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·Review·

Regulation of alternative splicing of tau exon 10

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The neuronal microtubule-associated protein tau is abnormally hyperphosphorylated and aggregated into neurofibrillary tangles in the brains of individuals with Alzheimer's disease and related neurodegenerative disorders. The adult human brain expresses six isoforms of tau generated by alternative splicing of exons 2, 3, and 10 of its pre-mRNA. Exon 10 encodes the second microtubule-binding repeat of tau. Its alternative splicing produces tau isoforms with either three or four microtubule-binding repeats, termed 3R-tau and 4R-tau. In the normal adult human brain, the level of 3R-tau is approximately equal to that of 4R-tau. Several silent and intronic mutations of the tau gene associated with FTDP-17T (frontotemporal dementia with Parkinsonism linked to chromosome 17 and specifically characterized by tau pathology) only disrupt exon 10 splicing, but do not influence the primary sequence of the tau protein. Thus, abnormal exon 10 splicing is sufficient to cause neurodegeneration and dementia. Here, we review the regulation of tau exon 10 splicing by *cis*-elements and *trans*-factors and summarize all the mutations associated with FTDP-17T and related tauopathies. The findings suggest that correction of exon 10 splicing may be a potential target for tau exon 10 splicing-related tauopathies.

Keywords: alternative splicing; tau; tau exon 10; tauopathies

Introduction

Tau is a major neuronal microtubule-associated protein, which promotes the assembly of microtubules and stabilizes the microtubule network[1]. As a phosphoprotein, the degree of phosphorylation of tau regulates its biological functions. However, abnormally hyperphosphorylated tau easily aggregates into neurofibrillary tangles (NFTs) and deposits in the affected neurons in the brains of individuals with Alzheimer's disease (AD)[2, 3]. It is believed that hyperphosphorylation of tau is responsible for its loss of biological function, gain of toxicity, and aggregation into NFTs[4-6].

In addition to AD, aggregation of hyperphosphorylated tau in the brain has been found in other neurodegenerative diseases, such as corticobasal degeneration (CBD), Down syndrome (DS), frontotemporal dementia with Parkinsonism linked to chromosome 17 and specifically characterized by tau pathology (FTDP-17T), Niemann-Pick disease, Pick's disease, postencephalitic Parkinsonism, and progressive supranuclear palsy (PSP). These sporadic and familial neurodegenerative disorders are defined as "tauopathies"[7, 8]. The discovery of tau gene (*MAPT*) mutations causing FTDP-17T provides clear evidence that aberrant tau can cause neurodegeneration by itself without amyloid plaques[9].

Human tau is encoded by a single gene located on chromosome 17q21, which is composed of 16 exons. By alternative splicing of exons 2, 3, and 10, six isoforms of tau are expressed in the normal adult human brain[10]. Exon 10 encodes the second microtubule-binding repeat. Thus,

its alternative splicing generates tau isoforms with three or four microtubule-binding repeats, termed 3R- or 4R-tau[10, 11], which have different functions in the polymerization and stabilization of neuronal microtubules. Due to an extra microtubule-binding repeat, 4R-tau binds more effectively to microtubules and stimulates the assembly of microtubules more strongly than 3R-tau[6, 12]. The normal adult human brain expresses approximately equal levels of 3R-tau and 4R-tau[10, 13]. However, almost half of tau gene mutations associated with FTDP-17T and related tauopathies disrupt this balance and cause neurodegeneration, suggesting that a 1:1 ratio of 3R-tau to 4R-tau is essential for maintaining normal brain function, while dysregulation of exon 10 splicing is able to cause neurodegenerative disorders[14]. Besides FTDP-17T, an alteration of the 3R-tau:4R-tau ratio has been reported in several other tauopathies[14, 15]. In this article, we review advances in the research on alternative splicing of exon 10 and the related tauopathies.

