Variable tau accumulation in murine models with abnormal prion protein deposits

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1. Introduction

Accumulation of host-encoded protein aggregates in the brain is the hallmark of a group of neurodegenerative diseases, including Alzheimer's disease (AD), Parkinson's disease (PD) and prion diseases [1–4]. Traditionally, the misfolding of specific proteins has been used to define different human neurodegenerative diseases. These include amyloid-β (Aβ) and hyperphosphorylated microtubule-associated-protein tau (p.tau) in AD; α-synuclein in PD; and misfolded prion protein (PrP) in prion diseases. Prion diseases differ from other protein misfolding diseases due to their infectious aetiology. The infectious agent is thought to be a misfolded conformer of PrP, which propagates by binding to and converting normal cellular PrP (PrPC) into the abnormal aggregated form [5]. Prion agents exist as a number of different natural and laboratory derived strains, which show characteristic differences in incubation time and histopathology [6,7]. While the heterogeneous nature of prion diseases is well recognized, the underlying mechanisms remain poorly understood.

We have shown that PrP amyloid plaques can be formed in mouse brain in the absence of prion agent replication, suggesting that not all misfolded PrP is infectious [8]. Thus, proteinopathies similar to AD and PD can also occur in mice when PrP misfolds [8]. In this manuscript we will use PrP\textsuperscript{ISE} to refer to accumulation of abnormal PrP in cases with prion infectivity, and misfolded PrP to denote the formation of abnormal PrP in cases that are not transmissible via an infectious mechanism. Despite the lack of an infectious aetiology for AD and PD [9], all of these protein misfolding diseases show some degree of overlap resulting in a spectrum of disorders with accumulation of more than one protein in the brain [10]. Therefore, while triggers of disease are diverse the basic mechanisms driving the formation and spread of misfolded proteins, and the progression of neurodegeneration may be similar [11–15].

Deposits of p.tau forming neurofibrillary tangles (NFTs) are characteristic of AD and some human prion diseases with PrP amyloid plaques in the brain [16]. P.tau is also seen in acquired and familial prion disease in the form of neuronal and glial inclusions, and as extracellular dots and rods [10]. In Gerstmann-Sträussler-Scheinker disease (GSS), variant Creutzfeldt-Jakob disease (vCJD) and some forms of sporadic CJD (sCJD), p.tau is mostly seen in the vicinity of amyloid plaques [17]. P.tau deposition has also been observed in mouse models of prion disease [18–20]. Despite these observations, analysis of knockout [21] and overexpression [22] tau mouse models suggests that tau is not essential for the development of prion disease. However, it has been proposed that the formation of toxic Aβ or PrP aggregates leads to the
formation of p.tau and subsequent aggregation as NFTs or smaller extracellular aggregates. It is therefore apparent that prion diseases show a spectrum of tau pathologies, and that these may be linked with its heterogeneity. We therefore aimed to assess the correlation between p.tau and PrP aggregation in models of infectious murine prion disease and non-infectious PrP proteinopathy. This will determine whether prion infection/agent replication or misfolded PrP accumulation are important in determining disease phenotype.

2. Materials and methods

2.1. Animal models

All tissues examined in this project were produced in previous transmission experiments [23–25] performed under licence from the UK Home Office in accordance with the Animals (Scientific Procedures) Act 1986. Archive blocks were re-cut to produce sections for analysis of p.tau, PrP and amyloid accumulation. Frozen tissue from the mouse models used in these studies was not available for biochemical analysis.

The severe tauopathy seen in squirrel monkeys infected with classical bovine spongiform encephalopathy agent (SQ-BSE) [26] led us to analyze the phenotype associated with disease in knock-in transgenic mice expressing bovine PrP with the 6-octapeptide repeat region (Bov6) [27] inoculated intracerebrally with classical BSE (C.BSE), H-type (H.BSE) and bovine amyloidogenic spongiform encephalopathy (BASE) [24]. To explore the correlation between PrP amyloid and p.tau we used the following models: (i) Wt mice injected with 87V murine adapted scrapie (87V-VM) [28]; (ii) transgenic mice that overexpress used the following models: (i) Wt mice injected with 87V murine adapted scrapie (87V-VM) [28]; (ii) transgenic mice that overexpress agent (ME7-C57BL) [25,31]. Four animals per group were analyzed.

