TAU PROTEIN AND HUMAN TAUOPATHIES: AN OVERWIEV*

PROTEIN TAU IN HUMANE TAUPATIJE: PREGLED PODROČJA

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Abstract *The growing knowledge of the molecular mechanisms of neurodegenerative diseases is unveiling their common characteristics, enabling their classification according to the pathologically changed protein that aggregates in the diseased central nervous system.*

Due to aggregation of hyperphosphorilated microtubule associated protein tau in a large group of neurodegenerative diseases, mostly dementias, these diseases have been collectively called tauopathies. In the healthy adult brain, tau protein is found in six isoforms that contain either three or four microtubule-binding domains, which divides them in two groups, accordingly. In the pathological tau filaments, all six isoforms can be found, although their representation in the filaments varies among the diseases, as does the structure of the filaments, which can be paired helical, straight or random coiled. This allows for the classification of tauopathies into five classes, according to the tau isoforms composition and structure of filaments. The filaments aggregate intracellularly, forming the so-called fibrillary tau inclusions (FTI).

Today, the accurate diagnosis of tauopathies is possible only post mortem, when the spread of FTI across the brain is observed. The form and distribution of FTI differs among the diseases. They are detected by several neuropathological techniques, which differ in their efficacy to label tangles from different diseases. The causes for this differential labelling are still not understood.

There is no cure for tauopathies, but better efficacy of some drugs that may slow down the cognitive decline in the early stages of the diseases and the need for monitoring the drug effects are calling for early pre mortem diagnostic tools. New imaging techiques employing molecular labels for pathological tau aggregates promise to provide a sensitive diagnostic tool. In order to make it sufficiently specific, differential binding characteristics of molecular imaging probes to different forms of pathological tau should be carefully assessed and considered in developing imaging techniques for diagnosing tauopathies in vivo.

Key words *tau; fibrillary tau inclusions; tauopathies; diagnosis*

Izvleček *Odkrivanje molekularnih mehanizmov nevrodegenerativnih bolezni razkriva njihove skupne značilnosti. To omogoča njihovo klasifikacijo glede na patološko spremenjeni protein, ki agregira v bolnem živčevju. Pri veliki skupini nevrodegenerativnih bolezni – gre predvsem za demence – se odlaga z mikrotubuli povezani protein tau; zato to skupino bolezni poimenujemo taupatije. V možganih odraslega je tau prisoten v šestih izoformah, ki imajo bodisi tri bodisi štiri domene za vezavo na mikrotubule. To jih deli na dve skupini. V patoloških filamentih tau je lahko prisotnih vseh šest izoform, vendar se njihova zastopanost od bolezni do bolezni razlikuje. Prav tako se med boleznimi razlikuje tudi struktura filamentov, ki so lahko parno-helikalni, ravni ali naključno zviti. Filamenti se odlagajo znotraj celic in tvorijo tako imenovane fibrilarne inkluzije tau. Na podlagi zastopanosti izoform in oblike odlagajočih se filamentov delimo taupatije v pet razredov.*

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Natančna diagnoza taupatij je mogoča šele po smrti, ko opazujemo razširjenost fibrilarnih inkluzij tau v možganih. Njihova oblika in razporeditev po možganih se med boleznimi razlikujeta. Fibrilarne inkluzije tau zaznamo z različnimi nevropatološkimi tehnikami, ki različno uspešno označijo inkluzije v različnih boleznih. Vzroka za različno označevanje še ne poznamo.

Za taupatije še nimamo zdravila, toda nekatera zdravila lahko upočasnijo odlaganje tau v zgodnjih fazah bolezni. Pravočasna prepoznava bolezni in spremljanje njenega zdravljenja zahtevata zgodnja zaživljenjska diagnostična sredstva. Nove metode slikanja, ki uporabljajo molekularne sonde, ki se vežejo na patološke odlage tau, bi lahko postale občutljivo diagnostično sredstvo. Da bi bilo dovolj specifično, je potrebno natančno preučiti značilnosti različnih označevanj posameznih oblik patoloških odlag tau. To bi lahko omogočilo razvoj diagnostičnih tehnik za zaživljenjsko diagnozo taupatij.

