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Research Report

CRBL cells: Establishment, characterization and susceptibility to prion infection

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ABSTRACT

The cerebellum is involved in complex physiological functions including motor control, sensory perception, cognition, language, and emotion. Humans and animals with prion diseases are characterized clinically by ataxia, postural abnormalities and cognitive decline. Pathology in the cerebellum affected by prions includes spongiform degeneration, neuronal loss, and gliosis. To develop an *in vitro* model system for studying prion biology in cerebellar cells, we established and characterized an immortal cell line (CRBL) isolated from the cerebellum of mice lacking expression of a protein involved in cell cycle arrest. The characteristics of the cells include morphological heterogeneity, rapid proliferation, serum responsiveness during growth, and a change in the number of chromosomes. CRBL cells expressed both neuronal and glial cell markers as well as a considerable level of cellular prion protein, PrP^{C}. Upon *in vitro* infection, CRBL cells exhibited selective susceptibility to prions isolated from different sources. These cells chronically propagated prions from SMB cells. Strain-specific prion infection in CRBL cells was not due to instability of the cell line, allelic variance, or mutations in the *PrP* gene. Molecular properties of prions derived from SMB cells were maintained in the infected CRBL cells. Our results suggest that the specific interaction between a prion strain and hosts determined the selective susceptibility of CRBL cells, which reflects the conditions *in vivo*. In addition to the future studies revealing cellular and molecular mechanism involved in prion pathogenesis, CRBL cells will contribute to the studies dealing with prion strain properties and host susceptibilities.

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Abbreviations: PrP, prion protein; PrP^{C}, cellular prion protein; PrP^{Sc}, scrapie prion protein disease; CJD, Creutzfeldt-Jakob; $p53^{-/-}$, p53 null; CRBL, a cell line established from the cerebellum; N2a, Neuro2a; ScN2a, scrapie-infected N2a; SMB, scrapie-infected mouse brain cells; SMB-PS, SMB cells cured by pentosan sulfate; PK, proteinase K; Bt_2AMP, N6, 2′-*O*-dibutyryladenosine 3′,5′-cyclic monophosphate; PMSF, phenylmethylsulphonyl fluoride; GFAP, glial fibrillary acidic protein; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; ORF, open reading frame

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1. Introduction

The cerebellum has been recognized as the structure involved in motor coordination. It is also known to contribute to non-motor functions such as sensory perception, cognition and emotion (Bastian and Thach, 2002; Ghez and Thach, 2000). The cerebellum contains more neurons than all the other structures of the brain and retains functionally well-defined organization where the networks of neural cells convey millions of bites of information related to cerebellar functions to and from many other regions of the brain and the spinal cord (Bastian and Thach, 2002). This complex network is essential for the roles of the cerebellum in motor, sensory, and cognitive functioning. For this reason, cerebellar lesions typically exhibit deficits during movement execution, difficulties in maintenance of posture and balance, and dysfunctions in eye movement and speaking (Topka and Massaquoi, 2002).

Prion diseases are fatal neurodegenerative disorders caused by the proteinaceous pathogen, prions (Prusiner, 1998). Prions are composed of β-sheet rich, disease-associated prion protein (PrP^{Sc}) that underwent conformational transition from α-helix rich cellular prion protein (PrP^{C}) (Prusiner, 1998). Due to conformational changes, PrP^{Sc} becomes hydrophobic and partially resistant to proteinase K (PK) digestion. Humans and animals with prion diseases exhibit abnormalities in coordination of muscle movement, lack of balance, disturbance of gait, over reactive sensory perception, loss of language, dementia, and irritable demeanor (Collinge, 2001). These diseases accompany pathological lesions in the brain structures including the cerebellum (DeArmond et al., 2004; Ferrer, 2002). Patients with Creutzfeldt–Jakob disease (CJD) frequently exhibit accumulated prion plaques in the cortex, a widespread microvacuolar spongiform change in the molecular layer, neuronal loss in the granular and Purkinje cell layers, and gliosis in astrocytes of the cerebellum (Armstrong et al., 2001a, 2002, 2001b; Ferrer et al., 2000; Jarius et al., 2003; Schulz-Schaeffer et al., 1996).