Alternative Splicing

Alternative splicing is a co- or post-transcriptional process, by which multiple mRNA variants are generated from a single gene. In this process, particular exons of a gene may be included within, or excluded from the pre-mRNA transcribed from that gene. In humans, it is believed that ~95% of multi-exonic genes are alternatively spliced[16, 17]. Remarkably, alternative splicing allows the human genome to produce 100 000 proteins, ~4-fold more than expected from its 20 000 protein-coding genes[16, 18, 19].

Both constitutive and alternative splicing are carried out by the spliceosome, which is composed of five small nuclear RNA (snRNA) molecules (U1, U2, U4, U5 and U6) and >150 proteins. Each snRNA together with several proteins forms a small nuclear ribonucleoprotein particle (snRNP). These five snRNPs bind correspondingly to pre-mRNA and catalyze the removal of each intron and the ligation of the flanking exons. Alternative splicing is regulated by a system of *trans*-acting factors that bind to *cis*-elements. A *cis*-element is a region of RNA that regulates the splicing located on the pre-mRNA itself. Depending on the locations (exon and intron) and on how they affect the spliced exon, *cis*-elements are divided into exonic splicing enhancers (ESEs), exonic splicing silencers (ESSs), intronic splicing enhancers (ISEs), and intronic splicing silencers (ISSs). *Trans*-acting factors are a group of proteins conserved in eukaryotes that include serine- and arginine-rich (SR) proteins and heterogeneous nuclear ribonucleoproteins (hnRNPs), as well as tissue-specific factors[20]. Both hnRNPs and SR proteins are involved in the regulation of alternative splicing[21, 22]. In addition, hnRNPs participate in pre-mRNA transport, RNA stability, and translational regulation.

SR proteins have one or two RNA-recognition motifs at the N-terminus, which determines the specificity of RNA binding, and an arginine-serine-rich (RS) domain at the C-terminus, which promotes protein-protein interactions in the splicing complex[23, 24]. Recently, SR proteins were renamed to match as closely as possible the existing gene nomenclature[25] and are listed in Table 1. They are involved in both constitutive and alternative splicing. In constitutive splicing, SR proteins are required for the formation of the early pre-spliceosomal complex to stabilize U1, the snRNP particle, and U2AF[26, 27]. In alternative splicing, SR proteins modulate the 5' weak splice-site in a concentration-dependent manner.

Some splicing factors promote, while others suppress, the inclusion of the alternative exon. However, in many cases, splicing factors act as either repressors or activators

Table 1. Serine- and arginine-rich (SR) proteins and their roles in exon 10 splicing of the tau gene

SR proteins		Target	Effect on exon
Old name	New name	*cis*-element	10 splicing
ASF, SF2, SRp30a	SRSF1	PPE	Inclusion
SC35, PR264, SRp30b	SRSF2	SC35-like	Inclusion
SRp20	SRSF3	ND	Exclusion
SRp75	SRSF4	ND	Exclusion
SRp40, HRS	SRSF5	ND	No effect
SRp55, B52	SRSF6	ND	Inclusion
9G8	SRSF7	ISS	Exclusion
SRp46 (human only)	SRSF8	ND	ND
SRp30c	SRSF9	ND	Inclusion
SRrp40, TASR1, SRp38	SRSF10	ND	ND
P54, SRp54	SRSF11	PPE	Exclusion
SRrp35	SRSF12	ND	ND

ND, not determined.

depending on the location of their binding site[28]. For example, hnRNP I can promote or suppress the inclusion of alternative exons depending on the location of its binding site relative to the exons[29]. The functions and localizations of splicing factors are highly regulated by post-translational modification[30-33]. Thus, the proteins involved in posttranslational modifications such as kinases and phosphatases, also participate in alternative splicing *via* modulating *trans*-acting proteins.

Due to more than one weak 5' and/or 3' splice-site, several alternative splicing patterns have been identified, which include exon skipping or cassette exons, mutually exclusive exons, alternative 5' splice-sites, alternative 3' splice-sites, intron retention, alternative promoter sites, and alternative polyadenylation sites (Fig. 1).