Ključne besede *tau; fibrilarni vključki tau; taupatije; diagnoza*

Introduction

The growing knowledge about the molecular mechanisms of numerous human diseases is unveiling their common characteristics, enabling us to form new classifications and develop new strategies for their diagnostics and/or treatment. This is especially true for neurodegenerative diseases, which were initially described at the beginning of the twentieth century and classified according to the gross and microscopic pathological findings in the brain *post mortem,* but somehow remained marginal and mysterious, possibly due to the fact that they predominantly affect older population. Prevalence of dementia is still not known in many world regions.[1] But the ageing of the population and the growing insight into their molecular characteristics and pathophysiology are drawing neurodegenerative diseases into the centre of the modern biomedical research.

Although neurodegenerative diseases have different causes and are characterized by different morphological changes in the brain and clinical pictures, they all share some important common characteristics. They are progressive diseases which mostly affect the elderly and have two major hallmarks: the deposition of pathologically changed proteins in the central nervous system and the cell death of specific population of neurons. Due to the former characteristic, the neurodegenerative diseases fall into the family of the so-called conformational disorders.[2]

The most frequent neurodegenerative diseases are dementias, especially Alzheimer disease (AD), which affects 1 % of people aged 65 to 69 years and 20 % to nearly 50 % of people older than 85 years.[3]

Accumulation of pathologically modified proteins inside and in between the cells of the central nervous system is pathogenetically important. Normally there are one or two types of protein that predominate in every disease type. This has enabled a classification of the neurodegenerative diseases according to the predominant protein that characteristically accumulates in the affected brain, which is being increasingly accepted. The most common proteins that are pathologically modified in neurodegenerative diseases are microtubule-associated protein tau, beta-amyloid, alpha-synuclein and prion protein. The diseases with the accumulation of pathological form of at least one of these proteins are thus classified as being tauopathies, amyloidopathies, alpha-synucleinopathies, and/or prion diseses, accordingly. There is no effective treatment for neurodegenerative diseases.[4] We are currently inable to make an accurate diagnosis of sporadic and infectious neurodegenerative diseases *pre mortem.*[5]

The present review focuses on a major group of neurodegenerative diseases, the tauopathies, briefly presenting characteristics of tau pathology and possible novel ways of their diagnosis.

Microtubule associated protein tau

Microtubule associated protein tau is predominantly expressed in nerve cells where it is important in neurogenesis, axonal maintenance and axonal transport.[6] Tau is translated from mRNA and located predominantly in the cell body and the axons.[7] Its first known function is to promote microtubule assembly and stabilization.[8] Apart from microtubule-stabilising function, there appear to be also other functions of tau in the cell: it interacts with the plasma membrane[9] and actin filaments,[10] participates in signal transduction trough Src family tyrosine kinases[11] and regulates the multiple-motor based transport of cargoes along microtubules in the axon.[12]

When unbound to mictotubules, tau is a highly soluble protein without a secondary structure.[13]

There are six isoforms of tau in adult human brain which are generated by the alternative splicing of mRNA (Figure 1[14]). The transcripts from the tau gene located on the chromosome 17 that contains 16 exons differ in whether they contain exon 10, exon 2 and exon 3 (Figure 1). The resulting proteins differ in the presence or absence of the fourth microtubule binding domain (coded for in the exon 10) and the presence or absence of one (exon 2) or two (exons 2 and 3) N-terminal inserts.[15] The six isoforms are classified according to the number of microtubule binding domains (MBD) they contain into two functionally different groups: the ones with three MBD, called three repeat tau (3R-tau), and the ones with four MBD, called four repeat tau (4R-tau).[16] The smallest of the