2.2. Neuropathology

Formalin fixed, paraffin embedded brain sections (6 μm) were processed and stained using published protocols [26]. For light microscopy sections were stained with haematoxylin-eosin (HE) and immunostained for the detection of PrP with monoclonal antibody 6H4 at 5 μg/ml (Celtic diagnostics). Antibodies 01–100 that recognizes residues 144–152 of mouse PrP [32]. Hyperphosphorylated tau was detected with antibodies AT8 at 4 μg/ml (Thermo MN1010) and Thr231 (AT180) at 0.05 μg/ml (Thermo MN1040). Antibody AT8 recognizes a triple phosphorylated peptide (pS202, pT205 and pS208) present in animals with large amounts of widespread non-amyloid (diffuse) PrP TSE deposits we used C57-BL mice inoculated with ME7 murine adapted prion agent (ME7-C57BL) [25,31]. Four animals per group were analyzed.

After developing clinical disease or at the end of their expected lifespan. Non-inoculated Bov6, C57BL, VM, 101LL and 129Ola mice served as controls.

3. Results

3.1. P.tau and PrP TSE in typical and atypical BSE infected Bov6 mice

Hyperphosphorylated tau has been described in experimental models following transmission of C-BSE agent [18]. However, no information is available in transmission experiments using atypical BSE agents. Here, we used Bov6 mice inoculated with C.BSE, H.BSE and BASE and observed widespread p.tau immunopositivity forming dots and rods in multiple areas of the brain, including cerebral cortex, septum, hippocampus, thalamus, hypothalamus, colliculus and brainstem (Table 1). Similar patterns and distribution of immunoreactivity were observed in adjacent sections stained with antibodies AT8 and Thr231. Abundant deposits and strong reactivity was consistently seen in the thalamus of Bov6 mice inoculated with BASE (Fig. 1). Tau immunopositivity was identical to that described in SQ-BSE [26], in some human prion diseases [17,46] and primary tauopathies [47]. The thalamus of Bov6 mice inoculated with C.BSE showed larger amounts of PrP TSE than the thalamus of Bov6 mice inoculated with BASE or H.BSE (Fig. 1a–c) (Table 2). However, p.tau positivity was most prominent in the thalamus of BASE infected mice (Fig. 1e) (Table 1). In contrast, the superior colliculus of Bov6 mice exposed to BASE (with severe spongiform degeneration) showed prominent accumulation of both PrP TSE (Fig. 1h) and p.tau (Fig. 1k) (Tables 1 and 2). Bov6 mice inoculated with H.BSE accumulated the smallest amounts of both PrP TSE and p.tau (Fig. 1c,f,l). The absence of thioflavin-s fluorescent plaques in the colliculus indicates that p.tau is not associated with PrP-amyloid in this brain area. However, PrP TSE amyloid and p.tau were seen in the thalamus and brainstem. Confocal images of isolated immunopositive deposits (Fig. 2a–c) show co-localization between PrP TSE and p.tau. In
addition, p.tau is also observed in the vicinity of the PrP^{TSE} central core. Imaris 3D reconstruction (Fig. 2d–f) confirm the previous observation and revealed p.tau deposition throughout the PrP plaque in C-BSE and H-BSE (Fig. 2d–f). No immunoreactivity was observed in brain sections of non-inoculated aged matched Bov6 probed with antibodies AT8 or Thr231 or in BSE infected Bov6 mice incubated without primary antibody. Therefore, widespread accumulation of p.tau is present in the brain of Bov6 mice infected with typical and atypical BSE agents.

|       | Med | Cer | Coll | Hypo | Thal | Hippo | Sep | Cin.Cx | Ret.Cx |
|-------|-----|-----|------|------|------|-------|-----|--------|--------|
| Bov6-H.BSE | ++ | − | + | + | + | ++ | ++ | + | +++ |
| Bov6-C.BSE | ++ | − | + | + | ++ | ++ | + | ++ | + |
| Bov6-BASE | ++ | − | + | + | ++ | ++ | + | ++ | + |
| 87V-VM | + | − | − | + | + | + | + | + | ++ |
| ME7-C57BL | + | − | + | + | + | + | + | + | + |
| GSS-22 | + | − | − | − | − | − | − | − | − |
| 1011L-rec.Wt-PrP | − | − | − | − | − | − | − | − | − |
| 1011L-rec.PrP-101L | − | − | − | − | − | − | − | − | − |

Table 1
Scoring of p.tau deposition in experimental mouse models.

