Review article

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Prion protein (PrP) deposits in the tectum of experimental Gerstmann-Sträussler-Scheinker disease following intraocular inoculation

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Abstract

The abnormal misfolded isoform of prion protein (PrP^d; "d" for disease) is considered as a surrogate marker for infectivity in the transmissible spongiform encephalopathies (TSEs) or prion diseases, including Creutzfeldt-Jakob disease (CJD). In this experiment, we used intraocular inoculation to study PrP^d deposition in the visual system of the brain of mice infected with the Fujisaki (K.Fu) strain of Gerstmann-Sträussler-Scheinker (GSS) disease. We report here that PrP^d is deposited in the superior colliculus following contralateral intraocular inoculation and thus follows neuronal connections when it spreads into the brain. Until 26 weeks postinoculation, no PrP^d-specific immunostaining was observed in the brain. At 27 weeks postinoculation, PrP^d targeted to the contralateral superior colliculus as delicate granular synaptic deposits located in the superficial part of this structure. As already reported, a few spongiform vacuoles were visible in the same area by conventional H&E staining. In several other sections, vacuoles were visible but no PrP^d staining could be detected.

Key words: *prion, prion diseases, Gerstmann-Sträussler-Scheinker disease, spreading of prions.*

Introduction

The abnormal misfolded isoform of prion protein (PrP^d; "d" for disease) is considered as a surrogate marker for infectivity in the transmissible spongiform encephalopathies (TSEs) or prion diseases [30], including Creutzfeldt-Jakob disease (CJD) [3,6,31]. In this experiment, we used intraocular inoculation to study PrP^d deposition in the visual system of the brain of mice infected with the Fujisaki (K.Fu) strain of Gerstmann-Sträussler-Scheinker (GSS) disease. We report here that PrP^d is deposited in the superior colliculus following contralateral intraocular inoculation and thus follows neuronal connections when it spreads into the brain [32].

Material and methods

The experiment was designed to recapitulate that of targeting scrapie pathology to the contralateral superior colliculus following intraocular injection [32]. To this

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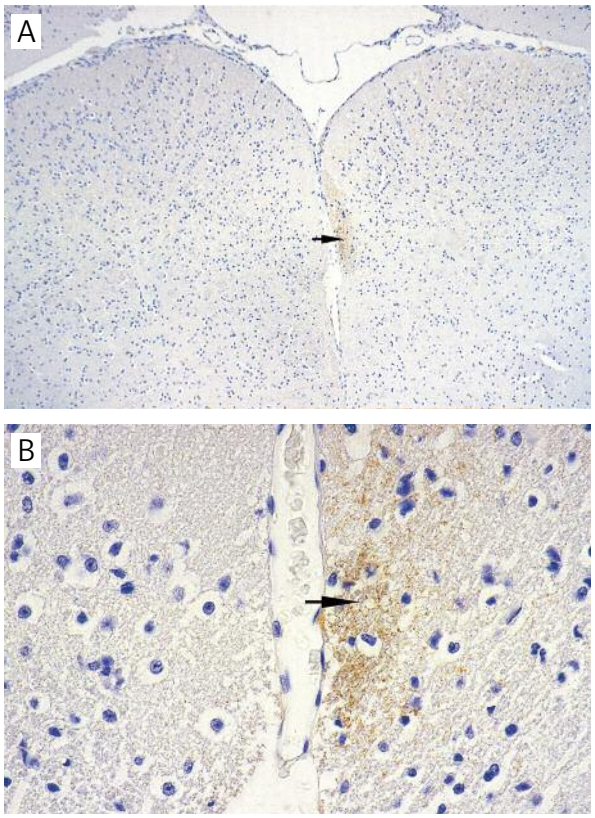

Fig. 1. Low (**A**) and high (**B**) magnification of expression of PrP (arrows) in superior colliculus 27th week postinoculation.

