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Research report

Quantitative analysis of tau isoform transcripts in sporadic tauopathies

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Abstract

A number of neurodegenerative diseases, including Alzheimer's disease (AD), are characterized by intraneuronal accumulation of the tau protein. Some forms of FTDP-17 are caused by mutations in the tau gene affecting exon 10 splicing. Therefore, dysregulation of tau pre-mRNA splicing may be a contributing factor to sporadic tauopathies. To address this question, we devised a real-time RT-PCR strategy based on the use of a single fluorogenic probe to evaluate the ratio between tau isoforms containing or lacking exon 10 (4R/3R ratio) in post-mortem brain samples. We found a two- to six-fold increase in the 4R/3R ratio in cases of FTDP-17 linked to a splice site mutation, hence confirming the validity of the strategy. The difference in the 4R/3R ratio in the superior temporal and superior frontal gyri between AD and control brains was not statistically significant. Similarly, there was no significant difference in the 4R/3R ratio between Pick's disease cases and controls, indicating that the predominance of tau3R protein in PiD reflects post-translational modifications of specific isoforms. This study indicates that post-translational events are likely to be the main factors controlling tau isoform composition in sporadic tauopathies and highlights the benefit of quantitative RT-PCR in the assessment of splicing abnormalities in tauopathies.

Theme: Disorders of the nervous system
Topic: Degenerative disease: Alzheimer's—miscellaneous

Keywords: Alternative splicing; Tau; Alzheimer's disease (AD); Pick's disease (PiD); FTDP-17; Tauopathies

1. Introduction

Tau is a microtubule-associated protein mainly expressed in neurons and enriched in axons; tau binding promotes microtubule polymerization and stabilization [2]. A number of neurodegenerative diseases are characterized by inclusion bodies immunoreactive for the tau protein. These diseases include Alzheimer's disease (AD), frontotemporal dementia with parkinsonism linked to chromosome 17 (FTDP-17), Pick's disease (PiD) and progressive supranuclear palsy (PSP) and are collectively referred to as tauopathies [2,6,11,21].

Human tau is encoded by a single large gene of about 100 kb comprising 16 exons, on chromosome 17 [1]. Alternative splicing of exons 2, 3 and 10 in the tau pre-mRNA results in the expression of six isoforms in the brain. Exon 10 encodes the second of four imperfect microtubule-binding repeats in the C-terminal half of the tau protein. Inclusion or exclusion of exon 10 gives rise to tau isoforms with three (tau3R, exon 10^-) or four (tau4R, exon 10^+) microtubule-binding repeats [14]. Furthermore, inclusion or exclusion of exons 2 and 3 produces tau isoforms with zero (0N), one (1N) or two (2N) inserts near the N-terminus. Adult rodent brain expresses tau4R only, but approximately

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equal amounts of tau3R and tau4R are expressed in adult human brain. Interestingly, an abnormal composition of tau protein isoform is associated with some sporadic tauopathies. For instance, tau4R predominates in progressive supranuclear palsy (PSP) [5,7,19,25], although there is some heterogeneity between cases [13]. By contrast, tau3R is in excess in PiD [9,10]. Tau isoform imbalance in PSP is at least in part the result of aberrant splicing of the tau pre-mRNA leading to increased incorporation of exon 10 and elevated 4R/3R tau mRNA ratio (4R/3R ratio) [25]. A balance of tau4R and tau3R might be essential for normal microtubule function in adult human brain and, thus, an altered isoform ratio may result in microtubule dysfunction and tau aggregation.

The importance of a proper balance in tau isoforms is highlighted by the existence of mutations in the tau gene in some cases of FTDP-17 that affect tau splicing. Tau mutations in FTDP-17 are either missense or silent mutations or are 5′ splice site mutations that increase the incorporation of exon 10 [18,23,24]. Exon 10 retention in FTDP-17 results in a two- to six-fold excess of tau4R mRNA over tau3R mRNA [12,18,24]. Two exonic missense mutations in exon 10, and two silent mutations also result in an increase in tau4R by affecting several splicing silencer and enhancer elements in exon 10 [8,12,17]. Finally, the deletion Δ280K results in complete exon 10 skipping [12]. Taken together, these data raise the possibility that dysregulation of tau splicing may be a contributing factor to sporadic tauopathies, including AD.