Tau deposition scored as the following (−) no deposition; (+) low deposition; (++) moderate deposition; (+++) heavy deposition, results based on mean of 4 mice per group. Med-medulla, Cer-cerebellum, Coll-colliculus, Hypo-hypothalamus, Thal-thalamus, Hippo-hippocampus, Sep-septum, Cin.Cx-cingulate cortex, Ret.Cx-retrosplenial cortex.

Fig. 1. PrP^{TSE} and p.tau in Bov6 mice challenged with typical and atypical BSE. Large, moderate and low amounts of PrP^{TSE} were observed in the thalamus of Bov6 mice inoculated with C.BSE, BASE and H.BSE respectively (a–c). Large amounts of p.tau immunopositivity were observed in Bov6 mice inoculated with BASE (e) and lower amounts of p.tau were observed in Bov6 mice inoculated with C.BSE (d) and H.BSE agents (f). Large amounts of PrP^{TSE} (h) and p.tau (k) were observed in the superior colliculus of Bov6 mice inoculated with BASE; large amounts of both PrP^{TSE} and p.tau were observed in Bov6 mice inoculated with C.BSE (g, j) and H.BSE (i, l). Images obtained after staining with anti-PrP antibody 6H4 (a–c, g–i), anti-p.tau antibody AT8 (d–f, j–l) and counterstained with haematoxylin. Scale bar in f = 100 μm (corresponding to panels a–f). Scale bar in l = 50 μm (corresponding to panels g–l).
3.2. P.tau in mice inoculated with different scrapie strains

ME7-C57BL and 87V-VM are well characterized mouse passaged scrapie strains with different time courses of disease (160 days/ME7-C57BL, 320 days/87V-VM) and pathologic phenotypes. ME7-C57BL infected mice show large amounts of widespread, diffuse (i.e. non-amyloid) PrPTSE accumulation and few plaques (Fig. 3a,b and Table 2), with severe astro- and micro-gliosis in all areas of the brain [25,31]. In contrast, 87V-VM infected mice show widespread unicentric amyloid plaques, and coarse and fine-punctate PrPTSE deposits in the brain (Fig. 3e,f and Table 2). 87V-VM mice also show selective targeting of the CA2 region of the hippocampus with spongiform degeneration and fine-punctate PrPTSE [25,48]. ME7-C57BL mice (160 dpi) show limited amounts of p.tau in most areas of the brain at terminal disease (Table 1). This pattern of p.tau accumulation was seen in regions with spongiform degeneration and PrPTSE accumulation (Fig. 3c,d). In 87V-VM infected mice, p.tau was observed within and around PrPTSE amyloid plaques at terminal disease (320 dpi) (Fig. 3h). P.tau was also observed in regions with diffuse PrPTSE deposits (Fig. 3g) Table 1.

Table 2
Scoring of PrP deposition in experimental mouse models.

|            | Med | Cer | Coll | Hypo | Thal | Hippo | Sep | Cin.Cx | Ret.Cx |
|------------|-----|-----|------|------|------|-------|-----|--------|--------|
| Bov6-H.BSE | +++ | −   | −    | +    | −    | −/−   | +   | +      | +      |
| Bov6-C.BSE | +   | +   | +    | +    | +    | +++   | +/− | ++     | +      |
| Bov6-BASE  | +++ | +/− | +/−  | +/−  | +/−  | +/−   | +   | +/−    | +/−    |
| 87V-VM     | +   | +   | +    | +/−  | +/−  | +     | +   | +/++   | +/++   |
| ME7-C57BL  | +++ | +++ | +++  | +++  | +++  | +++   | +++ | +++    | +++    |
| GSS-22     | ++  | +   | +/−  | +/−  | +/−  | +/−   | +   | +      | +      |
| 101LL-rec.Wt-PrP | −   | −   | −    | −    | −    | −     | +   | −      | −      |
| 101LL-rec.PrP-101L | −   | −   | −    | −    | −    | −     | +   | −      | −      |

PrP deposition scored as the following (−) no deposition; (+) low deposition; (+++) moderate deposition; (++++) heavy deposition, results based on mean of 4 mice per group. Med-medulla, Cer-cerebellum, Coll-collicolus, Hypo-hypothalamus, Thal-thalamus, Hippo-hippocampus, Sep-septum, Cin.Cx-cingulate cortex, Ret.Cx-retrosplenial cortex.