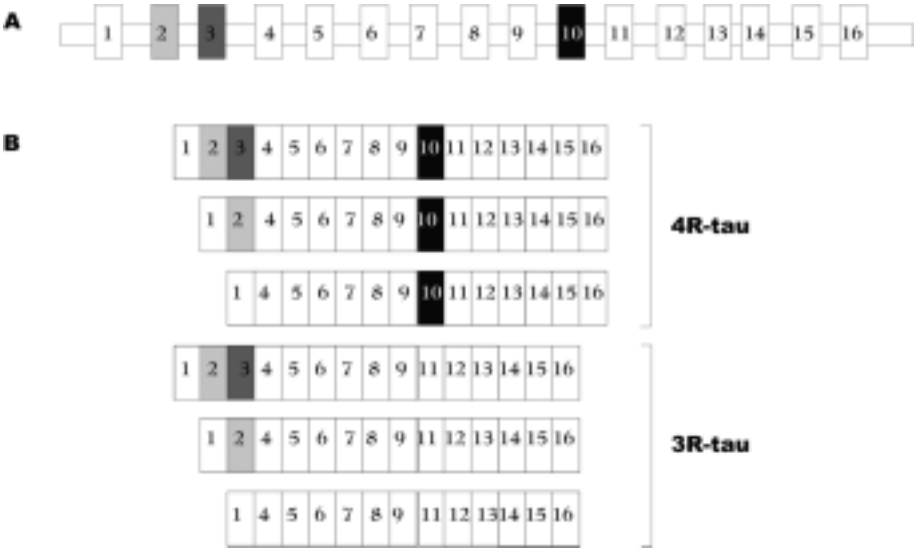

Figure 1. *In an adult brain, 6 tau isoforms are expressed due to alternative splicing.* **A** *– A schematic representation of tau gene. Exons are presented as rectangles. The picture is not in proportions.* **B** *– Alternative splicing of mRNA produces 6 tau isoforms. If the exon 10 is included, there are 4 microtubule-binding domains in tau (4-repeat tau (4R-tau)), if the exon 10 is missing, it results in 3 microtubule-binding domains (3-repeat tau (3R-tau)).*

six isoforms is expressed in the foetal brain and during the first days after birth.[17] Subsequently, the longer isoforms are expressed and their post-translational modification results in a reduced phosphorylation.[18] The longest isoforms of tau, the 4R tau, have a stronger affinity for microtubule binding. In a healthy adult there is an equal ratio of 3R/4R-tau. Changes in this ratio might result in neurodegeneration.[19]

Pathologically changed tau forms filaments

Tau is a phosphoprotein: the extent of its phosphorylation determines its binding abilities to microtubules and plasma membrane; the higher the phosphorylation the weaker the binding.[20] However, the aberrant phosphorylation of tau results in an increase of the concentration of the unbound phospho-tau in the cytosol, its accumulation and the formation of filaments which form the pathologic deposits in the form of intraneuronal filamentous inclusions observed in the diseased brain *post mortem.*[6] These intraneuronal filamentous inclusions were first revealed by different protocols of silver staining and were called neurofibrillary tangles (NFT) (21). While some authors reserve the name NFT only for the intracellular deposits of tau of typical shape as seen in AD,[22] other use it for all intracellular fibrillar tau deposits.[21, 23] In the present text, we will refer to these structures as fibrillary tau inclusions (FTI). Apart from FTI in neurons, fillaments of pathologically modified tau are present also in astrocytes and oligodendroglia that surround the FTI.[24]