Effect of *cis*-elements on Tau Exon 10 Splicing

Exon 10 of the tau gene, which is flanked by an unusually large 13.6-kb intron 9 and a 3.8-kb intron 10, has a weak 5' splice-site and a weak 3' splice-site[34-36]. Thus, exon 10 can be included or skipped to produce tau proteins with or without exon 10, depending on the action of *trans*-acting proteins on the *cis*-elements located on exon 10 or introns 9 and 10.

Several short *cis*-elements in exon 10 and intron 10 of the tau gene regulate the exon 10 splicing by modulating the use of the weak 5' and 3' splice-sites[14, 37]. There is an SC35-like element (TGCAGATA) at the 5' end of exon 10 (Fig. 2). Deletion of this element promotes exon 10 exclusion in cultured cells[38]. Mutation of adenine (A) to

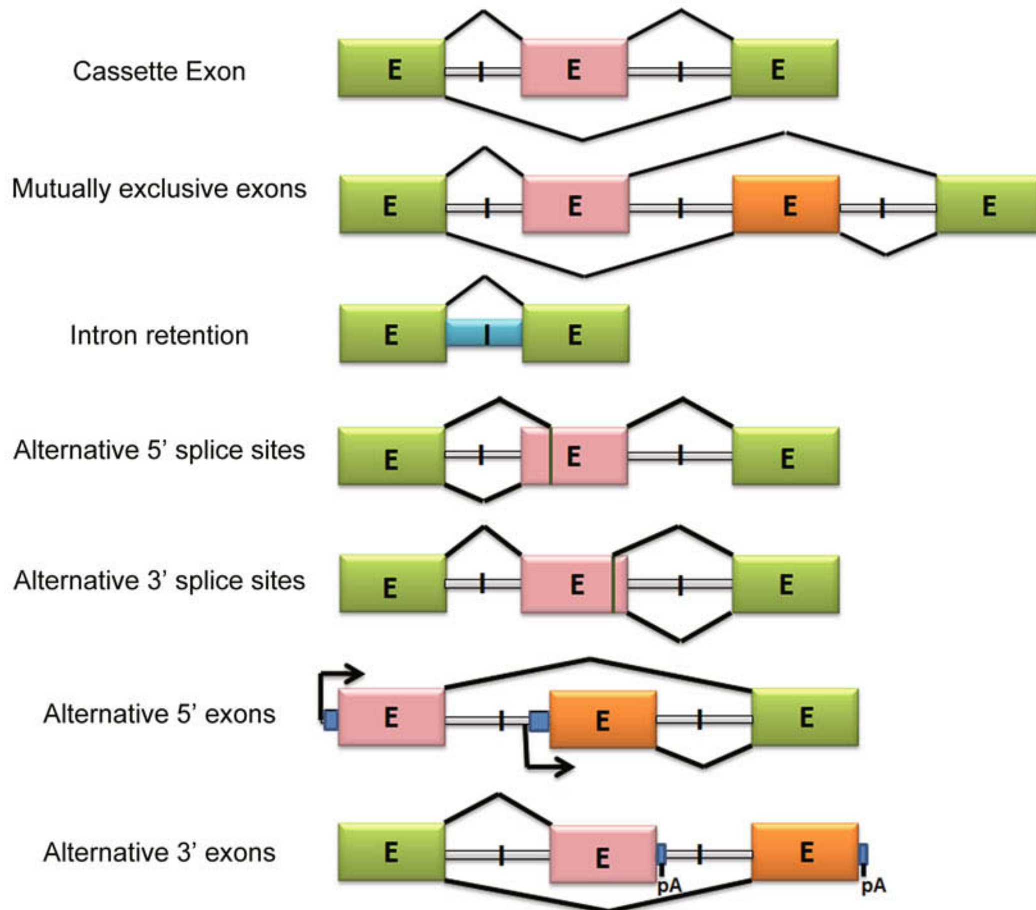

Fig. 1. Alternative splicing patterns have been identified. Boxes represent exons (E) and lines represent introns (I). Exons in green are constitutively spliced and those in pink or orange are alternatively spliced.

guanine (G) at base 4 of this element (A4G) promotes exon 10 inclusion, but G5A mutation suppresses its inclusion[38], suggesting that the adenine may be important for the enhancer. However, so far, no disease-related mutations have been reported in this element.

