pivotal role in Wnt signaling during embryogenesis (Peifer et al., 1991, 1994; Clevers, 2006). Previous studies have illustrated that β-catenin-dependent canonical Wnt signaling is required for early hippocampal development and granule cell generation (Zhou et al., 2004; Li and Pleasure, 2005). An interesting extension of this study might investigate whether β-catenin-dependent canonical Wnt signaling plays a role in hippocampal cell differentiation at a later stage of hippocampal development. Here we have shown that hippocampal cells in CA2 and CA3, which lack β-catenin expression at P7, still express NRP2 (double arrows in Fig. 3B), which suggests that a deficiency of β-catenin in hippocampal neurons does not disrupt their differentiation at this time. Although the ectopic cells in the hippocampus fail to express some hippocampal markers such as NRP2 and Prox1 (Fig. 3D, F), we cannot exclude the possibility that the differentiation arrest of these cells is a secondary defect caused by ectopic migration of the hippocampal cells.

**CONCLUSIONS**

In summary, we conditionally knocked out β-catenin, a key factor of canonical Wnt signaling and also a component of adherens junctions, in forebrain cells during the perinatal stage and examined the role of β-catenin in perinatal hippocampal development. Disorganization of radial glial scaffold and ectopic migration of hippocampal cells were observed as early as E18 in β-catenin CKO hippocampus, which suggests that β-catenin is required for maintaining hippocampal primordium morphology.

**AUTHOR CONTRIBUTIONS**

X.Z., H.Z., Z.J. conceived the idea. H.Z. and W.H. designed the experiment and analyzed the data. W.H., Z.L., C.Q. and L.J. performed the histological analysis. Z.X. bred the mice and conducted genotyping. H.Z., W.H. and L.T wrote the manuscript. All authors read and approved the final manuscript.

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