Tau mis-splicing in the pathogenesis of neurodegenerative disorders

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INTRODUCTION

Tau proteins, which stabilize the structure and regulate the dynamics of microtubules, also play important roles in axonal transport and signal transduction. Tau proteins are missorted, aggregated, and found as tau inclusions under many pathological conditions associated with neurodegenerative disorders, which are collectively known as tauopathies. In the adult human brain, tau protein can be expressed in six isoforms due to alternative splicing. The aberrant splicing of tau pre-mRNA has been consistently identified in a variety of tauopathies but is not restricted to these types of disorders as it is also present in patients with non-tau proteinopathies and RNAopathies. Tau mis-splicing results in isoform-specific impairments in normal physiological function and enhanced recruitment of excessive tau isoforms into the pathological process. A variety of factors are involved in the complex set of mechanisms underlying tau mis-splicing, but variation in the cis-element, methylation of the MAPT gene, genetic polymorphisms, the quantity and activity of spliceosomal proteins, and the patency of other RNA-binding proteins, are related to aberrant splicing. Currently, there is a lack of appropriate therapeutic strategies aimed at correcting the tau mis-splicing process in patients with neurodegenerative disorders. Thus, a more comprehensive understanding of the relationship between tau mis-splicing and neurodegenerative disorders will aid in the development of efficient therapeutic strategies for patients with a tauopathy or other, related neurodegenerative disorders. [BMB Reports 2016; 49(8): 405-413]

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TAU ALTERNATIVE SPlicing AND ISOFORM-Specific FUNCTIONS

The tau protein is encoded from the MAPT gene, which is located at chromosome 17q21 (14). There are 16 exons in the MAPT gene and exons 2, 3, 4A, 6, 8, and 10 can be alternatively spliced (11). Exons 4A, 6, and 8 are not transcribed in the brain; thus, six isoforms are produced in the brain through different combinations of the splicing of exons 2, 3, and/or 10 (Fig. 1). Exons 2 and 3 are translated into the N1 and N2 aspects of the N-terminal microtubule-binding repeat domain, respectively (15), which play important roles in signal transduction and membrane interactions (16, 17). The encoding region of exon 10 is the second aspect of the C-terminal microtubule-binding repeat domain, R2, and the resulting tau proteins become either 3R or 4R tau, which differ in the number of repeats depending on the splicing of exon 10 (15). Because the microtubule-binding repeat domain of tau is its binding site to a microtubule (18), it is essential for the ability of the tau protein to maintain the stability, and regulate the dynamics, of microtubules (19), as well as to support axonal transport (1).

The difference in the number of repeats determines the strength of the binding of the tau protein to microtubules; 4R tau binds to microtubules more tightly than 3R tau, which is better for stabilizing the microtubule (20), but the extra repeat makes it more likely that 4R tau will aggregate (21, 22). Additionally, the dynamics of both retrograde and anterograde axonal transport are higher for the 3R isoform than the 4R isoform (23). The tau isoform-dependent differences in microtubule-binding capacity and axonal transport may explain the benefits of changes in tau isoforms during various developmental stages; 3R tau is the main isoform present in the fetal stage, during which the dynamic nature of the axon is an important requirement for synaptogenesis and establishing neural pathways (24). On the other hand, the overall ratio of 3R to 4R in the mature human brain is maintained at 1:1 (11) even though the relative amounts of these isoforms vary according to brain region and cell type. For example, granule cells in the hippocampal only express the 3R tau isoform (24, 25) and this difference is thought to provide the cells in this region with a particular resistance or susceptibility to specified tauopathies (25-27).

Alternative splicing of the tau protein can also occur at exons 2 and 3 to produce the 0N, 1N, and 2N tau isoforms, which differ in the number of amino-terminal (N-terminal) inserts. Interestingly, exon 3 is spliced only when exon 2 is present (11, 13); thus, the 1N isoform is produced from a combination of exon 2+ exon3− but not from exon2− exon3+. The relative amounts of the N-terminal isoforms are regulated in the human adult brain such that the 2N isoform is the most abundant (28). This difference does not seem to have a direct impact on microtubule assembly (15) but it was recently suggested that the N-terminal projection domain plays an active role during the regulation of microtubule stabilization (29). When the tau protein is truncated at Gln124 in the N-terminal due to the deletion of repeat inserts, there is an increase in its binding affinity to the microtubule compared to the full-length tau protein (29).