Following the SC35-like element at the 5' end of exon 10 is a polypurine enhancer (PPE). Two FTDP-17T mutations, N279K and Δ280, have been identified in this element[39-41] (Fig. 2). Deletion of the AAG bases seen in the Δ280 mutation impairs PPE and a change of thymine (T) to guanine (G) in the N279K mutation enhances PPE (Fig. 2), resulting in suppression and promotion of exon 10 inclusion, respectively[39-41]. The third element in exon 10 is an A/C-rich enhancer (ACE). Mutation of T to cytosine (C) in L284L strengthens ACE, thus promoting exon 10 inclusion, resulting in an increase of the 4R-tau:3R-tau ratio in human brain[42]. In addition, there is a silencer and an enhancer at the 3' end of exon 10. Many FTDP-17T-associated mutations have been found in these elements (Fig. 2)[14, 37].

At the 5' end of intron 10, there is an ISS and an intronic splicing modulator (ISM) (Fig. 2). The ISM is not an enhancer by itself, but functions only in the presence of the ISS and counteracts the ISS-mediated inhibition of the 5'splice-site. Reverse effects of the ISS and ISM on E10 splicing have been shown by deletion assay[36].

A high degree of self-complementarity leads to the formation of a stem-loop at the exon-intron interface at the 3' end of exon 10 and the 5' end of intron 10 (Fig. 2). Disruption of this self-complementarity or destabilization of this stem-loop structure makes this region more available for U1 snRNP, resulting in exon 10 inclusion and 4R-tau expression. In rodents, this stem-loop structure is destabilized by the replacement of adenine (A) with guanine (G) at position E10+13, which is also seen in FTDP-17T (Fig. 2)[36]. Thus, this replacement might explain why 4R-tau is predominantly expressed in the brains of adult mice and rats. To date, eleven mutations causing FTDP-17T have been found to be clustered in this stem-loop region. Most of them disrupt the complementarity of the stem-loop and promote exon 10 inclusion (Fig. 2)[36].