Several studies have attempted to evaluate the relative levels of tau3R and tau4R mRNA in AD but have provided conflicting results. For instance, a study has suggested an increase in tau4R mRNA in AD [29], but other studies reported no changes in relative isoform composition or a change in some cases only [3,7,27]. However, these studies were based on RNA analysis methods that were only semi-quantitative. To address this question, we have devised a real-time RT-PCR strategy to directly evaluate the 4R/3R ratio in individual post-mortem brain samples. Precise quantitative estimate of the 4R/3R ratio is a reliable reflection of exon 10 splicing, independently of the absolute mRNA levels for individual isoforms, the evaluation of which is complicated by the variability of the extent of neuronal loss between cases. We have applied this strategy to compare the 4R/3R ratio in post mortem brains from sporadic AD and PiD.

2. Materials and methods

2.1. Cases

Ten AD brains (mean age 75.2 years), four FTDP-17 brains with intronic +16 (C→T) mutation (mean age 58.8 years), three PiD brains (mean age 68.7 years) and 10 control brains (mean age 71.7 years) were obtained from the London Neurodegenerative Diseases Brain Bank, Department of Neuropathology, Institute of Psychiatry, London. Tissue donation and use was approved by the local Ethics Committee. AD patients were assessed clinically using the criteria of the National Institute of Neurological and Communicative Disorders and Stroke and the AD and Related Disorders Association (NINCDS-ADRDA) [26]. Both the AD and control cases were diagnosed neuropathologically according to the Consortium to Establish a Registry for AD (CERAD) criteria [22]. The PiD cases were reviewed extensively and conformed to the European Concerted Action on Pick's Disease (ECAPD) diagnosis (ECAPD 1998).

For Alzheimer's disease cases, immunohistochemical analysis was performed on the superior frontal gyrus (at the level of the genu of the corpus callosum), the superior temporal gyrus (at the level of the lateral geniculate body) and the cerebellum using an antibody to tau (DAKO). The number of tau-positive inclusions was determined for each case. Cases were classified as moderate if they had less than 20 inclusions/mm^2, and severe if the density of tau-positive inclusions was higher than 20 inclusions/inclusions/mm^2.

2.2. RNA extraction and analysis

Total RNA was extracted from 0.2 to 0.5 g of frozen brain tissue using 2 ml Trizol® reagent (Invitrogen) and treated with RNase-free DNAseI. Quantitative PCR was carried out using TaqMan®method (PE Applied Biosystems). Total brain RNA and RNA standards were reverse transcribed and incubated with AmpliTaq Gold DNA polymerase (PE Applied Biosystems) at 0.025 U/μl, the fluorescent probes and the tau3R or tau4R-specific pairs of primers (Fig. 1). PCR cycles were performed at 50 °C for 2 min, 95 °C for 10 min, followed by 40 cycles at 95 °C for 15 s for denaturation and 60 °C for 1 min for annealing and extension. The fluorescent signal was captured using the ABI Prism 7700 sequence detection system. The 4R/3R ratio was determined by converting the threshold cycle (Ct) values using a standard curve of in vitro transcribed tau RNA, taking into account the difference in amplicon length. Between 5 and 10 experiments were performed for each brain sample and statistical analysis was performed using an unpaired t test for 2 independent sample means. To synthesize RNA standards, tau3R- and tau4R-specific PCR products were generated from full-length tau cDNAs and cloned into pBluescript II KS$^+$. RNA was generated by in vitro transcription using T7 RNA polymerase and the Riboprobe® in vitro Transcription System (Promega).

2.3. Tau dephosphorylation and Western blotting

Soluble tau and a fraction enriched in aggregated tau were obtained from approximately 0.2 g of brain tissue following a previously published protocol [16]. Proteins were separated in 7.5% (w/v) SDS-PAGE gels and trans-