The 1N isoform, which contains an N1 insert from exon 2, enhances the self-aggregating tendency of the tau protein (30) while the N2 insert from exon 3, which has an additional N-terminal domain, attenuates the aggregation-promoting effects of the N1 insert (30). The N-terminal repeat inserts interact with various molecules in the human brain that are involved in synaptic signaling, energy metabolism, and cytoskeletal function. When the interaction proteins were analyzed according to the individual N-terminal inserts using bioinformatics with biological process enrichment, the N2 insert is the least expressed while the 1N isoform is the most abundant (28). The findings demonstrate that the N0, N1, and N2 isoforms behave differently according to brain region and cell type. For example, granule cells in the hippocampus only express the 3R tau isoform (24, 25) and this difference is thought to provide the cells in this region with a particular resistance or susceptibility to specified tauopathies (25-27).

MECHANISMS UNDERLYING THE SPlicing REGULATION OF THE TAU TRANSCRIPT

The assembly of the spliceosome, which is a multi-protein complex to the cis-acting pre-mRNA sequence, is an essential step in the splicing process (32). The cis-acting element is a short and diverse sequence that can be located in either the exon or intron; its influence differs depending on location and sequence. Based on their effects on splicing, cis-elements are classified as splicing enhancers, silencers, or modulators (32).
Table 1. Factors regulating the splicing of exon 10 of MAPT pre-mRNA

<table>
<thead>
<tr>
<th>cis-elements</th>
<th>Sequence</th>
<th>Effect on E10</th>
</tr>
</thead>
<tbody>
<tr>
<td>SC35-like enhancer</td>
<td>E10, TGCAGATA</td>
<td>Inclusion</td>
</tr>
<tr>
<td>Polypyrimidine enhancer (PPE)</td>
<td>E10, AAGAAGCTG</td>
<td>Inclusion</td>
</tr>
<tr>
<td>A/C-rich enhancer (ACE)</td>
<td>E10, AGCAAAGTCCAGTCC</td>
<td>Inclusion</td>
</tr>
<tr>
<td>Exonic splicing silencer (ESS)</td>
<td>E10, TCAAAGGATAATATCAA</td>
<td>Exclusion</td>
</tr>
<tr>
<td>Exonic splicing enhancer (ESE)</td>
<td>E10, CACGTCCCGGAGGCGGC</td>
<td>Inclusion</td>
</tr>
<tr>
<td>Intronic splicing silencer (ISS)</td>
<td>I10, tcacacgt</td>
<td>Exclusion</td>
</tr>
<tr>
<td>Intronic splicing modulator (ISM)</td>
<td>I10, cccatgcg</td>
<td>Exclusion</td>
</tr>
<tr>
<td>SR proteins</td>
<td>Effect on E10</td>
<td></td>
</tr>
<tr>
<td>SRSF1: ASF, SF2, SRp30a</td>
<td>Inclusion</td>
<td></td>
</tr>
<tr>
<td>SRSF2: SC35, PR264, SRp30b</td>
<td>Inclusion</td>
<td></td>
</tr>
<tr>
<td>SRSF3: SRp20</td>
<td>Exclusion</td>
<td></td>
</tr>
<tr>
<td>SRSF4: SRp75</td>
<td>Exclusion</td>
<td></td>
</tr>
<tr>
<td>SRSF6: SRp55, BS2</td>
<td>Exclusion</td>
<td></td>
</tr>
<tr>
<td>SRSF7: SRSF3 9G8</td>
<td>Exclusion</td>
<td></td>
</tr>
<tr>
<td>SRSF9: SRp50</td>
<td>Exclusion</td>
<td></td>
</tr>
<tr>
<td>SRSF11: P54, SRp54</td>
<td>Exclusion</td>
<td></td>
</tr>
<tr>
<td>Other RNA binding proteins</td>
<td>Effect on E10</td>
<td></td>
</tr>
<tr>
<td>RBM4</td>
<td>Inclusion</td>
<td></td>
</tr>
<tr>
<td>Tra2β</td>
<td>Inclusion</td>
<td></td>
</tr>
<tr>
<td>DDX5 (RNA helicase p68)</td>
<td>Inclusion</td>
<td></td>
</tr>
<tr>
<td>hnRNPE2 and hnRNPE3</td>
<td>Inclusion</td>
<td></td>
</tr>
<tr>
<td>hnRNP-G</td>
<td>Exclusion</td>
<td></td>
</tr>
<tr>
<td>SWAP</td>
<td>Exclusion</td>
<td></td>
</tr>
<tr>
<td>CELF2</td>
<td>Exclusion</td>
<td></td>
</tr>
<tr>
<td>CELF3, TNRC4</td>
<td>Inclusion</td>
<td></td>
</tr>
<tr>
<td>PTBP2</td>
<td>Inclusion</td>
<td></td>
</tr>
<tr>
<td>PSF</td>
<td>Exclusion</td>
<td></td>
</tr>
<tr>
<td>miRNAs</td>
<td>Target SF</td>
<td>Effect on E10</td>
</tr>
<tr>
<td>miR-9</td>
<td>PTBP1</td>
<td>Exclusion</td>
</tr>
<tr>
<td>miR-124</td>
<td>PTBP1</td>
<td>Exclusion</td>
</tr>
<tr>
<td>miR-132</td>
<td>PTBP2</td>
<td>Exclusion</td>
</tr>
<tr>
<td>Kinases</td>
<td>Target SF</td>
<td>Effect on E10</td>
</tr>
<tr>
<td>CLK2</td>
<td>9G8</td>
<td>Exclusion</td>
</tr>
</tbody>
</table>

