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Research Paper

Prion-Seeding Activity Is widely Distributed in Tissues of Sporadic Creutzfeldt-Jakob Disease Patients

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ABSTRACT

Human prion diseases are neurodegenerative disorders caused by abnormally folded prion proteins in the central nervous system. These proteins can be detected using the quaking-induced conversion assay. Compared with other bioassays, this assay is extremely sensitive and was used in the present study to determine prion distribution in sporadic Creutzfeldt-Jakob disease patients at autopsy. Although infectivity of the sporadic form is thought to be restricted within the central nervous system, results showed that prion-seeding activities reach 10^6/g from a 50% seeding dose in non-neuronal tissues, suggesting that prion-seeding activity exists in non-neural organs, and we suggested that non-neural tissues of 10^6/g SD50 did not exist the infectivity.

1. Introduction

Transmissible spongiform encephalopathies, also called 'prion diseases,' are caused by abnormally accumulated prion protein (PrP-res) in the central nervous system (CNS). The causative agent is thought to be solely composed of amyloid prion proteins and is not inactivated by standard and popular procedures. Iatrogenic Creutzfeldt-Jakob disease (CJD) can be caused by the reuse of neurosurgical instruments or contamination of biomaterials, such as dura mater graft material (Thadani et al., 1988; Bernoulli et al., 1977; Will & Matthews, 1982). However, extensive investigations have concluded that accidental prion transmission, as well as sporadic CJD (sCJD), which is the idiopathic form of CJD, is not likely the consequence of spontaneous somatic mutations. The proteinase K (PK)-resistant (PK-res) PrP in sCJD has been shown to be limited to cases with biological materials from the CNS or cornea. Additionally, in sCJD, prion infectivity has not been detected in extracerebral organs in studies using animal models, suggesting that infectious prions are restricted to the CNS. However, recent studies used Western blotting analysis to detect PrP-res in the spleen of a sCJD patient, although the PrP^{Sc} levels were lower by a factor of approximately 10^{-4} than in brain tissue (Glatzel et al., 2003). These studies highlight the need to elucidate prion distribution in humans to reduce the risk of accidental prion infection. Various studies have already detected PK-resistant PrP in peripheral tissues in natural and experimental sCJD cases. For instance, Glatzel M, et al. (Glatzel et al., 2003) used Western blotting to show the presence of PrP^{Sc} in the spleen, as well as in the muscle of some sCJD patients. Additionally, Rubenstains et al. detected PrP^{Sc} in tonsil and lymph node tissues of sCJD patients (Rubenstein & Chang, 2013). Experimentally, Herzog et al. infected non-human primates with the sCJD agent (among others) to investigate the involvement of peripheral organs (Herzog et al., 2005). They also confirmed the presence of PrP^{Sc} in lymphoreticular organs and muscles.

A technique recently developed for *in vitro* amplification of prions, the RT-QuIC (quaking-induced conversion) assay, is a highly sensitive and specific technique that can detect small amounts of prion-seeding

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activity (Takatsuki et al., 2015). In our study, the detection limit of the RT-QuIC assay was approximately 0.12 fg of PrP-res (Herzog et al., 2005). Combined with an endpoint dilution, it was possible to quantify prion-seeding activity and the 50% seeding dose (SD_{50}), which correlated well with PK-res PrP. These results encouraged us to re-evaluate the human prion in various tissues in sCJD patients.

2. Materials & Methods

2.1. Patients

A total of four female patients were diagnosed with classical-type sCJD, which was histopathologically confirmed after autopsy. To avoid contamination of brain tissue, other organs were separately harvested. The tissue specimens were immediately stored at −80 °C until further use. Western blotting analysis of the PK-res PrP fragment and genotyping at codon 129 of the *PRNP* gene was conducted as previously described by the reference laboratory of the Japan CJD Surveillance Unit. Non-CJD tissues were purchased from Proteo Genex (Culver City, *CA*, USA). The protocol was approved by the Ethics Committee of Nagasaki University Hospital (ID: 100428423) and the use of specimens was also granted ethical approval by the Japan CJD Surveillance Unit. The study was registered with the University Hospital Medical Information Network (ID: UMIN000003301). Informed consent was obtained from patient families and/or patients.

2.2. Tissue Homogenate Preparation

Brains, spleens, kidneys, lungs, livers, and adrenal glands were subjected to RT-QuIC for evaluating SD_{50}. To prevent contamination of brain tissues into other samples, we used single-use disposable tubes and beads, and all procedures were performed on different days. Tissue samples of brain, spleen, kidney, lung, liver, and adrenal gland were homogenized in 10% (w/v) ice-cold phosphate-buffered saline supplemented with a protease inhibitor mixture (Roche, Mannheim, Germany) using a multi-bead shocker (Yasui Kikai, Osaka, Japan). The samples were clarified by centrifugation at 6000 rpm for 2 min and stored at −80 °C.