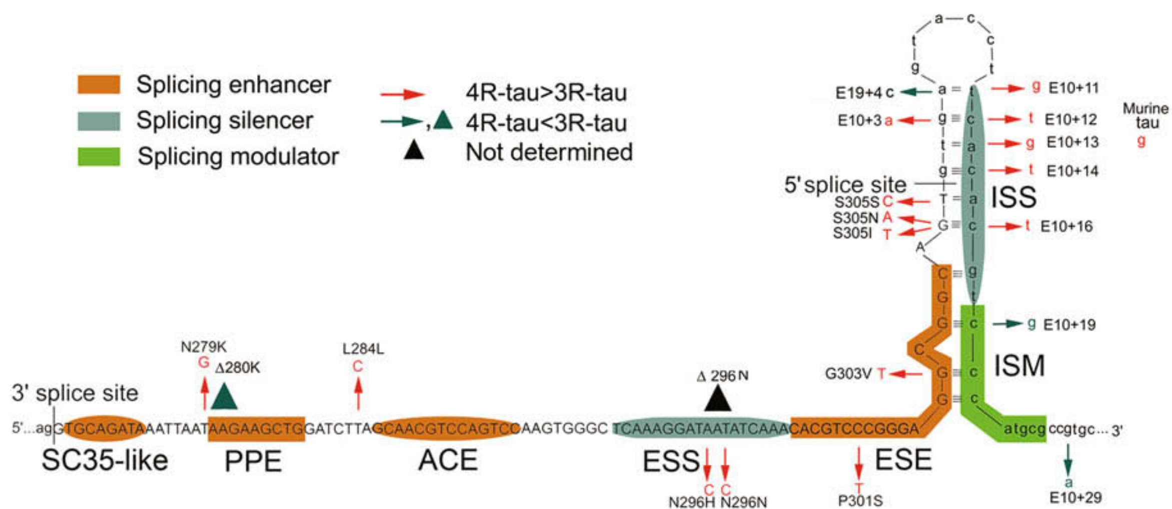

Fig. 2. Structure of exon 10 and intron 10 of the tau gene. Exon 10 is shown in capital letters and part of the flanking introns 9 and 10 are shown in lower case. There are three ESE (SC35-like enhancer, PPE, and ACE) at the 5' end of exon 10 and bipartite *cis*-elements (an ESS and an ESE) at its 3' end. Intron 10 elements include an ISS and an ISM. There is a stem-loop structure at the interface between exon 10 and intron 10. Mutations that cause an increase (red), decrease (dark green), or a not yet known change (black) in the 4R-tau:3R-tau ratio are indicated. Triangles indicate deletion mutations. ACE, A/C-rich enhancer; ESE, exonic splicing enhancer; ESS, exonic splicing silencer; ISM, intronic splicing modulator; ISS, intronic splicing silencer; PPE, polypurine enhancer.

Regulation of the Alternative Splicing of Exon 10 by SR proteins

SR proteins play important roles in the regulation of tau exon 10 splicing. However, different SR proteins modulate the alternative splicing differently. Several studies have shown that SRSF1 (ASF/SF2), SRSF2 (SC35), SRSR6 (SRp55), and SRSF9 (SRp30c) promote exon 10 inclusion, but SRSF3, SRSF4, SRSF7, and SRSF11 suppress its inclusion[43-50]. SRSF1 (ASF/SF2) is well-studied in terms of the element on which it works to promote exon 10 inclusion. SRSF1 acts on the PPE element of exon 10. Deletion or site-mutation of this element abolishes its role, suggesting it plays a critical and regulatory role in exon 10 inclusion[36].

SRSF2 (SC35) acts on an SC35-like element located at the 5' end of exon 10 (Fig. 2). Overexpression of SRSF2 promotes exon 10 inclusion of a mini-tau gene, pCI/SI9-SI10 or pCI/SI9-LI10[38, 51]. Knockdown of SC35 significantly inhibits 4R-tau expression[38]. Deletion of GCAGATA in the SC35-like element in the mini-tau gene, SI9-SI10, suppresses exon 10 inclusion, suggesting a critical role of this element in exon 10 inclusion[38]. Overexpression of SC35 fails to promote exon 10 inclusion in the mutant mini-tau gene in which SC35-like element has been deleted[38]. Thus, SC35 promotes exon 10 inclusion mainly by acting on SC35-like elements.

In contrast, 9G8 (SRSF7) represses exon 10 splicing and induces its exclusion[50, 52]. It has been reported that 9G8 may interact with the proximal downstream intron of exon 10 directly and suppress its inclusion[50]. The cytosine (C) at position +14 of intron 10 is thought to be critical for the interaction between 9G8 and tau pre-mRNA. Mutation of C to T, named M14, which is also an FTDP-17T-related mutation, abolishes 9G8 action in COS7 cells[50]. However, our recent study found that 9G8 still suppresses exon 10 inclusion of the mini-tau gene with M14 in HEK-293 cells[52], suggesting that in addition to the ISS of intron 10, 9G8 may also work on other elements to suppress exon 10 inclusion.

In addition to the above SR proteins, other RNA-interacting proteins may also be involved in tau exon 10 splicing. It has been reported that Tra2β works on PPE and promotes exon 10 inclusion[49]. Transient occlusion of the middle cerebral artery causes a decreased level of Tra2β leading to exon 10 exclusion and 3R-tau expression in cortical neurons[53]. hnRNP E2 and E3 moderately activate exon 10 splicing[54, 55]. RNA-binding motif protein 4 (RBM4) interacts with an ISE located in intron 10 and stimulates tau exon inclusion[56]. RNA helicase p68 regulates exon 10 splicing by modulating the stem-loop structure at the interface region of exon 10 and intron 10 in an RBM4-dependent manner[57]. However, another RNA/DNA-binding protein, polypyrimidine tract binding protein-associated splicing factor[58], interacts with the stem-loop structure and promotes exon 10 exclusion[59].