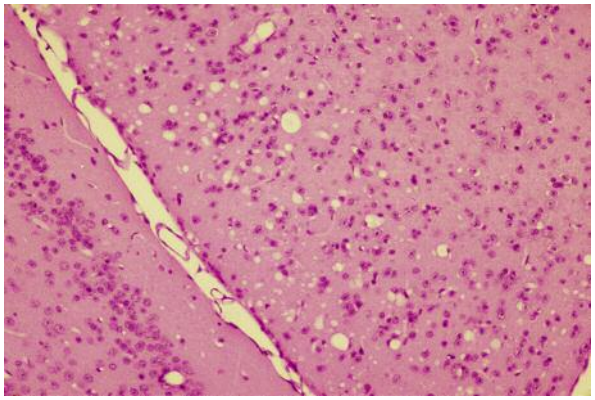

Fig. 2. The same region stained with H&E.

end, four- to five-week-old NIH Swiss mice of both sexes were inoculated into the right anterior eye chamber with 0.01 mL of a 10% (w/v) clarified brain suspension of the Fujisaki strain of GSS [33,34]. Brains were collected beginning at 18 weeks postinoculation and the experiment was terminated when unequivocal spongiform changes appeared in the contralateral superior colliculus. The latter part of the experiment has been already published [32]. Two mice were sacrificed weekly by decapitation under light anaesthesia with ketamine and brains were immediately fixed in 10% buffered formalin. Brains were then transected coronally to include the mesencephalon with superior colliculi and lateral geniculate bodies. For immunohistochemistry, paraffin-embedded sections were immersed in 100% formic acid for 15 minutes, then equilibrated in digestion buffer (50 mM TRIS-HCL, 1 mM ethylenediaminetetraacetic acid, 0.1% Tween 20, pH 7.8) for 5 minutes and treated with proteinase K (1 μg/mL in digestion buffer) for 15 minutes at 37°C followed by autoclaving in distilled water for 15 minutes (121°C) in a pressure cooker. We used the 6H4 monoclonal anti-PrP antibody (Prionics, Zurich) or 3F4 antibody (DAKO) and the ABC technique with the Vectastatin Elite ABC-kit, using DAB as chromogen as previously described [27].

Results

Until 26 weeks postinoculation, no PrP^d-specific immunostaining was observed in the brain. At 27 weeks postinoculation, PrP^d targeted to the contralateral superior colliculus as delicate granular synaptic deposits located in the superficial part of this structure (Fig. 1A-B). As already reported [32] (Fig. 2), a few spongiform vacuoles were visible in the same area by conventional H&E staining. In several other sections, vacuoles were visible but no PrP^d staining could be detected.

Discussion

The involvement of the visual system is well recognised in prion diseases. First, if scrapie is inoculated intraocularly, infection travels along the optic nerves to target the superior colliculi and lateral geniculate bodies [16-18,24,25,32,37-40]. Second, scrapie replicates in the eye [11,23] and PrP^d is expressed in the retina [12] and its mRNA is detected in the optic tectum [19]. In variant CJD, PrP^d is expressed in the retina and the proximal part of the optic nerve at levels of 2.5% and 25% of the brain concentration, respectively [20,43]. Third, the retina degenerates in several models of scrapie and CJD in rodents as a result of apoptosis of ganglion cells [7-10,14,15,21,23], and cases of CJD and GSS with retinal or geniculate body degeneration or optic nerve atrophy have already been reported [1,2,26,29,36,41,42]. Of note, in a GSS case with bila-

teral optic atrophy, the retina was normal [41]. Fourth, iatrogenic CJD cases following corneal transplantation have been described [4].

Here, we report that following intraocular inoculation, both spongiform change and PrP deposition targeted the superior colliculus as it has been showed by classical neuropathologic methods [32]. This targeting of PrP to the superior colliculus showed that PrP^d and spongiform change follow structured neural pathways of the visual system of the brain, as already suggested in studies on the spread of infectivity from the periphery to the central nervous system [32]. Of note, spongiform change in the superior colliculus was not always accompanied by PrP deposition, and this discrepancy has also been noticed, albeit infrequently, by other investigators [28]. Alterations in the glycosylation pattern have been studied following inoculation into the superior colliculus in two models (the ME7 and the 139A) of scrapie in rodents [35]. The non-glycosylated PrP band increased first in the retina and then in the optic nerves while the deglycosylated band decreased concomitantly. Thus, alterations of the glycosylation pattern follows the structure of the optic pathways – these changes appear first in the cell bodies and then in their respective axons. These data, along with our immunohistochemical studies, strongly suggest spreading of infectivity and its surrogate marker PrP^d through connections of the visual system via fast axonal transport [5]. Indeed, the mutation at the first glycosylation site results in the accumulation of PrP^d within the cell bodies [13].

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