3.3. Dissociation between misfolded PrP and p.tau in mice overexpressing PrP P101L

GSS-22 mice overexpressing PrP-P101L spontaneously develop severe spongiform degeneration, abundant PrP amyloid plaques and gliosis in the brain, but do not replicate prion infectivity and do not transmit prion disease to Wt or 101 LL mice [23,29]. Therefore, GSS-22 provide a model to study the possible correlation between PrP aggregates and p.tau in animals that express mutant PrP throughout their lifespan but are not exposed to the trauma of intracerebral inoculation,
or develop a transmissible disease [23,29]. We observed that despite the severe spongiform degeneration (Fig. 5d) and large accumulation of PrP plaques in the brain (Fig. 5b,e and g), these animals show almost complete absence of p.tau in all brain areas at terminal disease (Tables 1 and 2). This unexpected finding was confirmed by confocal microscopy (Fig. 5h). Similar results were obtained in sections probed with both antibodies AT8 and Thr231. Therefore, we observed a striking dissociation between severe spongiform degeneration and abundant PrP amyloid formation, and deposition of p.tau.

3.4. No p.tau is seen in 101LL mice following seeding of PrP amyloid plaques

We recently described a model of seeded PrP proteinopathy following the inoculation of rec.Wt-PrP and rec.101L-PrP amyloid fibrils into knock-in mice homozygous for the proline to leucine mutation at PrP codon 101 (101LL) [8]. Inoculated 101LL mice did not develop clinical signs of prion disease, spongiform degeneration of the brain, or replication of infectious prions, but accumulated large PrP amyloid plaques in the area of inoculation and vicinity (i.e., the corpus callosum and hippocampus) [8]. This model could support the analysis of the correlation between misfolded PrP and p.tau without potential artifacts due to overexpression of PrP, or pathogenic mechanisms associated with prion agent replication and the formation of spongiform encephalopathy. In addition, inoculation of recombinant PrP fibrils allows analysis of p.tau formation in the absence of other components present in brain extracts that could enhance or inhibit the formation of p.tau isoforms. Although multiple PrP amyloid plaques were observed in the hippocampus and the corpus colloseum of 101LL mice inoculated with Wt-rec or 101L-rec PrP fibrils (Fig. 6a, b), immunolabelling with antibodies AT8 and Thr.231 showed no p.tau in any area of the brain in both models (Fig. 6c, d). Thus, despite the co-localization of p.tau with PrPTSE amyloid plaques in 87V/VM scrapie, and association with plaques described in other studies [19,49,50] results obtained in GSS-22 mice (overexpressor) and 101LL mice inoculated with recombinant PrP fibrils show that PrP-amyloidogenesis is not invariably associated with the formation of p.tau isoforms (Table 3).
4. Discussion

Aggregates of tau protein are observed in several neurodegenerative diseases, and are thought to be responsible for neurotoxicity and cell death [51,52]. Studies in mice showed that expression of human tau protein is not essential for the formation of Aβ plaques, but that it is necessary for neurotoxicity [53,54]. The role of p.tau in prion disease is unknown. A recent study stated that the frequency of tau pathology is not unusually high in sCJD (the most common prion disease in humans) and that it does not relate to PrP^TSE deposition [10]. However, the spectrum of tau pathologies in prion diseases suggest that p.tau could be a factor in the heterogeneous presentation of these disorders. To study whether p.tau deposition was associated with prion replication and neurodegeneration, or occurred as a result of PrP misfolding and aggregation we analyzed mouse models with (i) accumulation of PrP^TSE either in the form of diffuse deposits or amyloid plaques that are transmissible via an infectious mechanism as shown by serial passage; and (ii) non-infectious PrP proteinopathy, in which PrP amyloid plaques are seeded in the absence of agent replication.

