Tau alternative splicing in familial and sporadic tauopathies

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Abstract

Six tau isoforms differing in their affinity for microtubules are produced by alternative splicing from the MAPT (microtubule-associated protein tau) gene in adult human brain. Several MAPT mutations causing the familial tauopathy, FTDP-17 (frontotemporal dementia with parkinsonism linked to chromosome 17), affect alternative splicing of exon 10, encoding a microtubule-binding motif. Advanced RNA analysis methods have suggested that levels of exon 10-containing MAPT mRNA are elevated in Alzheimer’s disease. Furthermore, the MAPT H1 haplotype, associated with Alzheimer’s disease, promotes exon 10 inclusion in MAPT mRNA. Thus, an accurate regulation of tau alternative splicing is critical for the maintenance of neuronal viability, and its alteration might be a contributing factor to Alzheimer’s disease. Tau alternative splicing could represent a target for therapeutic intervention to delay the progression of pathology in familial as well as sporadic tauopathies.

Introduction

The human tau protein is encoded by the MAPT (microtubule-associated protein tau) gene at chromosome locus 17q21.3. Six tau isoforms resulting from alternative splicing of exons 2, 3 and 10 (E2, E3 and E10) in the MAPT pre-mRNA and ranging from 48 to 56 kDa are expressed in adult human brain. Inclusion or exclusion of E2 and E3 produces tau isoforms with different N-termini. E2 and E3 encode short inserts in the N-terminal projection domain of tau that may interact with the plasma membrane. Tau binds to microtubules through a C-terminal microtubule-binding domain comprising four repeated 31–32-amino-acid microtubule-binding motifs. E10 encodes the second of these repeats; consequently, tau isoforms have either three (3R tau, E10−) or four (4R tau, E10+) microtubule-binding motifs. Owing to the presence of an additional microtubule-binding domain, 4R tau has a higher affinity for microtubules than 3R tau and is also a more potent promoter of microtubule assembly in vitro [1,2]. Alternative splicing of E10 is therefore an important regulator of the affinity of tau for microtubules. E10 splicing is developmentally regulated; isoforms lacking E10 (i.e. 3R tau) are the only ones expressed at early stages of development, and both 3R tau and 4R tau are expressed in adult human brain in approximately equal amounts. A number of MAPT mutations affecting E10 splicing regulatory elements cause some forms of the familial tauopathy FTDP-17 (frontotemporal dementia with parkinsonism linked to chromosome 17), hence demonstrating that an accurate regulation of tau isoform balance is critical for the maintenance of neuronal viability.

Abnormal tau splicing in FTDP-17

FTDP-17 MAPT mutations are either exonic missense, deletion or silent mutations, or intronic mutations [3]. FTDP-17 mutations cause diverse clinical phenotypes, including movement disorders and memory problems. Neuropathological examination of the brain of FTDP-17 cases shows abundant filamentous phosphorylated tau pathology; however, the morphology of the tau filaments is determined, to a significant degree, by the specific mutation involved. Several intronic or exonic FTDP-17 MAPT mutations affect E10 splicing, resulting in an increase in the E10+/E10− MAPT mRNA ratio (4R/3R ratio) from approximately one in normal individuals to up to 2.5–3 in disease. The regulation of E10 splicing and how it is affected by FTDP-17 mutations has been the subject of several reviews (see [4–6] and references therein). The main findings are summarized below.

Most intronic MAPT mutations are clustered at the exon 10/intron 10 junction that is predicted to form a stem–loop structure protecting the 5′ splice site. FTDP-17 mutations +3 to +16 destabilize this stem–loop, resulting in an increase in E10 inclusion. The stem–loop structure is also destabilized by the exonic S305N mutation. A mutation at position −10 in intron 9 promotes E10 inclusion, suggesting that it is located in a regulatory element [7]. Trans-acting factors binding to intronic elements in MAPT pre-mRNA and stimulating the incorporation of E10 include RBM4 (RNA-binding motif protein 4), binding to a sequence in intron 10, and CELF (CUG-binding protein, Elav-like family member) 3 and CELF4. The latter are members of the CELF family of RNA-binding proteins that have been implicated in a number of diseases.

Key words: alternative splicing, Alzheimer’s disease, familial tauopathy, sporadic tauopathy, tau. 
Abbreviations used: AD, Alzheimer’s disease; A5τ, alternative splicing factor; CBS, corticobasal degeneration; CELF, CUG-binding protein, Elav-like family member; E, exon; E3, exonic splicing enhancer; FTDP-17, frontotemporal dementia with parkinsonism linked to chromosome 17; GWAS, genome-wide association study; MAPT, microtubule-associated protein tau; NFT, neurofibrillary tangle; PiD, Pick’s disease; PSP, progressive supranuclear palsy; RT, reverse transcription; SF2, splicing factor 2; SMaRT, spliceosome-mediated RNA trans-splicing; SNP, single nucleotide polymorphism.