Regulation of Tau Exon 10 Splicing by Kinases

SR proteins contain many serine and threonine residues that can be phosphorylated. The phosphorylation level tightly regulates its localization and activity[60-63]. It has been shown that phosphorylation is required for the translocation of SR protein from the cytoplasm to the nucleus[64, 65] and the recruitment of SR proteins from nuclear speckles to nascent transcripts for splicing[33, 66]. SR protein kinase (SRPK) and cdc-like kinase (CLK/Sty) phosphorylate SR proteins and control their functions. SRPK1 phosphorylates the N-terminal region of the RS domain of SF2/ASF and leads to its translocation from the cytoplasm to nuclear speckles[30, 33], whereas CLK/Sty phosphorylates the C-terminal region of the RS domain and causes translocation of SF2/ASF within the nucleus[30]. In addition, several kinases have been reported to phosphorylate the SR proteins and modulate the alternative splicing, including DNA topoisomerase I[67], cAMP-dependent protein kinase (PKA)[68], dual-specificity tyrosine-phosphorylated and regulated kinase 1A (Dyrk1A)[38, 51, 52, 69], glycogen synthase kinase-3β (GSK-3β), and AKT[70, 71].

Dyrk1A is a proline/arginine-directed Ser/Thr protein kinase. It lies at the Down syndrome critical region of chromosome 21[72]. We recently found that Dyrk1A phosphorylates ASF/SF2 (SRSF1) at Ser227, Ser234, and Ser238 in its RS domain and represses SRSF1-promoted tau exon 10 inclusion, resulting in increased 3R-tau expression[51]. Phosphorylation of SC35 (SRSF2) or SRp55 (SRSF6) by Dyrk1A also suppresses its function[38, 69]. Overexpression of Dyrk1A in Down syndrome due to an extra copy of the gene may dysregulate exon 10 and result in increased 3R-tau, leading to the early-onset tau pathology in individuals with Down syndrome[51]. In addition, Dyrk1A participates in the regulation of the

splicing of other pre-mRNAs and delays the switching of isoforms of Ca^{2+}/calmodulin-dependent kinase IIδ[73] and troponin T (unpublished observation) from fetal to adult forms in the Ts65Dn animal model of Down's syndrome, suggesting that Dyrk1A may be involved in the regulation of heart development.

GSK-3β is a key protein kinase that interacts with several proteins such as tau and APP involved in the etiology of AD[74]. GSK-3β interacts with and phosphorylates SC35. Inhibition of GSK-3β with LiCl in cultured neurons increases tau exon 10 inclusion[44]. Aβ promotes exon 10 exclusion *via* activation of the GSK-3β-SC35 pathway[75]. Thus, upregulation of GSK-3β in the AD brain may dysregulate exon 10 splicing, resulting in an increase of 3R-tau, which may contribute to the tau pathology in this disease.

PKA also regulates exon 10 splicing. It phosphorylates ASF/SF2 and SC35 and enhances their functions in the splicing. However, the action of PKA on SF2/ASF shows a catalytic subunit-specific effect. PKA-Cα, but not PKA-Cβ, interacts with SF2/ASF and enhances its splicing activity in exon 10 splicing[68]. All isoforms of PKA-C enhance SC35-promoted exon 10 inclusion[76]. In the AD brain, down-regulation of PKA due to calpain I activation is correlated with increased 3R-tau expression[68]. Therefore, dysregulation of protein kinases may not only phosphorylate tau, but also dysregulate exon 10 splicing and contribute to tau pathogenesis.