Previous studies showed p.tau in animals inoculated with C-BSE [18]. Whether experimental animals exposed to atypical BSE (i.e., BASE or H-BSE) develop similar phenotype is unknown. Here, we observed that B0v6 mice infected with typical and atypical BASE agents showed widespread accumulation of p.tau in brain areas with spongiform degeneration, gliosis and PrP^TSE at terminal disease. The largest amounts of p.tau were seen in mice inoculated with BASE. P.tau has recently been described in bovines with Idiopathic Brainstem Neuronal Chromatolysis (IBNC) showing that tau pathology might be a widespread phenomenon in the animal kingdom [55]. In mice infected with rodent-adapted prion strains, p.tau deposition varied depending on PrP^TSE deposition type. In ME7/C57BL p.tau was detected mainly in areas with diffuse PrP^TSE and spongiform degeneration. However in 87 V infected mice, p.tau was mainly associated with PrP amyloid plaques but was also seen in areas with spongiform degeneration and non-amyloid PrP^TSE deposition. The molecular mechanisms underlying p.tau accumulation remain poorly understood. Previous studies have shown that the N-terminus (amino acids 1–19) and tandem repeats region (amino acids 186–283) of tau protein interact with PrP. In addition, the octapeptide repeat region of PrP is involved in the binding activity of PrP with tau [56]. The reported molecular interactions between tau and PrP highlight the potential role of tau in PrP function and its possible involvement in the pathogenesis of prion diseases.

GSS-22 mice overexpressing PrP 101L [29] and 101LL mice inoculated with recombinant PrP amyloid fibrils [8] represented animal models without infectious prion disease, but which formed PrP amyloid plaques in the brain. In contrast to the plaque associated p.tau staining observed in 87V infected mice, p.tau deposits were exceptionally rare in GSS22 mice, and completely absent from 101LL mice inoculated one possibility is that PrP amyloid might not induce p.tau recognized by antibodies AT8 and Thr231. This possibility is unlikely because these antibodies are extensively used for the detection of abnormal tau in humans and animals. Another possibility is that GSS22 animals did not live long enough (180 days) to accumulate readily detectable p.tau by immunohistochemistry. However, p.tau was present in multiple brain areas of ME7/C57 mice at 160 dpi suggesting that its accumulation is not a phenotypic trait in GSS 22 mice. Importantly, 101LL mice inoculated with rec-PrP fibrils with large PrP-amyloid plaques show complete absence of clinical disease and p.tau up to 500 dpi. Therefore,
GSS-22 and 101LL rec-PrP mouse models show dissociation between misfolded PrP and p.tau. Future studies should be geared towards understanding why a misfolded protein has the potential to aggregate and lead to the development of a complex proteinopathy that can be transmitted between hosts posing a risk to public health. In conclusion, our studies indicate that p.tau is a phenotypic feature of prion diseases and might be a factor in the heterogenous spectrum of these disorders. The data presented here suggest that replication of prions, or prion induced neurotoxicity rather than the physical conformation of PrP is crucial for the abnormal processing of tau.

Compliance with ethical standards

All applicable international, national and institutional guidelines for the care and use of animals were followed. All procedures performed in studies involving animals were in accordance with the ethical standards of the institution at which the studies were conducted. The article does not contain any studies with human participants performed by any of the authors.

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Conflict of interest

The authors declare no conflict of interest.

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6H4/PrP

AT8/tau

Table 3
Summary of findings in experimental mouse models.

| i.p | p.tau | spong. deg. | PrP deposits |
|-----|-------|-------------|--------------|
| Bov6-BSE | 590 | + | + | Diffuse plaques |
| Bov6-CBSE | 540 | + | + | Diffuse plaques |
| Bov6-BASE | 635 | + | + | Diffuse plaques |
| 87V-VM | 320 | + | + | Diffuse plaques |
| M7-C57BL | 160 | + | + | Diffuse |
| GSS-22 | 198 | – | – | – |
| rec. PrP-101LL | 686 | – | – | Plaques |
| rec. PrP-Wt | 681 | – | – | Plaques |

Note: incubation period (i.p) corresponds to the mean of 4 mice.

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