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of neurological diseases [8]. In addition to intronic mutations, several missense, silent or deletion mutations within E10 also affect E10 splicing. The N279K mutation strengthens a well-characterized ESE (exonic splicing enhancer) by increasing the affinity of the splicing factors Tra2β and SF2 (splicing factor 2)/ASF (alternative splicing factor) for the ESE. Tra2β binding is antagonized by the SR (serine/arginine-rich) protein, SRp54, and this provides an additional level of regulation. E10 also contains an ESE near its 3’ end and an ESS (exonic splicing silencer), which is disrupted by the N296H/N and L284L mutations.

The codon at position 280 (K280) mutation is located in the same ESE as the N279K mutation, but reduces the affinity of Tra2β, Δ1SF2/ASF for the ESE. Thus the ΔK280 mutation has the opposite effect to the N279K mutation and inhibits the incorporation of E10 and, consequently, reduces rather than increases the 4R/3R ratio. Tau lesions in the brain of patients harbouring the ΔK280 mutation contain exclusively, or, at least predominantly, 3R tau [9,10]. The reduction in the 4R/3R ratio resulting from the ΔK280 mutation suggests that neurotoxicity is not caused by the increase in 4R tau, but by the imbalance in 3R and 4R isoforms.

**Tau splicing in sporadic tauopathies**

Impairment of tau E10 splicing can clearly be the cause of pathogenesis in FTDP-17; alteration of MAPT pre-mRNA splicing can also be a contributing factor to sporadic tauopathies, in particular AD (Alzheimer’s disease). Tau inclusions in several tauopathies, such as PSP (progressive supranuclear palsy), are predominantly composed of 4R tau [11]. Tau isoform imbalance in PSP is at least in part the result of aberrant splicing of E10, leading to an excess of 4R tau over 3R tau [12]. In contrast, Pick’s disease, where tau inclusions, or Pick bodies, have a distinctive rounded morphology, are composed predominantly of 3R tau [13], although both 3R and 4R has been found in PiD [14,15].

In AD, PHFs (paired helical filaments) in NFTs (neurofibrillary tangles) are consistently found to contain both 3R and 4R tau [16], with some exclusively 4R NFTs and threads in limbic regions such as the presubiculum [17]. Another consistent finding is that ghost tangles, also referred to as extracellular tangles, that are NFT remnants from neurons that have died, are almost always positive for 3R tau only; this may be due to proteolysis of 4R tau [18] or that the specific epitope for the 4R tau-specific antibodies used is buried inside the structure or degraded [19]. Tau splicing analysis by quantitative RT (reverse transcription)–PCR or semi-quantitative RT–PCR have, in most cases, failed to show differences in 4R/3R ratios between AD patients and controls [20–24]. Many of these studies also found no differences at the protein level [22–24]. There are, however, some studies where increases in 4R tau transcripts have been found [25,26]. One possible explanation for these discrepancies is that particular neurons with tau inclusions have altered 4R/3R ratios, but these differences are averaged out over a typical tissue sample. To test whether differences in the 4R/3R ratio exist at the level of single-tangle-bearing neurons, a microarray analysis of RNA isolated from single neurons by microaspiration found a reduction in 3R tau transcripts in both AD and MCI (mild cognitive impairment) patients [27]. However, another study, also using another single-cell-based approach, laser-capture microscopy followed by quantitative RT–PCR, found no differences in tau isoform ratios [23].

Conventional PCR-based methods may not be accurate enough to detect small differences in the 4R/3R ratio which have a theoretical maximum difference of 25–33% [23]. Conventional RT–PCR methods measure E10 content irrespective of the specific tau isoform they originate from, i.e. whether they have none, one or two N-terminal inserts. In contrast, Conrad et al. [28] used the polony profiling method, in which a single cDNA molecule gives rise to a colony of amplification products, allowing for the quantification of individual isoforms [29]. Using this method, these authors found a 1.3-fold increase in 4R transcripts in AD cases compared with controls, with the 0N4R isoform, in particular, accounting for the increase. In addition, there was a 1.6-fold decrease in E2-containing transcripts with 2N3R and 1N3R isoforms being significantly reduced in AD cases. The splicing factor Tra2β, which differentially regulates both E10 and E2, was decreased in the AD samples analysed and this, the authors suggest, provides a mechanism for the co-ordinated up-regulation of E10 and down-regulation of E2 [29].

These results suggest that tau isoform ratios may be altered in NFT-bearing neurons. Regional populations or neuronal types may be particularly vulnerable to tau pathology and immunohistochemistry with antibodies directed to either 3R or 4R tau have been used to determine whether particular populations of neurons in AD harbour specific tau isoform inclusions [16,17,19,30,31]. Espinoza et al. [19] examined the hippocampus of advanced AD cases as well as brains with milder neurofibrillary pathology consisting mostly of NFTs and pre-tangles, which are non-fibrillar accumulations of tau and considered to be early NFTs. In NFTs from the less severe cases, 4R and 3R tau were detected with the specific antibodies ET3 and RD3 respectively. More variability was observed, with some cases showing mainly 3R staining in the subiculum, CA1 and entorhinal cortex in the severe end-stage cases. Ghost tangles were predominantly 3R and neither antibody was effective in detecting pre-tangles.