Three types of tau filaments have been observed in different FTI: paired helical (PHF), straight (SF) and randomly coiled filaments (RCF).[21] The characteristic secondary structure of protein aggregates in neurodegenerative disease is beta pleated sheet, but the secondary structure of these filaments has not been conclusively determined, yet. PHF are the most thoroughly studied, as they are present in the NFT of AD, which has itself been the most thoroughly studied tauopathy. PHF have been successfully formed *in vitro* from various forms of recombinant tau protein (for a review on pathways of tau fibrilization see 25). The *in vitro* experiments suggest a beta-structured core of PHF[26, 13] which would make tau filaments a part of the large group of beta-structure rich fibrils, typical for various neurodegenerative diseases. However, there are contradictory results on the structure of PHF purified from Alzheimer disease-affected brain: some researchers are suggesting a beta-structured core of PHF[13] while others propose an alpha-helix rich core of PHF.[27] If the latter claims were experimentally confirmed, it would mean that non-beta-structured type of fibrils also can play a role in neurodegeneration.[6] Results of a study with synthetic fragments of tau seem to support this view.[28]

Tauopathies

Neurodegenerative diseases in which tau-containing FTI are found are called tauopathies. Different tauopathies are characterized by different types of tau filaments in the tangles as well as by a different combination of tau isoforms with specific phosphorylation pattern.[23] The forms of glial tau filaments resemble those in the neurons of the particular disease.[24]

Due to differences in tau phosphorylation and its isoform composition among the diseases a specific pattern of western blot results is obtained for every disorder, a sort of a taupathies »bar code«[23] (Figure 2). Each pattern of hyper phosphorylation of a certain tau isoform means a different band on the electrophoretic gel, as it has its own specific molecular mass. According to the bar code, tauopathies could be classified into five different classes.[23]

Class 0 is characterized by a loss of tau protein expression. Therefore no tau aggregates are observed. Frontal lobe degeneration (non-Alzheimer, non-Pick) is a representative of this class of disorders. It is the second most common pre-senile dementing disorder in Europe after AD.[23]

B

Class of tauopathy	Tau isoforms present and	Structure of tau filaments
0	loss of tau expression	
1	3R and 4R	PHF
2	4R	SF
3	3R	SF, RCF
4	3R	

Figure 2. *Tauopathies bar code. (Adapted from 23.)* **A** – *A schematic representation of electrophoretic distribution of tau isoforms characteristic for different classes of tauopathies. The tau bands are named after their molecular weight (in kDa).* **B** – *Tauopathy class characteristics (number of microtubule binding domain-repeats and structure of fibrilar aggregates): 3R: 3-repeat tau; 4R: 4-repeat tau, PHF: paired helical filaments, SF: straight filaments, RCF: random-coiled filaments.*

Table 1. *List of tauopathies with their classification. Adapted from 23.*

Tautopathy	Classification
Alzheimer disease	1
Amyotrophic Lateral Sclerosis parkinsonism-dementia complex of Guam	1
Argytrophylic grain disease	2
Cerebral ageing (elderly over 75 years)	1
Corticobasal degeneration	2
Dementia pugilistica	1
Down's syndrome	1
Familial British dementia	1
Frontal lobe dementia non Alzheimer non Pick	0
Fronto-temporal dementia with parkinsonism linked to chromosome 17	1, 2, or 3
Myotonic dystrophy type I	4
Niemann-Pick disease type C	1
Pallidonigroluysiab atrophy	2
Parkinson with dementia of Guadaloupe	1
Pick disease	3
Postencephalitic parkinsonism	1
Prograssive supranuclear palsy	2

Class 1 is characterized by three major bands at 60, 64 and 69 kDa and a minor one at 72/74 kDa. The bands represent all six tau isoforms. The majority of tauopathies belong to this class. The representative disease is AD.[23] PHF and straight filaments of tau are found in AD.[21, 24] Normal ageing of the human brain[29] and mild cognitive impairment (MCI), which might progress into AD,[30] are also classified as Class 1 conditions.