SR proteins play essential roles in alternative splicing. Phosphorylation of SF2/ASF by SRPK1 and CLK/Sty promotes its translocation and engagement in splicing. Dephosphorylation of SR proteins is required for maturation of the spliceosome and further steps in RNA processing. Compared with the phosphorylation of SR proteins by kinases, little is known about their dephosphorylation. It has been reported that protein phosphatase 1 (PP1) binds to the conserved RVDF sequence located on the RNA recognition motif of splicing factors, such SF2/ASF, Tra2-β1 and SRp30c, and dephosphorylates them[77]. PP1 removes phosphates from SF2/ASF in a preferred N-to-C-terminal manner[78]. We recently also found that PP1, but not PP2A, PP2B, and PP5, interacts with SF2/ASF, and overexpression of PP1 suppresses exon 10 inclusion (unpublished data).

Tau Gene Mutations in Tauopathies

To date, at least 59 mutations in human tau gene have been identified in exons 1, 2, 4, 9, 10, 11, 12, and 13 and introns 8, 9, and 10 (Table 2). Most of the mutations are associated with FTDP-17T[79], and several cause other types of tauopathy, such as PSP, Pick's disease and CBD[80-84]. These mutations can be divided to two groups. (1) Missense or deletion mutations that influence the interaction between tau and microtubules. There are 43 missense mutations located in the coding regions in exon 1 (R5H and R5L), exon 2 (G55R and V75A), exon 4 (Q124), exon 7 (A152T), exon 9 (K257T, I260V, L266V, G272V, and G273R), exon 10 (N279K, ΔK280, ΔN296, ΔN296H, P301T, P301L, P310S, G303V, G304S, S305N, and S305I), exon 11 (L315R, K317M, S320F, S320Y, and P332S), exon 12 (G335S, G335V, Q336R, V337M, E342V, S352L, S352V, S356T, V363I, V363A, P364R, G366R, and K369I), and exon 13 (G389R, R406W, N410H, and T421M). (2) Silent or intronic mutations that do not change the primary sequence of tau, but alter exon 10 splicing and consequently change the 3R-tau:4R-tau ratio. These mutations include L284L, N296N, S305S, L315L, E9-15, E9+33, E10+3, E10+4, E10+11, E10+12, E10+13, E10+14, E10+16, E10+19, and E10+29. In addition, some missense and deletion mutations also disrupt normal exon splicing of the tau gene, including two deletion mutations (Δ280K and Δ296N) and eight missense mutations located in exons 10, 12, and 13. Most of the disease-related tau mutations promote exon 10 inclusion, leading to increased expression of 4R-tau. However, a few mutations, such as Δ280K, E9-15, E10+4, E10+19, and E10+29, enhance exon 10 exclusion, resulting in increased expression of 3R-tau. Moreover, the L266V and G272V mutations are associated with 3R-tau deposition in Pick bodies[85, 86], which may also be due to the increased 3R-tau expression even though it has not been reported whether they alter the exon 10 splicing. The discovery of splicing mutations in FTDP-17T demonstrates that neurodegeneration and dementia can be caused merely by disruption of the 3R-tau:4R-tau balance alone. Equal levels of 3R-tau and 4R-tau are important for maintaining normal brain functions. The characterization of FTDP-17T firmly places tau and, specifically, its splicing, directly upstream of the process that causes neurodegeneration and dementia[79, 87].

Table 2. Mutations in the tau gene associated with FTDP-17T and related tauopathies

Exons	Mutations	Introns	Mutations
1	R5H, R5L		
2	G55R, V75A		
4	Q124E		
7	A152T	8	E9-15
9	K257T, I260V, L266V, G272V, G273R	9	E9+33
10	N279K, ΔK280, L284L, L284R, N296N, N296H, ΔN296, P301T, P301L, P301S, G303V, G304S, S305I, S305N, S305S	10	E10+3, E10+4, E10+11, E10+12, E10+13, E10+14, E10+16, E10+19, E10+29
11	L315L, L315R, K317M, S320F, S320Y, P332S		
12	G335S, G335V, Q336R, V337M, E342V, S352L, S356T, V363I, V363A, P364R, G366R, K369I		
13	G389R, R406W, N410H, T427M		

Underlined: mutations that alter exon 10 splicing or are associated with specific isoform deposition.