**MAPT haplotypes and tau splicing**

A 900 kb inversion in the q21 region of chromosome 17, which includes the MAPT gene, results in two major haplotypes referred to as H1 and H2. The two haplotypes are defined by a series of SNPs (single nucleotide polymorphisms) spanning the whole MAPT gene [32,33]. In addition to SNPs, H2 also has a 238 bp deletion between positions −951 and −713 in intron 9. In addition to the H1 and H2 haplotype-defining SNPs, there are also SNPs that vary only within the H1 background, defining several
H1 sub-haplotypes. Among H1 sub-haplotypes, H1c is most strongly associated with PSP and CBD (corticobasal degeneration) [34,35]. By contrast, no association between MAPT haplotypes and PiD has been found [36]. The association of MAPT haplotypes with AD is more complex, with both positive and negative associations having been found. A recent large GWAS (genome-wide association study) that combined a number of datasets found no significant association of SNPs in the APP (amyloid precursor protein), PSEN1 (presenilin 1), PSEN2 (presenilin 2) and MAPT genes, which all play a role in AD [37]. Genome-wide analysis of the dataset in which all SNPs located within 20 kb of these genes were tested for association showed that MAPT was significantly associated with AD. This analysis, in which all the risk-conferring variations within the MAPT gene found in the initial GWAS analysis were combined [38], suggests a complex pattern of association with AD where many MAPT SNPs of weak effect are associated with AD. The H1c SNPs most strongly associated with PSP and CBD span a region from the large 5′ region of intron 9 which encompasses regions potentially involved in transcription and splicing regulation respectively. To determine whether tau splicing and/or transcription was altered in individuals carrying the H1c haplotype, Myers et al. [39] measured total MAPT mRNA from AD and control cases homozygous for the MAPT haplotypes by quantitative RT–PCR. Individuals analysed had an H1bH1b, H1cH1c, H1eH1e or H2aH2a genotype. An increase in tau transcripts was found in H1cH1c homozygotes. Although the sample size used in this study was large (296 cases and 128 controls), homozygotes are quite rare and therefore these authors also tested whether the H1c allele increased tau transcription in H2aH1c homozygotes compared with all other non-H1c-containing homozygotes. This analysis showed that the H1c allele increased both total tau expression as well as E10 inclusion. An independent allele-specific expression analysis of post-mortem human brain and cultured cell lines confirmed that more E10+ MAPT mRNA was expressed from the H1 haplotype than from the H2 haplotype [40]. Also, a region-specific difference in the E10+ transcripts produced from H1 and H2 was found, the difference being the highest in the globus pallidus, a region severely affected in PSP. Thus elevated production of 4R tau may explain the susceptibility to neurodegeneration conferred by the H1 haplotype. Taken together, these results suggest that up-regulation of 4R tau contributes to pathogenesis. Haplotype differences in the expression of MAPT E2 and E3 has been found in both neuroblastoma cells and normal human brain [41]. Tau transcripts that include E2 and E3 (known as 2N tau) are increased in the H2 haplotype compared with H1 and this may contribute to a protective effect conferred by the H2 haplotype. The change in E10 and E2/E3 splicing in opposite directions observed between H1 and H2 haplotypes are consistent with the increase in E10 inclusion and reduction in E2 inclusion in AD cases measured by single-molecule profiling analysis [28].

Therapeutic strategies for tau splicing
A high-throughput cellular screen using a fluorescent reporter identified several compounds that modulate E10 inclusion [42]. Reduction of E10 inclusion has been achieved in rat phaeochromocytoma PC12 cells that express predominantly E10+ tau, by using modified antisense oligonucleotides targeting the 5′ or 3′ splice junction of E10 and blocking the access of the splicing machinery to MAPT pre-mRNA [43]. SMaRT (spliceosome-mediated RNA trans-splicing) reprogramming can offer a versatile and selective way of correcting aberrant E10 splicing. SMaRT creates a chimaeric mRNA by a trans-splicing reaction between the 5′ splice site of an endogenous target pre-mRNA and the 3′ splice site of an exogenously delivered pre-trans-splicing RNA molecule. Both E10− into E10+ and E10− into E10− MAPT RNA conversion have been obtained by SMaRT using minigenes in heterologous systems. Furthermore, trans-splicing can overcome the effects of FTDP-17 mutations affecting E10 splicing [44,45]. Thus SMaRT, combined with viral delivery of pre-trans-splicing molecules to the brain, would offer a distinctive therapeutic advantage over other methods such as drug-based therapies, including local specificity of delivery, long-term benefits and limited side effects.

In conclusion, as with many neurological diseases [46,47], aberrant RNA processing is involved in the pathogenesis of tauopathies, including, at least as a contributing factor, sporadic tauopathies. Tau alternative splicing could represent a target for therapeutic intervention to delay the progression of pathological changes in a number of tauopathies.

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References