Class 2 has two bands at 64 and 69 kDa. They are formed of 4R tau. The examples of class 2 diseases are progressive supranuclear palsy (PSP), corticobasal degeneration (CBD) and argyrophilic grain dementia (AGD).[23] In this class of tauopathies pathological tau fibrillates in the form of straight filaments,[21, 24] although some random-coil filaments are present in CBD.[24]

Class 3 has a major tau doublet at 60 and 64 kDA that are composed of 3R tau. The only neurological disorder of this class is Pick disease,[23] where we find both straight and random-coil filaments.[21, 24]

Class 4 has one major tau band at 60 kDa, composed of the shortest tau isoform. The only disease in this class is myotonic dystrophy type I.[23]

Frontotemporal dementias with Parkinsonism linked to the chromosome 17 are familiar forms of tauopathies caused by the mutations in *tau* gene.[31] They may belong to classes 1, 2 and 3, depending of their tau aggregates characteristics.[23]

The common feature of tauopathies is that they are progressive. Therefore, different stages of the diseases can be observed based on the distribution and extent of brain pathology in diseases of all classes.

Diagnosing tauopathies

Currently, the most useful instruments for the assessment of dementias are neuropsychological tests.[32] However, they alone are insufficient to diagnose AD[33] and other diseases.

As different causes produce similar clinical picture of dementia, the accurate diagnosis of tauopathies is currently possible only *post mortem.*[34] The diagnosis and staging of the diseases is performed at brain autopsy. Although there is a considerable inter-observer variation in the identification of intraneuronal inclusions,[35] examining the spread of FTI in different brain regions is diagnostically highly reliable.[35] In AD, the so-called Braak staging is used.[36] There is a stereotyped, sequential, hierarchical pathway of spreading of NFT across the brain in AD and in brain ageing starting at transenthorinal cortex and following through enthorinal cortex, hippocampus, anterior temporal cortex, inferior temporal cortex, medium temporal cortex, polymodal association areas, unimodal areas, primary motor or sensory areas and finally all neocortical areas.[29] It might be asimptomatic until it reaches the polymodal association areas.[29]

The most accurate methods of pathological tau detection are silver staining methods and immunohistochemistry.[37] Labelling the aggregates with beta-structure-specific fluorescent labels has been proposed as a quicker and easier alternative. An example of such label is Thioflavin S (ThS), which has been shown to label NFT in AD comparably to the Gallyas silver staining method.[38] The mechanisms of label binding to tau aggregates have not yet been determined. However, it seems that ThS needs a large array of beta-sheet structure in order to be fluorescent, while Gallyas silver staining labels the fibrils in another (still unknown) manner.[38]

Because their labelling differs due to their different composition and structure, tau aggregates have not been consistently labelled by any method. It has been reported that different silver staining methods pre-

ferentially label tau-positive deposits according to their isoform: the Gallyas method detects 4R-tau, whereas the Campbell-Switzer method detects 3R-tau.[39, 40] Apart from isoform composition, the form of filaments present in FTI also affects their labelling: ThS labels PHF better than SF.[41] The variable labelling of tau aggregates from different tauopathies with different fluorescent molecular probes has been observed also in our laboratory. In our experience ThS most consistently labels FTI from PSP, a tauopathy of class 2, with 4R tau aggregates. NFT from AD, a representative of the class 1 of tauopathies, where both, 3R and 4R tau is aggregated, are labelled less extensively with ThS. Interestingly, ThS does not bind 3R tau containing Pick bodies in PiD, a tauopathy of class 3. Since ThS needs an array of beta-sheet structure to be fluorescent, this variability in probes binding to different tau depositions could mean their different secondary structure. It has been suggested that tau might form also α-helical aggregates *in vivo* and independently of amyloid formation.[42]

Emerging methods of *pre mortem* diagnosis

Evidence shows that medications are more effective in slowing the cognitive decline if administered early in the course of dementia. This finding emphasizes the importance of early *pre mortem* diagnosis and longitudinal investigations of therapeutic interventions.[43] The most promising emerging diagnostic approaches are relatively non-invasive and aim to detect the disease in its early symptomatic stage. They were initially developed for AD, and are being expanded to other tauopathies.