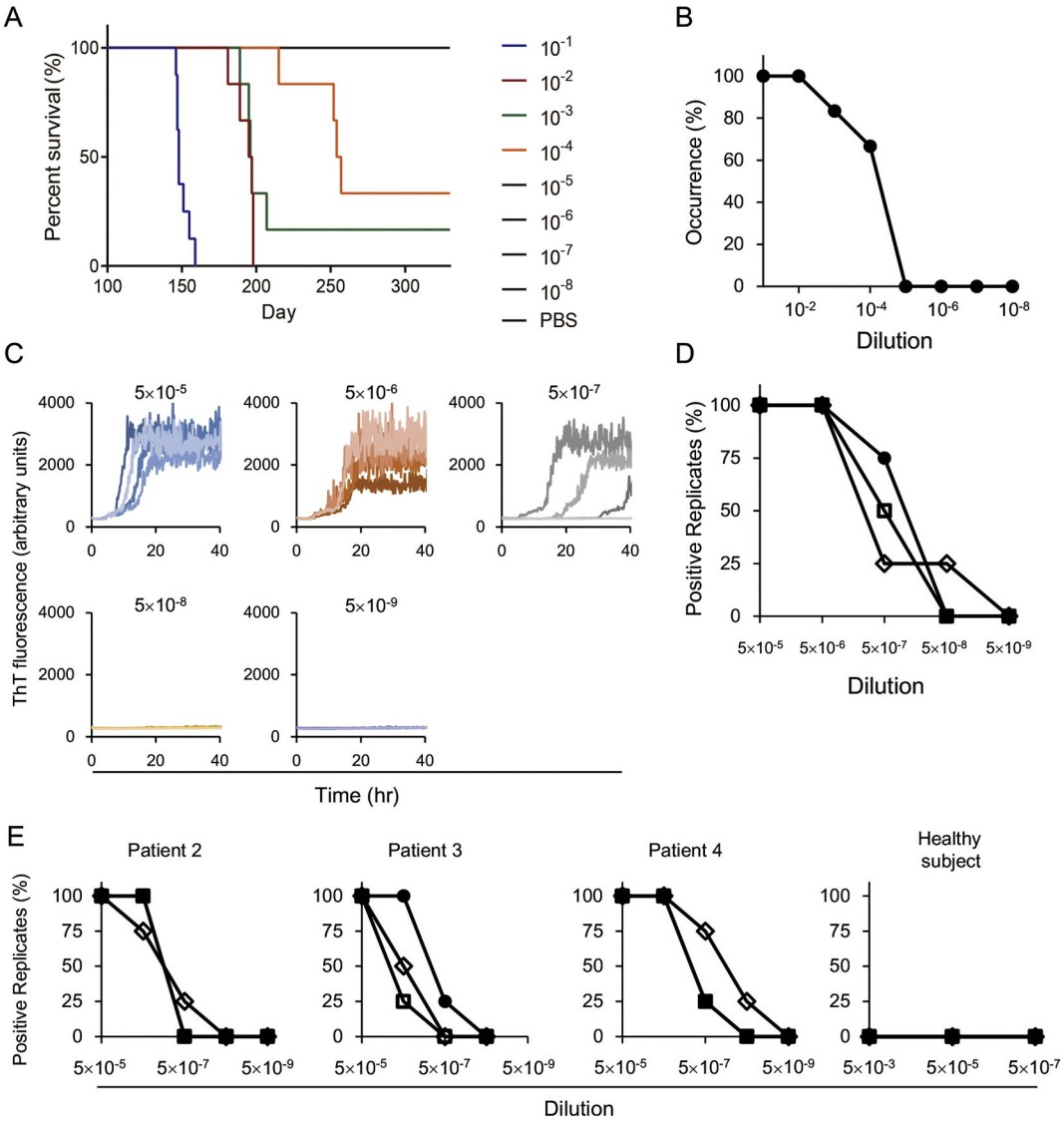

Fig. 1. Analysis of brain tissues from patients with sporadic CJD using bioassay and endpoint RT-QuIC assay. (A, B) Transmission of human prions to Ki-ChM mice. (A) Survival curve for Ki-ChM mice inoculated with a serially diluted brain homogenate from Patient #1. (B) Endpoint titration of brain tissue from a sCJD patient (Patient #1) using the bioassay. Each value represents the percentage of occurrence of symptoms caused by prion disease. (C, D) Endpoint RT-QuIC assay of brain tissue from Patient #1. (C) Brain tissue was diluted and the endpoint RT-QuIC assay was used to evaluate prion-seeding activity. (D) Each value represents the percentage of positive reactions for each dilution rate. The experiments were performed in triplicate for each sample. (E) Endpoint RT-QuIC assay of brain tissues from patients with sCJD (Patients #2–4). The experiments were performed in triplicate for each sample.

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