Fourteen mutations within six elements (PPE, ACE, ESS, ESE, ISS, and ISM) have been identified in individuals with tauopathies, and they promote or inhibit exon 10 inclusion. Among them, 11 FTDP-17T mutations are clustered in the stem-loop region residing in the exon-intron interface at the 3' end of exon 10 that displays a high degree of self-complementarity.

In addition to FTDP-17T, abnormal exon 10 splicing in both familial and sporadic cases may lead to other human neurodegenerative disorders such as PSP, Pick's disease, and CBD. Only 3R-tau inclusions have been found in the brains of both familial and sporadic cases of Pick's disease[88, 89]. In most of the cases of PSP and corticobasal degeneration, 4R-tau is up-regulated[88]. Tau pathology is present in Down syndrome cases ~20 years earlier than in sporadic AD. The increased 3R-tau:4R-tau ratio in the Down syndrome brain suggests that the imbalance in tau isoforms may have an effect on early-onset tau pathology[51].

Several studies on the mRNA of tau isoforms in sporadic AD showed either an increase in 4R-tau[90, 91] or no change[92, 93]. Thus, it is believed that the alternative splicing of exon 10 is not disrupted in the AD brain. However, by immunohistochemical staining, Espinoza and colleagues found that some advanced AD cases had a large amount of 3R-tau-positive, but not 4R-tau-positive, NFTs that were positive for thioflavin-S. More severe pathology appears in association with more abundant 3R-tau-positive tangles[94]. These findings suggest that aggregation and deposition of 3R-tau may be associated with more advanced stages in AD.

Thus, the 1:1 ratio of 3R-tau:4R-tau bound to microtubules is critical for maintaining the normal dynamics of microtubules in mature human neurons. Dysregulation of exon 10 splicing results in redundant amounts of either 3R-tau or 4R-tau, leading to increased free 3R-tau or 4R-tau in the cytoplasm. Compared with the microtubule-bound tau, free tau is more vulnerable to hyperphosphorylation and aggregation into NFTs[95]. In addition, the tau isoforms might be phosphorylated differentially. *In vitro* 4R-tau is a more favorable substrate for phosphorylation by rat brain protein kinases and is phosphorylated faster and to a greater extent than 3R-tau, at multiple sites including Ser199, Ser202, Thr205, Thr212, Ser214, Thr217, Thr231, Ser235, Ser262, Ser396, Ser404, and Ser422[96]. However, we still cannot conclude whether 3R-tau or 4R-tau is more toxic. It seems more likely that the disrupted 4R-tau:3R-tau ratio is the key for tau-related neurodegeneration.

Concluding Remarks

The microtubule-associated protein tau plays critical roles in neuronal microtubule dynamics. The adult human

brain expresses six isoforms of tau by alternative splicing of exons 2, 3, and 10. Alternative splicing of exon 10 produces 3R-tau or 4R-tau. Several mutations of the tau gene in FTDP-17T and related tauopathies lead to abnormal exon 10 splicing and consequent alteration of the 3R:4R-tau ratio, resulting in neurodegeneration and dementia. This indicates that abnormal exon 10 splicing is sufficient to induce neurodegeneration and dementia. Alternative splicing of exon 10 is regulated by *trans*-acting factors acting on c*is*-elements located mainly on exon 10 and intron 10. The function of the splicing factors is tightly regulated by phosphorylation, suggesting that kinases and phosphatases may also be involved in the regulation of exon 10 splicing. Dysregulation of either kinase or phosphatase activity may cause abnormal expression of 3R-tau and 4R-tau *via* altered exon 10 splicing and contribute to tau pathogenesis.

To date, many crucial questions about tau remain to be answered. How does the imbalance of 3R-tau and 4R-tau result in neurodegeneration? Which tau isoform is neurotoxic? What is the molecular mechanism underlying the regulation of exon 10 splicing? Answers to these questions will provide new insights into the mechanisms underlying these tauopathies and help to identify new therapeutic targets for these disorders.

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