One approach is to determine disease-specific brain atrophy or/and changes in brain function using different brain-scanning techniques. The structural changes can be observed with computer tomography[44] or magnetic-resonance imaging.[45] Due to the dying of specific neuronal populations, characteristic patterns of atrophy can be observed in tauopathies. However, the amount of brain atrophy does not always mirrror the extent of cognitive decline. In mice with induced brain atrophy and cognitive decline by tau-overexpression, there was a cessation of cognitive decline and even an amelioration of spatial memory when the overexpressing tau-gene was shut down.[46] Thus the relationship between cognitive function and brain atrophy in tauopathies requires additional experimental clarification.

Another diagnostic approach employs indirect biomarkers to detect pathological changes of proteins in the living brain of affected individuals. As the changes of the brain are reflected in the cerebrospinal fluid (CSF) which is in direct contact with the extracellular space of the brain, changes of tau can be observed in CSF.[47] In AD the decreased levels of beta amyloid 1–42 (one of the two fragments of amyloid precursor protein that accumulate in amyloid plaques of AD) and increased levels of total tau, are observed together with the increased level of phospho-tau in CSF. These, however, do not reflect the clinical course of the disease over time.[48] Novel possible biomarkers are being searched for among proteins found in CSF.[30]

Clinical course of the disease could be matched to the conformational pathology by brain-scanning techniques which use brain perfusion with molecular probes that bind to pathologically aggregated proteins in the brain. These techniques were first developed for beta-amyloid imaging but due to ultrastructural similarities between the protein deposits they may detect tau as well. In this way, the spread of amyloid-beta and tau pathology across the brain can be observed, similar to *post mortem* neuropathological staging. The emerging field of this type of scanning is called molecular neuroimaging.[49] It mainly employs the techniques of positron emission tomography (PET) and single photon emission computed tomography (SPECT). For an introductory review to these techniques, see Smid and Bresjanac[50] in this issue of Zdravniški vestnik.

Although the majority of AD diagnostic approaches thus far have been developed primarily for the detection of amyloid plaques, formed by beta-amyloid, which have long been considered the main target for AD neuroprotection and treatment, novel data suggest that pathological tau is a key culprit causing neurodegeneration and should thus be targeted in diagnostic as well as treatment attempts.[51, 52] Due to the similar ultrastructural characteristics of the fibrils of pathological beta amyloid and tau deposits in the brain some of the same molecular neuroimaging probes are being tested for detecting both.[53] However, as indicated by the *in vitro* labelling and ultrastructural studies, tau may require a more selective approach. Different tau isoforms may combine between themselves and with other molecules to build different supramolecular structures like PHF, SF and random coiled filaments. This is the likely reason why no single diagnostic approach has been able to successfully label all pathological tau aggregates, or – alternatively – to reliably distinguish between them. Novel probes are being developed that seem to specifically label tau aggregates while not labelling beta-amyloid senile plaques in AD.[54] A recent study employing a nonradioactive analogue of a PET probe [^{18}F]FDDNP confirmed the need for thorough assessment of labels for each disease: fluorescent FDDNP labelled 31 % of NFT in AD brain tissue samples, while it was not found to label FTI from PSP or PiD samples.[55]

Conclusions

Alternative splicing of tau mRNA produces 6 tau isoforms, which have different roles in neuronal physiology. These six isoforms are also differently represented in pathological fibrillar deposits of tau. In this way, a single protein pathogenetically contributes to an array of different diseases of the CNS. Most of these disorders, called tauopathies, are clinically manifested as dementias. They share many common characteristics but differ in the combination of tau isomers their

FTI are composed of and the type of the filaments they form. The differences they bear make them challenging to detect either neuropathologically or by *in vivo* diagnostic tools. On the other hand the noted differences offer a potential for differential diagnosis and may also allow the development of a cause-specific therapy

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