The MAPT gene mutations that are close to, or within, the cis-acting elements result in FTD with Parkinsonism linked to chromosome 17 (FTDP-17) and other tauopathies associated with mis-splicing (12). Many serine- and arginine-rich (SR) proteins possess a specific affinity for the cis-element of the MAPT gene and regulate the splicing of exon 10 (33) (Table 1). In addition to these proteins, several non-SR proteins also play a role in the splicing of exon 10. For example, RNA-binding motif protein 4 (RBM4) (34), Tra2β (35), RNA helicase p68 (36), heterogeneous nuclear (hn) RNP E2 and E3 (37, 38), and CUG-binding protein (CELF) (39, 40) are known to be involved. The variants that depend on the cis-element and splicing factors likely act cooperatively to determine the efficacy and direction of the splicing of exon 10 in the MAPT gene.

The regulation of alternative splicing processes is further complicated by variables arising from the altered expression and activity of splicing factors following modifications at the transcriptional, post-transcriptional, and post-translational levels. miRNA-132 is known to regulate the splicing of exon 10 via the inhibition of the expression of the PTBP2 protein, which is a splicing factor (41). Additionally, various kinases, including cyclic AMP-dependent protein kinase (PKA) (42, 43), dual-specificity tyrosine-phosphorylated and regulated kinase 1A (DYRK1) (44-47), and GSK-3β (48), regulate the activities of splicing proteins via phosphorylation and, thereby, exon 10 splicing. This issue has been well described in a recent review article (33). From an epigenetic point of view, the regulation of splicing by DNA methylation may also be involved in this process (26). The speed of RNA polymerase II during the elongation and reposition of splicing factors to alternative exons by heterochromatin protein 1 (HP1) is controlled by DNA methylation (49). Considering that a CpG island is present in exon 9, it is the possible that DNA methylation plays a role in regulating the alternative splicing of the MAPT.
gene (26) but there is a lack of studies investigating this issue.

Relative to the processes associated with the splicing of exon 10, the regulatory mechanisms underlying the alternative splicing of exons 2 and 3 are less clear. Several spliceosomal proteins involved in exon 10 splicing also regulate exon 3 splicing. For example, SRSF1, SRSF2, SRSF3, SRSF9, SWAP, Traβ, and Nova 1 decrease the inclusion of exon 3 while SRSF4 and SFSF6 enhance its inclusion (13). According to the linkage disequilibrium of nucleotide polymorphisms, the MAPT gene has two major haplotypes, H1 and H2, and the alternative splicing processes exhibit different patterns depending on haplotype. The H2 haplotype of the MAPT gene tends to include the exon 3 (27, 50). Furthermore, the transcriptional efficacy and DNA methylation patterns of the H1 and H2 haplotypes, which are described in detail below, also differ. In conjunction, the differential roles of the haplotypes are thought to contribute to haplotype-dependent tauopathies (51, 52).