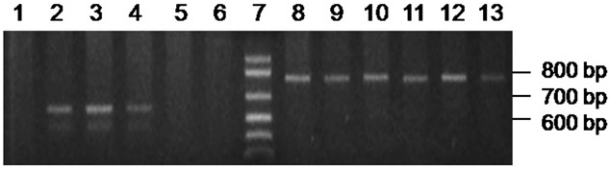

Fig. 1 – Screening of p53 null mice. p53 null homozygote (lanes 1, 5 and 6) and heterozygote (lanes 2, 3, and 4) mice were screened by PCR. Genomic DNA from mouse tail snips were purified by gDNA isolation kit (Qiagen) after PK and RNase treatment. The p53- and neo-specific amplicons were generated by the combination of different forward primers (p53 forward primer: 5′- gacaagttatgcatccataca -3′ and neo forward primer: 5′- gaacctgcgtgcaatccatct -3′) and the common reverse primer (5′- ctcctcaacatcctggggcag -3′). The PCR condition for p53-specific amplicon was 94 °C for 2 min; 40 cycles of 94 °C for 1 min/60 °C for 2 min/72 °C for 3 min; 72 °C for 15 min. The PCR condition for neo-specific amplicon was 94 °C for 3 min; 35 cycles of 94 °C for 45 s/61 °C for 25 s/72 °C for 30 s; 72 °C for 15 min. The amplicons were separated on the 0.8% agarose gel. Lanes 1–6: amplicons from p53 forward and p53 reverse primers, Lane 7: 1 kb plus DNA marker (Invitrogen), Lanes 8–13: amplicon from neo forward and p53 reverse primers.

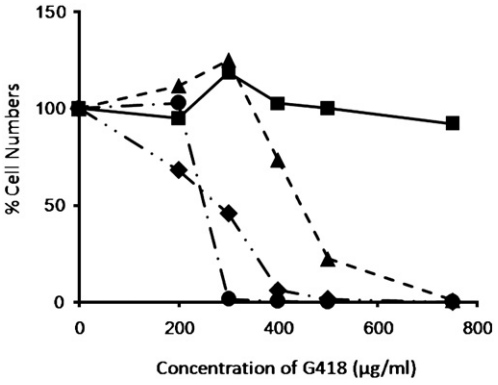

Fig. 2 – Resistance of CRBL cells against G418 treatment. The cells were seeded at 1×10^4 cells/well, cultured with 0–750 μg/ml G418 for 7 days, and surviving cells were counted after trypan blue staining. The numbers of live cells were normalized with the cell numbers obtained from the untreated cells, which differ from ~2 to 9×10^5 cells depending on cell types. CRBL (squares, solid line); 2.0×10^5 cells, N2a (triangles, dashed line); 5.6×10^5 cells, ScN2a (diamonds, double-dot line); 6.5×10^5 cells, SMB (circle, single-dot line); 8.7×10^5 cells. The viability of CRBL was not significantly affected by treatment with G418.

PrP^{C} appears to play an important physiological role for neurons in the cerebellum (Herms et al., 2000, 2001; Katamine et al., 1998; Laine et al., 2001; Legname et al., 2002). The major events leading to pathogenesis also occur in the cerebellum when PrP^{C} is converted to PrP^{Sc} (Ferrer, 2002). Despite the increasing number of studies, the mechanisms involved in both physiology and pathology of PrP isoforms in the cerebellum is still poorly understood. In order to understand the cellular and molecular mechanisms involved in prion diseases, it is necessary to investigate the roles of the cerebellar cells in a well-characterized *in vitro* model system that mimics the conditions *in vivo*.

The present study describes the establishment and characterization of the immortalized mouse cerebellar cells termed CRBL, and susceptibility of these cells to prions. CRBL cells will be a useful model system for the research involved in prion biology of the cerebellum.

2. Results

2.1. *Establishment of the CRBL cells*

The normal diploid cells obtained from wild type animals undergo senescence losing their ability to divide when they are cultured *in vitro*. To establish a cell line that mimics the physiological conditions in the cerebellum and is continuously dividing *in vitro*, we obtained cells from the cerebellum of the mice lacking expression of the *p53* gene. Since the *p53* gene product arrests progression of the cell cycle when DNA damage is sensed (Harris and Levine, 2005), the loss of *p53* gene expression results in perpetual cellular division and