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Research Report

Hydrocephalus and abnormal subcommissural organ in mice lacking presenilin-1 in Wnt1 cell lineages☆

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ABSTRACT

Presenilin-1 (PS1) is a transmembrane protein that is in many cases responsible for the development of familial Alzheimer's disease. PS1 is widely expressed in embryogenesis and is essential for neurogenesis, somitogenesis, angiogenesis, and cardiac morphogenesis. To further investigate the role of PS1 in the brain, we inactivated the PS1 gene in Wnt1 cell lineages using the Cre–loxP recombination system. Here we show that conditional inactivation of PS1 in Wnt1 cell lineages results in congenital hydrocephalus and subcommissural organ abnormalities, suggesting a possible role of PS1 in the regulation of cerebrospinal fluid homeostasis.

1. Introduction

Presenilin-1 (PS1) is a transmembrane protein expressed ubiquitously in human and mouse tissues (Sherrington et al., 1995; Lee et al., 1996) and is in many cases responsible for the development of early-onset familial Alzheimer's disease (Cruts and Van Broeckhoven, 1998a,b). Full-length PS1 undergoes endoproteolytic cleavage, generating stable N- and C-terminal fragments (NTF and CTF) that interact with other proteins to form a macromolecular complex with γ-secretase activity. This enzyme is required for the regulation of intramembrane proteolysis of amyloid precursor protein (APP), Notch, and cadherins (De Strooper et al., 1999; Marambaud et al., 2003; Koo and Kopan, 2004). PS1 also has an important role in the

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turnover of β-catenin, a molecule essential in Wnt signaling and cell adhesion (Kang et al., 2002; Gottardi and Gumbiner, 2004).

Earlier studies of PS1-knockout null mice have contributed to our understanding of the *in vivo* developmental functions of PS1 in neurogenesis, somitogenesis, angiogenesis, and cardiac morphogenesis (Shen et al., 1997; Wong et al., 1997; Handler et al., 2000; Koizumi et al., 2001; Yuasa et al., 2002; Nakajima et al., 2003, 2004). The role of PS1 in the perinatal and postnatal stages, however, has not been examined because PS1 null mice die perinatally. A new approach using the Cre–loxP system allows for the production of mice that conditionally lack PS1 and examination of the PS1 function during the perinatal and postnatal periods (Yu et al., 2001; Saura et al., 2004; Nakajima et al., 2009).

Hydrocephalus is typically divided into noncommunicating or communicating subtypes (Fishman, 1992). Noncommunicating hydrocephalus is caused by an obstruction within the ventricular system, such as a tumor, that prevents cerebrospinal fluid (CSF) proximal to the obstruction from draining into the subarachnoid space, where it is reabsorbed into the venous sinuses. Communicating hydrocephalus results from impaired absorption of CSF despite patent CSF pathways. Both communicating and noncommunicating hydrocephalus occur congenitally or are acquired secondary to trauma, tumor, hemorrhage, or infection (Guyot and Michael, 2000; Yoshioka et al., 2000). The development and progression of congenital hydrocephalus are not yet well understood. Only one hydrocephalus gene, *L1CAM*, has been identified in humans (Jouet et al., 1993). The mutations are distributed over the functional protein domains of L1CAM protein. The exact mechanisms by which these mutations cause a loss of L1CAM function remain unclear.

In the present study, we examined the role of PS1 using mice conditionally lacking PS1 in the neural crest cell lineages and found a hydrocephalic pathology in the mutant mice. Our findings suggest a possible role of PS1 in the regulation of CSF homeostasis.

2. Results

2.1. *Congenital hydrocephalus in Wnt1-cre PS1-conditional knockout (cKO) mice*

In the previous study, we crossed floxed PS1 and Wnt1-cre mice (Danielian et al., 1998; Yu et al., 2001; Saura et al., 2004) and generated mice lacking PS1 in the neural crest cell lineages (Nakajima et al., 2009). In contrast to PS1 null mice, which die perinatally (Shen et al., 1997; Koizumi et al., 2001; Nakajima et al., 2003, 2004), the Wnt1-cre PS1-cKO mice are viable with no obvious phenotypic abnormalities for several days after birth. Although 20% to 40% of mice exhibit reduced weight at 5 weeks of age, the remaining mice mature and do not show abnormal phenotypes in appearance (Nakajima et al., 2009). In the present study, we observed that nearly all mutant mice developed hydrocephalus at 5 weeks of age (Fig. 1), irrespective of their weight or sex. Histologic analyses revealed enlargement of the lateral and third ventricles in the mutant brains (Fig. 1B and D). Analyses of young mutant mice revealed enlarged ventricles in three of four brains at 4 days of age and three of three brains at 7 days of age (data not shown), indicating that the hydrocephalus of the mutants is congenital. No gross histologic abnormalities were detected, however, other than hydrocephalus.

2.2. *Hydrocephalus is associated with abnormalities of the subcommissural organ (SCO) and the Sylvian aqueduct in Wnt1-cre PS1-cKO mice*

Because the SCO and the Sylvian aqueduct are often affected in mice exhibiting hydrocephalus, we examined the structures in Wnt1-cre PS1-cKO mice. The SCO is located in the roof of the third ventricle at the entrance of the Sylvian aqueduct and spans the rostral part of the aqueduct (Rodríguez et al., 1998;

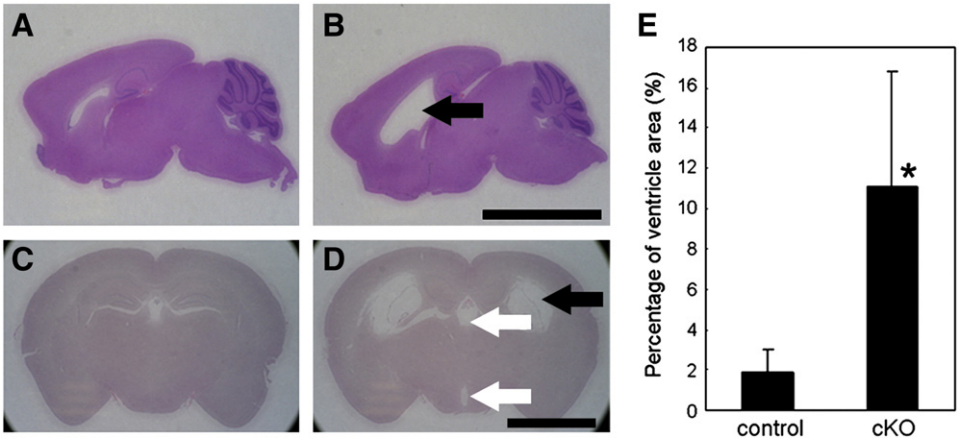

Fig. 1 – Dilated ventricles in Wnt1-cre PS1-cKO brains. Parasagittal (A, B) and coronal (C, D) brain sections of control (A, C) and Wnt1-cre PS1-cKO (B, D) mice, 5 weeks old, were stained with hematoxylin and eosin. Note the dilation of the lateral ventricles (black arrows in B and D) and third ventricles (while arrows in D). (E) Ventricle areas (lateral ventricles and third ventricles) and total brain areas of coronal sections from control ($n=6$) and Wnt1-cre PS1-cKO ($n=8$) mice were measured with Image J and the percentage of ventricle areas relative to the total brain areas was calculated. Values are mean ± SD. *The percentages differed significantly between control and Wnt1-cre PS1-cKO brains ($p<0.005$). Scale bar = 5 mm (A, B), 3 mm (C, D).