TAU MIS-SPlicing AND NEURODEGENERATIVE DISORDERS

Genetic mutations in the MAPT gene

Genetic mutations in the MAPT gene can result in PSP, CBD, PiD, and FTDP-17. These pathogenic mutations of the MAPT gene are primarily located within exons 9-13 (Fig. 2) but are not limited to point missense and deleitional mutations in exons. In fact, silent, and even intronic, mutations can induce a tauopathy (12). The boundary of exon10/intron 10 includes an RNA sequence that forms a stem-loop due to self-complementary bindings at the stem and this region is a hot spot for MAPT gene mutations. Most of the pathogenic intronic mutations are clustered in the stem-loop where mutations induce the mis-splicing of exon 10 by decreasing stem-loop stability, which in turn increases the inclusion of exon 10. Subsequently, the altered RNA structure enhances the accessibility of the splicosome to this region and results in mis-splicing (36, 53). Ultimately, these intronic mutations clinically manifest as FTD in most cases, via increases in either the 3R or 4R tau isoform; IVS9-10 G>T, IVS10+3 G>A, IVS10+11 T>C, IVS10+12 C>T, IVS10+13 A>G, IVS10+14 C>T, IVS10+15 A>C, and IVS10+16 C>T tend to increase 4R tau (53-58) while IVS9-15 T>C, IVS10+4 A>C, and IVS10+19 C>G inhibit the inclusion of exon 10 and increase 3R tau (59, 60). L284L (CTT to CTC), N296N (AAT to AAC), and S305S (AGT to AGC) are silent point mutations that result in tauopathy, FTD, or PSP (61-63). Their location is close to the exon 10/intron 10 interface and increases the 4R tau isoform by enhancing the inclusion of exon 10. The mechanisms underlying the neurodegeneration caused by perturbations of the 3R-4R tau balance remain elusive but the isoform-dependent differences in the propensity for aggregation are thought to be pathologically when the 3R-4R balance is disordered (64).

Changes in the tau protein sequence due to exonic missense and deletion mutations do not always cause alterations in the ratio of the 3R and 4R tau isoforms. Instead, amino acid substitutions alter the tau structure into pathological forms (65). Interestingly, despite the fact that exon 10 is not mis-spliced, distinctive isoform-specific pathologies have been noted. RSH (66), RSL (67), L260V (68), P301L (57), G303V (69), and K317N (70) result in an increased propensity for aggregation and filament formation of 4R tau proteins without altering the 3R-4R tau ratio. The dominance of 4R tau isoform-specific pathologies in the absence of mis-splicing suggests that the 4R tau isoform is susceptible to becoming pathological following a mutation of the MAPT gene.

It is rare that pathogenic MAPT gene mutations will lead to the mis-splicing of exons 2 and 3. The E342V mutation in exon 12 causes an increased splicing of exon 10, but the reduced inclusion of exons 2 and 3 (71), and the tau inclusions in the R5L mutation of exon 1, primarily consist of 4R tau with either no insert or the N1 insert (ON4R or 1N4R) (67). The possible role of an altered number of N-terminal inserts in tauopathies can be considered based on the biological effects of N-terminal inserts in modifying tau aggregation and signaling pathways (16, 17, 30, 31). However, further studies are needed to clarify this issue.

Without genetic mutations in the MAPT gene

Isoform-specific tau pathologies are also observed in the absence of MAPT gene mutations in sporadic cases of PSP, CBD, PiD, AGD (4R tau), PiB (3R tau), and FTD (mixture of 3R and/or 4R tau). Overt tau mis-splicing can occur in these sporadic cases (72) and it has been suggested that the preference of haplotype for specified splicing is the mechanism underlying the alterations in alternative splicing (12). The H1 haplotype, particularly the H1c sub-haplotype, is
thought to increase the risk of PSP and CBD by increasing exon 10 splicing (73-76). However, a recent study that included a large sample size of brains found opposite results for the H1 haplotype but a protective influence of the H2 haplotype against PSP, CBD, and PD via increases in exon 3 (27), as has been previously suggested (50). Various combinations of haplotype-dependent genetic variations are known to modulate DNA methylation (77), transcription, and mRNA splicing (75, 78). Thus, the complicated interactions of these factors are thought to cooperatively determine the direction of tau exon splicing.

