Neuroscience 284 (2015) 273–282

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 *CamKIIα-iCre; β-catenin*$^{flox/flox}$ 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.

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

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Abbreviations: BCIP, 5-bromo-4-chloro-3-indolyl-phosphate; BSA, Bovine Serum Albumin; CA, Cornu Ammonis; CKO, conditional knockout; DG, dentate gyrus; EDTA, ethylenediaminetetraacetic acid; GFAP, glial fibrillary acidic protein; ISH, *In situ* hybridization; NBT, nitro blue tetrazolium chloride; Ngn2, neurogenin 2; NRP2, neuropilin-2; PBS, Phosphate buffered saline; PCR, polymerase chain reaction; SSC, Saline-sodium citrate buffer.

http://dx.doi.org/10.1016/j.neuroscience.2014.08.055
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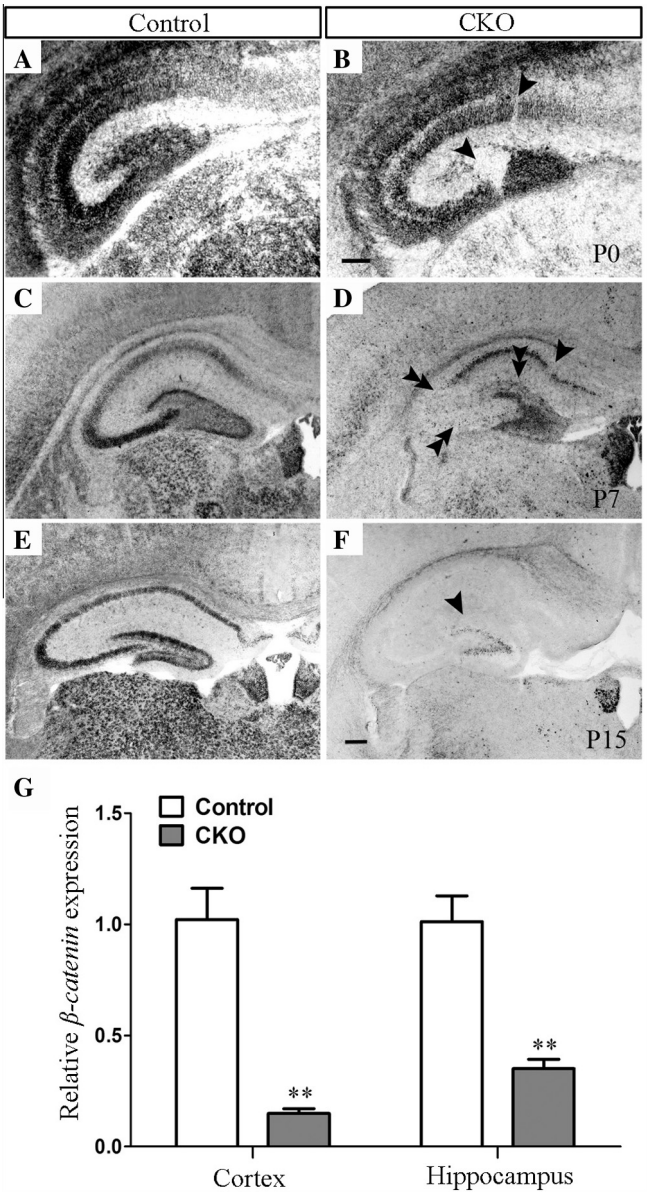

Fig. 1. Expression analysis of *β-catenin* from P0 to P15 in control and *β-catenin* CKO mice. (A–F), *In situ* hybridization revealed that, as age increases, so does the extent to which *β-catenin* mRNA expression is inactivated in the hippocampus of CKO mice. (A, B), In P0 CKO mice compared to controls, *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. $**$, $P \leqslant 0.01$ compared with control mice. Scale bars = 100 μm in B (for A–B), 200 μm in F (for C–F).

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*$^{flox/flox}$ 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.