Differences in the expression and activity of spliceosomal proteins result in aberrant splicing and contribute to the manifestation of a tauopathy. In PSP patients, increases in SRSF2 and Tra2β in the locus coeruleus are associated with increases in the 4R tau isoform (79). And the decreases of miR-132 thereby increase of PTBP2 was shown to enhance 4R tau pathology in the PSP brain (41). The pathogenic role of tau mis-splicing in AD is controversial and has been previously reviewed (12), but recent reports have raised the possibility that its contribution to AD is due to increased DYRK2 activity in the brain, which continuously increases 3R tau expression and tau pathology (44).

Recent, accumulating evidence suggests the there is a discriminative relationship between tau isoforms and neurodegenerative disorders, which means that certain tau isoforms are more vulnerable to specific pathogenic factors and explains why there are isoform-specific pathologies and regional selectivity in tauopathies (27, 80). The relative ratio of tau isoforms varies across cell types and brain regions (27, 80) and specified cells and/or regions that contain greater amounts of specified tau isoforms tend to be more easily affected by corresponding disorders (7, 81). For example, the quantity of the 4R tau isoform is higher in the globus pallidum, which may explain why this region is particularly affected by the pathological processes of PSP (81). Likewise, granule cells in the hippocampus exclusively express 3R tau isoforms and the 3R tau-positive pick body is most abundant in PiD patients (25).

Tau mRNA mis-splicing may develop as a co-phenomenon of widespread RNA dysregulation during neurodegenerative processes. As a prime example, 4R tau aggregates have been identified in the striatum and cortex of Huntington’s disease (HD) patients (82) while 0N3R tau inclusions are found in DM1 patients (83, 84). DM1 and HD are caused by CTG (HD) patients (82) while 0N3R tau inclusions are found in the striatum and cortex of Huntington’s disease (25). In pathological conditions, the inclusion of FUS and TDP-43 as RNA processing proteins results in impaired physiological processes. The altered splicing of exons 3 and 10 in tau pre-mRNA has been observed in FUS-related proteinopathies (94) and it is thought that decreases in the propensity of FUS to directly bind to tau pre-mRNA alters the regulation of the splicing of exons 3 and 10 (94). In contrast, the TDP-43 proteinopathy does not impair tau pre-mRNA alternative splicing (95) despite the extensive RNA mis-processing exerted by the aggregation of TDP-43 proteins, which hinders its normal function as an RNA binding protein (96). Thus, the perturbation of tau pre-mRNA processing by neurodegenerative disorders is determined by the types of proteinopathies and RNAopathies. Future investigations will provide a clearer understanding of the relationship between tau mis-splicing and individual neurodegenerative disorders.

CONCLUSIONS

The pathogenic contributions of tau mis-splicing are likely highly correlated with the manifestation of neurodegenerative disorders via tauopathies as well as non-tau proteinopathies. This type of mis-splicing leads to an imbalance of tau isoforms that impairs isoform-specific, normal physiological function and enhances vulnerability to pathological processes. Current understanding of the relationship between tau mis-splicing and neurodegenerative disorders is originated from cases of MAPT gene mutations, which widened existing knowledge about the mechanisms underlying tau splicing. Several trials corrected exon 10 mis-splicing in MAPT gene mutations using small molecules (97), modified antisense oligonucleotides (98), or spliceosome-mediated RNA trans-splicing (99) but none of these studies progressed to clinical trials.

A variety of complex factors are involved in the regulation of the alternative splicing of tau. Differences in the integrity of the cis-element, methylation of the MAPT gene, genetic polymorphisms, quantity and activity of spliceosomal proteins, and patency of other RNA binding proteins appear to cooperatively impact alternative tau splicing. In sporadic cases of tauopathy that present with tau-isoform specific pathologies, these variables operate together to influence tau mis-splicing; thus, therapeutic strategies should be much more delicately designed. Current understanding of tau mis-splicing remains limited, especially in terms of its pathological role in non-tau proteinopathies, RNAopathies, and sporadic cases. Further studies should be performed to develop efficient therapeutic strategies for the treatment of these disorders.
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